WWMR2010

Joint EUROMAR 2010
and 17th ISMAR Conference

Book of Abstracts

Florence, July 4-9, 2010

This book has been edited by
Stefano Chimichi
Claudia Andreini
Francesca Cantini
Angelo Gallo

We would like to thank those who have assisted in the editing of this book of abstracts:
Enrico Morelli, Gabriele Cavallaro, and all of the 'CERMians'

Electronic Version 1.1.1

Foreword

480 participants asked the organizers to provide a printed book of abstracts; I therefore take the opportunity to welcome the 1190 persons registered at the time of this writing as active participants. Let's hope that the conference will be satisfactory for all of us. Over fifty people have been involved in the organization, from the electronic set-up, to the collection of abstracts, to fundraising and the organization of events such as the romantic sunset reception at Boboli Gardens. The scientific breadth of the conference is extensive and I hope that everyone will find his/her niche of interest as well as a broad vision of the field. I would like to remind you that the first chemistry conference was held in Karlsruhe in 1860 and that there were 140 participants. At that conference Cannizzaro brought Avogadro's principle, which allowed the determination of molecular weights, to the attention of the participants. Since then the atomic weight of carbon moved from 6 to 12 and that of oxygen from 8 to 16. Of course Mendeleev, who was a participant, enjoyed the conference. Here we risk being 1200, and we risk missing the great achievements. The conference will therefore be closed by three speakers, who will try to summarize the main achievements in their field at the plenary level.

This book, besides the abstracts of the various actors, contains the names of the members of the Program Committee. There are fifteen of them, and they have contributed to suggesting a group of invited speakers with a wide range of competences. There is also a National Welcoming Committee, which has encouraged us, the local organizers, to overcome those difficulties that are of course encountered when you undertake big challenges. Then there are the various committees composed of colleagues and pupils from the University of Florence who, as mentioned, have taken care of many important things as well as many details. As far as this book is concerned, I would like to highlight the Abstract Committee (Stefano Chimichi, Francesca Cantini, Claudia Andreini, and Angelo Gallo). Most if not all of you have interacted with Francesca Morelli. She has been the soul of the organization. Another soul has been Paola Turano, who took care of every activity involving more than 1 Euro, from the young participant grants to the renting of the convention center. Then of course there are Claudio Luchinat and Lucia Banci, who have not done much because they assisted me, and I haven't done anything. Still, I hope that we contribute to providing a pleasant, fruitful, and stimulating atmosphere.

Ivano Bertini
Chair

Florence, June 16th, 2010

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Contents

1 Ampere, ISMAR and Andrew Prize Lectures

Ampere Prize Lecture

Energy Storage and Conversion: Using Local Structural Probes to Understand and Optimise the Functioning of Battery and Fuel Cell Materials

Baris Key[a], Rangeet Bhattacharyya[a], Lucienne Buannic[a], Frederic Blanc[a] and Clare P. Grey[a,b]

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The application of new NMR approaches to correlate structure and dynamics with function in materials lithium-ion batteries and solid oxide fuel cells will be described. A particular focus is the development of methodology to allow these systems to be investigated *in-situ*, i.e., under realistic operating conditions. This allows processes to be captured, which are very difficult to detect directly by ex-situ methods. For example, we can detect side reactions involving the electrolyte and the electrode materials, and processes that occur during extremely fast charging and discharging. The approach will be demonstrated for the anode materials silicon and lithium metal. Lithium-ion batteries (LIBs) containing silicon have been the subject of much recent investigation, because of the extremely large gravimetric and volumetric capacity of this anode material. This material undergoes a crystalline-to-amorphous phase transition on electrochemical Li insertion into crystalline Si, during the first discharge, hindering attempts to link structure in these systems with electrochemical performance. We apply a combination of static, *in-situ* and magic angle sample spinning, *ex-situ* ^{7}Li and ^{29}Si nuclear magnetic resonance to investigate the changes in local structure that occur in the actual working LIB. In another example, we use the skin-depth effects associated with metals to develop a methodology to distinguish between the dendritic and bulk lithium ion metal that is plated and stripped during lithium-ion battery cycling. In the second area, we illustrate the use of NMR to investigate the nature of the defects in materials that have been proposed for use as electrolytes that operate via either oxygen-ion or protonic conduction in solid oxide fuel cells. For example, $BaZrO_3$ or $BaSnO_3$ can be doped with Y^{3+} or Sc^{3+} to create oxygen vacancies. These vacancies can be filled with H_2O, the water molecules dissociating to form mobile ions that contribute to the long-range ionic transport in these systems. NMR experiments are used to examine the local structure, the locations of the vacancies and how this affects protonic/oxygen ion motion in these systems.

ISMAR Prize Lecture

High Field Dynamic Nuclear Polarization – The Renaissance

Robert G. Griffin

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Dynamic nuclear polarization (DNP) is rapidly moving to high fields and frequencies and evolving as an approach to significantly increase sensitivity in MAS and solution NMR and imaging experiments. This renaissance is due to advances on several fronts including new instrumentation, new polarizing agents tailored for specific experiments, and magnetic resonance methodology. In this presentation we review new developments in each of these areas. Advances in instrumentation include frequency tunable gyrotron sources that circumvent the requirement of a superconducting sweep coil and developments in low temperature probe technology. New polarizing agents include molecules that more effectively exploit the cross effect DNP mechanism or are more efficient at polarizing certain nuclear spin species such as low-γ nuclei. New methods for performing DNP involve time domain experiments. In addition, we discuss applications of these techniques to structural studies of amyloid and membrane proteins.

Andrew Prize Lecture

Solid-state Magic-Angle Spinning NMR methods for tensor measurements and protein structure refinement using chemical shift tensors

Benjamin J. Wylie[a], W. Trent Franks, Lindsay J. Sperling, Andrew J. Nieuwkoop, Donghua H. Zhou, Heather L. Frericks Schmidt, Charles D. Schwieters[b], Eric Oldfield[c] and Chad M. Rienstra[c]

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Solid-state NMR possesses the unique ability to measure structurally dependent anisotropic properties, including chemical shift and dipolar tensors. Here we present multidimensional SSNMR experiments to measure tensor magnitudes and orientations in the streptococcal β-1 immunoglobulin binding domain of protein G (GB1). Experimental techniques presented include: three-dimensional experiments that recouple ^{13}C or ^{15}N chemical shift tensor (CST) powder lineshapes, slow magic angle spinning analysis of highly-^{13}C,^{15}N-enriched solid proteins using 2D heteronuclear correlation at 750 MHz, and precise distance measurements using TEDOR. This work culminates in the first structure of a solid protein solved and refined using Cα CST magnitudes and orientations to constrain backbone conformation. This is achieved by comparing the experimental CST elements to *ab initio* chemical shielding calculations as a function of local conformational degrees of freedom. To this end, a customized CST force field was generated and used as a restraint class in the XPLOR-NIH simulated annealing algorithm. By combining CST information with vector angles and TEDOR distances we generated a family of highly precise (~0.18 Å backbone RMSD) and accurate (up to 0.5 Å relative to the 2QMT crystal structure) protein structures. These results demonstrate that *de novo* structure calculations utilizing Cα CST data can yield atomic-resolution structures of solid proteins.

2 Preconference Lecture

Speed

Ray Freeman[a] and Ēriks Kupče[b]

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Multidimensional methods have revolutionized NMR spectroscopy, ever since the early initiative of Jean Jeener. The one fly in this particular ointment has been the long duration of some of the experiments, particularly when several dimensions are used. Several ways for speeding up these measurements are discussed – multiple parallel receivers, Hadamard spectroscopy, spatially-encoded single scan methods, and projection-reconstruction. Applied to biological macromolecules such as proteins, these investigations tend to favour higher and higher dimensions in order to combat spectral overlap and to accommodate carbon-13 and nitrogen-15 isotopic enrichment. The concept of hyperdimensional spectroscopy has been introduced to pave the way for high dimensionalities, for example spectra from the ten-dimensional spin systems of two adjacent aminoacid residues.

3 Plenary Lectures

NMR on Surfaces

Lyndon Emsley

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Catalysts are the key actors in many chemical processes. The precise understanding of their active sites is the key to controlling these complex systems and improving their design in a rational way. When the catalyst is grafted on a surface, many characterization methods are compromised. We have shown over the last few years that multi-dimensional magic-angle-spinning solid-state NMR spectroscopy can play a major role in characterizing single site heterogeneous catalysts, obtained by grafting organometallic compounds onto an oxide support.

We will present new methods for studying heterogeneous organometallic systems using ultra-fast magic angle spinning (>60 kHz). Ultra-fast MAS is found to profoundly change 1H spectra in these systems, which in turn allows the development of a range of new approaches to characterization and structure determination.

The first direction is the study of diamagnetic systems. One example here will be the effect of ultra-fast MAS on the assignment of the 1H NMR spectra of phenolic species grafted in the interior of mesostructured organic-inorganic hybrid materials. The change in resolution between moderate and fast MAS in these materials is spectacular, allows the implementation of multi-dimensional 1H-1H correlations, and is determinant for the full characterization of the structures.

The second direction we pursue is the complete characterization of paramagnetic metal complexes and materials. Here we will demonstrate how to determine full crystal structures by using both the isotropic pseudo-contact shifts and the anisotropy of the paramagnetic shifts. This requires ultra-fast MAS and the development of sophisticated adiabatic pulse schemes, including “single-band” methods for obtaining perfect inversion in highly paramagnetic systems.

Finally, methods for sensitivity enhancement in organometallic systems will be investigated.

Why NMR and MRI, as useful as they are for analyzing art, are sometimes outperformed by other techniques, such as RAMAN

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NMR is a great tool, also in the arts. However, it requires special circumstances to render it applicable. As is well known, its major handicaps are low sensitivity and size-limitations of the objects that can be handled by conventional high-sensitivity and high-spectral-resolution techniques. Obviously, the NMR mouse is the method of choice for exploring arbitrarily large objects, however again with severe sensitivity and resolution limits. NMR mouse investigations are focusing usually on the study of bulk materials, mostly by relaxation measurements, providing information on molecular mobility. Plaster, gypsum, concrete, and other bulk materials can be studied very efficiently by NMR. – Another approach would be sample taking from artworks for conventional NMR studies, for example, sampling pigment layers on a painting. But in nearly all cases, the degree of destruction of the artwork would by far exceed the potential information gain.

X-ray based techniques, especially X-ray fluorescence analysis (XRF) are valuable non-destructive alternatives for obtaining rather specific local information, primarily on the elemental composition of pigment layers and of the supporting material. However, a full chemical identification of compounds with similar elemental composition is often not possible. Also depth information for multi-layer paintings is not easily obtainable. To a limited extent, also Fourier-transform infrared (FTIR) can be applied nondestructively.

A nearly ideal technique is RAMAN microscopy. It can be applied nondestructively; it has high spatial resolution, it is surface-specific, and it is fully characteristic for a wide range of chemical compounds, such as pigments. - In the lecture, optimized instrumental set-ups and techniques are described and fascinating applications to Central Asian, in particular Tibetan scroll paintings are presented.

Molecular Dynamics and Neurodegeneration as seen by NMR spectroscopy

N.-A. Lakomek[a], O. F. Lange[b], K. F. A. Walter[a], M. Funk[a], D. Ban[a], D. Lee[a], H. Schmidt[a], K. Seidel[a], C. Farès [c], D. Egger[d], P. Lunkenheimer[d], R. Kree[e], J. Meiler[f], Ö. Poyraz[g], M. Kolbe[g], A. Zychlinsky[g], H. Grubmuller[b], X. Salvatella[h], R.B. Fenwick[h], S. Becker[a], B. de Groot[b], P. Karpinar[a,j], S. Ryazanov[a,j], M. Babu[a,i,j], H. Heise[a,j], J. Wagner[m], C.O. Fernandez[k], A. Fischer[j,l], N. Wender[k,l], N. Resaei Ghaleh[a,j], S. Eimer[j,l], H. Jäckle[i], A. Leonov[a,j], A. Giese[m], J. Schulz[j,n], A. Lange[a], M. Baldus[a,j], M. Zweckstetter[a,j] and Christian Griesinger[a,j]

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The possibility to explore dynamics of proteins and other biomolecules will be presented based on the accurate measurement of anisotropic parameters such as residual dipolar couplings.[1] Rates of interconversion between ensembles will be measured by low temperature relaxation dispersion and related to other biophysical methods. The amount of correlated motion will be characterized by cross correlated relaxation.[2] The impact on protein recognition will be discussed. In a second part, folding and refolding upon aggregation in infection biology and neurodegeneration will be described on two examples that require a combined approach of liquid and solid state NMR.[3] It is shown how the information from NMR is used for drug development.[4]

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Structural and Dynamic Basis for the Assembly of Protein Machineries by NMR

Charalampos Kalodimos

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We will discuss the application of NMR spectroscopy to characterizing supramolecular protein systems. Our lab is actively applying NMR to determine the functional mechanisms of many machineries: the protein translocase, the transcription machinery and the type III secretion machinery. Recent results will be discussed.

In vivo applications of MR physiological and metabolic imaging

Sarah J. Nelson

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With the availability of higher field whole body MR scanners, in vivo studies are increasingly integrating anatomic with physiological and metabolic imaging. When combined with the development of multi-channel radiofrequency coils, this has provided 2 to 10-fold increases in sensitivity and spectral resolution of the MR data being acquired. For patients with neurological diseases, this has been used to either decrease the acquisition time for routine 3T scans by a factor of 4 or to improve the coverage and/or spatial resolution of the data being acquired. This is critical for predicting whether patients are likely to respond to therapy, for planning focal therapy and for evaluating the effectiveness of new treatments. Preliminary results using high resolution angiography and phase imaging with 7T whole body scanner have underlined the potential for improved visualization of vascular and anatomic lesions in Multiple Sclerosis and other neurodegenerative diseases. The use of hyperpolarized C-13 agents is a promising method for improving the sensitivity and specificity of metabolic imaging. DNP polarization has been shown to provide a >10,000 fold signal enhancement for detecting ^{13}C probes of endogenous, nontoxic substances and have the potential for monitoring fluxes through multiple biochemical pathways such as glycolysis, the citric acid cycle and fatty acid synthesis. Preliminary studies performed using a whole body MR scanner in pre-clinic models of prostate, liver and brain cancers have provided promising results in terms of using this technology to assess disease severity and response to therapy.

Developments and Applications of Multi-Extreme THz ESR System

Hitoshi Ohta[a,b,c], Eiji Ohmichi[b], Susumu Okubo[a] and Takahiro Sakurai[c]

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Developments of our multi-extreme THz ESR system[1] and its applications are presented. Our ESR system, which covers the frequency range of 30 GHz to 7 THz using Gunn oscillators, backward wave oscillators (BWO) and far-infrared laser, can be operated under multi-extreme conditions, such as the pulsed magnetic field up to 55 T,[1] the temperature down to 1.8 K, the pressure up to 1.4 GPa[2] and the detection of micrometer order sample.[3] The pulsed magnetic field is applied using reinforced Cu-Ag wire magnet and 300 kJ (10kV) capacitor bank. The examples of high spectral resolution measurement of finite Haldane chain antiferromagnet[4] and the magnetic phase transition measurements of magnon Bose-Einstein condensation system will be shown. The pressure is applied using the clamped type piston cylinder pressure cell, which enables the transmission of electromagnetic wave using sapphire or zirconia pistons. The pressure dependence of spin gap in $KCuCl_3$ dimer antiferromagnet will be shown. Finally, highly sensitive THz ESR measurement system using micro-cantilevers will be shown, and we achieved the sensitivity of 10^{11} spins/G[3].

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Force-detected nanoscale MRI: recent progress and challenges ahead

Daniel Rugar

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Motivated by the quest for a molecular structure microscope, we are working to dramatically enhance the resolution of magnetic resonance imaging (MRI) using a technique called "magnetic resonance force microscopy" or MRFM. MRFM achieves a 100 million-fold improvement in sensitivity over conventional MRI by replacing the traditional inductive pickup with ultrasensitive detection of magnetic force. By combining this sensitivity improvement with novel methods for spin manipulation, we have successfully detected individual electron spins and nanoscale ensembles nuclear spins. By carefully measuring the magnetic force from nuclear spins as a function of position, 3D images of nuclear spin density can be reconstructed with resolution better than 10 nm.[1]

In this talk we will review the basic principles of nanoscale MRFM. The measurement of statistical spin polarization, double resonance in statistically polarized ensembles and techniques for MRFM signal multiplexing will be discussed. Prospects for pushing the resolution below 1 nm and turning this technique into a useful tool for structural biology will be addressed. A key challenge will be overcoming near-surface force noise at close tip-sample distances.

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Resolving antimicrobial and amyloid peptides in membranes

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The results of solid-state NMR studies aimed at determining the orientation and location of antimicrobial peptides obtained from Australian tree frogs and amyloid peptides in phospholipid membranes will be discussed. The detailed structure of these peptides in membranes is difficult to determine as they disrupt the phospholipid bilayer. Solid-state NMR techniques are being used to determine the conformation and mobility of these pore-forming peptides in order to understand the mechanisms by which they exert their biological effect that leads to the disruption of biological membranes. Both static and magic angle spinning techniques have been applied to antimicrobial peptides in a range of model membranes, which reveal that the peptide activity is strongly dependent on the lipid composition of the bilayer and correlate with the selectivity for bacterial membranes. Similarly, the membrane interactions and structural changes of Aβ(1-42) and Aβ(1-40) from Alzheimer's disease are dependent on the presence of cholesterol and metal ions, which have been implicated in the disease. The data from both the amyloid and antimicrobial peptides reveal the importance of using appropriate membranes systems for studying membrane-active peptides.

4 Session Lectures

Solid-State NMR Analysis of H^+-ATP Synthase Subunit c-Ring in Membranes

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The subunit *c*-ring of H^+-ATP synthase (F_oc-ring) plays an essential role in the proton translocation across membranes driven by the electrochemical potential. To understand its structure and function, we have carried out solid-state NMR analysis of membrane-reconstituted F_oc-rings under magic-angle sample spinning. The uniformly [^{13}C, ^{15}N]- and specifically labeled F_oc from *E. coli* (EF_oc) and thermophilic bacterium PS3 (TF_oc) were reconstituted into lipid membranes. AFM images of TF_oc/membranes revealed ring structures. Reconstitution of the ring to F_oF_1 complexes recovered the proton-translocation activity coupled with ATP hydrolysis. The high-resolution two- and three-dimensional spectra were obtained, and the ^{13}C and ^{15}N signals were assigned. The obtained chemical shifts suggested that EF_oc and TF_oc take on hairpin-type helix-loop-helix structures in membranes. The results on the magnetization transfer between the EF_oc and deuterated lipids indicated that Ile55, Ala62, Gly69 and F76 were lined up on the outer surface of the ring. This is in good agreement with the cross-linking results previously reported. Distance analysis of the ^{13}C nuclei distance of [3-^{13}C]Ala24 and [4-^{13}C]Asp61 in the F_oc-ring did not agree with the model structures proposed for the EF_oc-decamer and dodecamer. Interestingly, the carboxyl group of the essential acidic amino acid in the membrane-embedded F_oc-ring turned out to be protonated as COOH even at neutral pH.

New developments in low field Nuclear Magnetic Resonance

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We report about advances in high resolution low field NMR spectroscopy. Three fundamental issues are addressed: 1) the problem of limited access to chemical shift information in low field, 2) the signal to noise problem (SNR) for small number of spins and 3) the dual correspondence between high and low field NMR spectroscopy.

With respect to problem 1, ^{1}H chemical shift differences of small organic molecules in the ppm range can be measured down to the ultimate limit of the static field, where the chemical shift differences correspond to the line width. We show that this limit can be broken if different hetero-nuclear *J*-couplings for the corresponding chemical groups are present.

We tackled the problem of insufficient SNR in low magnetic fields by application of continuous flow PHIP technology, resulting in the ability to detect a few nl of samples in a single scan and with mobile low field NMR spectrometers.

Concerning issue 3 we derived a theory describing the structure of strongly and weakly J-coupled NMR spectra over the entire B-field range. This can be delineated into two weakly and one strongly J-coupled regime. The theory predicts the existence of boundaries Bi where the complexity of J-coupled NMR spectra changes in terms of the number of lines. Moreover the spectra of the two weakly coupled regimes at high (~ 1 T) and low field (~ 10-7 T) are in dual correspondence to one another in the sense that in both cases the chemical structure may be unambiguously determined.

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New developments in dissolution-DNP for in vivo imaging

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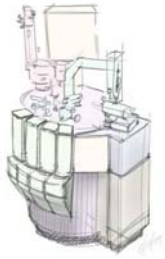
Magnetic Resonance continuous to make great progress on several technological fronts, and sensitivity has been improved dramatically over the last decades. However, despite these advancements the sensitivity of MR is still limited by a low thermal polarization. During the last decade several techniques for hyperpolarizing nuclear spins in molecules in solution have become available; in particular para-hydrogen induced polarization (PHIP) and DNP in the solid state followed by dissolution (dissolution-DNP). These methods offer many new possibilities in MR.

This talk will summarize some of the recent developments in the field of dissolution-DNP for in vivo imaging (MRI). Dissolution-DNP is being developed for MRI and is currently in clinical development. To make this a viable clinical method several technological and scientific advancements will have to take place. The talk will focus on four main areas:

1) Strategies for reaching the highest nuclear polarization by DNP: Doping with Gd-chelates, new radicals and magnetic field and temperature dependence
2) Strategies for sample formulation to enable new compounds
3) Dissolution physics: How to retain polarization and control relaxation; optimize fluid dynamics for complete sample recovery and achieve a homogenous liquid sample
4) Clinical polarizer technology: How to ensure a safe and sterile solution by appropriate in-line quality control, improve sample throughput for patient flow, automate process, eliminate liquid cryogen handling and large mechanical pumps



Dissolution-DNP is a young and immature field with still many scientific questions to be addressed and resolved. Despite of this it has progressed rapidly into clinical development.

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Interaction of cisplatin with transport proteins: solution and in-cell NMR studies

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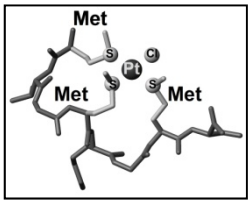
NMR spectroscopy has two distinctive features that make this technique an excellent tool for structural studies of biological macromolecules: i) the sensitivity of the chemical shift of an NMR-active nucleus to changes in its chemical environment; ii) the ability to gather information about molecules under physiological or near-physiological conditions. Thanks to these features, NMR allows to study the interaction of biological macromolecules with several classes of binding partners, including clinically relevant drugs and metal ions. In particular, NMR can address key biological and mechanistic issues related to cellular processes involving metal ions. In vitro studies are essential to provide a high resolution structural characterization of the metal-protein adducts, the overall conformation of the protein in solution as well as the coordination geometry and the nature of the ligands around the metal center. For this purpose, tailored isotope labeling strategies are applied; in some cases the metal nucleus can be directly detected. Furthermore, the non-invasive character of NMR spectroscopy makes it ideal to probe the binding mode and interactions of proteins with metal ions inside living cells.[1] It is crucial for in-cell biomolecular studies to distinguish the resonance frequencies of the macromolecule of interest from those of all other cellular components crowding the sample. Solution and in-cell NMR spectroscopy is used to monitor the interaction of the anticancer drug cisplatin with proteins involved in copper trafficking.[2] Membrane transporters and soluble chaperones of copper ions also mediate cellular uptake of and resistance to platinum-based drugs.[3]



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Structural genomics of chromatin interacting proteins

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We are taking a protein family approach to understand how human protein domains act as recognition modules for specific histone tail sequences and their post translational modifications (PTMs). Covalently modified histones within chromatin are the "language" of the histone code that determines whether associated genes are transcriptionally active or not. A variety of biophysical techniques (peptide arrays, ITC, fluorescence polarization, differential scanning fluorimetry) together with structural studies (NMR and x-ray crystallography) help explain mechanisms of binding selectivity for these proteins. NMR has played a crucial role in elucidating the mechanism of recognition of these modules in several cases where crystallography provides an incomplete picture. I will discuss the role of NMR in structural genomics including our current strategies for rapid, parallel protein structure determination by NMR as well as hybrid approaches for more challenging systems. Strategies that will be discussed include non uniform sampling of multidimensional spectra processed with multidimensional decomposition (MDD)[1,2] and protein resonance assignment and solution structure determination using ABACUS[3,4] and CS ROSETTA. Structural genomics allows one to objectively assess the advantages and disadvantages of these approaches and has helped drive the development of methods for evaluation of solution structures by various techniques.

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Structure and dynamics of bitopic and politopic membrane helical proteins

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Helical membrane proteins are a major class of membrane proteins that are essentially involved in key processes including bioenergetics, signal transduction, ion transmission, catalysis, and so on. This class of proteins is characterized by the presence of highly hydrophobic stretches of ca. 20 amino acids, which span the membrane in a helical conformation. Helical membrane proteins can exist as simple structures, with just one or a few helices spanning a membrane, as well as large oligomeric complexes with many transmembrane helices.

The lecture will present our experience with high-resolution NMR study of structure and dynamics of several homo and hetero dimers of transmembrane domains of EphA and ErbB families of bitopic receptor tyrosine kinases and four-helical voltage-sensing domain of the archaeal potassium channel KvAP. The following issues will be discussed: a) effective peptide/protein expression, b) choice of solubilization media and prove of the native fold, c) search for NMR constraints, d) specificity and details of helix-helix interactions and e) what can we learn at present about functioning of the membrane proteins based on NMR data.

Investigating Disorder in Ceramics: Multinuclear Solid-State NMR and First-Principles Calculations

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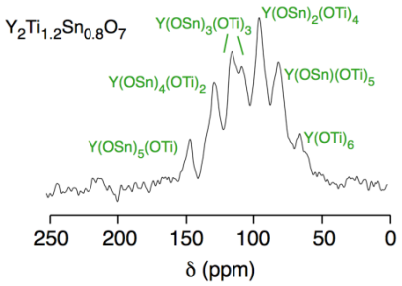
NMR spectroscopy provides an element-specific probe of local structure and dynamics in solids, without any requirement for long-range order. Whilst techniques such as magic-angle spinning (MAS) can achieve high-resolution spectra in many cases, for disordered systems we typically see a distribution of NMR parameters and corresponding broadening or splittings in the spectrum, hindering analysis. There has been considerable recent progress in the calculation of NMR parameters from "first principles" in periodic systems, aiding both spectral assignment and interpretation. Here, we combine high-resolution NMR experiments with DFT calculations to investigate disorder in ($Y_2Ti_{2-x}Sn_xO_7$) pyrochlore ceramics. These materials are of particular interest for their application in the long-term storage of radioactive waste.



In addition to ^{89}Y and ^{119}Sn MAS NMR, we also compare experimental and calculated values of the $^{89}Y/^{119}Sn$ chemical shift anisotropy (CSA). These are measured using a two-dimensional approach, where an amplified CSA is reintroduced in the indirect dimension, whilst retaining the practical advantages of faster MAS. Our calculations provide insight into spectral interpretation and assignment, allowing us to probe B-site cation disorder in these materials.

Remotely Detected Magnetic Resonance Imaging and Velocimetry

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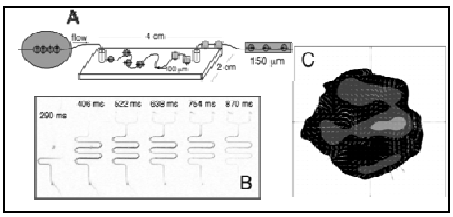
MRI can elucidate the interior structure of an optically opaque object in unparalleled detail but is ultimately limited by the need to enclose the object within a detection coil; acquiring the image with increasingly smaller pixels reduces the sensitivity because each pixel occupies a proportionately smaller fraction of the detector's volume. Here, we overcome this limitation using *remotely detected MRI*: images of fluids flowing in an object are encoded into the phase and intensity of their NMR signals and decoded by a single volume-matched detector after they flow out of the sample. Using remote detection, we accelerate MRI acquisition in microfluidic devices by 10^6, obtaining microscopic (up to 10 μm) images of flow and velocity distributions. In the context of remote detection, we introduce compressive sampling techniques that further reduce the time required to acquire these images, particularly when *a priori* data about the flow geometry can be incorporated into the image reconstruction algorithm. Finally, we provide illustrative examples of remotely detected MRI velocimetry in microporous systems such as packed bead microreactors and chromatography columns. Our results illustrate the facile integration of MRI with microscale assays and suggest generalizations to other systems involving microscopic flow, including microvasculature in living organisms.



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Selective membrane transport systems investigated by solid-state NMR spectroscopy

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Cellular membranes are critically involved in elementary cellular functions such as partitioning, signal transduction or molecular transport. In our contribution, we describe recent progress to dissect the structural details of ion channel transport and inactivation.[1] Recent ssNMR studies in our group also reveal a remarkable interplay between protein structure and dynamics in the permeability barrier of nuclear pore complexes (NPCs). These protein networks control all molecular trafficking between the nucleus and the cytoplasm in eukaryotic cells. Yet. the structural details of their function has remained elusive. Using ssNMR, we identified specific transient hydrophobic interactions between Phe and methyl side chains as well as intermolecular β-sheets between Asn-rich spacer regions in the 62 kDa FG/FxFG repeat domain of the yeast nuclear pore complex protein Nsp1p. These results not only provide novel insight into the structural aspects of nucleo-cytoplasmic exchange but also establish a functional link between molecular trafficking and Amyloidosis.[2]

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Magnetic resonance in semiconductor nanostructures: EPR, ESE, ENDOR and ODMR studies

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High-frequency electron paramagnetic resonance (EPR), electron spin echo (ESE), electron-nuclear double resonance (ENDOR) and optically detected magnetic resonance (ODMR) spectroscopy is shown to be excellent tools for the investigation of the electronic properties of semiconductor nanostructures. Results are presented on doped ZnO and CdS quantum dots (QDs). Shallow donors (SDs) have been identified in this material. The shallow character of the wave function of the donors is evidenced by the multitude of ENDOR transitions. The spatial distribution of the electronic wave function of a SDs in a ZnO semiconductor QDs has been determined in the regime of quantum confinement, the effect of confinement on the *g*-factor of SD's in ZnO as well as in CdS QD's are observed. Hyperfine interactions as monitored by ENDOR spectroscopy quantitatively reveal the transition from semiconductor to molecular properties upon reduction of the size of the nanoparticles. An almost complete dynamic nuclear polarization (DNP) of the ^{67}Zn nuclear spins in the core of ZnO quantum dots and the ^{1}H nuclear spins in the $Zn(OH)_2$ capping layer can be achieved by saturating the EPR transition of the SDs with resonant high-frequency microwaves at low temperatures. DNP manifests itself as a hole and an antihole in the EPR absorption line of the SDs in the QDs and a shift of the hole. Spin-dependent electron-hole recombination has been studied by monitoring tunnelling afterglow and ODMR in ZnO QD's.

The results are reported of application of ODMR and level anticrossing (LAC) spectroscopy for the investigations and local diagnostics of GaAs/AlAs and GaAs/AlGaAs quantum wells and superlattices and self-organized oriented semiconductor nanocrystals embedded in crystalline matrix. ODMR, ESE and EPR have been applied for detection of nitrogen-related centers: isolated N donors and nitrogen-vacancy (NV) defects in nanodiamonds.

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HF-EPR study of Magnetic anisotropy in Tetrairon(III) Single-Molecule Magnets

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Molecules showing slow relaxation of the magnetization at low temperature, known as Single Molecule Magnets (SMMs), have represented a major breakthrough in nanomagnetism. Especially, they exhibit classical and quantum effects in the dynamics of the magnetization. HF-EPR spectroscopy has demonstrated to be a key tool to characterize these quantum systems and provide information on their magnetic anisotropy. SMM are complexes displaying a large spin ground state S associated to an Ising type magnetic anisotropy, leading to the presence othe reversal of the magnetization at low temperature. Axial anisotropy terms govern the height of the barrier whereas transverse magnetic anisotropy terms influence the quantum tunneling of the magnetization.

Tetrairon(iii) complexes with a propeller-like structure, of formula $[Fe_4(L)_2(dpm)_6]$, are providing an important class of Single Molecule Magnets displaying synthetic flexibility and ease of functionalization (H*dpm* = 2,2,6,6-tetramethyl-heptane-3,5-dione). We report on a series of derivatives prepared by using tripodal bridging ligandsL. HF-EPR spectra at low temperature have been collected on polycrystalline samples of several complexes in order to determine the zero-field splitting (zfs) parameters in the ground $S = 5$ spin state. In all these compounds, a remarkable correlation is found between the axial zfs parameter D and the pitch γ of the propeller-like structure. The origin of the relationship will then be directly illustrated on the newly synthesized heterometallic complex $[Fe_3Cr(L)_2(dpm)_6]$, with H_3L = 2-phenyl-2-hydroxymethyl-propane-1,3-diol, together with its Cr- and Fe-doped Ga_4 analogues, which contain chromium(III) in the central position (for the Cr doped Ga_4) and iron(III) in two magnetically-distinct peripheral sites (for the Fe doped Ga_4). The doped Ga_4 complexes allow establishing the single ion contributions to the magnetic anisotropy of the parent SMM complex.

The sticky fingers of influenza visualized by modern solution NMR

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All but five of the N-terminal 23 residues of the HA2 domain of the influenza virus glycoprotein hemagglutinin (HA) are strictly conserved across all 16 serotypes of HA genes. The structure and function of this HA2 fusion peptide (HAfp) continues to be the focus of extensive biophysical, computational, and functional analysis, but most of these analyses are of peptides that do not include the strictly conserved residues Trp^{21}-Tyr^{22}-Gly^{23}. The heteronuclear triple resonance NMR study reported here of full length HAfp of sero subtype H1, solubilized in dodecylphosphatidyl choline (DPC), reveals a remarkably tight helical hairpin structure, with its N-terminal α-helix (Gly^{1}-Glu^{11}) packed tightly against its second α-helix (Trp^{14}-Gly^{23}), with six of the seven conserved Gly residues at the interhelical interface. The structure is stabilized by multiple interhelical C□H to C=O hydrogen bonds, characterized by strong interhelical H^N-H^α and H^α-H^α NOE contacts. ^{15}N relaxation analysis at high pH (7.4) indicates the structure to be highly ordered on the nanosecond time scale, and NOE and paramagnetic relaxation enhancement analysis indicates HAfp is located at the water-lipid interface, with its hydrophobic surface facing the lipid environment, and the Gly-rich side of the helix-helix interface exposed to solvent. Although the structure is predicted to change by lowering the pH, no such structural transition is observed and pH 4 RDC and NOE data fit well to the high pH structure. However, relaxation experiments at pH 4 indicate low populations of an alternate conformer in rapid exchange with the dominant conformation, and likely a key intermediate in the fusion mechanism.

Studies of Dynamic Nuclear Polarization (DNP) in Liquids: Understanding the Overhauser Mechanism for New Experimental Designs

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Dynamic nuclear polarization of nuclei coupled to paramagnetic centers has been known since the early years of magnetic resonance to obtain information about molecular motion and electron-nuclear spin relaxation. In the past few years, this technique has experienced a renaissance because of recognition that it could provide a means to overcome the sensitivity limits in solution and solid state NMR towards studies of macromolecular complexes. EPR spectroscopy at low and high fields represents the essential tool to investigate the conditions to develop an experimental set up for DNP. Enhancement of the nuclear spin polarization via DNP requires optimized pumping (saturation) of the electron spins with highly efficient microwave irradiation, suitable polarizing agents and knowledge about electron-nuclear spin relaxation. This contribution summarizes some recent efforts towards understanding the physical and instrumental aspects of DNP in aqueous solutions using nitroxide radicals as polarizers at 9 as well as 94 GHz EPR frequencies.[1-6] This studies allow optimization of an experimental design for liquid DNP that opens the door to applications in NMR spectroscopy of biological samples.

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Principles and practice of projection-decomposition tools for resonance assignments and protein structure

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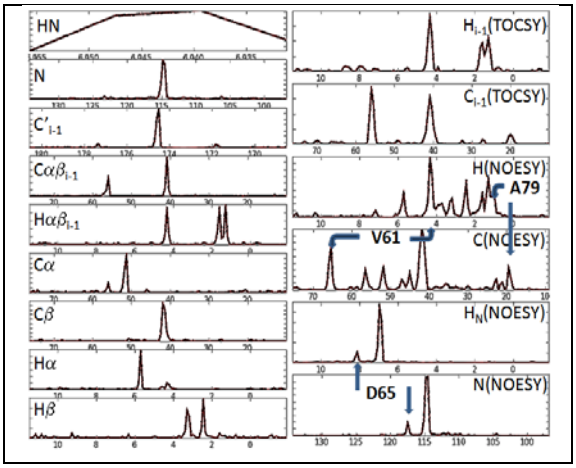
Projection spectroscopy followed by computational decomposition for resonance assignments and structure determination of proteins is reviewed.[1] Principles of the approaches are discussed and exemplified by applications covering various proteins: choice of spectral projections and artefacts related to this choice, 'model-free' approach of the decomposition algorithm, and the option of combining several experiments (triple-resonance, NOESY …) for simultaneous decomposition.

The figure shows an example (HN of Phe 66 of a histone protein) resulting from five projection series, including two triple-resonance experiments ($H_{\alpha\beta}C_{\alpha\beta}CONH$, $H_{\alpha\beta}C_{\alpha\beta}NH$), two NOESYs ($^{13}C$ and ^{15}N resolved) and one TOCSY. From the 109 possible input projections, 54 planes were used in a simultaneous decom-position. The resulting 'shapes' (figure) provide chemical shifts for the selected HN, for its neighbouring five carbons (αβ of F66 and D65) and attached six aliphatic hydrogens, as well as for the entire preceding side chain (TOCSY) and all spatially neighbouring hydrogens (NOESYs; illustrated for V61 and D65).



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EPR/ENDOR on complex metal centers in enzymes – from single crystals to whole cells

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Multi-nuclear metal sites are the catalytic cores of many enzymes. In Photosystem II (PSII), the oxygen-evolving complex (OEC) consists of four Mn and a Ca ion as the site of photosynthetic water splitting. Spectroscopic information on this metal center is important, as the crystallographic model cannot provide the details necessary for a mechanistic understanding. The center cycles through different oxidation states with two paramagnetic intermediates S_0 and S_2. However, EPR spectroscopy on theses states is challenging as their signals cover a spectral width beyond 0.1 T. The situation is even worse for ^{55}Mn-ENDOR spectroscopy with a spectral width of the ENDOR signals covering about 100 MHz with little structure of spectra. In order to increase the spectral resolution and to correlate magnetic interaction axis with molecular axes we recorded orientation dependent ^{55}Mn-ENDOR spectra on the S_2 state in PSII single-crystals. The analysis of the data allows an assignment of the largest Mn hyperfine coupling, i.e. likely the ion in the Mn(III) state, to two out of four Mn positions in the structural model.

Instead of spectroscopy on the extreme protein environment in a single-crystal we went to the similarly challenging situation of EPR/ENDOR in whole cells for investigation of the [NiFe] center in oxygen tolerant hydrogenases catalyzing the reversible cleavage of molecular hydrogen. By comparing the hyperfine structure of the protons coupled to the metal center in an oxygen tolerant membrane bound hydrogenase (MBH) with standard, oxygen-sensitive hydrogenases we could show a high similarity of the metal centers and that the oxygen insensitivity of this enzyme is very likely not determined by alterations of the catalytic core. For a soluble oxygen-tolerant hydrogenase (SH) the "in cell" experiments again show a standard catalytic core in variance to earlier experiments on isolated protein and thereby questioning a model invoking a modified metal center as origin of the oxygen tolerance of this enzyme.

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Measurement of complex diffusion in the micro-sec time scale and 10 nm length scale by electron spin resonance

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The critical roll of diffusion in the mechanism of various biological and chemical processes has led researches to an ongoing search for methods to quantify the diffusion coefficient. The measurement of diffusion occurring over relatively long distances (of at least few microns) can be carried out with techniques such as nuclear magnetic resonance (NMR) or fluorescence recovery after photo-bleaching (FRAP), and are well-established. However, methods for the direct measurement of diffusion over short distances (in 10-100 nm range), occurring in the micro-sec time scale, have not yet been developed. Here we show that by utilizing the well known NMR pulse sequence pulse gradient spin echo (PGSE), in an electron spin resonance (ESR) experiment; one can measure the diffusion coefficients over such short distance and time scales. In order to adapt PGSE to ESR we had to develop high sensitivity micro-resonators and a capability to generate very intense and short gradient pulses of ~1 micro-sec in length and > 100 T/m in magnitude. Our preliminary work included the measurements of the non-restricted isotropic diffusion coefficient of three types of radical solutions: trityl radical in water, N@C_{60} in chloronaphthalene and N@C_{60} in CS_2. The experimental results were compared to the theoretical diffusion coefficient calculated by the Stocks-Einstein equation, and revealed an excellent agreement.[1] This preliminary work was recently extended to the measurement and characterization of restricted diffusion of N@C_{60} and trityl solutions in a porous media made of deep sub-micron sized spheres. The effects of restricted diffusion in such type of porous media were well observed in the PGSE ESR data.[2] Possible applications and future directions of this methodology will be discussed.

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Maltose and vitamin B12 importers: modeling the conformational changes during transport with interspin distance restraints

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In this work we applied site directed spin labeling EPR to two types of ABC transporters: the maltose importer from *E.coli* ($MalFGK_2$-E) and the vitamin B12 importer from *E.coli* (BtuCD-F). The first belongs to the class I of importers, where the alternating access mechanism of substrate import has been established. For the second (class II) a distinct mechanism of action has been proposed.[1] Crystal structures are available for the two bacterial importers in different states with different degrees of resolution. The study aims to unveil the molecular details of the unresolved regions of the class I importer during the transport cycle and to elucidate the distinct mechanisms of action in the two classes of importers.

In the case of $MalFGK_2$-E the study focused on the periplasmic MalE and MalF-P2 loop. The reciprocal communication between MalK and MalF-P2 loop meditated by MalE gave insights into the stimulatory effect of MalE on the ATPase activity.[2] Based on a set of interspin distance constraints a first model of the periplasmic region during the complete transport cycle is presented. The periplasmic and cytoplasmic gates of the vitamin B12 importer BtuCD-F investigated with pulse EPR techniques provided clear evidences for the distinct displacement of the transmembrane subunits suggested from the crystal data. A mechanism of substrate import in the class II importers is proposed.

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Optimizing the relaxivity of macromolecular MRI contrast agents

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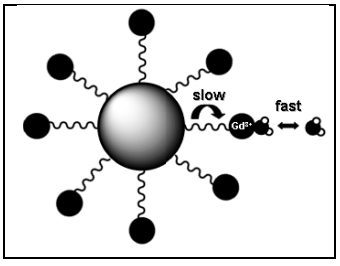
The use of macromolecular constructs, including polymers, dendrimers, micelles and liposomes, chemically modified viral capsids and silica nanoparticles is an emerging technology for the development of high relaxivity Gd-based MRI contrast agents.[1] These systems possess high molecular relaxivity (r_1) resulting from both the additive effect of all of the active Gd^{III} centers and the reduced tumbling rate that enhances the r_1 of each complex. However, in spite of the high relaxivities per particle found for these systems, the r_1 values for the individual gadolinium centers are typically modest [10-20 $mM^{-1}s^{-1}$ at 298 K, 0.47 T] and well below theoretical expectations. The two major limiting factors are the use of neutral Gd complexes (DTPA bisamides and DOTA monoamides) exhibiting slow water exchange ($k_{ex} = 1/\tau_M$) and/or fast local rotation of the Gd^{III} complex around its linker to the nanoparticle.[2] An optimisation of several physico-chemical parameters (hydration number q, water exchange rate and rotation flexibility of the chelate) allows to obtain relaxivity enhancement up to 200% without compromising the stability of the paramagnetic building blocks.



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Can MRI be Used to Improve Microreaction Technology?

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Micro-structured reactors that consist of multiple parallel channels may some advantages: higher selectivity and yield, better control of the reaction parameters, less waste and increased safety. It is unclear if the current technology has reached its limits of efficiency or not. We explore the possibility of optimizing the fluid distribution inside the reactor. MRI is capable of providing measurements of various physical parameters in operating catalytic reactors.[1] In a chemical reactor the reaction product originates from the porous catalyst bed and travels downstream due to mass transport and its flow is affected by obstacles. We model the reacting flow velocities defined at each cell on a grid. Each cell is a vertex, each of which can have any number of streams running through it. Adjacent vertices are connected by edges. Cells are labeled using Strahler indexing, except that when two streams diverge, their index remains the same. This preserves the memory of the branching pattern, and allows us to index a tree which branches both in and out. A dynamic tree is defined in which the streams merge or break apart according to the streaming velocity. This tree has a different hierarchy and topology than the static one. Depending on the dynamics, some of the static-tree branches might be completely cut off, either due to a blockage that prevents transport along these branches or due to the absence of conditions for downstream transport. At the outlet of the reactor, the collection of all Strahler indices forms a statistical distribution which reports on the complexity of the flow inside the reactor. I will discuss how this can be used to optimize reactor topology.

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High-Resolution Pulsed EPR: Separating and Connecting Peaks

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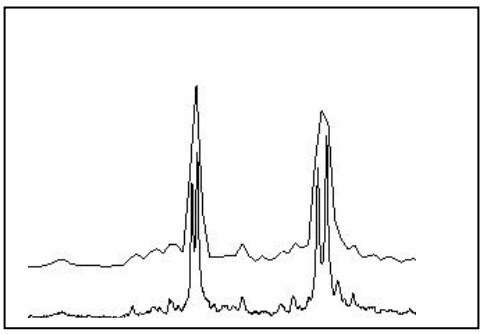

The ability to accurately measure resonance frequencies, and to assign and correlate transitions lies at the heart of much of magnetic resonance spectroscopy. Fast relaxation times and strong spectral overlap despite large spectral widths present challenges for high-resolution pulsed EPR spectroscopy of most paramagnetic species. Two-dimensional and multiple resonance approaches can enhance the resolution of pulsed EPR with little or no penalty in sensitivity.

A two-dimensional alternative to the classic field swept echo detected EPR spectrum provides an EPR spectrum with higher resolution and sensitivity by using a skew projection of data measured at several field steps. The effective resolution can be increased in ENDOR or HYSCORE spectra by spectral subtraction to remove overlapping lines from nuclei that are not of interest. Reference spectra can be obtained from samples with altered isotopic composition or with minor chemical perturbations in metalloprotetins.

The same two-dimensional Mims ENDOR spectrum provides both TRIPLE and ENQOR spectra when properly normalized and processed. Correlations between peaks in the spectra aid in the assignment of transitions to different sites, different species and different electron spin manifolds.

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Detection of brown adipose tissue using intermolecular zero quantum coherences

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Spatial resolution limits in magnetic resonance imaging can be overcome by exploiting signal that is refocused by intermolecular dipolar coupling. Because these couplings yield sub-voxel imaging resolution, the technique can be used to differentiate Brown Adipose Tissue (BAT) from the more abundant White Adipose Tissue (WAT).

BAT is thought to have a large impact on long-term energy balance, thanks to its capacity to burn calories.[1] Despite its importance, this tissue is very hard to detect *in vivo* and to differentiate from the more abundant white fat.

BAT can be differentiated from the more abundant WAT through measurement of the hydrolipidic ratio with CSI-based methods,[2,3] but the partial volume effect, which arises from the limited spatial resolution available in magnetic resonance, limits this capability *in vivo*. Signals from intermolecular zero quantum coherences between water and fat spins that are separated by around 100 microns offer a solution to this problem. Our *in vitro* data shows that the signal from these coherences is characteristic only of BAT tissue and it can be used to detect the presence of BAT depots that are scattered over different regions and that are impossible to detect using standard NMR techniques. We use this signal to localize BAT depots in mice, and the water-fat iZQC maps that we obtain are well correlated with more conventional BAT maps obtained with ^{18}FDG-PET scans.

Since this signal is intrinsically insensitive to local magnetic field inhomogeneities at length scales exceeding the selected correlation distance, it can be used to analyze large volume samples without a need for localization, shimming, or water suppression. We also show how this method is sensitive to temperature shift, and could thereby be used to track BAT activity, providing a non-invasive alternative to ^{18}FDG-PET for the detection of BAT activation.

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Detecting tumour responses to treatment using hyperpolarized ^{13}C magnetic resonance spectroscopic imaging

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Patients with similar tumour types can have markedly different responses to the same therapy. The development of new treatments would benefit significantly, therefore, from the introduction of imaging methods that allow an early assessment of treatment response in individual patients, allowing rapid selection of the most effective treatment. We have been developing methods for detecting the early responses of tumours to therapy. This has included a targeted MRI contrast agent for detecting tumour cell death and MR imaging of tumour cell metabolism using hyperpolarized ^{13}C-labelled cellular metabolites. We showed that exchange of hyperpolarized ^{13}C label between lactate and pyruvate, in the reaction catalyzed by the enzyme lactate dehydrogenase, could be imaged in tumours and that this flux was decreased in treated tumours undergoing drug-induced cell death.[1] We compared this method for detecting treatment response with measurements of fluorodeoxyglucose uptake.[2] We have shown, more recently, that hyperpolarized [1,4-^{13}C]fumarate can be used to detect tumour cell necrosis post treatment.[3] We have also shown that tissue pH can be imaged from the ratio of the signal intensities of hyperpolarized $H^{13}CO_3^-$ and $^{13}CO_2$ following intravenous injection of hyperpolarized $H^{13}CO_3^-$.[4]

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High-resolution solid-state NMR methods for the structural characterisation of organic solids

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^{1}H double-quantum (DQ) spectroscopy is being increasingly applied as a probe of proton-proton proximities across a range of applications.[1] Enhanced resolution as compared to MAS alone can be achieved in a ^{1}H DQ CRAMPS experiment.[2] For example, ^{1}H DQ CRAMPS spectra have identified that the anhydrous and not the hydrous form of an active pharmaceutical ingredient is present in a tablet formulation.[3] Based on applications to systems with known crystal structures, a rule of thumb has emerged whereby the observation of ^{1}H DQ peaks is indicative of a H-H proximity within 3.5 Å.[1] Quantitative information about H-H proximities can be obtained from the build-up of DQ peak intensity in ^{1}H DQ CRAMPS spectra recorded with increasing numbers of POST-C7 recoupling elements,[4] which allow the reliable determination of relative H-H distances, even in dense networks of many dipolar-coupled spins.

The disaccharide β-maltose represents a challenging case because of the 24 distinct protons (14 aliphatic and 10 OH) having ^{1}H chemical shifts that all fall within a narrow range of approximately 3 to 7 ppm. Nevertheless, the ^{1}H resonances due to the 24 distinct protons can be assigned from ^{1}H DQ CRAMPS spectra and ^{1}H (DQ)-^{13}C correlation spectra obtained with a new pulse sequence that correlates a high-resolution ^{1}H DQ dimension with a ^{13}C single quantum (SQ) dimension using the refocused INEPT pulse-sequence element to transfer magnetization via one-bond ^{13}C-^{1}H *J* couplings, with the assistance of first-principles chemical shift calculations based on the GIPAW (Gauge Including Projector Augmented Waves) plane-wave pseudopotential approach.[5]

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Protein Dynamics, NMR, and Force Fields

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Every NMR parameter of a protein reflects to a certain extent both underlying structural and dynamic properties. This makes NMR uniquely suited for the detailed assessment of molecular ensembles generated via computer simulations and modeling. The utility of spin relaxation parameters, dipolar couplings, and scalar J-couplings has been demonstrated for a number of proteins. By contrast, the most prevalent type of NMR parameter, the protein chemical shift, has not been systematically harnessed for this task. We will describe the quantitative assessment of molecular dynamics ensembles of wide range of folded proteins generated by different force fields, including Amber ff99, ff99SB, and ff03, based on their back-calculated Cα, Cβ, and C' chemical shifts in comparison with NMR experiment.[1] For the latest generation of force fields, a substantial improvement is found for ensemble-averaged chemical shifts over individual snapshots. Explicit inclusion of protein dynamics provides the largest improvement for Cβ chemical shifts, which are dominated by the φ, ψ, and χ_1 dihedral angle distributions. NMR chemical shifts are available for a vast number of proteins via the BMRB repository, which makes it now feasible to quantitatively certify molecular dynamics simulations on an unprecedented scale. Moreover, the systematic exploration of differences between experimental and calculated chemical shifts opens up the possibility to directly improve molecular mechanics force fields.

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Electron Paramagnetic Resonance and the graphite world

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Electron paramagnetic resonance has long provided a valuable probe of the electronic properties for carbon-based materials with extended π-electron systems. Since the first EPR studies the attention has been focused on itinerant π-carriers in the graphitic conduction band and more recently on the magnetic properties of the electron spins localized on open edges of nanographenes. In this communication we report the results of an EPR study on nanographites obtained by grinding for different time graphite by a ball-milling equipment. A parallel Raman study has been done on the same samples.[1] The EPR spectra of the samples, given by ensembles of layered structures of graphene sheets of various dimensions and thicknesses, show how the milling affects progressively the mobility of the carriers and the density-of-states at the contact point between the valence π and the conduction π* bands. By an accurate simulation of the EPR signals inhomogenously broadened we obtained the g tensors, the lineshapes and the homogeneous linewidths of the spin packets. We have been able to separate the EPR contributions due the mobile electrons with paths in the range of μm (dysonians broad bands) from those in smaller particles (gaussians broad bands) and from the non bonding electrons on the edges (lorentzians). The lorentzians are exchange narrowed by the interaction with the itinerant electrons. We have established a clear correlation between the amounts of defects created by the grinding with the spin lattice relaxation rate and the g anisotropy. These results confirm that for nanoparticles the main relaxation mechanism is due to the scattering of the electron momentum by the edges, and that the g anisotropy in graphitic samples increases by increasing the degree of stacking disorder of the graphitic layers. A sound correlation between EPR and Raman results has been found.

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Novel NMR tools for the study of folded and unfolded proteins

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Enhancing spectral resolution and sensitivity, while preserving reasonable experimental times are important challenges for the study of proteins and protein complexes of increasing complexity, low concentration, or limited life time. This is especially true for intrinsically disordered proteins (IDPs) that are characterized by high frequency degeneracy and thus low spectral resolution. Here we present spectroscopic methods (BEST, SOFAST) for enhancing steady-state proton spin polarization in multidimensional NMR experiments of proteins. Polarization enhancement techniques allow faster repetition rates of the pulse sequence while preserving, and often significantly improving the overall experimental sensitivity both for folded and unfolded protein states. We also demonstrate how magnetization that is "lost" during the pulse sequence by spin relaxation can be partly recovered using fast-pulsing experiments. Amino-acid type editing in ^{1}H-^{15}N correlation spectra is another powerful tool for the study of proteins. Sequential and intra-residue HADAMAC experiments present an additional useful tool for sequential resonance assignment of IDP's.

Biological Interface Dynamics from Magnetic Relaxation Dispersion

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The spin-lattice relaxation of nuclear spins with I = ½ is driven by magnetic noise created by relative motions of the interacting spins. The magnetic field dependence of the relaxation rate constants provides a map of the power spectrum created by these motions that include molecular rotation, translational diffusion, intramolecular conformation fluctuations, and chemical exchange events. Relaxation dispersion measurements at high frequency provide a characterization of the molecular dynamics of the interfacial region of biological macromolecules. Although the diffusive exploration is biased by the excluded volume of the large molecule, the average interfacial translational correlation time for water at the protein interface is approximately 30 ps, about a factor of 3 slower than in pure water.[1, 2] In proteins labeled with a paramagnet, the electron-nuclear dipolar coupling dominates the relaxation rate constant, but similar translational correlation times are found. However, when a protein is spin-labeled with a nitroxide radical with a long T_{1e}, the apparent strength of the electron-nuclear coupling to water protons in solution experiments may be larger than expected because of contributions from long range dipolar couplings between the electron spin and long-lived protein bound water molecules that are rotationally correlated.[3] In tissues, the magnetic field dependence of the apparent spin-lattice relaxation rate constant is complex because it represents the superposition of effects from several classes of molecular interactions. Nevertheless, measurements on excised tissues over from 10 kHz to 300 MHz show that the relaxation is generally described by a power law over this range of Larmor frequencies suggesting that the underlying dynamics that drive the relaxation are common over this range.

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Detection of nuclear magnetization with optical magnetometers: from remote-detection imaging to measuring J-couplings at zero field

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In this talk, we will describe the approach to low-field NMR and MRI based on remote detection utilizing all-optical magnetometers that our group has been pursuing in collaboration with the group of Prof. Alexander Pines (UC Berkeley, Chemistry). An imaging system based on atomic gradiometer has been demonstrated,[1] which achieved sum-mm resolution[2] with pre-polarization with a permanent magnet and encoding at the Earth's field. The system was applied to imaging of porous metal[3] and a variety of other NMR and non-NMR studies.

More recently, in collaboration with the group of Dr. John Kitching (NIST, Boulder), we have been using microfabricated atomic sensors for such applications, which has resulted in the demonstration of the first microfluidic device with NMR-detection capabilities.[4] We are currently using such device for a systematic study of zero-field scalar ("J") coupling in a variety of organic molecules in the liquid phase.[5] In the talk, we will present the results of the recent attempts to combine this approach with "non-brute-force" hyperpolarization techniques, such as the use of parahydrogen (allowing one to do away with the pre-polarization magnet altogether), promising a significant boost in the signal-to-noise ratio and a much expanded applicability of the method. A complete up-to-date bibliography of our group's work can be found at http://budker.berkeley.edu.

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Multi-component Protein: Protein Complexes: The Impact of Long-range Restraints Derived from PRE, PCS, RDCs, Intermolecular NOE, and SAXS Data

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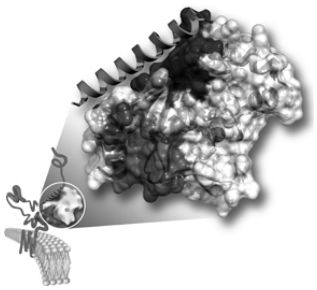

Our laboratories are engaged in a multi-disciplinary investigation of the ubiquitination process and the role of protein regulation via proteosomal degradation. In particular, we are interested in the multi-protein complexes associated with the E2:E3 recognition and ubiquitin transfer to substrate. Our previous studies[1] demonstrated that the E3 ubiquitin ligase gp78 uses a novel binding region to establish a high affinity complex with the E2 ubiquitin conjugating enzyme Ube2g2. The result of this binding complex is to allosterically increase the binding affinity between another domain of the E3, a RING domain, for the E2, resulting in more efficient ubiquitination. In order to understand this complex machinery, we have employed a combination of NMR methodology and complemented it with crystallography and molecular biology. This presentation will focus on the NMR aspects of examining the structure and interactions of these multi-component complexes by combining the use of intermolecular NOEs, long-range and intermolecular paragmagnetic relaxation enhancements (PRE) and pseudocontact shifts (PCS), residual dipolar couplings (RDCs), and small angle X-ray scattering (SAXS) data. We utilize tagging of the protein to introduce paramagnetic spin labels and lanthanide ions. In addition to the solution structures determined using this combined approach, we have determined crystal structures of some complexes and integrate all of the data with molecular biology in order to develop a global understanding of the molecular machine.

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Conformational Exchange and Dynamics in Membrane Transporters Determined by Site-Directed Spin Labeling

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Site-directed spin labeling (SDSL) and EPR spectroscopy have been used to investigate structural transitions that accompany ligand binding in a series of outer-membrane bacterial transport proteins. The measurements reveal order-to-disorder transitions that appear to initiate the transport process, as well as the dynamics and structural changes at ligand-binding sites. However, the structures and structural transitions observed by SDSL are often not consistent with high-resolution crystal structures. These differences appear to be due to environment, including the precipitants (or osmolytes) that are used to crystallize membrane proteins. The crystal lattice also plays a role in modulating conformational exchange, as revealed by both EPR and diffraction of membrane protein crystals. Regions of membrane proteins that are dynamic are generally modified by environment, while sites on proteins that are well-structured are not. A combination of CW and pulse EPR spectroscopy has been used to quantitate the conformational exchange in these proteins and to measure the effect of environment on the protein energy landscape.

Intracellular activation of integrin membrane receptors

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Cell migration depends on the formation of transient self-assembling complexes called integrin adhesions (IAs). Integrins are membrane spanning adhesion receptors that can send signals from inside to out and outside to inside the cell.[1] The protein components of IAs are generally constructed from multi-domain proteins, often with flexible linkers; as well as integrins, they include the extracellular matrix protein fibronectin,[2] numerous intracellular proteins, including talin,[3,4] kindlin[5] and filamin.[6] Integrin activation is an essential biological process that can be triggered via interactions of intracellular proteins with short cytoplasmic tails.[1,3] These interactions are regulated by phosphorylation,[7,8] protein-protein[4] and protein-membrane interactions.[9] I will review our recent studies of some of the dynamic complexes formed by IA proteins using NMR and other methods. The general features of such the complexes will be discussed, including the common use of unstructured peptide regions.

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Mechanisms of Intermolecular Recognition and Drug Design by INPHARMA: Theory and Applications

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Small molecules play a fundamental role in the regulation of the function of proteins, nucleic acids and molecular machines. The development of specific binders that selectively alter the function of one or a few cellular targets relies on the availability of structural information for the target active site and its mode of interaction with low affinity ligands, typically identified in screening experiments. When this structural information is not available, the rationale design of selective drugs is impossible and the process of drug development has to rely on the screening of large libraries of molecular fragments accompanied by lengthy, parallel routes of chemical synthesis. Recently we have developed a new NMR methodology, INPHARMA,[1,2] which provides access to the relative binding mode of low-affinity ligands to a common target. The method is based on the observation of interligand, spin diffusion mediated, transferred-NOE data, between two ligands A and B, binding competitively and weakly, to a macromolecular receptor T. In accordance with existing SBDD workflows, the INPHARMA NOEs are used to select the correct binding mode among many possible binding orientations obtained by molecular docking.[3] Here we show further developments of INPHARMA and benchmark its performance with respect to the parameters and information used in the evaluation of the data.[4] Finally, we demonstrate the application of INPHARMA to the design of ligands targeting membrane proteins.

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Solid-State NMR Study of the Formation of Steric Zipper in Amyloid Fibrils

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Steric zippers, where the residues of two neighboring beta-sheet layers are tightly interdigitated, have been proposed as fundamental structural units of amyloid fibrils by Eisenberg and co-workers. The steric zipper formed by polypeptides containing the palindromic sequence AGAAAAGA has a distinctive feature that the distance between two interdigitated beta-sheet layers is comparable to the inter-strand distance within an individual beta-sheet layer,[1] which presents a particular challenging case in structural characterization of amyloid fibrils. In this work,[2] a solid-state NMR strategy exploiting the multiple-spin effect in homonuclear dipolar recoupling has been developed to probe the molecular structure of the amyloid fibrils formed by the peptide fragment, A113 to G127, of Syrian hamster prion protein (SHPrP). Although the polypeptide sequence contains hydrophobic residues only, the SHPrP(113-127) fibrils do not form any in-register parallel beta-sheet structure. On the contrary, the target fibrils adopt the structural motif of class 7 steric zipper, which is formed by stacking of two antiparallel beta-sheet layers. Our structural model reflects a compromise among the extent of the zipper region and the number of hydrogen bonds between $SHPrP_{113–127}$ molecules. Because the AGAAAAGA sequence is highly conserved in prion proteins of different species, the steric zipper formed by this palindromic sequence (alanine zipper) may play a vital role in the formation of scrapie prion proteins.

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Structure, Dynamics and Ca^{2+}-Binding Properties of Intrinsically Unfolded And Folded βγ-Crystallins

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βγ-Crystallins belongs to a superfamily of diverse proteins having members from prokaryotes and eukaryotes, which have similar topology as that of lens β- and γ-crystalllins. Structurally, each domain comprises of an eight stranded β-sandwich made of two *Greek key* motifs. These motifs consist of a partially conserved signature sequence "Y/FXXXXY/FXG" that interacts with a generally conserved serine at the start of the fourth β-strand in each motif, which provides the stability to the scaffold. The functions of members of this family are not yet known.

In this backdrop, we investigated diverse members of putative βγ-crystallin superfamily, one from the genome sequence of the archaebacterium *Methanosarcina acetivorans,* a methanogen (named as M-crystallin), and another from the genome sequence of *Hahella chejuensis,* a marine bacterium which secretes a red pigment that has lytic activity against a red-tide dinoflagellates (named as Hahellin).

In this presentation, we provide structural and dynamic characterization of M-crystallin and Hahellin. Our study demonstrates that M-crystallin has the features of the lens βγ-crystallin fold and structurally nearest to the vertebrate lens βγ-crystallins. The observed structural resemblance of M-crystallin with vertebrate lens βγ-crystallins and phylogenetic analysis suggest that evolutionarily, lens βγ-crystallins descended from the archaeal crystallins (M-crystallin). This study allowed us to demonstrate the presence of βγ-crystallins in all the three domains of life, and to classify them as archaeal, microbial and eukaryotic βγ-crystallins. On the other hand, Hahellin shows unusual characteristics generally not seen in βγ-crystallins. βγ-crystallin domain of Hahellin is natively unfolded in apo form and folds upon binding Ca^{2+}, thus providing a clue for its function in the bacterial survival under high saline conditions. This prompts us to propose that such intrinsically unstructured βγ-crystallins constitute a separate sub-class of the superfamily. Thus, the members of βγCrystallin superfamily, which have evolved from primordial organisms to vertebrates to function mainly as structural proteins, indeed show significant functional role.

^{29}Si-^{29}Si Scalar and Dipolar Couplings as Constraints for Determining Complicated Silicate Structures

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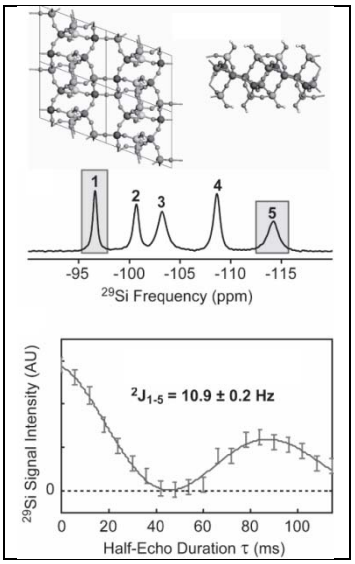
Solid silicates often exhibit subtle combinations of short- and long-range structural order and disorder, as manifested by solid-state 1D and 2D ^{29}Si NMR and X-ray scattering measurements.[1,2] Such features generally arise in the presence of cationic species that strongly interact with and direct the formation of co-assembling silicate networks, whose resulting structures are often difficult to establish. Nevertheless, homonuclear ^{29}Si{^{29}Si} scalar and dipole-dipole interactions, in conjunction with molecular modeling, can be used to establish the interconnectivities and local bonding environments among different sites in ordered silicate frameworks.[3,4] In combination, these provide important constraints on candidate structures that yield new insights for analyses of zeolites, layered silicates, and surfactant-templated silicate networks, several examples of which will be presented.



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Biophysical and structural approaches in fragment-based screening: probing molecular recognition for chemical biology and drug discovery

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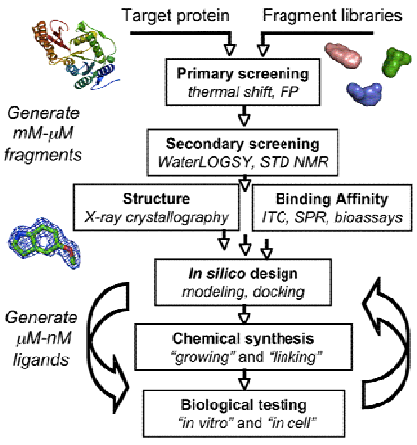
Fragment-based screening is now established as a new paradigm for the discovery of high-quality ligands for chemical biology and drug discovery. Crucial to fragment-based approaches is the ability to identify *bona-fide* fragment "hits" that bind weakly to the target protein, and to validate these from both structural (binding mode) and energetic (ligand efficiency) stand-points. My talk will focus on how we have implemented ligand-based NMR spectroscopy, including WaterLOGSY and STD techniques, for fragment screening, and how these are integrated within a cascade of biophysical and structural methods, including thermal shift, isothermal titration calorimetry, and protein X-ray crystallography (Figure).[1]



I will present how fragment-based approaches were applied and developed *a)* to probe "hot spots" at protein binding sites, and to mature new concepts, e.g. group efficiency;[2] *b)* to design new leads for antimicrobials against TB, by combining and contrasting both fragment-growing and fragment-linking strategies;[3] *c)* to optimize methodologies, e.g. ILOE NMR.[4] We are now extending our interests in new areas, for example targeting protein-protein interactions. I will present recent work using fragments to interrogate the plasticity and druggability of functional protein interfaces within the context of complex multi-protein assemblies.

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Hybrid structure determination methods, paramagnetic relaxation and differential relaxation

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This talk will focus on recent developments in our lab on three topics. (1) The use of hybrid strategies to solve the structures of large multimeric proteins and complexes based solely on dipolar couplings and SAXS/WAXS data coupled with conjoined rigid body/torsion angle/cartesian simulated annealing. This will be illustrated with regard to the structure determination of the 128 kD enzyme I dimer and the 146 kDa Enzyme I-HPr complex from the bacterial phosphotransferase system. (2) The use of PRE titration measurements to probe for heterogeneity in protein-protein encounter complexes illustrated with regard to the interaction of the N-terminal domain of enzyme I with HPr. (3) Differential relaxation measurements to probe exchange between monomeric proteins/peptides and large oligomeric/fibrillar structures.

NMR studies of protein-protein interactions

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Systematic approaches to the structural features of protein interfaces require new methodological developments in NMR, prioritized by leading edge systems of practical biological interest. Such interfaces have been characterized by NMR spectroscopy mostly by using chemical shift perturbations and cross-saturation via intermolecular cross-relaxation. Although powerful, there remains a need for unambiguous estimates of distances between interacting proteins. We have developed an alternative approach[1] to do so with greater accuracy using multiple sites, based on monitoring the cross-relaxation from a source protein (or from an arbitrary ligand that need not be a protein) with high proton density to a target with low proton density by using isotope-filtered NOESY. This technique is illustrated for two different protein–protein complexes.

The role of ‘unstructured' regions can be assessed using heteronuclear nOe's and improved measurements have been developed.[2] Segmental labeling permit the synthesis of isotopically labeled complex proteins like tyrosine kinases.[3]

The roles of multiple PDZ domains in the sodium/ hydrogen exchange regulatory factor 1(NHERF1) had remained unclear. Detailed structural characterization of the PDZ2 domain indicates a much extended structure compared to ‘canonical' PDZ domains. By combining NMR and small angle scattering experiments, a conformational transition in PDZ2 CT(C-terminal) section and release of the intramolecular domain-domain couplings between the PDZ2 and CT domains upon binding to ligand proteins was found.[4]

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Structure-activity studies of cyclotides:ultrastable plant proteins with applications in drug design

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Cyclotides are topologically unique proteins in that they have a head-to-tail cyclised peptide backbone and a cystine knotted arrangement of disulfide bonds. This makes them exceptionally stable to chemical, thermal and enzymatic treatments and, indeed, they are amongst nature's most stable proteins. They occur in plants from the Rubiaceae (coffee), Violaceae (violet) and Cucurbitaceae (cucumber) families of plants. Because of their exceptional stability and well-defined structures cyclotides make excellent templates for drug design applications. NMR has played a vital role in characterising their structures and examples of their applications in drug design will be described.

This presentation will describe the discovery of the cyclotides in plants, their structural characterization, evolutionary relationships and their applications in drug design. Their stability and compact structure makes them an attractive protein framework onto which bioactive peptide epitopes can be grafted to stabilize them. The structures of cyclotides will be related to other examples of cyclic peptides from bacteria, plants and animals.

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Sorting Structural Reality from Among the Artifacts: The M2 Proton Channel

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Anfinsen's thermodynamic hypothesis states "that the native conformation (of a protein) is determined by the totality of inter-atomic interactions and hence by the amino acid sequence in a given environment." Too often these last four words are ignored. For membrane proteins the membrane environment can have a substantial impact on the protein structure. Here, I will discuss structures of the M2 proton channel from Influenza A virus that is a proven drug target. With solid state NMR we have characterized both the transmembrane domain (residues 22-46) with and without the antiviral drug, amantadine, and we have recently characterized the longer conductance domain (residues 22-62) that has very similar electrophysiology to that of the full length protein. These structures were all characterized in a native-like liquid crystalline lipid bilayer environment. In comparison the transmembrane domain has been characterized by x-ray crystallography, both with and without amantadine where the crystals were formed out of an octyglucoside solution. In addition, the conductance domain has been characterized by solution NMR in DHPC micelles. Both the solution NMR and x-ray crystallography show significant structural distortions as a result of the membrane mimetic environment that prevent achieving a functional understanding of the protein.

The solid state NMR conductance domain represents a highly constrained backbone structure with key sidechain restraints. In restrained Molecular Dynamics a detailed model of the key HxxxW sequence responsible for acid activation, gating and conductance has been achieved at neutral pH. Based on this model it has been possible to develop a mechanism for proton conductance showing in detail how the charge on the histidine residues is stabilized and how protons are shuttled from the N-terminal pore to the C-terminal pore of this protein. The mechanistic details explain a broad range of chemical and physiological data.

Probing Novel Electronic States in Strongly Correlated Electron Materials Using NMR and NQR

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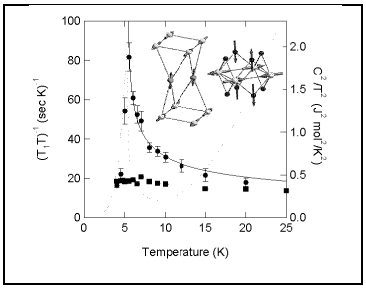
In the last two decades several new materials have been discovered which exhibit strong electron-electron interactions that lead to novel ground states such as superconductivity, coexisting antiferromagnetism and superconductivity, and ”hidden” order. NMR/NQR are ideal probe of these new states, several of which only emerge under extreme conditions in high magnetic fields, low temperatures and high pressures. By taking advantage of the hyperfine interaction, NMR/NQR can provide detailed information about order parameters and their dynamics throughout the phase diagram of these systems. Furthermore, NMR provides a local spectroscopy of the response of these systems to impurity doping. Several heavy fermion and iron pnictide materials will be discussed.



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Fluorophilic protein environments probed with ^{19}F NMR-based fragment screening

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The biological activity of lead compounds can be affected dramatically by the presence of a fluorine moiety that is placed in a particular position within the molecule. This is sometimes the result of an improved membrane permeability and/or metabolic stability of the fluorinated compound. However, the presence of a fluorine moiety can also strengthen the interactions of the molecule with the desired target. This is due to the favourable interactions of the fluorine moiety with the fluorophilic protein environments. ^{19}F NMR-based fragment screening, in combination with computational chemistry and X-Ray structure determination, represents a powerful and sensitive approach for identifying these fluorophilic spots on the proteins. For this purpose we have generated a library of fluorine fragments, known as LEF library, composed of fragments with different chemical environment around the fluorine moiety.[1] The library is screened in mixtures with ^{19}F NMR spectroscopy. Proper set-up of the experiments, according to rules derived from theoretical simulations, allows the identification of very weak-affinity ligands and the simultaneous detection of multiple ligands contained within the same tested mixture. In addition, a modification of the pulse sequence of the used NMR experiments allows for rapid acquisition resulting in the screening of few thousand fragments in just one day. The identified NMR-hits are then used in the FAXS experiments for the fragment optimization process via fluoroscan, for the follow-up screening and for binding constant measurements.[2,3] The principles of the combined approach and a selected application to the identification of fluorinated fragments binding to trypsin, a serine protease enzyme, will be presented and discussed.

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Self-assembling natural and artificial light-harvesters

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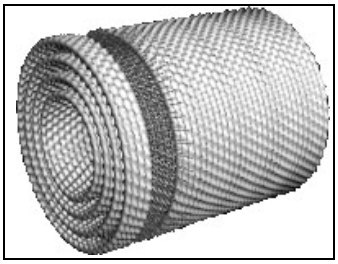

Solid state NMR is combined with quantum mechanical calculations and cryo-EM imaging or X-ray diffraction to image the stacking and supramolecular organization of chlorophyll light harvesters. By constructing a triple mutant, the heterogeneous BChl *c* pigment composition of chlorosomes of the green sulfur bacteria *Chlorobaculum tepidum* was simplified to nearly homogeneous BChl *d*. Computational integration of two different bio-imaging techniques, solid-state NMR and cryoEM, revealed a previously undescribed *syn-anti* stacking mode and showed how ligated BChl *c* and *d* self-assemble into coaxial cylinders to form tubular-shaped elements. Helical H-bonding networks form the basis for ultrafast, long-distance transmission of excitation energy. The structural framework is robust and can accommodate extensive chemical heterogeneity in the BChl side chains for adaptive optimization of the light-harvesting functionality in low-light environments. In a next step, we used this knowledge to generate biomimetic systems and study their structure by proton MAS HETCOR NMR experiments, which provided the resonance assignments of the chlorin rings, allowing for readings of ring currents. Density functional theory calculations revealed that in the biomimetic system the chlorins self-assemble in anti-parallel π-stacks in planar layers in the solid-state, while X-ray powder diffraction measurements revealed the 3D lattice of the packing.

NMR approaches for studying intermediate dynamics in organic solids and their applications in the study of electroluminescent polymers

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Chain relaxation due to molecular motions produces major effects on the properties of organic macromolecular solids such as polymer materials, affecting their softening, toughness, creep and solid-state processability features, as well as their dielectric and conducting behaviors. For instance, it has been shown that the conformation and dynamics of the polymer chains are especially important for the luminescent and transport properties of conjugated polymers used as active layers in polymer diodes, transistors and solar cells. Particularly, motions occurring in the range of hundreds of milliseconds to some microseconds (the so called intermediate regime) deserve special attention because the onset of molecular rotations in this regime usually triggers important modifications in the chains packing and conformation, which affects the materials properties. Although there are many NMR approaches capable of probing intermediate regime motions, the study of chemically and structurally complex materials often require some development and adaptation for either improving their performance or increasing the amount of information they provide. The acquisition of ^{13}C natural abundance high resolution MAS spectra is desired, but the use of MAS decrease the precision of the dynamic information, for example as compared with ^{2}H static spectra. In this lecture we will discuss recent developments and adaptations of ^{1}H-^{13}C separated local-field NMR experiments dedicated to obtain quantitative information on intermediate regime motions in organic solids. We will explore two well-known experiments namely Dipolar Chemical Shift Correlation (DIPSHIFT) and Lee-Goldburg Cross-polarization (LG-CP), using a combination of dynamical spin dynamics simulations and an analytical treatment to estimate the correlation times and activation energies of intermediate regime molecular rotations. Furthermore, we will present some applications of these methods for studying molecular rotations in electroluminescent polymers and, by comparing the results with many electro-optical characterizations, their effect on the desired materials properties, specifically the photo- and electro-luminescence and carrier mobility.

Molecular basis of viral pathogenicity

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Rabies Virus (RABV) infects exclusively neurons and causes lethal encephalitis. Pathogenic RABV strains favor neuronal survival, whereas non-pathogen strains lead to neuronal apoptosis. The use of recombinant RABV showed: (i) the G protein determined the induction of the survival or death phenotypes; (ii) the last 4 C-terminal amino acids of the G protein cytoplasmic domain (CytoG) are critical. These residues form a binding site for PDZ domain (PDZ-BS). Only one of the 6 amino acid that differ between survival and death G-proteins is located in this PDZ-BS. Results of two-hybrid experiments indicated that the CytoG of the two strains, pathogenic and non pathogenic, recruit host proteins containing PDZ domains. $CytoG_{survival}$ was found to interact with serine-threonine kinases (MAST), while $CytoG_{death}$ interacts also with three additional PDZ containing host proteins, predominantly with the tyrosine phosphatase PTPN4. To understand the fine structural basis for the specificity of the PDZ-CytoG complexes, we determined the 3D structures of the MAST-CytoG and PTPN4-CytoG complexes both by NMR and X-rays. The structures, as well as the affinities and kinetics parameters, of the MAST2 PDZ complexes with the two viral peptides are similar. We conclude that a single amino acid change in the PDZ-BS between the two strains cannot drastically modify the interaction with MAST2-PDZ, in agreement with the double hybrid data and confocal microscopy imaging on neuronal cells. The differential interaction of $CytoG_{death}$ with the cellular partner PTPN4 blurs the pro-survival signals engaging the infected cells through apoptotic trails.

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Spatial Proximity of Acid Sites in Microporous Zeolites as Studied by ^{1}H and ^{27}Al DQ MAS Solid-state NMR Spectroscopy

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The widespread application of microporous zeolites in petrochemical industry is mainly attributed to their acid-catalyzed activity. Many investigators found that a mild hydrothermal treatment of zeolites, which results in the release of aluminum from the zeolite framework and the formation of extra-framework aluminum (EFAL) species, can highly enhance the acid-catalyzed activity.[1] Generally, Brønsted acid sites are usually associated with framework tetrahedral Al and bridging hydroxyl group (SiOHAl), while Lewis acid sites correspond to the EFAL species in zeolites. Although direct experimental evidence was absent, the increase of catalytic activity was ascribed to the enhanced acidity due to the Brønsted acid and Lewis acid interaction or synergy.[2] The information on the spatial proximity of acid sites is crucial for understanding the synergetic effect. In this work, we employed ^{1}H and ^{27}Al DQ MAS solid-state NMR to probe the detailed spatial proximity of differeent acid sites in various zeolites. In combination with DFT calculations, the Brønsted/Lewis acid synergy was studied as well.

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Non linear spin-dynamics of dissolved hyperpolarized xenon

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Large magnetization in liquids induces the presence of two types of non-linearity which affects the spin dynamics. They result from the coupling between the rf coil and the magnetization (radiation damping) and from the distant dipolar fields. For very large magnetization, several effects such as NMR instabilities or spectral clustering have been reported.[1] Exploring this type of effects using dissolved laser-polarized xenon appears appealing since we benefit from variable concentrations, very high polarization (up to 0.5), and variable coupling between the coil and the magnetization.

We have recently reported the observation of spontaneous multiple chaotic maser emissions in such a spin system.[2,3] For better understanding this unexpected behaviour, we have developed alternative approaches for its investigation. In particular we have introduced spin-noise measurements of hyperpolarized species[4] which allows the monitoring of the spin dynamics without applying rf pulses which destroy and alter the transient magnetization. More recently an experiment based on multiple dipolar echoes was developed for allowing a direct local characterization of the magnetization.[4] These experiments performed at very low spin temperature (about 10mK for a magnetic field of 11.7T) have driven us to question the existence of a spin-temperature in such an ex-situ hyperpolarized system[5] or to develop a protocol for ensuring a perfect tuning of any probe, leading to a general method for NMR signal improvement.[6]

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Recent Progress in Clinical Hyperpolarized ^{129}Xe MRI

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After the introduction of hyperpolarized (HP) gas MRI using ^{129}Xe,[1] the field quickly transitioned to using ^{3}He, which offered simpler and more mature polarization technologies,[2] a large magnetic moment, and absence of physiologic effect.[3] Despite an extraordinary array of new methodologies and scientific insights, dissemination of HP ^{3}He MRI has been hampered by its dwindling supply and skyrocketing cost.[4] Therefore, to make HP gas MRI a sustainable technology, the field must transition from ^{3}He to the more readily abundant isotope ^{129}Xe.

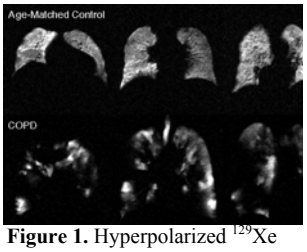

Figure 1. Hyperpolarized ^{129}Xe MRI

Here, we describe recent progress in hyperpolarized ^{129}Xe MRI made during a phase I clinical trial sponsored by GE Healthcare. Subjects received 3-4 doses of ^{129}Xe polarized to 6-8%. ^{129}Xe ventilation MRI showed remarkably good resolution ($3\times3\times15mm^3$) and SNR (25-35). Moreover, the addition of diffusion weighting[5] clearly delineates regions of emphysema in COPD subjects, and reveals age-related changes and postural gradients in healthy subjects. The most interesting property of ^{129}Xe is its solubility and large chemical shift, which we have now exploited to image alveolar-capillary gas transfer in human subjects.[6] This newest technique appears to be exquisitely sensitive to normal lung physiology and perfusion heterogeneity, which are altered in subjects with disease. Coupled with emerging new preclinical contrast ideas,[7] the future of hyperpolarized ^{129}Xe MRI appears to be looking up.

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Transfer of *para*hydrogen derived spin order sensitizes MRI and NMR measurements by three orders of magnitude

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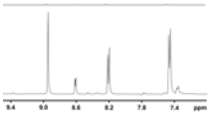
*Para*hydrogen as exemplified by PASADENA corresponds to a 'hyperpolarization' method.[1] When *para*hydrogen and a substrate to be polarized are brought into temporary contact via a suitable transition metal complex, polarization is transferred from the hydride ligands to the bound substrate over the period of a few seconds (Fig 1).[2] Substantial increases in NMR signal strengths are seen in the substrate without the need for its chemical modification (Fig 2). These hyperpolarized signals are employed in NMR and MRI procedures to demonstrate the ability of the method to probe molecular environments. The efficiency of this process and the nature of the magnetization created depend on the strength of the magnetic field where polarization transfer occurs and the lifetime of the interaction.[3]



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Folded-globule states of proteins detected by NMR

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Well-folded proteins give distinctive NMR spectra, with ^{1}H resonances that are well dispersed. Unfolded proteins also show less ^{1}H dispersion, but resonance linewidths are sharper. Intermediate states such as molten globules and other partly-folded species frequently combine the less-favorable features of the spectra of folded proteins (broader lines) and unfolded proteins (low dispersion). In addition, exchange processes on an intermediate time scale compared to the NMR chemical shift timescale can result in resonance broadening and disappearance. Nevertheless, a considerable amount of information can be obtained from NMR studies of these partly-folded states. We have now documented two cases, the inhibitor IκBα of the transcription factor NF-κB[1], and the p53 DNA-binding domain, a client protein of the chaperone Hsp90. For IκBα, the spectrum of the free protein shows resonance broadening and disappearance associated with the presence of motion on an intermediate time scale in one part of the protein; these resonances reappear in the complex. In the second system, parts of the ^{1}H-^{15}N HSQC spectrum of the client protein are broadened when Hsp90 is added, while the free protein gives a well-dispersed complete spectrum characteristic of a well folded protein domain. These results have led us to suggest the presence of a "folded globule" state, which contains secondary structure indistinguishable from that of the free protein, but consists of a manifold of states in intermediate exchange on the NMR time scale, thus causing resonance broadening and disappearance. Although NMR observations relevant to partly folded states such as the folded globule are characterized more by the absence of signals rather than their presence, the comparison of these proteins under various conditions can give information on the nature of partly-folded states that is not available by any other means.

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New Methods for Solid-State NMR Simulations and Studies of Bio-mimetic Apatite-Formation from Mesoporous Bioactive Glasses

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We will present results in the following areas of technique-developments and applications of solid state NMR:

(1) New approach to spectral interpolation: The simulation of magic-angle-spinning NMR spectra from powders may be very time-consuming, as the calculations need to be repeated for many crystallite orientations. We have previously demonstrated accelerated simulations by combining Gaussian spherical quadrature (GSQ) orientations with Alderman-Solum-Grant spectral interpolation.[1,2] Here we present an alternative interpolation strategy that restricts the explicit matrix-calculations to a small grid of GSQ-orientations; this information is exploited to construct the NMR spectrum representative of a much larger set of crystallites.

(2) Bio-mimetic apatite-formation of mesoporous bioactive glasses (MBGs): Thanks to their ability to bond both to soft and hard tissues, these silica-based materials are promising candidates for improved bone and tooth implants. The "bioactive" feature stems from the formation of a hydroxy-carbonate apatite layer on the MBG surface upon its immersion in a (simulated) body fluid. We recently proposed a structural model of the MBG pore-wall that improves the insight into the high bioactivity of MBGs.[3] The MBG surface reactions leading to apatite formation will be revealed by using an array of ^{31}P, ^{29}Si, ^{23}Na and ^{13}C NMR experiments.

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The Merging of Metabolomics and Natural Products: Applications in Chemical Communication of Nematodes

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The nematode *Caenorhabditis elegans* is one of the best-studied animals in the world. It was the first metazoan to have its genome sequenced. Its entire cell lineage from a single fertilized egg to an adult is known and has been related to the animal's anatomy, and its anatomical ultrastructure has been comprehensively described by thin-section electron microscopy. *C. elegans* is particularly tractable for genetic studies, and as a result many signal transduction pathways have been identified. Six people have shared three Nobel Prizes for developing the animal into a model organism, for discoveries of apoptosis and RNAi in *C. elegans*, and for the development of *in vivo* green fluorescent protein applications. We have recently discovered that *C. elegans* employs an overlapping set of pheromones to regulate both dauer formation and mating.[1] These pheromones all have an ascarylose sugar with a variety of fatty acid-like groups. At picomolar concentrations, at least three ascarosides act synergistically to attract males. At nanomolar to micromolar concentrations, these same compounds induce dauer formation. More than 10 ascarosides have now been identified in *C. elegans*, suggesting a potential for multiple physiological roles.[2] We are now working on the identification and characterization of pheromones and other small molecule metabolites in two other nematode species, *Panagrellus redivivus* and *Pristionchus pacificus*. I will describe new approaches to compare the small signaling molecules.

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From eukaryotes to prokaryotes (or vice versa?): single classical zinc fingers as DNA binding domains

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In eukaryotic organisms, the abundant Cys_2His_2 zinc finger domain consists of less than 30 amino acids and the zinc ion is essential to stabilize the ββα fold. In prokaryotic organisms, the first Cys_2His_2 zinc finger domain has been identified in the transcriptional regulator Ros from *Agrobacterium tumefaciens.*[1] We have structurally and functionally characterized Ros DNA-binding domain and shown that the prokaryotic Cys_2His_2 zinc-finger domain, though having a similar zinc coordination sphere, possesses a novel protein fold, which is very different from that of the eukaryotic counterpart.[2,3] A large number of Ros homologues have been found in different bacteria, having mostly a high sequence identity with Ros protein, which, surprisingly, does not comprise the zinc coordination sphere. The results here presented indicate that the prokaryotic zinc-finger domain, which in Ros protein tetrahedrally coordinates Zn(II) through the typical Cys_2His_2 coordination sphere, in Ros homologues can either change the coordination sphere or lose the metal while still preserving the DNA binding activity.[4] In light of our findings an evolutionary link between the prokaryotic and eukaryotic zinc-finger domains, based on bacteria-to-eukaryota horizontal gene transfer hypothesis, is discussed.

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New insights into structure and dynamics of riboswitch and telomerase RNAs

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RNA pseudoknots are commonly occurring secondary structural elements that provide long-range tertiary interactions in folded RNAs, and they can have essential roles in both structure and function. We recently determined the solution structure of the aptamer domain of the preQ1 riboswitch from *Bacillus subtilis*. Riboswitches are a class of regulatory mRNA elements that bind specific metabolites, and are most often found in bacterial operons coding for protein products that produce or transport the related metabolite. The $preQ_1$ riboswitch, found in the 5' UTR of bacterial genes involved in synthesis of the queuosine precursors $preQ_0$ and $preQ_1$, contains the smallest known aptamer domain. The modified nucleotide queuosine (Q) is almost universally found in the anticodon wobble position of specific tRNAs. The preQ1 riboswitch aptamer domain forms a unique compact pseudoknot with three loops and two stems that encapsulates $preQ_1$ at the junction between the two stems. The pseudoknot only forms in the presence of $preQ_1$, and the 3' A-rich tail of the aptamer domain is an integral part of the pseudoknot. In the absence of $preQ_1$ the A-rich tail forms part of the antiterminator. These structural studies provide insight into riboswitch transcriptional control of $preQ_1$ biosynthesis. Two related crystal structures of this riboswitch aptamer have been published, and reveal both similarities and differences in the structure. We report NMR studies of the dynamics of the riboswitch binding pocket and effect of cations on structure that provide new insights into 'catch and capture' of the preQ1 ligand. New results on structure and dynamics of telomerase RNA pseudoknot/core domain will be presented as well.

Magnetic Resonance Imaging in Real Time

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The purpose of this presentation is to introduce a novel method for real-time magnetic resonance imaging (MRI) that yields high-quality images with acquisition times as short as 20 ms and movies with 50 frames per second.

The approach combines two major principles: (i) a fast low-angle shot (FLASH) MRI technique using radial trajectories for spatial encoding,[1] and (ii) a regularized nonlinear inversion for image reconstruction.[2] The former allows for rapid, continuous and motion-robust imaging, and ensures insensitivity to off-resonance artifacts and moderate tolerance to data undersampling. The latter exploits the advantages of parallel imaging with multiple receive coils and enhances the degree of radial undersampling in an hitherto unexpected manner by another order of magnitude. Studies of healthy subjects were performed on an unmodified 3 T MRI system (Trio Tim, Siemens AG, Erlangen, Germany).

Apart from preliminary studies of joint motion and speaking processes, first applications focused on cardiovascular MRI during free breathing and without synchronization to the electrocardiogram. Real-time MRI movies based on T1-weighted radial FLASH images (TR/TE = 2.0/1.3 ms, flip angle 8º) were obtained at 1.5 to 2.0 mm in-plane resolution along anatomically defined orientations and with 20 to 30 ms temporal resolution (11 to 15 spokes per image). The images exhibit a high signal of the myocardial wall, good blood-tissue contrast, and excellent temporal fidelity.

The proposed real-time MRI technique pushes MRI towards its technological limit. It is expected to revolutionize many applications in the biomedical sciences as well as in diagnostic imaging.

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Pulse Dipolar ESR and Protein Superstructures: Signaling Apparatus in Bacterial Chemotaxis and Varying Structures of alpha-Synuclein

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Bacterial chemotaxis refers to the mechanism of bacterial movement in response to gradients of nutrients and repellents. In bacterial chemotaxis, autophosphorylation of the histidine kinase, CheA, is regulated by chemoreceptors and an adapter protein CheW. CheA phosphorylates CheY; Phospho-CheY interacts with the flagellum motor and switches the sense of rotation. One of the fundamental questions of this complex assembly is the ternary structure of the signaling complex of the CheA dimer, two CheW's, and the receptor dimer. Whereas each individual sub-unit protein could be studied by crystallography or NMR, neither technique can address this six protein complex. However, we have succeeded for the first time in determining the structure of this complex. We have shown that the receptor binds and stabilizes the regulatory domains of CheA. Our direct distance measurements by pulsed dipolar ESR (PDS) between the P3 domain (of CheA) receptor have shown that the two interact with their helical axis running anti-parallel to each other.

Alpha-synuclein (αS) is a highly conserved presynaptic protein that participates in synaptic strength maintenance and dopamine homeostasis. However, accumulation of αS amyloid fibrils was implicated as the major reason in the development of Parkinson's disease. It was shown by NMR that in detergent micelles the protein adopts two extended surface-bound helices separated by a non-helical linker and the helices are oriented in an antiparallel fashion. In previous PDS distance measurements of αS bound to different size micelles we showed that the helices splay further apart on the surface of larger micelles. We have used PDS to measure large distances (up to 8.7 nm) in αS bound to lipid vesicles, rod-like micelles, and isotropic lipid bicelles, all of which present the protein with a more extensive, less highly curved surface than spheroidal micelles. Distances measured for αS between labels are in close agreement with those expected for a single continuous helix, which argues strongly for a single, unbroken helix. Conditions which favor one or the other conformers will be discussed.

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Payback Time: Spatially Encoded NMR as a Novel MR Imaging Modality - Principles and Prospects

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The robustness of single-scan MRI is affected by the type of environment being addressed. This facet is well known in functional MRI experiments, Spin-Echo Echo-Planar Imaging (EPI) and Gradient-Echo EPI sequences are endowed with very different sensitivities and specificities. Recent developments of new NMR methodologies based on spatial, rather than temporal, encoding, provide new approaches to imaging with alternative features for both single- and multi-scan MRI.[1-4] Compared to EPI counterparts, these schemes will be shown to provide higher robustness to field inhomogeneity artifacts. Although initially suffering from lower a-priori spatial resolution, this study also will show how a post-processing algorithm based on super-resolution principles can resolve these resolution issues while retaining the fMRI activation data resulting from the BOLD effect. At the same time, it will be shown how it becomes possible to address via spatial encoding, rapidly susceptibility-changing regions that are not amenable to typical Spin-Echo MRI approaches.

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Wood NMR and MRI: molecules, interactions, and motion

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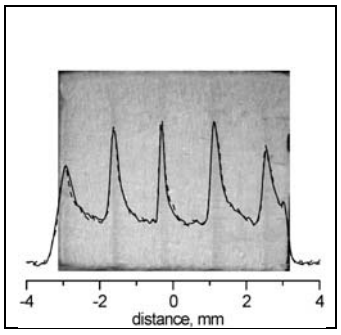
Wood has a composite structure with hierarchical and non-random porosity and with a rich chemistry. It has an enormous potential as renewable material for a large variety of applications which often call for improved properties such as dimensional stability, moisture insensitivity, and durability. To achieve these, bulk chemical modifications and coatings can be used. Alternatively, separating and re-engineering wood macromolecular components such as cellulose or lignin can yield completely new materials and properties. Irrespective of the particular sub-field, methods for characterizing the molecular states of different molecular components and their spatial distributions are in demand. The distribution and dynamics of adsorbed water is a particularly important area.[1] Various MRI approaches are to be presented, as illustrated here by one-dimensional radial ^{1}H (solid line) and ^{2}H (dashed line) MRI profiles depicting, respectively, macromolecular and water density in wood with 12 w% D_2O content (optical image overlaid). Besides high-field MRI, low field portable open access (unilateral) magnets are explored for in situ and non-invasive monitoring of local moisture content in extended wood specimens. Chemical changes and molecular immobilization/grafting in chemically modified wood are investigated both by multinuclear MRI and by ^{13}C and ^{1}H MAS NMR spectroscopy.



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Insights into structure, dynamics, and interactions in multidomain systems

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Many cellular proteins have modular architecture, i.e. consist of several well-folded domains connected by (often unstructured and flexible) linkers. Interdomain interactions and conformational flexibility play a key role in molecular recognition events and functional regulation involving multidomain proteins. For example, numerous cellular processes are regulated by tagging substrate proteins with a chain of covalently linked ubiquitin molecules, called polyubiquitin. The outcome of polyubiquitination depends on the length of the polyubiquitin tag and the specific lysine involved in the ubiquitin-ubiquitin linkage, and range from targeted protein degradation to DNA repair to inflammatory response. Knowledge of the conformational properties of polyubiquitin is essential for understanding the determinants of the amazing functional diversity and linkage-specificity in polyubiquitin signalling. Using polyubiquitin as a paradigm of a multidomain system, I will highlight the challenges in determining the structure and dynamics of such systems and their recognition by various receptors, and discuss some recent NMR-based approaches designed to address these issues and the recent results in this direction.

Mapping Inhibitor Binding Sites on a Large Enzyme by Electron Spin-Spin Derived Distances

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Finding where organic ligands dock on a “large” biological molecule is a theme common to many experiments. The experimental approach discussed here is a variation of long-range distance measurements by electron-electron dipolar interactions, in which a both the organic ligand and the protein to which it binds bear a spin label at selected sites. The approach is applied to determine how fatty acid derivatives inhibit lipoxygenases. Despite efforts, x-ray crystallography has not solved this problem, although the resting enzyme structure is well established. Lipoxygenases have a centrally located iron atom that performs highly selective hydrogen atom abstraction. The iron is surrounded by ~70 kDa of a roughly spherical protein. Five residues near the protein surface, with distal side chain atoms 20-29 Å from the iron, were selected for Cys mutagenesis and spin labeling. Inhibitors chosen were a TEMPOCHOLINE spin labeled lysolecithin and the well-known DOXYL-stearates. Locations of the spin labeled protein side chains were determined by DEER measurements on doubly-labeled proteins, in collaboration with Peter Borbat and Jack Freed at ACERT (Cornell). Then distances between single protein sites and spins on fatty acid inhibitors were examined, also by pulsed dipolar spectroscopy. Biochemical characterization, iron-induced saturation and solution EPR were carried out with Miles Bradshaw, Stephen Frausto and Fayi Wu at Florida State. Findings include that lipoxygenases readily solubilize lysolecithin micelles, that bound fatty acid derivatives have regions with variable microsecond dynamics, and that lipoxygenases have a substrate-interactive region of structure that is likely the entrance to an internal cavity.

Pulsed EPR studies of decoherence in rare earth ions and coupled systems

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Quantum bits (Qubits) are the basic building blocks of any quantum computer. They are two state systems which obey time-dependent quantum mechanics and, in particular, are able to produce several coherent oscillations before damping occurs. For this purpose, it is essential that the phase of the wave function be protected from noise. Maximization of coherence times requires a detailed understanding of decoherence mechanisms.

In principle there is a very broad choice of Qubits available because any sufficiently small system obeys quantum mechanics. In practice, however, difficulties associated with decoherence and implementation (scalability) reduce this choice considerably. Qubits based on S=1/2 electronic spins exhibit very interesting properties, in particular very long coherence times and have been intensively studied.

Recently, we, among others, found that electronic spin Qubits based on different systems could have promising properties. The presentation will cover the decoherence properties of some of these new Qubits, in particular based on:
- the total angular momentum of rare-earth ions, which includes an orbital contribution with strong spin-orbit and crystal-field effects. We called these qubits “Spin-Orbit QuBits” (SOQBs)- the collective spin of a molecular magnet.

For each system, the relaxation times (T1 and T2), and the single Qubit figure of merit (QM) as a function of different parameters will be shown to be key properties to understand decoherence mechanisms.

Frequency-Swept MRI: No Sound or Echoes

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A fundamentally different approach to MRI is described called SWIFT (sweep imaging with Fourier transformation).[1,2] SWIFT exploits time-shared RF excitation and signal acquisition, allowing capture of signal from spins with extremely short transverse relaxation time, T_2. MR signals are acquired in gaps inserted in a broadband frequency-swept excitation pulse, which results in acquisition delays of only 1 – 2 μs. With its ability to capture signals from ultrashort T_2 spins, SWIFT promises to expand the role of MRI into areas of research and medicine where MRI previously played no or negligible role, such as dentistry and imaging of lung parenchyma (Fig. 1). SWIFT is a quiet imaging technique with an ability to acquire 3D images in scan times similar to and, in some cases, faster than conventional 3D gradient echo sequences. Broadband frequency-swept excitation and extremely short acquisition delay make it possible to preserve frequency-shifted signals near metallic implants. In addition, magnetically labeled nanoparticles (e.g., SPIOs), which cause signal avoids in images acquired with conventional gradient echo sequences, give rise to positive contrast (bright spots) in SWIFT images, to improve MRI tracking of targeted molecules and cells.[3]

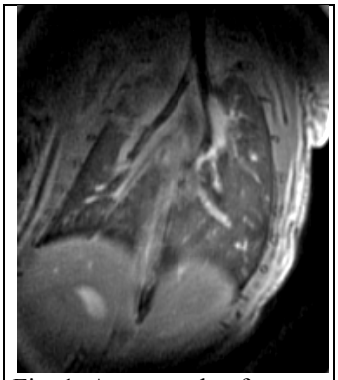

Fig. 1: An example of imaging short T_2 water spins in mouse lung in vivo with SWIFT.

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EMR of iron molecular nanomagnets

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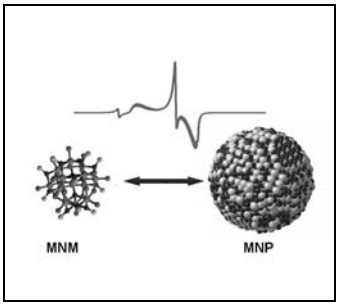

Molecular Nanomagnetism is producing many new systems which are relevant to many different scientific fields like surface science, spintronics, quantum computing, biology and biomedicine etc. One of the original goals of Molecular nanomagnetism was that of producing aggregates of magnetic centres of increasing size in order to explore the field where the transition from quantum to classical regime occurs. Molecular Nanomagnets (MNM) and magnetic nanoparticles (MNP) can now be obtained almost of the same size using synthetic molecular approaches.

In this communication it is shown how magnetic and EPR techniques provide evidence of the emergence of bulk functionalities like internal fields and shape effects in iron clusters prepared using suitable blocking ligands and proteic shells provided by ferritins and Dps.[1]

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Structure/Function of Radical Enzymes

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Enzymes utilize amino acid based radical species to catalyze reactions which would be difficult to achieve using two-electron chemistry. The enzymes use a variety of metals, either as endogenous ligands or as bound cofactors, to generate these radicals. Studies are presented in which EPR techniques, primarily High Frequency EPR, are used to identify protein-based radical species in several systems. Systems for which radical intermediates have been investigated include *Lactobacillus leichmannii* ribonucleoside triphosphate reductase (RTPR), prostaglandin H_2 synthase (PGHS), *Mycobacterium tuberculosis* catalase-peroxidase (KatG), and prostacyclin synthase (PGIS).

Insights into Allostery: The Hsp70 Molecular Chaperone

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Hsp70 molecular chaperones participate in chaperone networks essential to cellular homeostasis and are involved in both normal and disease states, leading to their emergence as possible therapeutic targets. Hsp70s have the capacity to bind extended hydrophobic segments of partially folded proteins and to release their clients in an ATP-dependent manner. Hsp70s are made up of an N-terminal nucleotide-binding domain (NBD) with an actin-like fold and a C-terminal substrate-binding domain (SBD) comprised of a distorted β-sandwich followed by a helical lid sub-domain and an intrinsically unfolded region. A highly conserved hydrophobic linker connects the SBD and NBD. Data from our lab and others support independence of the two domains in the ADP-bound state, with the SBD having high affinity for substrate. Upon binding of ATP, the two domains undergo internal rearrangements and dock onto each other to form one contiguous structure with lower affinity for substrate. To elucidate the role of the NBD as a nucleotide-modulated switch, we analyzed NMR chemical shift diversity of 12 forms of the NBD of the *E.coli* Hsp70, DnaK. Our results reveal detailed features of NBD conformational changes during the allosteric cycle and provide a structural description of the crucial ATP-bound state. We find that the interdomain linker specifically binds to the NBD in a nucleotide-dependent fashion, acting as a signal transduction element and leading to a conformation favorable for ATP hydrolysis. Chemical shift perturbations identify an allosteric intradomain network, which then communicates to the SBD to mediate interdomain allostery. Nucleotide binding to all four NBD sub-domains plays a central role by linking the nucleotide-binding site with sub-domain interfaces. In turn, perturbation of interdomain interfaces, for example upon linker binding, is communicated to the nucleotide-binding site. This model for Hsp70 allostery serves as a paradigm for other allosteric systems.

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Optimal Control of Spins Systems: Robust Pulses, Coherence Transfer and Decoupling

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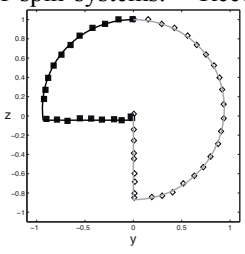
Based on principles of optimal control theory, the physical limits of quantum control can be explored and robust time-optimal and relaxation-optimized pulse sequences can be designed to control the dynamics of spin systems.[1-3] Recent advances include: (A) A general solution for the optimal control of a single spin with limited rf amplitude and in the presence of relaxation has been found. This is illustrated for the saturation problem in the absence of B_0 or B_1 gradients, where the optimal solution[4] (black curve) is up to 2.5 times faster than conventional sequences based on inversion recovery (grey curve). (B) A set of robust 90° and 180° pulses has been numerically optimized, which makes it possible to simply replace rectangular pulses in multi-dimensional experiments in a straight-forward way, providing increased bandwidth and tolerance with respect to rf inhomogeneity. (C) In heteronuclear spin systems, the optimal creation of multi-spin order, e.g. for out and back coherence transfer, has been studied. (D) The application of optimal control methods to the problem of heteronuclear decoupling[5] yields unprecedented flexibility and decoupling quality.



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Nanometer scale distance measurements in proteins using Gd^{3+} spin labeling

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Methods for measuring nanometer scale distances between specific sites in proteins are essential for analysis of their structure and function. In this work we introduce Gd^{3+} spin labeling for nanometer range distance measurements in proteins by high field pulse EPR. To evaluate the performance of such measurements we carried out four pulse DEER (double electron electron resonance) measurements on two proteins, p75ICD and τ_C14, labeled at strategically selected sites with either two nitroxides or two Gd^{3+} spin labels. In analogy to conventional site directed spin labeling using nitroxides, Gd^{3+} tags that are derivatives of dipicolinic acid were covalently attached to cysteine thiol groups.

Measurements were carried out on X-band (~9.5 GHz, 0.35 T) and W-band (95 GHz, 3.5 T) spectrometers for the nitroxide labeled proteins and at W-band for the Gd^{3+} labeled proteins. In the protein p75ICD, the orientations of the two nitroxides were found to be practically uncorrelated and therefore the distance distribution could as readily be obtained at W-band as at X-band. The measured Gd^{3+}-Gd^{3+} distance distribution had a maximum at 2.9 nm as compared to 2.5 nm for the nitroxides. In the protein τ_C14, however, the orientations of the nitroxides were correlated and the W-band measurements exhibited strong orientation selection that prevented a straightforward extraction of the distance distribution. The X-band measurements gave a nitroxide-nitroxide distance distribution with a maximum at 2.5 nm and the W-band measurements gave a Gd^{3+}-Gd^{3+} distance distribution with a maximum at 3.4 nm. The Gd^{3+}-Gd^{3+} distance distributions obtained are in good agreement with expectations from structural models that take into account the flexibility of the tags and their tethers to the cysteine residues. These results show that Gd^{3+} labeling is a viable technique for distance measurements at high fields that features an order of magnitude sensitivity improvement, in terms of protein quantity, over X-band pulse EPR measurements using nitroxide spin labels. Its advantage over W-band distance measurements using nitroxides stems from an intrinsic absence of orientation selection.

Hyperpolarisation: Possibilities and Impossibilities

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A large number of hyperpolarisation experiments with C-13 organic compounds have been described. Focus has been on either in vitro NMR analytical or in vivo MR Imaging studies. The studies reveal that the hyperpolarisation process has been improved and that polarization of more than 50% has been obtained by using the DNP method. A review of these studies will be given.

If the time between dissolution of the frozen polarized solid and the NMR in vitro analyses can be shortened significantly the method opens up for interesting possibilities for following chemical reactions (metabolism) and catalytical processes with previously unrevealed information. So far, the most interesting in vivo imaging results have been obtained using small endogeneous molecule like acetate, pyruvate, alanine and bicarbonate.

The experiments in animals (mice, rats, rabbits and pigs) have for the first time shown the possibilities for doing real metabolic imaging studies, in contrast to all PET studies where only the localization of a radioactive tracer can be imaged!

The experimental metabolic imaging performed with the hyperpolarized compounds indicates that future clinical use will allow improved medical diagnosis of major diseases like cancer and heart infarct.

The strong signal enhancement obtained by the hyperpolarisation process also opens up for the possibility of doing MRI interventional procedures where the catheters can be imaged and the effect of treatment can be studied real time.

The main obstacle for the hyperpolarisation imaging technique is the short T1 of most of the carbons in larger molecules. Imaging of small molecules may also be impossible if the small molecule is bound to proteins or other large molecules.

Use of other atoms than C-13 has been described in the literature. A review of this will also be given.

Pathway Symmetries in Magnetic Resonance

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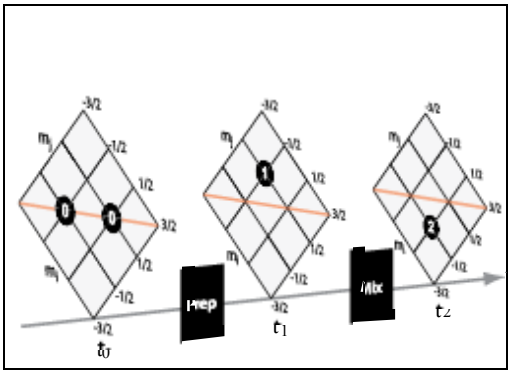
When internal spin couplings are much larger than the rf coupling we can no longer rely on the symmetries of the orthogonal rotation subgroup as a guide to designing new NMR experiments. In this case, the use of Average Hamiltonian Theory to design time reversal sequences can become challenging. This fact alone, may explain why solid-state NMR of quadrupole nuclei had developed at a slower pace than coupled spin 1/2 nuclei. Despite this difficulty, it is indeed possible to design time reversal experiments for nuclei experiencing strong couplings, and over the years a systematic approach has developed in many labs around the world. In this talk we attempt to distill these approaches down to their essential elements and present them in a single consistent framework. We expand the concept of coherence transfer pathways to ``symmetry pathways''. We give illustrative examples of how such pathways can be used to describe experiments that selectively eliminate specific contributions to an NMR spectrum, particularly for quadrupole nuclei. We also review the use of affine transformations to separate frequency contributions with different symmetries into different dimensions of a multidimensional spectrum.



Comparing Longitudinal and Transverse detection of EPR

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Longitudinal detection (LOD), which employs a coil to inductively measure the change of the longitudinal spin magnetization, is a largely complementary technique to the commonly used heterodyne detection for EPR spectroscopy. In most instances, heterodyne detection is significantly more sensitive than LOD, but LOD is more robust and can be used under unfavorable experimental conditions that prevent heterodyne detection.[1] A comparison of the features, the sensitivity, and complementary applications of the two methods is presented. The sensitivity discussion follows the well-known route outlined by Abragam,[2] taking into account different practical design alternatives available for LOD. This analysis is complemented by numerical simulations to estimate the spin magnetization transient that is inducing the signal.

Since LOD directly measures the change of electron spin polarization, it is particularly well suited for measuring longitudinal relaxation. Various different techniques have been suggested. Continuous wave (cw) schemes have proved successful to measure very short longitudinal relaxation times when the Bloch equations provide an adequate description of relaxation.[3] However, especially at low temperatures, solid samples commonly show a distribution of relaxation times, and transient methods are required for an accurate characterization.[4] Different experimental schemes are compared and discussed with respect to their practicality. Saturation–recovery with point-by-point or low-power cw detection can be implemented with transverse as well as with LOD detection. On the other hand, direct detection of the transient longitudinal magnetization is possible only with LOD. The feasibility and performance of this technique are discussed, along with possible methods for sensitivity improvements.

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The amyloid β peptide involved in Alzheimer´s disease: molecular interactions, secondary structure conversions and aggregation

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The amyloid β peptide consists of 39-43 residues and is the major component of neuritic plaques in the brains of Alzheimer's disease patients. We study the structure conversions and aggregation properties of the Aβ(1-40) peptide using high resolution NMR spectroscopy. At low concentrations, low temperatures and low ionic conditions in an aqueous solution, Aβ(1-40) is monomeric. Metal ions like Cu^{2+} and Zn^{2+} bind to amino acid ligands in the N-terminus of the peptide and induce increased order in the N-terminus.[1] Cyclodextrin or covalently linked cyclodextrin dimers interact with the aromatic sidechains of Aβ(1-40), and mediate inhibition of peptide aggregation.

By gradually adding the detergent lithium dodecyl sulphate (LiDS) or SDS to a dilute aqueous solution of Aβ(1-40), secondary structure conversions of Aβ(1-40) can be observed.[2] An initial transition involves conversion of the weakly structured peptide to β-sheet structure, concomitant with formation of large aggregates. At LiDS concentrations close to the CMC or above, a second transition makes the peptide rearrange to form a partly α-helical structure, concomitant with disaggregation and formation of normal LiDS micelles which apparently partly dissolve the aggregates. This α-helical structure is similar to that previously observed by NMR at high SDS concentrations.[3] Detergents like Congo red interfere with the peptide structure conversions, giving rise to changed aggregation pathways of Aβ(1-40).[4]

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Probing semiquinone binding to nitrate reductase A by pulsed EPR spectroscopy

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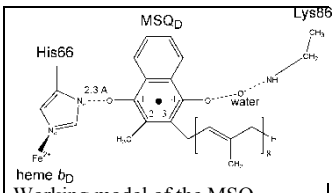
E. coli Nitrate reductase A (NarGHI), a membrane-bound respiratory complex, has the ability to utilize as substrate both menaquinol and ubiquinol usually associated with anaerobic or aerobic growing conditions, respectively. However, due to the absence of quinone in the crystal structure of NarGHI, the number and location of the quinol binding sites were largely debated.



Working model of the MSQ environment in *E. coli* NarGHI

To understand the molecular basis of the unusual stability of the EPR-detectable menasemiquinone (MSQ) intermediate located in the membrane subunit NarI, a multifrequency HYSCORE study was directly undertaken on NarGHI-enriched inner membrane vesicles (IMVs).[1] Analysis of the ^{14}N and ^{15}N hyperfine couplings reveals that MSQ is specifically H-bound to a nitrogen atom which was assigned to the N_δ imidazole nitrogen of the heme b_D axial ligand His66. Moreover, the EPR study of NarGHI-enriched IMVs purified from a menaquinone-deficient *E. coli* strain shows that endogenous ubisemiquinones (USQ) can also be detected. The use of ^{14}N HYSCORE enabled us to distinguish the USQ radicals bound to different membrane-bound enzymes, and to clearly identify the USQ species bound to NarGHI. Noticely, MSQ and USQ bind in a single site of the NarGHI complex in a similar mode involving H66 N_δ.[2] Overall, these results allow us to address at the molecular level the question of the adaptation of an anaerobic enzyme to oxygenic conditions.

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Synergy between NMR and cryo-EM - Novel Findings for HIV Capsid Function

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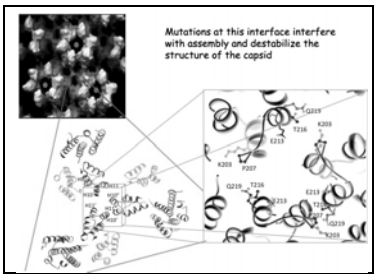
Mature HIV-1 particles contain a conical-shaped capsid that encloses the viral RNA genome and performs essential functions in the virus life cycle. Previous structural analysis of two- and three-dimensional arrays provided a molecular model of the capsid protein (CA) hexamer and revealed three interfaces in the lattice. We will present a high-resolution NMR structure of the CA C-terminal domain (CTD) dimer and a cryoEM study of a tubular assembly of CA. In the solution dimer structure, the monomers exhibit different relative orientations compared to previous X-ray structures. The solution structure fits extremely well into the EM density map, suggesting that the dimer interface is retained in the assembled CA. We also identified a novel CTD-CTD interface at the local three-fold axis in the cryoEM map and confirmed its functional importance by mutagenesis. In the tubular assembly, CA intermolecular interfaces vary slightly, accommodating the asymmetry present in tubes. This provides the necessary plasticity to allow for controlled, asymmetric virus capsid assembly.



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Ultra-high field imaging and spectroscopy at 14Tesla

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An increase with a magnetic field strength has been associated with gains in sensitivity and specificity. We took delivery of a horizontal bore 14.1Tesla scanner approximately two years ago. This presentation provides an account of the experiences made and improvements noted.

Our initial results further indicated that increasing magnetic field strength to 14.1 T enhanced spectral resolution in ^{1}H NMR spectroscopy and allowed the quantification of the neurochemical profile in rodent brain with ~50% improved accuracy and precision. The measurement of the neurochemical profile with microliter spatial resolution is routinely achieved in murine brain, allowing for a quantitative measurement of more than 20 biomarkers, covering membrane metabolism, energy metabolism, neurotransmitters, antioxidants and osmolytes. Such a highly quantitative, sensitive approach is likely to yield important insights into the function of many genes and mouse models of disease.

On another line of investigation, indirect ^{13}C label detection using short-echo ^{1}H NMR localization was developed and implemented. Under infusion of glia-specific substrate - [2-^{13}C] acetate, a ~50% improvement of the sensitivity and enhanced spectral dispersion was noted at 14.1T especially for J-coupled metabolites such as glutamate and glutamine. Therefore, time courses of GluC3 and GlnC3 were reported for the first time by ^{1}H-[^{13}C] NMR spectroscopy, which should greatly improve the ability to study neuron-glial metabolism using ^{1}H observed ^{13}C edited NMR spectroscopy. Modeling the time course of ^{13}C label incorporation from acetate suggest that in addition to the glial TCA cycle rate, the label exchange across the inner mitochondrial membrane in glia can be measured and glial glutamate concentration can be estimated.

Characterization of unfolded and folded protein states by novel NMR methods

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We extensively use residual dipolar coupling (RDC), paramagnetic labeling (PRE, PCS), scalar couplings and other NMR parameters to characterize to characterize the conformational ensembles of unfolded and folded protein states.[1-5] To obtain a minimal model of the unfolded state according to such data we have developed new modules for the use of steric alignment RDCs and PREs as constraints in ensemble structure calculations.[3] The results show that only a small number of about 10 conformers is necessary to fully reproduce a large set of 419 RDCs, 253 PREs and the average radius of gyration of urea-denatured ubiquitin. C^{α} contacts determined on 400 10-conformer ensembles show significant (10-20%) populations of native and non-native conformations that are similar to ubiquitin's A-state. A statistical analysis indicates that the present methods provide reliable detection of subconformations in the unfolded ensemble at population levels of a few percent. We have extended the characterization of unfolded proteins to the side-chain rotameric states by optimized detection of $^{3}J_{H\alpha H\beta}$, $^{3}J_{NH\beta}$ and $^{3}J_{C'H\beta}$ scalar and $^{1}D_{C\beta H\beta}$ RDCs.[4] For urea-denatured ubiquitin and protein G up to six ^{3}J-couplings to $^{1}H^{\beta}$ are detected, which define the χ_1 angle at very high precision. Interpretation of the ^{3}J couplings and RDCs by a model of mixed staggered χ_1 rotamers yields excellent agreement and also provides stereo assignments for $^{1}H^{\beta}$ methylene protons. The experimental χ_1 rotamer populations are in the vicinity of averages obtained from coil regions in folded protein structures. However, individual variations from these averages of up to 40 % are highly significant and indicate sequence- and residue-specific interactions.

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Cancer Metabolomics: from Diagnostics to Drug Discovery

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Cancer metabolomics represents a fast growing analytical science. It is based on the detection of small changes in metabolite concentrations in response to cancer. Metaboloic changes in cancer have been recognised early, Warburg associated the cause of cancer with an altered metabolism. Recent work sheds new light on this fundamental hypothesis and opens new avenues for diagnostics and therapy.

The ultimate goal of cancer metabolism would be early diagnosis by a non-invasive test using blood or urine metabolite fingerprints. Such an analysis may also include the monitoring of disease progression or treatment responses. We have recently shown that metabolomics clearly detects small head and neck tumours from blood serum and can even differentiate between disease states. Similar results were also obtained for colon cancer, myeloma and for pancreatic cancer. These results hold great promise for cancer metabolomics as a diagnostic tool.

Moreover, we have used metabolomics to study the effect of drugs in cancer cell lines, specifically in acute myeloid leukaemia cell lines. In this application we were able to derive a mechanism of a combined bezafibrate (BEZ) and medroxyprogesterone acetate (MPA) therapy associated with the generation of reactive oxygen species (ROS) within the tumour cells. Moreover, we have studied the effect of hypoxia on the proposed mechanism of action of this combination of drugs. These results demonstrate how metabolomics can support cancer drug discovery.

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Shielding and quadrupole coupling parameter of intermetallic compounds: NMR experiment and quantum mechanical calculations

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Intermetallic compounds are without any doubt an important class of materials due to their technological relevance. Considering this, our present knowledge about chemical and physical properties of intermetallic compounds is not sufficient. In order to archive a situation where well-directed synthesis of compounds with desired properties becomes possible basic research has to be done. Especially a better understanding of the chemical bonding in intermetallic compounds is desired. We focus on development of strategies for systematic investigation of these materials. At present a combined application of diffraction methods, quantum mechanical calculations and NMR spectroscopy seems to be promising. The link of NMR spectroscopy and quantum mechanical calculations of NMR coupling parameter turned out to be useful for two reasons: First, it can be used as experimental validation of the quantum mechanical calculations. Second, structural information of disordered materials can be obtained. For this reason the approach can be used to compensate the failures of diffraction methods.

Two branches of intermetallic compounds were used as model systems to evaluate the strategy. Gallides of the alkaline earth metals feature metallic conductivity, thus quadrupole coupling becomes the only reliable source of information for NMR spectroscopy.[1] The semiconducting silicides of the alkali metals and Ba_2Si_4 were used to study the influence of chemical shielding for intermetallic compounds.[2,3] A comparison with Ba_3Si_4 possessing metallic conductivity will be given.[4]

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Insights into Metal Ion Mutagenesis and Catalysis of Dinuclear Mn Metallohydrolases Utilising EPR Spectroscopy

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Using a novel approach for the analysis of strain broadened EPR spectra from antiferromagnetically coupled dinuclear Mn centres, we have gained insights into the catalytic function of dinuclear metallohydrolases, in particular purple acid phosphatses (PAPs) and exonuclease. PAPs require a heterovalent dinuclear metal ion center for catalysis under acidic conditions. Sweet potato PAP is unusual in that it has a specific requirement for Mn^{II}, forming a unique Fe^{III}-μ-(O)-Mn^{II} center under catalytically optimal conditions.[1] Detailed EPR and kinetic studies have revealed that in this enzyme the chromophoric Fe^{III} can be replaced by Mn^{II}, forming a catalytically active, unprecedented antiferromagnetically coupled homodivalent Mn^{II}-μ-(H)OH-μ-carboxylato-Mn^{II} center in a PAP.[2] However, the enzyme no longer functions as an acid phosphatase, having optimal activity at neutral pH. Thus, PAPs may have evolved from distantly related divalent dinuclear metallohydrolases that operate under pH neutral conditions, by stabilization of a trivalent-divalent metal ion core. The present Mn^{II}-Mn^{II} system models these distant relatives, and the results make a significant contribution to our understanding of the role of the chromophoric metal ion as an activator of the nucleophile. The ε186 subunit of *Escherichia coli* DNA polymerase III is a binuclear metalloenzyme that requires divalent metal ions for its 3′–5′ exonuclease proofreading activity during DNA replication. Metal ion binding studies employing EPR spectroscopy reveal that only one Mn^{II} ion (Mn_A) binds to the active site in the absence of the substrate analogue and reaction product thymidine 5′-monophosphate (TMP). In the presence of TMP the affinity of Mn_A increases and a second Mn^{II} binds to the active site (Mn_B). The combined results indicate that catalysis by ε186 is regulated in a manner whereby the catalytically essential second metal ion only binds to the active site upon the addition of the substrate.

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In vivo multi-nuclear Magnetic Resonance

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The majority of Magnetic Resonance applications in biomedicine employ the resonance of protons in water, because of sensitivity reasons. With the introduction of MR systems at higher field strength the proton resonances of other compounds and resonances of other nuclei are increasingly being exploited. In this presentation we will demonstrate biomedical applications of the nuclei ^{31}P, and ^{19}F both in animals and in humans.

The use of ^{31}P is particular helpful in studies on energy metabolism and phospholipids. We have used ^{31}P - ^{31}P magnetization transfer and NoE to test the validity of the common calculation of free [ADP] in skeletal muscle, a major regulating metabolite. The results show that this is prohibited by macromolecular binding of ADP and we propose another model to explain the involvement of ADP in cellular processes. In addition we will show how ^{1}H – ^{31}P polarization transfer can be employed to increase the sensitivity in the detection of some major phospholipids involved in tumor metabolism.

The compound 5-fluoruracil is still an important part of several cancer treatments. We will demonstrate that its conversion can be assessed in a spatially resolved way. Hypoxia in tumors is related to bad prognosis and is a serious problem in the treatment of cancers. We developed a ^{19}F labeled bio-reductive compound to image hypoxia in tumors. Cell tracking using ^{19}F compounds as label in ^{19}F imaging is attractive because it allows quantitative assessments. We developed several approaches to label dendritic cells for ^{19}F imaging purposes.

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Statistical Spectroscopy: tools for metabolic profiling

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Metabolic profiling is used as a high throughput post-genomic tool for discovering diagnostics, probing metabolic pathways associated with pathological or physiological challenges, mapping the functional consequences of genetic modification and for predicting the response of individuals to nutritional or pharmacologic interventions. It has seen its biggest successes within the biomedical sciences and has been a core tool in many disease areas such as metabolic syndrome. The platform is dependent upon high-resolution spectroscopy, usually NMR spectroscopy or MS combined with powerful mathematical modelling tools relying on multivariate algorithms. However, much of the success of the modelling is reliant upon the optimization of the preprocessing of spectra and much effort has been applied to this field recently. Simple procedures such as baseline correction, spectral alignment or correcting for phase distortions can be challenging if automation is required to facilitate high throughput. However, perhaps the greatest challenge is to identify the chemical structure of spectral features designated as differentiating between two or more classes of sample. Here there has been a recent explosion in the development of methods associated with statistical spectroscopy. Statistical spectroscopy generally involves harnessing the power of multiple samples within each class to reconstruct whole or partial spectra using properties such as correlation or covariance.[1] This can be done using one data type e.g. standard one-dimensional ^{1}H NMR urine spectra to identify signals that covary systematically within a group of samples (Statistical Total Correlation SpectroscopY; STOCSY) or by utilising different types of spectra acquired for the same sample (Statistical HeterospectroscopY; SHY), for example ^{1}H and ^{19}F NMR spectra or ^{1}H NMR spectra and LC-MS spectra.

The identification of correlated signals can provide molecular structural information by identifying atoms on the same molecule in different chemical environments functioning as a traditional two-dimensional NMR experiment such as TOCSY but without being restricted by bond length. Alternatively, this type of statistical spectroscopy can also yield pathway information. The most striking examples here are for xenobiotic metabolites. Such methods show increasing promise as biomarker identification tools.

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Structure and Dynamics of the Influenza M2 Proton Channel by Solid-State NMR

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The M2 protein of influenza A viruses forms a tetrameric proton channel important for the virus life cycle. The M2 proton channel is activated at low pH and inhibited by amantadine and rimantadine. Elucidating the atomic structure of the M2 protein bound to lipid bilayers is not only important for developing new antiviral drugs but is also crucial for understanding the mechanism of proton conduction. We present solid-state NMR studies of the high-resolution structure of the M2 transmembrane (TM) domain in lipid bilayers in various states, including the drug-free apo state, the amantadine-bound state, and the low-pH open state. Magic-angle spinning 2D correlation experiments, dipolar recoupling experiments, and ^{1}H spin diffusion NMR allowed us to determine 1) the TM helix conformation and its perturbation by amantadine, 2) inter-helical distances in the four-helix bundle, 3) drug-protein distances that located the binding site, 4) water interaction with the channel, and 5) the conformation and dynamics of the key proton-sensing residue histidine 37. These results yielded detailed insights into the mechanisms of proton conduction by the M2 channel and how amantadine inhibit this function.

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Animal-detected EPR: cryptochromes as magnetic sensors

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Migratory birds travel vast distances each year using, *inter alia*, the Earth's magnetic field as a source of directional information. Although it has been known for 40 years that birds possess a magnetic compass, avian magnetoreception is still poorly understood. One of the two mechanisms currently under consideration invokes magnetically sensitive photochemical reactions of radical pairs in spatially ordered cryptochrome photoreceptor proteins in the bird's retina.[1]

The essential chemical requirements for detecting the direction of an Earth-strength (~50 μT) magnetic field will be outlined. Evidence for a radical pair magnetoreceptor in birds will be presented, with the emphasis on cryptochrome as the host molecule.[2] In particular, the use of radiofrequency magnetic fields as a diagnostic test for the operation of the radical pair mechanism will be described. Studies of migratory birds subjected to oscillating magnetic fields have revealed remarkably sensitive disorientation responses when the frequency of the applied field (~1.3 MHz) matches the EPR condition of a radical with $g \approx 2$ in the Earth's magnetic field (~47 μT).[3] The occurrence of such 'Zeeman resonances' can be understood if one of the radical pair partners is devoid of hyperfine interactions. Theoretical considerations suggest that such an asymmetric distribution of hyperfine interactions may be optimal for the chemical detection of weak magnetic fields. The possibility that the radical is either superoxide or dioxygen is examined with the conclusion that neither offers a very credible explanation for these *in vivo* EPR 'signals'.[4,5]

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High-field NMR as a powerful tool to study "exotic" phases in quantum spin systems

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We review recent NMR results obtained at the Laboratoire National des Champs Magnétiques Intenses in Grenoble on magnetic field induced physics in several frustrated antiferromagnetic quantum spin systems. They are all based on spin 1/2 dimers, and we observe them in the regime where magnetic field closes the singlet-triplet gap and induces "exotic" quantum phases such as magnetization plateaus, Bose-Einstein condensation (BEC) of triplet excitations and supersolid phases. When these phases appear at very high magnetic fields above 17 T, which are currently out of the reach of neutron and x-ray diffraction techniques, NMR provides the only access to determine their magnetic (super)structure.

In the two-dimensional "Shastry-Sutherland" $SrCu_2(BO_3)_2$ compound we present for the first time the magnetic structure determination in all the magnetization plateaus of this system.[1] In the azurite, $Cu_3(CO_3)_2(OH)_2$, which is a diamond spin chain, we determined the local spin polarization in the 1/3 plateau, and have evidence for the absence of the 2/3 plateau.[2] In $BaCuSi_2O_6$, regarded as an archetype for the BEC of triplets (hard-core bosons), we proved that in every second plane the spin dimers have different gap value, leading to an exotic modulated BEC system.[3] We will review these high magnetic field results paying special attention to the employed NMR techniques.

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Molecular Interaction between SUMO and the Death-Associated Protein-6 (Daxx)

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Post-translational modification by Small Ubiquitin-like Modifier (SUMO) is an important regulatory control used by the cells to modulate activity, stability, and localization of different intracellular proteins. An enzyme pathway, analogous to the ubiquitin pathway orchestrates the SUMO attachment; but unlike ubiquitination, sumolytion is very specific about selecting the target lysine site. Many mechanistic aspects of the substrate and lysine selection by the SUMO conjugating machinery are still poorly understood with recent studies describing specialized SUMO interaction motifs (SIM) which can recognize and recruit SUMO moiety by non-covalent interaction.

Death-associated protein-6, Daxx, is an important transcription corepressor, which represses the transcriptional potential of several sumolyted transcription factors. Two separate SIMs are situated in both terminal ends of Daxx. These SIMs mediate Daxx interaction with SUMO and hence the sumolyted transcription factors, which eventually lead to the sequestration of Daxx to the PML oncogenic domains (PODs). Thus the structural insights to be presented here, on how the SIM binds to SUMO are critical for understanding the regulation of transcriptional activity and sub-nuclear compartmentalization of Daxx. Furthermore, Daxx SIM is recently identified to be phosphorylated *in-vivo* and thus opens a probable new avenue whereby the Daxx activity is modulated by both sumolytion and phosphorylation. In this presentation we will describe the structural mechanism underlining the molecular basis of the interaction between SUMO and various Daxx fragments.

RDC-enhanced NMR structural determination of unnatural peptidic scaffolds with periodic hydrogen bonded patterns

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Design and structural determination of hybrid peptides comprised of unnatural cyclic β-amino acids, have emerged as new generation foldamers, which adopt well-defined and periodic hydrogen-bonded folded patterns. The specific three-dimensional shape and conformational stability of these heterogeneous backbone structures are provided by the cyclic β-amino acid motifs. Accurate structural determination of these molecules is very important for subsequent design and development of function oriented peptidic scaffolds.

Measurement of residual dipolar couplings RDCs in weakly aligned solvents, offers a powerful means to access a coherent and long range structural information, the technique that is now routinely employed for biomolecular NMR structural elucidation in aqueous alignment phases. The recent advent of polymer/organic solvent gel media, with tunable alignment features has provided new opportunities to access this rich structural information via hetero nuclear one-bond RDCs in small organic molecules as well. The present work discusses RDC-enhanced NMR structural determination of distinct hydrogen-bonded secondary folds in unnatural peptides, measured in organic solvent media. We will show that the conventional constraints, $^{3}J_{H}$ and NOE-derived distances alone do not allow the accurate structural elucidation even for rigid foldamers and emphasize the immense need and scope for RDC-based structure validation and refinement for unnatural peptides in particular and small organic molecules in general.

NMR shielding constants and nuclear magnetic moments - ab initio methods of quantum chemistry and experiment

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The NMR shielding constant, describing the shielding of a nucleus by all the electrons in a molecule, is given on an absolute scale with bare nucleus as the reference. In theoretical *ab initio* studies the absolute shielding constants are obtained, in agreement with this definition. In experiment using bare nuclei is practically impossible, and chemical shifts - defined with respect to an arbitrarily chosen standard – are applied. Once the absolute shielding of a given nucleus in one reference molecule is established, the shielding and chemical shift scales for this isotopic species can be related to each other. Increasing accuracy of ab initio calculations provides new possibilities to determine accurate absolute shielding scales. For small molecules one can analyze the basis set and electron correlation effects, include the relativistic effects and estimate the temperature dependence of the shielding constants. At the same time, gas-phase NMR experiments provide the zero-pressure extrapolated values, corresponding to the theoretical values computed for an isolated molecule.

Accurate *ab initio* results can be used to analyze the relation between shielding constants, resonance frequencies and magnetic dipole moments of different isotopic species. Applying this relation, experimental NMR frequencies and computed *ab initio* shielding constants one can obtain new, corrected values of nuclear magnetic moments.[1] When accurate magnetic moments are known, the same relation may be applied to determine absolute shielding constants. In this approach, shielding constants of all the isotopic species are related to a single reference scale, and nuclear magnetic shielding can be directly observed from NMR spectra. From purely theoretical point of view, atomic ^{3}He is preferable as the primary standard. Since the use of ^{3}He is not in general a practical alternative, one can transfer the reference standard, for instance to the ^{2}H signals. The accuracy of this scheme has now been demonstrated in practical applications.[2]

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Modeling of protein structural transitions from EPR constraints-Scope and caveats

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Large-scale domain motion is an important component of protein function, for instance in substrate uptake and release of enzymes and in active transport across membranes. In particular for membrane transporters, there is a lack of experimental methods for characterization of the corresponding structural transitions. Since such structural transitions can be well approximated by motion along only a few selected degrees of freedom, a small number of distance constraints on the relevant length scale should suffice to specify them. Indeed an algorithm has been proposed by Zheng and Brooks in 2006[1] that arrives at the final structure within 1…3 Å r.m.s.d. of the C^{α} atoms when given only the initial structure and 10 distance constraints between C^{α} atoms for the final structure. The distances generally fall into the range accessible with pulsed dipolar EPR measurements, for instance by the DEER (or PELDOR) experiment, with some distances in the range of CW EPR measurements.

Here we discuss the underlying approximations as well as the adaptation of the algorithm that is needed for application to measurements between spin labels. The Zheng/Brooks fitting algorithm is simplified by modification of the underlying elastic network model and the algorithm for site pair selection is extended to account for restrictions in site-directed spin labeling. Performance tests are based on *in silico* spin labeling[2] of proteins whose x-ray structures are known in two alternative states.

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^{1}H NMR based Metabolomics for Early Disease Diagnosis

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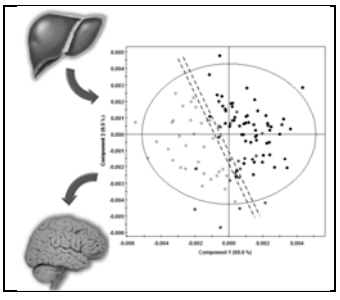
^{1}H NMR spectroscopy enables the simultaneous detection, quantitation and characterisation of multiple metabolites and small molecules in a variety of biological samples (e.g. biofluids, cells and tissues). Collectively, this approach can be refered to as metabolomics.[1] During the last couple of decades, the applications for metabolomics approaches have increased exponentially, especially in the biomedical field.



We have utilised ^{1}H NMR based metabolomics to study the serum metabolic profile of a number of diseases in the hope of identifying new tools for patient classification and the identification of relevant metabolite biomarkers. Firstly, we have examined differences in the serum metabolome of control and alcohol induced cirrhotic patients with or without Minimal Hepatic Encephalopathy (MHE).[2] Secondly, we have also studied serum metabolic differences between molecular subgroups of Chronic Lymphocytic Leukaemia (CLL) patients.[3] In both cases, our results indicate the usefulness of ^{1}H-NMR-based metabolomics as a potential non-invasive diagnostic tool as well as an approach to better understand the underlying biology involved in these disease pathways.

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NMR of Atomic and Small Molecular Probes in Anisotropic Liquids

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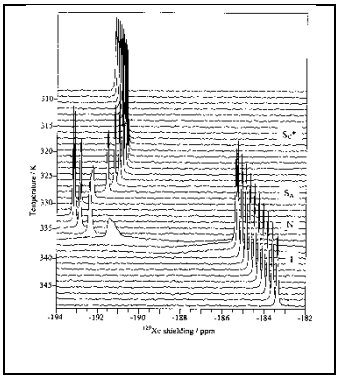
NMR spectroscopy of small molecules in anisotropic liquids (liquid crystals, LC) opens up a possibility to derive versatile information on the molecular properties: geometry,[1] nuclear shielding tensor,[2] spin-spin coupling tensor[3] and quadrupole coupling tensor.[4] On the other hand, NMR of atomic and molecular probes makes feasible also the determination of the physicochemical properties of the solvent LC. One of the most applicable atomic probes is noble gas xenon,[5] whereas isotopomers of methane, $^{13}CH_4$ and CD_4, are useful molecular probes.



The methods available within LC NMR for deriving molecular properties will be introduced but more emphasis is given on the utilization of ^{129}Xe and ^{131}Xe NMR of xenon and ^{13}C NMR and ^{2}H NMR of methanes in a thermotropic ferroelectric liquid crystal (TFLC). The figure shows as example ^{129}Xe NMR spectra of xenon in a TFLC at variable temperatures.

Second order quadrupole shift (SOQS) is observed in ^{131}Xe NMR spectra and is used to derive information on the LC phase structure.[6]

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Exploring the Missing NMR Information by Selective Isotope Labeling Methods

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The stereo-array isotope labeling (SAIL) method has been successfully applied for the structure determinations of relatively large proteins, which are difficult to handle by conventional NMR methods.[1] For these applications, however, we have to prepare protein samples that are exclusively composed of SAIL amino acids, using cell-free protein expression systems. This might impose a further barrier to the general use of the SAIL method by the NMR community. On the other hand, for the various other applications of SAIL amino acids to analyze the local conformations and dynamics of selected amino acid residues, in many cases conventional cellular protein expression systems are sufficient. For example, proteins selectively labeled with SAIL aromatic amino acids, which can be readily prepared by *in vivo E. coli* expression, were useful for the structural refinement of the hydrophobic core of a protein.[2] We have developed new applications of selectively labeled SAIL proteins to acquire information related to the dynamic aspects of the side-chain moieties of various amino acids, such as hydrogen-deuterium exchange of hydroxyl or sulfhydryl groups,[3,4] aromatic ring flipping, and disulfide bond isomerization. We are also trying to apply the selective SAIL methods for larger proteins, and will show some of the preliminary results, if time permits.

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Quantum-chemical computation of magnetic resonance parameters: from EPR of metalloenzymes to NMR of paramagnetic systems

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Recent progress in the quantum-chemical computation of NMR and EPR parameters of paramagnetic systems is highlighted. EPR spectroscopy of metalloenzyme sites and models benefits increasingly from accurate computations. This is exemplified by recent work on the S_2 state of the oxygen-evolving complex of photosystem II, where broken-symmetry DFT calculations followed by spin-projection procedures allow access to the hyperfine couplings not only of the ^{55}Mn nuclei but also of nuclei in bound protein residues and other ligands.[1] In particular, histidine ^{14}N hyperfine tensors provide support for a Mn^{III} oxidation state of the Mn_C center and thus for energy-optimized structural models suggested recently by Siegbahn.[2] The spin projection procedures are refined, taking the effects of zero-field splitting into account.

The accurate computation of NMR chemical shifts of paramagnetic transition-metal complexes is the most recent frontier of quantum-chemical methodology, going beyond the pure Fermi-contact shifts and taking spin-dipolar shifts and the effects of zero-field splitting into account in a consistent modern formalism.[3-6] Recent application examples include a mechanistic proposal for the enantioselective kinetic resolution of epoxides by Jacobsen-type catalysts.[7]

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From Biology to Business: combining EPR with thin films to create a sensor?

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Metallo-phthalocyanines (MPcs) are a group of organic semiconductor materials for applications such as large area solar cells due to their optoelectronic properties coupled with the possibility of cheaply fabricating thin films.[1,2] Many of the interesting properties of MPcs such as magnetism, light absorption and charge transport are highly anisotropic.[2,3] To maximise the efficiency of a device based on these materials it is important to understand the molecular layout in films to assess the influence of different growth conditions and substrate treatments. Here we present an EPR study using the anisotropy of the EPR spectrum of CuPc[4] to determine the orientation effects in CuPc films. We gain insight into the molecular arrangement of films of CuPc mixed with the isomorphous H_2Pc and with C_{60} in films similar to real solar cells. Finally, we discuss how these results could lead to the development of novel EPR-based devices such as sensors.

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Developing NMR Tools to Study Nanoliter Solids and Liquids Samples

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An overview of micro NMR probes and experiments will be presented that are currently under development in our lab. Miniaturized NMR detectors enhance sensitivity and allow structural investigations of mass-limited solid samples. The small sample size makes it possible to study single crystals or specifically aligned samples (e.g. fibers, thin films, etc). As has already been shown in the early days of NMR, studying single-crystals can supply additional information about the orientation of the NMR interaction tensors. However, single crystals of sufficient size to do NMR experiments are generally not available. We demonstrate the feasibility of μMAS experiments for determining quadrupolar tensor orientations using rotor-synchronised MAS NMR and MQMAS NMR of micro-sized crystals.

Considering the high-sensitivity of proton observation combined with the information content offered by proton chemical shifts, there is a continuous interest in achieving high-resolution proton NMR spectra of powdered samples under Magic Angle Spinning. However, because of the size and spin-dynamics of dipolar couplings between abundant protons, averaging of these interactions by either fast MAS and/or homonuclear decoupling sequences is very demanding. It will be shown that experimental optimization can be achieved on-spectrometer within hours using self-learning genetic algorithms. It proved possible to obtain ^{1}H spectra of 40 - 80 nl sample volumes in only a few scans. Furthermore, we demonstrate the feasibility of indirectly detecting low-sensitivity X-nuclei. With sample amounts in the nanoliter regime meaningful 2D NMR spectra can be obtained for natural abundance samples.

Microcoils are capable of generating high rf-field strengths with relatively low power. This can be exploited to efficiently excite large band widths or decouple strongly coupled spin systems. We are exploring the limits of T_2' values that can be obtained for strongly coupled carbons such as the α-C_2 carbon in glycine reaching improvements of a factor 5 over the previously published optimal values.

Choreographing an enzyme's dance –surprises exposed by NMR, crystallography and computation

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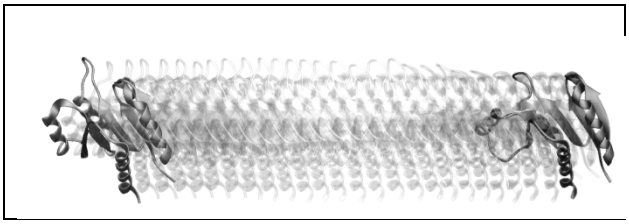
The synergy between structure and dynamics is essential to the function of biological macromolecules. While this is a widely accepted concept, key questions remain: Have proteins evolved so that substates necessary for activity are preferable accessible? How can a protein interconvert among folded substates while avoiding unfolding? What are the molecular pathways for conformational transitions? Can we characterize the entire structural ensemble including lowly populated states?

The talk will address these questions. We will quantitatively characterize the energy landscape of a signaling protein and reveal how its features explain allosteric activation. Surprises will be presented for transition pathways and transition state ensembles. Second, the energy landscape of an enzyme both during catalysis and in the absence of substrates is being characterized, which allows identification of dynamics that are linked to enzyme catalysis.

Both examples illustrate that motions in folded proteins are not random but preferentially follow the pathways, which create the configuration capable of proficient function. This situation is analogous to protein folding, which is biased so as to sample only a small portion of the energy landscape. The expansion of the concept of non-random sampling of conformational space for efficient biological function from folding to conformational rearrangements within the folded space combines both phenomena through the energy landscape. We hypothesize that lowly populated, "hidden"conformations are frequently the biological active structures, rather than the low-energy major conformations solved by traditional structural biology methods.



NMR in Neurological, Gastrointestinal and Liver Diseases, Infection and Open Heart Surgery

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The use of NMR as a noninvasive tool for studying anatomy, structure and *in vivo* metabolism is seeing exponential growth due to continuous advancements in NMR methodologies and instrumentation. However, developments of innovative techniques for understanding human diseases at molecular level are still in infancy. Variations in metabolic profile resulting from disorders and clinical intervention, at molecular level are more sensitive in identifying diseases in early stages and assessing the efficacy of the interventions. This is the prime objective of the talk.

Specifically results on Amyotrophic Lateral Sclerosis, obstructive jaundice and cholangitis, various liver diseases such as fatty liver disease and Fulminant hepatic failure, assessment of liver graft and sub-clinical infections in open heart surgeries will be presented. For such studies results obtained from bio-fluids such as urine, serum, bile acids, and pericardial fluid which have very complex metabolic profiles with numerous structurally similar metabolites will be described. Specific metabolic signatures for different diseases from such investigations will be illustrated.

Isotope effects in ^{195}Pt high-resolution NMR: Unambiguous assignment method of all $[PtX_{6-n}(H_2O)_n]^{n-2}$ (X = $^{35/37}$Cl/ $^{79/81}$ Br, *n* = 0-5) complexes in aqueous solution

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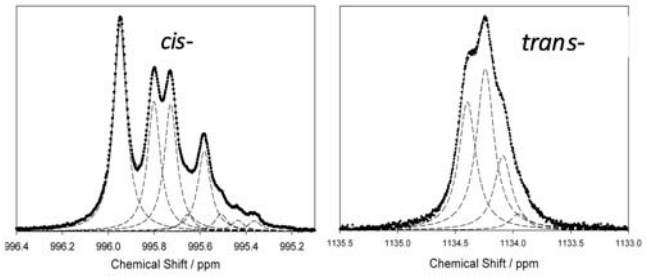
^{195}Pt NMR is a powerful tool with which to probe the solution structure and solvation shells of Pt(IV) complexes in halide rich aqueous solutions. We use experimental and computational ^{195}Pt NMR to better understand the fundamental nature of the ^{195}Pt NMR shielding of such 'simple' complexes,[1] as well as to gain insight into the nature of species in solution.[2] We here present a new method for the unambiguous assignment of the many possible Pt(IV) complex species in solutions designed to emulate real process solutions relevant to the PGM refining industry. By exploiting the ^{35}Cl/^{37}Cl (in addition to ^{16}O/^{18}O and ^{13}C) isotope effects on the ^{195}Pt chemical shifts of the complex anions $[PtCl_{6-n}(H_2O)_n]^{n-2}$ (*n* = 0-5),[3] $[PtBr_{6-n}(H_2O)_n]^{n-2}$ (*n* = 0-5) and related Pt(IV) chlorido-complexes, the ^{195}Pt resonance of each species can be de-convoluted into a distribution of *isotopologues and isotopomers*, which constitutes a unique 'fingerprint' for unambiguous identification of the species in solution. This method leads to the possibility of distinguishing the *cis/trans* or *fac/mer* Pt(IV) geometric isomers, shown here for the *cis*-$[PtCl_4(H_2O)_2]$ and corresponding *trans* isomer.



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Dissolution Dynamic Nuclear Polarisation NMR spectroscopy with an dedicated Spectrometer

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Dynamic Nuclear Polarisation (DNP) at cryogenic temperatures in conjunction with a fast temperature rise to ambient temperature can be used to generate highly polarised nuclear spin systems for NMR spectroscopy.[1] The scope of our DNP project is to develop this technique into a strategy for the investigation of molecular dynamics on a fast time scale. To this end we have focused on the optimisation of the solid state DNP process, on the minimisation of the shuttling time and the strategy by which two different liquids are mixed. In a major hardware design project we have built a dedicated DNP NMR spectrometer that is based on a two isocentre magnet.[2] Sample transfer after DNP at a temperature of 1.4K is carried out in solid state between the upper 3.4T and the 9.4T isocentres. Close to the 9.4T centre the sample is rapidly dissolved and injected into a high resolution NMR probehead.

Here we describe progress in understanding the DNP process and first applications of our novel DNP NMR spectrometer to studies of molecular dynamics. Furthermore, we will report on our attempts to achieve rapid mixing between a solution containing hyperpolarised spins and another solution containing receptor molecules. For the acquisition of spectra with fast time resolution we use of a multiple receive console and a probehead with two rf coils that makes it possible to acquire a second spectra from the same sample before the acquisition of the first is finished.

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Acknowledgments: We are grateful for the collaboration with R. Hunter and G. Smith, School of Physics & Astronomy, St. Andrews, Scotland and for support during the hardware design stage of our project by Oxford Instrument Molecular Biotools. Ltd (Abington, UK).

A Closer Look at Heterogeneous Catalysis: Applications of and Novel Hypersensitive Tools for the NMR/MRI Toolkit

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The first part of the presentation will describe the development and applications of the MRI techniques for the studies of heat and mass transfer processes important in heterogeneous catalysis. These include the studies of the preparation of supported catalysts,[1] the dynamics of redistribution of the liquid phase in the catalyst beds and individual pellets in an operating heterogeneous catalytic reactor,[2] and the spatially resolved NMR thermometry of the catalyst bed .[3] The second part will deal with the studies of parahydrogen-induced polarization in gas-solid and gas-liquid-solid heterogeneous catalytic hydrogenation processes.[4-6] The objectives of this research are to develop the novel approaches for the facile production of catalyst-free hyperpolarized liquids and gases for technical and biomedical NMR/MRI applications and to create hypersensitive NMR tools for studying the mechanisms of heterogeneous catalytic processes.

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Joint analysis of NMRD and EPR data by slow-motion theory: two medium-sized Gd(III) complexes as an example

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The "Swedish slow motion theory"[1] developed originally for modelling NMRD (Nuclear Magnetic Relaxation Dispersion) profiles for solutions of transition metal ion complexes with $S \geq 1$ and recently compared with other approaches,[2] was extended to allow ESR spectral analysis. The extended theory was applied to interpret in a consistent way (within one set of parameters) NMRD profiles and ESR spectra at 95GHz and 237GHz for two Gd(III) complexes (S=7/2) denoted as P760 and P792 (hydrophilic derivatives of DOTA-Gd, with molecular masses of 5.6kDa and 6.5kDa, respectively). The goal was to verify the applicability of the commonly used pseudorotational model of the transient zero field splitting (ZFS).[3] According to this model, the transient ZFS is described by a tensor of a constant amplitude, defined in its own principal axes systems, which changes its orientation with respect to the laboratory frame according to the isotropic diffusion equation with a characteristic correlation time reflecting the time scale of the distortional motion. This unified interpretation of the high field ESR and NMRD leads to an acceptable agreement with the experimental data, provided that g-tensor anisotropy effects are included. Thus, we conclude that the pseudorotational model indeed captures the essential features of the electron spin dynamics.

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Multidimensional NMR beyond resolution limitations

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A variety of different methods was proposed to overcome the sampling limitation in multidimensional NMR spectroscopy. They could be utilized in two different ways, either to shorten the experiment duration without loss of resolution, or to perform experiments that are not obtainable conventionally, i.e. with significantly improved resolution and/or of high dimensionality. Most often first of these two, so called "Fast NMR" approach, is shown as the example of the utility of these methods, as it saves expensive spectrometer time. However, in many cases spectra featuring extraordinary resolution and high number of dimensions may be more interesting from scientific point of view as they reveal effects that are hidden, when spectral lines are broad, or enable resolving spectral ambiguities when peaks are overlapped. This second approach we refer to as "Accurate NMR". Owing to unique feature, that the artifacts level depends only on the number of sampled points, but not required frequency range nor maximum evolution times reached,[1] Discrete Fourier Transform of randomly sampled NMR data sets,[2,3] seems to be the method of choice for "Accurate NMR" type of applications. Its full potential is manifested when the overall experiment time is less important than a new information available from spectra of high dimensionality (4-6D)[4,5] or of high resolution approaching natural line-width.[6,7]

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NMR and Muon Spin Relaxation in molecular nanomagnets

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Since their discovery molecular nanomagnets, also called single-molecule magnets (SMM), have attracted a lot of scientists as they were promising systems for applications and offered the possibility to study fundamental physical properties in finite-size molecular systems whose units are replicated over a bulk quantity of sample. Among different experimental discoveries and theoretical treatments we recall the quantum tunneling of the magnetization, the evidence of the Berry phase, studies about quantum levels' crossing and many other issues regarding the spin dynamics in different temperature ranges. We report here a brief summary of the main MUSR and NMR studies on molecular nanomagnets in the last 15 years,[1-3] whose results cover most of the above cited research fields.

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The Development of A Gadolinium Based MR Contrast Agent for the Visualization of Malignant Micro-calcification in Human Breast Cancer

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We have synthesized and characterized a high-relaxivity Gadolinium based contrast agent that bind specifically and selectively to hydroxyapatite (HA). Hydroxapatite is the form of micro-calcification that is most commonly found in human malignant breast cancer. We have previously shown that pamidronate, a bisphosphonate can be used as a ligand for hydoxyapatite. In the present work we have synthesized a DOTA based analogue, which we call DOTA-ser-PAM. We will present the synthesis and binding characteristics of this agent. Wde will show both in vitro and in vivo data showing that this agent is both specific and selective for HA. The binding og Gd-DOTA-ser-PAM appears to obety the Langmuir isotherm. We will discuss potential applications of this agent to human studies.

Singlets, Triplets and Multipoles: Spin Rotational Symmetries in Solids and Liquids

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Nuclear spin states may be constructed that have the rotational properties of an atomic s-orbital (rank 0), an atomic p-orbital (rank 1), an atomic d-orbital (rank 2), and so on. Experiments may be devised that separate NMR signals according to their rotational properties, and that analyze them individually.

In the solution NMR of 2-spin-1/2 systems, the rank-0 component corresponds to singlet nuclear order. This order can be exceptionally long-lived, with lifetimes of more than 25 minutes observed in some systems. We show how the decay of nuclear singlet order depends on the neighbouring molecular geometry. The singlet order may be manipulated by using audio-frequency magnetic field sequences, in low static magnetic field. We will report a new method for converting nuclear Zeeman order into singlet order, and back again, outside the NMR magnet.

Nuclear spin orders of rank 1 and rank 2 are also found in the solution NMR of 2-spin-1/2 systems. We show how these states respond to the presence of paramagnetic species in solution. The relaxation properties of the spherical spin order components depend on the correlations of fluctuating local fields.

In solid-state NMR, the conversion of rank 1 order into rank 2 order is diagnostic of nuclear spin-spin couplings. Analysis of this rank-order conversion may be used to provide a quantitative measure of internuclear couplings, and hence internuclear distances. This analysis method is robust with respect to incoherent relaxation effects and may be conducted in samples with a high density of magnetic nuclei, such as uniformly labelled organic molecules.

Spin electric effects in molecular antiferromagnets

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Molecular nanomagnets show clear signatures of coherent behavior and have a wide variety of effective low-energy spin Hamiltonians suitable for encoding qubits and implementing spin-based quantum information processing [1,2]. At the nanoscale, the preferred mechanism for control of quantum systems is through application of electric fields, which are strong, can be locally applied, and rapidly switched. In this work, we provide the theoretical tools for the search for single molecule magnets suitable for electric control. By group-theoretical symmetry analysis we find that the spin-electric coupling in triangular molecules is governed by the modification of the exchange interaction, and is possible even in the absence of spin-orbit coupling. In pentagonal molecules the spin-electric coupling can exist only in the presence of spin-orbit interaction. This kind of coupling is allowed for both $s=1/2$ and $s=3/2$ spins at the magnetic centers. Within the Hubbard model, we find a relation between the spin-electric coupling and the properties of the chemical bonds in a molecule, suggesting that the best candidates for strong spin-electric coupling are molecules with nearly degenerate bond orbitals. We also investigate the possible experimental signatures of spin-electric coupling in nuclear magnetic resonance and electron spin resonance spectroscopy, as well as in the thermodynamic measurements of magnetization, electric polarization, and specific heat of the molecules. A most promsing candidate for such spin-electric coupling are Cu_3-rings where the chirality of the spin texture defines a scalable qubit that can be controled and measured by electric fields, e.g. by using an STM tip or a microwave cavity.

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Mind the gap: paramagnetic NMR studies of the trans-periplasmic bioenergetic chains linked to extracellular metallic ores

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Geological evidence suggests that iron respiration and iron based anoxygenic photosynthesis were some of the earliest forms of metabolism to emerge on the primordial Earth. The metabolic utilization of extracellular solids requires that electrons are delivered or received across the cell walls. Gram-negative proteobacteria are the focus of increasing interest because of the possibility to harness their metabolic capabilities for development of microbial fuel cells, applications in bioremediation of metal contaminated environments, and protection of submerged metallic structures. However, ATP production is typically associated with the internal membrane and therefore extracellular respiration requires electrons to bridge the periplasmic space, while maintaining the coupling to the phosphorylation of ADP. Multiheme c-type cytochromes are known to be major players in these respiratory chains. They play a variety of roles, from electron transfer across the periplasmic space, to terminal reductases or oxidases of metallic compounds.

Understanding the molecular mechanisms of the redox and/or catalytic activity of these proteins requires knowledge on their structure and on the specific properties of each of the redox centres. Hemes of type c contain an hexacoordinated iron with strong field ligands that ensure redox transitions from diamagnetic Fe(II) to low spin paramagnetic Fe(III). These transitions provide convenient spectroscopic handles and methods based on paramagnetic NMR experiments and redox titrations followed by visible spectroscopy can define detailed functional information for proteins weighting up to 64 kDa. The reduction potentials of the individual hemes can be determined as well as the redox interactions between pairs of hemes within the same molecule. Under conditions of fast intramolecular electron transfer and slow intermolecular electron transfer, kinetic experiments can provide information on the time dependent activity of the various redox centres in the multiheme cytochromes. Of the various aspects of development of microbial fuel cells, the molecular details of the electron transfer between the microbial component and the electrodes remains the least explored. Studies on the structure function relationship in multiheme cytochromes from organisms capable of powering microbial fuel cells will be presented.

Intermediates in Hydrogenase Catalysis Studied by Advanced EPR Techniques

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[NiFe] and [FeFe] hydrogenases contain bridged binuclear transition metal cores in their active sites which are tuned by a special ligand environment (thiolates, CN^-, CO) to fulfill the task of efficient di-hydrogen conversion to protons and electrons – or vice versa – via a heterolytic H_2 splitting mechanism.

Based on spectroscopic data derived from pulse EPR and ENDOR, complemented by FTIR experiments, the structures of the intermediates that play a role in the activation, inhibition and the catalytic cycle of the [NiFe] hydrogenase have been determined.[1,2] For [FeFe] hydrogenase similar measurements were performed on all paramagnetic states of the H-cluster, yielding the spin density distribution and the spin coupling of the active site and the iron oxidation states.[3] Evidence has been obtained for the presence of a nitrogen in the bridging dithiolate ligand of the di-iron subcluster, which is important for understanding the mechanism of this enzyme.[4]

The structural information obtained for the [NiFe] and [FeFe] hydrogenases from spectroscopy is supported by model system studies and DFT calculations and yields insight into the catalytic cycle of these enzymes. Possible differences and similarities of the two classes of enzymes are discussed.[5]

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Recoupling and Sensitivity Enhancement in Half-Integer Spin Quadrupolar Nuclei

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Several approaches have been introduced to enhance the sensitivity of the central-transition spectra of half-integer spin quadrupolar nuclei. We here present an overview of the fast amplitude modulated (FAM) schemes and its variants under both magic-angle spinning and static conditions of the samples. Recoupling of the dipolar interaction under magic-angle spinning between the protons and quadrupolar nuclei is essential for distance measurements between them. We demonstrate the use of symmetry-based recoupling scheme in combination with MQMAS and STMAS for the measurement of ^{17}O-^{1}H distance in a model system.

Towards an NMR spectrometer operating beyond 1GHz: Operation of a 500MHz high temperature superconducting NMR

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We have begun a project to develop an NMR spectrometer that operates at frequencies beyond 1GHz using a high temperature superconductor (HTS) innermost coil. As the first step, we developed a 500 MHz NMR with a Bi-2223 HTS innermost coil, operated in external current mode. The temporal magnetic field change of the NMR magnet after the coil charge was dominated by (i) the field fluctuation due to a DC power supply and (ii) relaxation in the screening current induced in the HTS tape conductor; effect (i) was stabilized by the ^{2}H lock, while effect (ii) increased with time reaching 0.01ppm/h on the 20th day after the coil charge, a value that was no larger than that of the persistent current mode of the low temperature superconductor (LTS) NMR magnet. The 2D-NOESY, 3D-HNCO and 3D-HNCACB spectra of ubiquitin acquired by the 500 MHz LTS/HTS NMR magnet were of a quality nearly equivalent to that achieved by a conventional LTS NMR magnet.[1] An external lock system using signals of an NMR microcoil was also developed for solid-state NMR operated in external current mode; the 2D-^{13}C solid-state NMR spectrum of isoleucine was recorded.

The innermost Nb_3Sn LTS coil of the 920 MHz NMR (21.6 T) is to be replaced by Bi-2223 HTS for 1.03 GHz operation; an HTS innermost coil has already been fabricated and the 1.03 GHz LTS/HTS NMR magnet will be installed (24.2 T) and charged in the National Institute of Materials Science within Fiscal Year 2010.

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Possible Role of Structural Modifications of DJ-1 under Oxidative Stress in Parkinson Disease

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The physiological role of DJ-1, a dimeric protein of 189 amino acids involved in rare familial forms of Parkinson disease (PD), is still controversial. Among various hypotheses, a sensor role for oxidative stress has been proposed, through oxidation of a conserved Cys residue (C106 in humans) to $CysSO_2H$. Modified in this way, the protein is able to translocate to mitochondria and to protect against toxicity.[1] The association of mutations of DJ-1 with PD suggests a loss of function, which specifically affects dopaminergic neurons. In fact, DJ-1 was found to be covalently modified by dopamine in both brain mitochondrial preparations and SH-SY5Y cells.[2]

Under oxidative conditions, highly reactive dopamine quinones (DAQs) can be produced, which can modify many potential protein targets especially through reactions with Cys residues. We analysed the structural modifications induced on DJ-1 by DAQs, through biochemical, spectroscopic, and computational techniques. The DJ-1 residues involved in the interaction with DAQs were identified through the study of various mutants and the effects of the DAQ modifications on the structure and its stability were investigated. The three Cys residues showed very different behaviour, in agreement with their proposed functional role.

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NMR Methodology in Food Analysis

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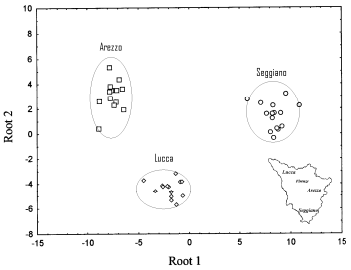
The increasing ability of high field NMR spectroscopy to solve spectra of complex mixtures and to recognize and quantify each component without chemical separation, has found a constantly increasing application in metabolomics and food chemistry. ^{1}H high field NMR spectroscopy has shown to be a valuable tool for the qualitative and quantitative analysis of the metabolic profiling of food stuff such as olive oils,[1] sea bass,[2] truffles,[3] lettuce,[4] tomatoes,[5] and mangoes.[6] The quantitative analysis of the metabolic profiling along with the application of a suitable statistical analysis has allowed food characterization in terms of geographical origin, genetic origin and farming. The potential of NMR spectroscopy to detect food adulterations has been also demonstrated.



Here, the NMR methodology used to study foodstuffs is discussed reporting some significant examples.

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NMR structural studies of bacterial virulence factor membrane proteins

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Approaches for NMR structure determination of membrane proteins in lipids will be discussed. Integral membrane proteins regulate major cellular processes in health and disease, including transport, signaling, secretion, adhesion, pathogenesis, and apoptosis, and therefore, represent important targets for structural and functional characterization. Solid-state NMR experiments with proteins in oriented bilayers, and solution NMR experiments with proteins in weakly oriented micelles, provide high-resolution orientation-dependent restraints, which can be combined for protein structure determination and refinement. Results will be presented for membrane proteins integral to the cell envelopes of *Mycobacterium tuberculosis* (Rv0899), the causative agents of tuberculosis, and *Yersinia pestis* (AIL), the causative agent of plague. The NMR structures characterized in lipids provide insights to their specific functions in each of these pathogens.

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NMR Investigations of the Rieske Protein from *Thermus thermophilus* Support a Coupled Proton and Electron Transfer Mechanism

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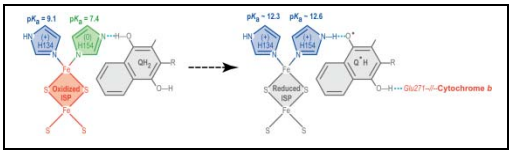

The Rieske protein component of the cytochrome *bc* complex contains a [2Fe-2S] cluster ligated by two cysteines and two histidines. We determined the pK_a values of each of the imidazole rings of the two ligating histidines (His134 and His154) in the oxidized and reduced states of the Rieske protein from *Thermus thermophilus* (*Tt*Rp). Knowledge of these pK_a values is of critical interest, because of their pertinence to the mechanism of electron and proton transfer in the bifurcated Q-cycle. To assign the paramagnetically broadened signals, we look advantage of the unique His-Leu (H134-L135) sequence and used residue-selective labeling to establish a key sequence-specific assignment. The pH dependence of assigned His $^{13}C'$, $^{13}C^{\alpha}$, and $^{15}N^{\varepsilon 2}$ signals from the cluster ligands showed that the pK_a of His134 changes from 9.1 in oxidized to ~12.3 in reduced *Tt*Rp, whereas the pK_a of His154 changes from 7.4 in oxidized to ~12.6 in reduced *Tt*Rp. This establishes His154, which is close to the quinone when the Rieske protein is in the cytochrome *b* site, as the residue experiencing the remarkable redox-dependent pK_a shift. Secondary structural analysis of *Tt*Rp based upon our extensive chemical shift assignments rule out a large conformational change between the oxidized and reduced states. Therefore, *Tt*Rp likely translocates between the cytochrome *b* and cytochrome *c* sites by passive diffusion. Our results are most consistent with a mechanism involving the coupled transfer of an electron and transfer of the proton across the hydrogen bond between the hydroquinone and His154 at the cytochrome *b* site.[1]

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Acknowledgments: Supported by NIH grants GM58667 and P41 RR02301.

Sorting out chemical and geometrical contributions in disordered materials

Dominique Massiot, Michael Deschamps, Franck Fayon, Sylvian Cadars, Valérie Montouillout, Nadia Pellerin, Pierre Florian, Laura Martel and Julien Hiet

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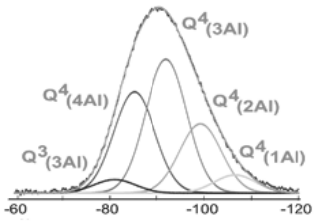
The occurrence of disorder in crystalline, amorphous or glassy materials translates into a broadening of the isotropic solid state lines of the observed nuclei due to distributions of isotropic chemical shifts (^{29}Si, ^{31}P) and to distributions of quadrupolar interaction (^{11}B, ^{17}O, ^{27}Al...) when dealing with quadrupolar nuclei. These distributions lead to overlap and loss of resolution of the signatures of the different chemical motifs which are usually resolved in spectra of perfectly ordered compounds.

The chemical disorder (nature of neighbouring spins and types of chemical bonds) and geometrical disorder (bond distances and angles) can be disentangled by putting to work experiments based on the existence of unresolved but still usable indirect *J*-couplings[1-4] or dipolar interactions in spin counting[3] or spectral editing experiments applied to dipolar and quadrupolar nuclei[5] that allows quantifying the different structural motifs and evidencing their individual geometrical disorders.



Multiple quantum edited ^{29}Si spectrum of a glass[3]

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Probing the physics of antiferromagnetic rings by EPR spectroscopy

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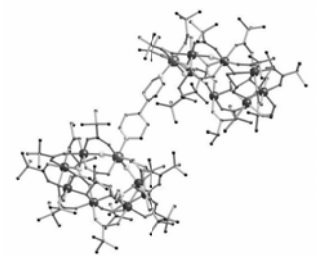
"Antiferromagnetic rings" are a class of spin clusters comprising a cyclic array of antiferromagnetically coupled transition ions. Until recently all examples were homometallic and even-membered giving total electronic spin $S = 0$ ground states. The first heterometallic examples were reported by Winpenny and co-workers in 2003, $(Me_2NH_2)[Cr_7MF_8(O_2C^tBu)_{16}]$ where M is a divalent metal ion; a second family was reported in 2008, $[Cr_7MF_3(glu)(O_2C^tBu)_{15}L]$ where H_5glu = N-Et-glucamine and L is a terminal ligand at M.[1] The presence of the heterospin leads to $S \neq 0$ ground states that can be tuned by choice of M, allowing systematic study of spin structure in complex but isostructural sets of clusters. This lecture will summarise our EPR studies of such species and then focus on recent efforts towards controlled covalent and electronic coupling of the rings in supramolecular clusters-of-clusters (see Fig).[2] EPR proves to be a very sensitive tool for detecting and measuring the very weak inter-cluster interactions.


Linked Cr_7Ni rings

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The Coordination Chemistry of Signal Amplification and Targeting for MR Probe Development

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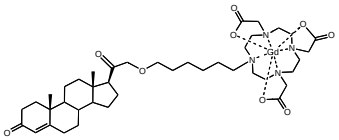
During the last decade there has been significant advances in MR contrast agent design and experimental testing. To overcome the limitations of previous generations of contrast probes, new agents have incorporated multiple chelation sites for Gd(III) while optimizing rotational correlation times, t_m, targeting, cellular uptake and responsive properties.[1] Our laboratory has focused on three aspects of probe development with the ultimate goal of cell patterning and recognition studies of the central nervous system. Our approach attempts to optimize has many of the parameters into relatively small molecule coordination complexes and include i. agents responsive to in vivo physiological or biochemical events ii. cell-permeable MR agents to increase local concentration (Figure 1), and iii. amplification of the MR signal by attachment to large molecules and the synthesis of multiply labeled conjugates. The strategy for the design of responsive or bioactivated contrast agents involves the modulation of one or more parameters (q, or τ_m) affecting relaxivity to produce distinct relaxation states before and after activation. Caged complexes were designed to coordinatively saturate the paramagnetic ion in the absence of specific enzymes or ionic signal transducers, and to allow water access in the presence of these species. These agents conditionally modulate the access of water through changes in the coordination environment of Gd(III) producing a conditionally activated complex.



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Hyperpolarized Krypton-83 Magnetic Resonance

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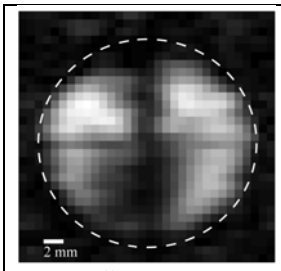

Fig.1. Hp ^{83}Kr FLASH transverse plane MRI of rat lung (no slice selection, NEX =4).

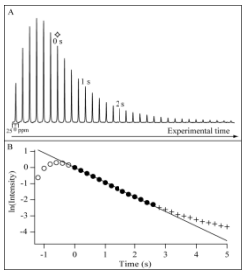

Fig.2.(A)Series of hyperpolarized ^{83}Kr NMR spectra of an excised rat lung acquired from a series of 12° RF pulses spaced evenly by 200 ms delay times. The signal rises initially as the lungs inhales and T_1 relaxation leads to the decay of the signal after the inhalation has stopped. (B)Semi logarithmic plot

In porous media, quadrupolar interactions during collisions with surfaces are typically the main cause for the relaxation of the noble gas isotope ^{83}Kr spin (I = 9/2)[1,2]. The nuclear relaxation of ^{83}Kr can therefore be utilized for surface studies[3] since it is susceptible to the surface-to-volume ratio, surface hydration, and temperature. A special ventilation chamber allowes for *in situ* hp ^{83}Kr MRI of excised rat lungs[4].Hyperpolarized (hp) ^{83}Kr with 6% spin polarization corresponding to 13,000 fold signal enhancement at 9.4 T (i.e.) was generated using a 25 % krypton in N_2/He mixture. The signal intensity was strong enough to allow for ^{83}Kr T_1 relaxation studies as a function of lung inflation and the first hp-^{83}Kr FLASH image of *in situ* rat lungs. Surprisingly, the relaxation in the alveolar space of ex vivo lungs is not affected by various stages of lung inflation despite the presumably changing surface to volume ratios in the alveoli. The measured relaxation of $T_1 \geq 1$s is slow enough to permit for future *in vivo* studies.

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Structural bile-ology

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This presentation refers to a long-time running project dedicated to the investigation of a central aspect of lipid homeostasis, namely the interactions of bile acids and other lipids with their intracellular chaperones (proteins belonging to the Fatty Acid Binding Protein family), with the aim of characterising the mechanism of lipid uptake, transport, and release within the cell. In this framework, NMR data will be presented describing the complex system composed of the bile acid binding protein, bile acids, and membrane mimetic systems, such as anionic liposomes.

The acquired knowledge on the structural and molecular determinants of bile acid binding to intracellular lipid binding proteins has served as a basis to study the interactions with exogenous ligands, such as Gd(III) derivatives, belonging to the class of most widely studied contrast agents for MRI. Such studies have been dealing with the structural characterisation of new potential hepatospecific contrast agents, undergoing active molecular transport in hepatocytes exploiting the enterohepatic circulation. These interaction studies have been extended to different proteins of the FABP family and lipophilic Gd(III) derivatives. The overall data on ligand binding provide a rationale for the future development of the project, related to the investigation of the protein within human living cells and its interaction with liposome-loaded contrast agents.

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Acknowledgments: The overall project has been conducted in collaboration with the groups of Silvio Aime, University of Torino, Laura Ragona, CNR Milano, Volker Dötsch, University of Frankfurt, Ulrich Gunther, University of Birmingham, David Fushman, University of Maryland.

New DOSY and pure shift NMR tools for the chemist

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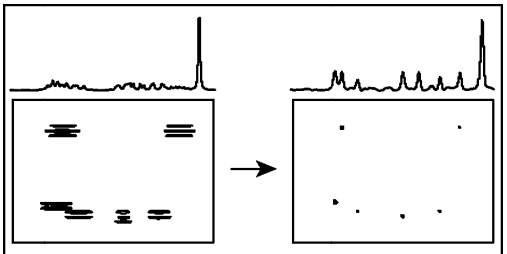
Diffusion-ordered spectroscopy (DOSY) sets out to distinguish between the NMR signals of different species in an intact mixture. Normally this requires first that the signals be well-resolved in the 1D spectrum, and second that the species have different hydrodynamic radii r_H. A range of experimental methods that can circumvent these two requirements will be presented. 3D DOSY methods[1] use the added dispersion of further spectroscopic dimensions to give high resolution in both spectral and diffusion domains, and can be extended to study phenomena such as amide proton exchange.[2] Matrix-assisted DOSY methods, e.g. using micellar or reversed micellar systems, use chemical discrimination to resolve mixtures of species with similar r_H, such as stereoisomers.[3] Signal overlap can also be defeated using multivariate statistical methods to decompose mixture spectra[4] and for the study of reaction kinetics using DOSY.[5] Finally, pure shift methods suppress the effects of homonuclear couplings to give 1D, DOSY and 2D homonuclear correlation spectra in which almost all signals appear as singlets, improving resolution by an order of magnitude or more.[6]



Double pure shift TOCSY suppresses the effects of homonuclear couplings, greatly improving resolution

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NMR and DOCKING Studies on Electron Transfer Complexes

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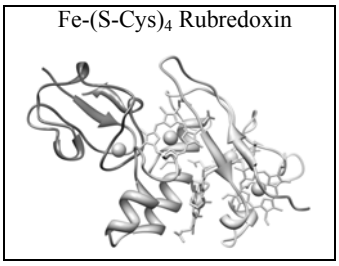
Chemera 3.0 is a molecular modelling software package that includes BiGGER (Bimolecular complex Generation with Global Evaluation and Ranking), a protein docking algorithm. This paper focuses on new features of Chemera 3.0, specially constrained docking, which allows the user to restrict the search for protein-protein complex models in a manner consistent with the ambiguity of some experimental data. This allows the user to take advantage of sets of experimental data obtained by NMR, site-directed mutagenesis, or other techniques, by specifying sets of potential contacts between the two proteins. This models the possibility of some of experimental results being due to effects other than proximity to the docking partner.



Examples were selected for probing the transient interaction between two proteins (A and B), where double (A and B), single (A or B) or no ^{15}N labelling is considered.

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Nuclear Spin Noise - Fundamental Insights and Applications

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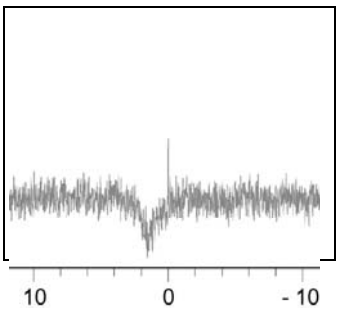
Bloch predicted[1] that even a spin system in equilibrium can give rise to a weak rf-signal due to statistically incomplete cancellation of randomly fluctuating magnetic moments. Experimental detection of spin noise was achieved much later via different approaches.[2-6] The theory of spin noise is based on Nyquist noise.[2] However it does not completely describe the experimental results on the tuning dependence of NMR noise. The spin contribution to noise power can be positive (i.e. it increases the noise power at the NMR resonance frequencies) or negative (one observes "dips" in the noise power spectrum, i,e. less than thermal noise as in the Figure). This "absorbed circuit noise"[6] is often not observed at the minimum of the tuning curve, as predicted. Instead the “dip” noise line shape is often observed tuning offsets of up to several 100 kHz).[3,4,6] Tuning for the noise "dip" substantial gains in the signal-to-noise ratio have been achieved[4]. Examples of spin noise spectra under a variety of conditions for liquids and solids (see Figure) underscore an application potential reaching beyond the tuning improvements.



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Acknowledgments:The project has been supported by FWF (Austrian Science Funds) Project No. P19635-N17. Access to Research Infrastructures was made possible in the 6th FP of the EC (Contract # RII3-026145, EU-NMR) and in the 7th FP of the EC (Contract # 228461, EAST-NMR).

Imaging Angiogenesis: Microenvironmental control of vascular remodeling

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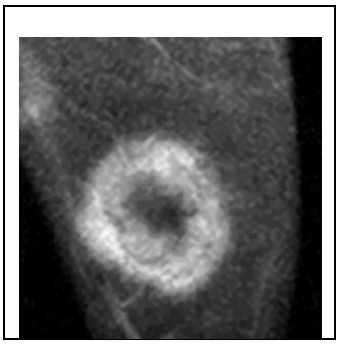
Blood and lymphatic vessels dynamically adapt to changing needs of the tissues so as to maintain physiological homeostasis. Acute changes in demand are addressed through a functional response. For blood vessels this entails vasoconstriction or dilation and changes in vessel permeability, affecting blood flow and molecular transfer rates respectively. For lymphatic vessels, acute changes in interstitial pressure result in changes in vessel permeability and increased lymphatic clearance.



Chronic changes in the tissues including fetal implantation and development, organ growth, wound healing, or tumor progression, require sustained adaptation through matching expansion (or regression) of the vascular bed. These changes must match needs, and such regulation is achieved through sets of feedback loops coupling the sensing of the changes in the microenvironment to orchestration of vessel growth.

MRI provides an important component in the multimodal efforts for imaging the dynamics and regulation of the changes in the vasculature. A panel of structural, functional, molecular and cellular imaging methods were developed for the adaptation of blood vessels in the early stages of fetal implantation and during the early stages of tumor growth. Differences in the controlled vascularization of the former and the unchecked irregular expansion of vessels in the later provide insight to the mechanisms affecting tumor progression.
Finally, imaging provided insight for the role of microenvironmental stress in modulating lymphangiogenesis.

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The challenges and opportunities of molecular imaging with MRI

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MR offers a diversity of non-invasive readouts that find extensive use in biomedical research and clinical diagnostics. PET and SPECT, however, have a much higher sensitivity than MR. Therefore, these techniques are presently preferred for the visualization of sparse molecular markers. Making MR suitable for molecular imaging applications, is a challenging task. Several approaches are explored to allow *in vivo* detection of sparse molecular markers with MR. One such strategy involves the use of targeted nano-particles. Nano-particles can readily be equipped with a high payload of MRI contrast agent. Four main approaches are used: (i) nano-particles loaded with Gd^{3+}-chelates for T_1 shortening;[1] (b) FeO nano-crystals for T_2-detection;[2] (c) fluorinated nano-emulsions for F-19 MR;[3] (d) nano-particles incorporating CEST agents.[4] Nano-particles also offer attractive features for multimodality imaging and incorporation of drugs for image-guided therapy. Nano-particles are primarily used for targeting of intravascular markers and of extravascular markers in case of enhanced vascular permeability, such as occurring in tumors, atherosclerosis and myocardial infarction.

The presentation will deal with the design and characterization of target-specific MRI contrast agents and their use in preclinical imaging studies on animal models of disease.

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New twists to dipolar recoupling in biological solid-state NMR: Optimal control, multiple-field oscillation, recoupling without decoupling, and resolution enhancement

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Novel approaches for systematic design of dipolar recoupling experiments are presented. The methods are designed analytically using mutiple-oscillating-field techniques,[1] numerically using optimal control,[2] or combinations of the two approaches.[3] The first part of the talk will address the concepts of multiple-oscillating field methods, and demonstrate the versatility of such methods for combined demodulation and modulation of internal parts of the nuclear spin Hamiltonian to provide efficient re- and decoupling of selected nuclear spin interations. Examples will include dipolar recoupling without decoupling,[4] homonuclear dipolar recoupling without dipolar truncation for measurement of long-range internuclear distances, and design of experiments for recoupling of native dipolar coupling Hamiltonians. The second part of the talk will addr ess the use of optimal control theory with constraints to emphasize certain nuclear spin interactions or specific coherence/polarization transfers while suppressing others. Examples will include recoupling methods offering higher robustness towards instrumental errors, band-selective dipolar recoupling, and methods improving the resolution of multipledimensional solid-state NMR spectra. The various methods will be described analytically and verified numerically and by experiments on biological samples, including ubiquitin, GB1, and amyloid fibrils of hIAPP(20-29).

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Solution-NMR structure determination of the seven-helical transmembrane protein sensory rhodopsin

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The study of seven-helical integral membrane proteins (7TM) remains a challenge for structural biology. We use the 7TM phototaxis receptor sensory rhodopsin pSRII to demonstrate the feasibility of such studies by means of solution NMR spectroscopy. For the first time we present the full 3D structure determination of a 7TM receptor using NMR spectroscopy. The size of the protein-detergent micelle complex under investigation is on the order of 70 kDa. The quality of the pSRII structure ensemble is extremely good (backbone root mean squared deviation of 0.48 Å). Based on our experience with pSRII we discuss the possibility of similar NMR structural studies with other 7TM proteins.

Structure and interactions of malaria surface proteins

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Proteins on the surface of the merozoite stage of the malaria parasite *Plasmodium falciparum* are excellent candidates for inclusion in a malaria vaccine and are potential drug targets. Here we describe studies of the 3D structure and interactions of two of the leading candidates, merozoite surface protein 2 (MSP2) and apical membrane antigen 1 (AMA1).

NMR assignments have been obtained for full-length MSP2 (23 kDa) in solution, allowing the residual secondary structure and backbone dynamics to be defined.[1] There are motional restrictions in the conserved C-terminal region and in two other regions, both of which display helical structure propensities; one of these helical regions is within the conserved N-terminal domain. Although MSP2 is highly disordered in solution, its conformation on the merozoite surface has not been determined. MSP2 associates with lipid micelles through the N-terminal region, while the C-terminus is GPI-anchored to the parasite membrane. Studies are underway to develop a model of MSP2 on the merozoite surface and, in particular, to understand its antigenic properties in that environment.

The structure of the AMA1 ectodomain (65 kDa), determined by X-ray crystallography, contains a substantial hydrophobic trough at its membrane-distal surface. Recent evidence from NMR[2] and mutational studies shows that peptides identified by phage display[3] bind to this trough and inhibit merozoite invasion of host red blood cells. This site therefore appears to be a promising target for anti-malarial development.

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Development of NMR Methods for Studying Membrane Proteins

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Membrane proteins are important but challenging systems for NMR spectroscopy. The development of NMR methods capable of describing the dynamics and determining the structures of membrane proteins requires parallel and integrated research in molecular biology and biochemistry, instrumentation, and experimental methods. The common features of membrane proteins are being identified through comparative studies of three systems, including the viroporins Vpu from HIV-1 and p7 from HCV, the mercury transport membrane proteins MerE, MerF, and MerT, and the G-protein coupled receptor CXCR1. Results from solution NMR studies of the proteins in micelles and isotropic bicelles are compared to those from solid-state NMR studies of the same proteins in aligned and unoriented samples in bilayers. Recent advances in the instrumentation and experimental methods for solid-state NMR are focused on triple-resonance methods to extend the studies to all carbon and nitrogen sites in backbone and side chains of the proteins.

The Structure of Human αB-Crystallin by Solid-State NMR and Small-Angle X-ray-Scattering, and some exciting Adventures with DNP

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Imbalanced protein homeostasis is detrimental to a large variety of vital biological functions in higher organisms, accordingly regulating protein systems are of high pharmacological interest. An important example is the small heat shock protein (sHSP) αB-crystallin (αB) which acts as an ATP-independent chaperone. Dysfunctions of human αB are associated with the occurrence of cataracts in the eye lens, multiple sclerosis, cardiomyopathies, and Alzheimer's disease. αB is a paradigm example of a polydisperse supramolecular complex whose inherent dynamics is connected to its temporal activation and inactivation. We present a mechanistic understanding of oligomer assembly and heterogeneity on a molecular and atomic level, applying small-angle X-ray scattering (SAXS) and solid-state NMR. As a basic building block we obtained a curved dimer. The C-terminal IXI motif and the N-terminal residues S59-W60-F61 interact with other dimers. We observe a pH-dependent modulation of the interaction of the IXI motif with β4/β8.

Dynamic nuclear polarization enables the investigations of new types of samples when a maximum of signal enhancement can be achieved. In this section of the presentation, first the effects of freezing on a number of samples will be discussed, and then strong enhancements observed on deuterated samples. Following this, applications to the detection of the nascent chain emerging from ribosomes and kinesins bound to microtubule will be presented, using concepts of differential labeling, including deuteration.

Lanthanide Tagging for Protein Structure Determination

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Paramagnetic lanthanide ions present outstanding opportunities to accelerate structural biology studies by nuclear magnetic resonance (NMR) spectroscopy.[1,2] In particular, pseudocontact shifts (PCS) and paramagnetic relaxation enhancements (PRE) from lanthanide labelled proteins provide valuable long-range structure restraints to assist 3D structure determinations of proteins, protein-protein complexes, and protein-ligand complexes. Accelerated protein structure determination can be achieved by PCS-ROSETTA using only backbone chemical shifts and PCSs. In order to make paramagnetic effects available for diamagnetic proteins, we developed different strategies for site-specific tagging of proteins with paramagnetic lanthanides. The $[Gd(DPA)_3]^{3-}$ complex is shown to allow measurements of PREs both close and far from the binding site of the DPA complex. As an illustration of the use of long-range PREs induced by $[Gd(DPA)_3]^{3-}$, elucidation of the quaternary structure of a leucine zipper is shown. Specific binding sites for the lanthanide-DPA complex can be generated by a simple mutation strategy. Finally, a number of new covalently binding lanthanide tags are presented that complement the existing range of lanthanide tags.[3]

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Joint Analysis of Conformational Dynamics in Ribonuclease H using NMR Spectroscopy and Molecular Dynamics Simulations

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NMR spectroscopy is a powerful experimental approach for characterizing protein conformational dynamics on multiple time scales, while molecular dynamics (MD) simulation is the only method capable of describing full atomistic details of protein dynamics. Homologous mesophilic (*E. coli*) and thermophilic (*T. thermophilus*) ribonuclease H (RNase H) enzymes serve to illustrate how changes in protein sequence and structure that affect conformational dynamic processes can be monitored and characterized by joint analysis of NMR spectroscopy[1] and molecular dynamics simulations.[2] A Gly residue within a putative hinge between helices B and C is conserved among thermophilic RNases H. Experimental spin relaxation measurements show that the dynamic properties of *T. thermophilus* RNase H are recapitulated in *E. coli* RNase H by insertion of a Gly residue between helices B and C. Additional specific intramolecular interactions that modulate backbone and sidechain dynamical properties of RNase H proteins have been identified using MD simulations and subsequently confirmed by NMR spin relaxation measurements. These studies emphasize the importance of hydrogen bonds and local steric interactions in restricting conformational fluctuations, and the absence of such interactions in allowing conformational adaptation to substrate binding.

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NMR based drug discovery: screening and design of novel chemical probes

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Fragment-based legend design is an emerging and powerful approach to the discovery and optimization of novel pharmacologically active molecules. Central to several FBLD methods, NMR represents a versatile technique for various aspects of the discovery and design aspect including fragment-hit identification, validation and optimization. Ultimately, these methods enable the identification of protein's hot spots by using small molecules, regardless of the knowledge of the function of the protein, and the development of a specific assay. Subsequently, such small organic molecules can be used in pharmacological assays to further delineate the molecular role of the target in a disease state. The approaches were applied to the identification of inhibitors of protein-protein interactions, for the discovery highly selective substrate competitive protein kinase inhibitors, and for the design of potent and effective inhibitors of protein tyrosine phosphatases.

Exploring stucture space – theory and experiment combined

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The prediction of solid state nuclear magnetic resonance (ssNMR) parameters of a given crystal structure, using the gauge including projector augmented wave (GIPAW) theory,[1] is becoming a relatively routine component of experimental investigations. While the accuracy of the predictions are typically acceptable, allowing contributions to be made to experimental design, assignment and interpretation, considerable challenges remain. The effects of quantum and classical dynamics[2] and disorder[3] have been investigated, but their treatment is computationally expensive, and the current approaches are unlikely to be widely adopted. But the greatest limitation is in the origin of the crystalline models on which the calculations are performed. They are typically taken from published structures obtained from scattering based techniques (x-ray or neutron). This severely limits combined ssNMR experimental/theoretical studies, tying them to those systems previously investigated by other methods. This is particularly unsatisfactory given that ssNMR has strengths that do not overlap with those of diffraction techniques. I will present a first principles approach to the generation of candidate structures. It is called *Ab Initio* Random Structure Searching (AIRSS)[4] and it has been extensively applied to the field of high pressure physics.[5,6] I will present attempts to combine AIRSS with GIPAW, applied to the perovskites and elemental phosphorous.

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DNA G-quadruplex structures and cation interactions

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DNA quadruplex structures play roles in key biological processes such as the maintenance of telomeres, regulation of gene transcription, DNA recombination and packaging of the retroviral genome. The formation of G-quadruplex, B-type duplex or nonB-DNA structures within the cell will depend on their relative stabilities. The basic building block of a G-quadruplex is the G-G-G-G quartet, which is composed of four hydrogen-bonded guanine nucleotides in a horizontal planar arrangement. G-quartets are linked together by eight hydrogen bonds in a Hoogsteen pairing geometry. Cations play a major role in stabilization of G-quartets by reducing repulsions amongst guanine carbonyl oxygen atoms. In addition, cations contribute to enhanced base-base stacking interactions in a G-quadruplex. The coordination of cations by the closely spaced carbonyl oxygen atoms of a G-quartet was postulated long before the first high-resolution structure of a G-quadruplex was determined. G-quartets interact with dehydrated cations via inner sphere coordination. It is therefore not surprising that formation, stability and structural details of G-quadruplexes are dependent on cation species and cation concentration. A rational design of quadruplex topologies relies on understanding the role of loop length, loop composition and effect of metal ions on loops.

Our insights into the possible biological roles of G-quadruplexes and into the origins of their topological sensitivity to cation species and concentration have benefited by recent studies on structure, stability and interactions of cations with constituent G-quartets. However, it is not currently possible to delineate general rules governing the folding of G-rich DNA sequences, and more specifically the role of cations in this process. Different G-rich sequences adopt distinct quadruplex topologies and, in addition, a given sequence can also fold into various different conformations, which can coexist. NMR studies utilizing $^{15}NH_4^+$ ions as a probe revealed that $^{15}NH_4^+$ ions move between different coordination sites and with bulk solution in a manner that is controlled by structural and other factors. $^{15}NH_4^+$ ions move faster between the interior of tetramolecular structures and bulk solution in comparison to monomolecular and bimolecular G-quadruplexes.

Long-Range and Orientational Constraints for Membrane Associated Complexes

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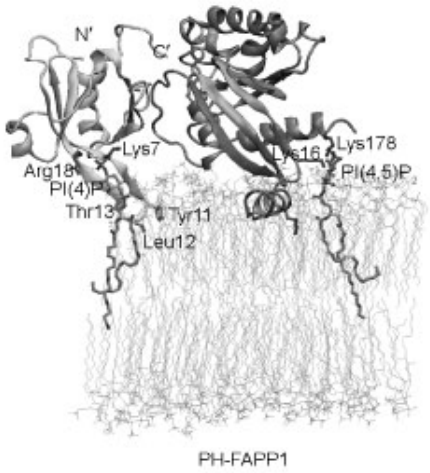
Structure determination of proteins in solution using NMR methods based solely on NOE-derived distance constraints has been enormously successful. However, as systems of interest become larger resolution and assignment of the myriad of proton resonances needed for NOE analysis becomes difficult. In these cases, supplementing NOEs with data that provide constraints over longer distances, and data that can be measured from backbone resonances only, becomes very important. In extreme cases it is even possible to reduce the number of labeled backbone sites, if new resonance assignment strategies can be devised. Among the useful long-distance constraints are residual dipolar couplings (RDCs) and paramagnetic relaxation enhancements (PREs). We illustrate the use of these constraints in a structural analysis of ADP ribosylation factor (ARF), a myristoylated GDP/GTP switch protein that is critical to intra-cellular vesicle trafficking. GTP-myrARF1 is in itself just 21kDa, but it is not soluble in the absence of membrane fragments. Its structure has now been solved as a bicelle complex of approximately 70 kDa. RDC and PRE constraints have proven essential in defining the structure and the mode of membrane association. Moreover, this basic structure and the techniques employed provide a basis for the examination of the higher order complexes essential to the recruitment of materials into the nascent vesicles that bud from the membrane surface in the vesicle trafficking process. Recent applications to the complex of myrARF1-GTP with the PH domain of FAPP1 will be discussed.



High field Dynamic Nuclear Polarization in Aqueous Solutions

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Sensitivity is a major limitation for structural studies of biomolecules by high-resolution NMR spectroscopy. Recently it was shown that dynamic nuclear polarization (DNP) can be used also at high magnetic fields to strongly enhance solid state NMR signal intensity.[1] The situation is more demanding for high-resolution NMR structural studies in solution. Firstly, microwaves mandatory to excite unpaired electrons are strongly absorbed by liquid water samples, and secondly, the Overhauser polarization transfer mechanism[2] effective for liquids was predicted to be ineffective at high magnetic fields.[3] We show here for the first time that unexpected high DNP enhancements of more than 30 can be achieved in liquid water samples at room temperature (RT) and at magnetic fields of 9.2 T (corresponding to 400 MHz ^{1}H NMR frequency and 260 GHz EPR frequency).[4,5] Our approach is to polarize liquid samples *in-situ* at high magnetic fields using a double-resonance structure, which allows simultaneous excitation of the NMR and EPR transitions. Possible reasons for these high enhancements will be discussed[6] and first applications to metabolites and proteins will be shown.

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Acknowledgments: We thank Frank Engelke and Alexander Krahn (Bruker Biospin) for technical support with the NMR probe.

Structural and dynamic determinants of the multistep bile salt binding to lipid binding proteins

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Bile acids are important signalling molecules that regulate a network of metabolic pathways including lipid, glucose, drug and energy metabolism. The specific mechanisms that couple intracellular lipid processing to biological targets and signalling pathways are not yet well understood. Bile acid binding proteins (BABPs), belonging to the intracellular lipid binding protein family, have been recognized to play central roles in driving bile flow, with complex regulation of activity and function in nucleus, cytoplasm and membrane. Structural and dynamical properties of a few BABPs and their ternary complexes have been previously characterized through different NMR and docking approaches by our group.[1] A number of factors have been shown to modulate ligand binding, namely the chemistry of the ligand, the nature of protein residues, the formation of disulphide bridges, which induce changes in local mobility and site-selectivity for the different bile acids. To clarify the structural and dynamical determinants of the molecular recognition processes different approaches have been followed and will be discussed: i) NMR structural studies combined with docking techniques for the determination of the ternary complex formed with the two most abundant natural bile salts; ii) kinetics studies based on NMR line shape analysis and relaxation dispersion experiments, which allow a reliable and sensitive investigation of binding events occurring on μs-ms time scales. A two-step binding mechanism was found to fit the behaviour of several residues. The overall results support the finding that the initial recognition process fits a conformational selection model, where the dynamics observed in the apo-form is essential for ligand uptake. The derived binding mechanism is discussed in light of the results obtained for other members of the lipid binding protein family.

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Molecular basis of FIR-mediated c-*myc* transcriptional control

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The *c-myc* proto-oncogene is a master regulator of cellular proliferation, growth and differentiation and is important in the re-programming of stem cells. *c-myc* up-regulation has been associated with many cancer pathologies[1]. The Far UpStream Element (FUSE) regulatory system is a fast, transcription-mediated mechanism of gene control that promotes a peak in the concentration of c-Myc during cell cycle. FUSE regulation is based on the binding of a transcriptional activator (FBP) to the FUSE DNA element and the subsequent recruitment of its specific transcriptional repressor (FIR). The interaction between FBP and FIR acts as an on/off *c-myc* transcriptional switch. Here we analyse the molecular basis of FIR recruitment and explain how the FUSE system achieves a precise regulation of the cell cycle-dependent peak in *c-myc* transcription.

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Optical Nuclear Hyperpolarization in Semiconductors

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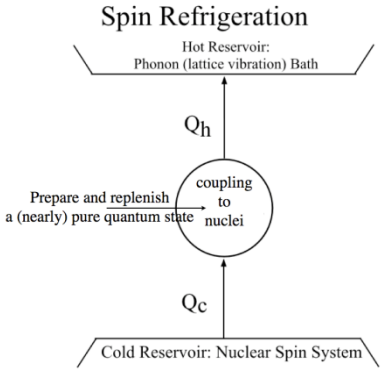
I will describe a generalized scheme for preparation of highly athermal nuclear polarization; the scheme consists of a nuclear spin refrigeration system in which the nuclear spin temperature is lowered by rejecting heat into a higher temperature reservoir using an "engine." The engine of choice is a replenishable pure quantum state that is coupled to the nuclei. I will review several examples of this strategy well known to the NMR community, then I will describe in more detail its application of hyperpolarized nuclei in GaAs, with particular attention to electrical control of the resultant polarization. I will identify a new method of optical hyper-polarization of carbon-13 nuclei in diamond imbibed with NV[-] defect centers. The polarization process for diamond has been modeled with a first-principles quantum mechanical treatment in conjunction with a spin diffusion transport model. The mechanism underlying this model is not dependent upon lattice energy (unlike GaAs), and thus is generalizable to room temperature pumping in bulk and in other materials.



The Relationship between the 3D Structures and Properties of Amyloids

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Amyloids are highly ordered cross-β-sheet containing protein aggregates associated with several dozens diseases including Alzheimer's, Creutzfeldt-Jakob and Parkinson's disease, but are also associated with functional states such as hormone storage in secretory granules and skin pigmentation in mammals. In contrast to soluble protein folds, the cross-β-sheet entity is an inter-molecular motif repetitive in nature almost indefinitely. The intermolecular repetitiveness is key for the multiple properties of amyloids: On the one hand the repeating motifs can translate a rather non-specific interaction into a specific one through cooperativity. On the other hand the cross-β-sheet entity can grow by recruitment of the corresponding amyloid peptide/proteins. Because of these two properties activities of amyloids are manifold including peptide storage, template assistant, loss of function, gain of function, generation of toxicity, membrane binding, infectivity etc. In this presentation, we will discuss the structure-function relationship of the HET-s prion of the filamentous fungus Podospera anserine and the role of the amyloid entity in the storage of hormones in secretory granules.

Assessment of Lung Function with Polarized MRI

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Improvements in quantitative assessment of structure, function and metabolic activity in the lungs, combined with recent enhancement of the spatial and temporal resolution of these techniques, have helped to enhance the quality of diagnosis and evaluation of pulmonary disorders. Radiological methods are among the most attractive techniques for the comprehensive assessment of the lung as they allow quantitative assessment of this organ through measurements of a number of structural, functional, and metabolic parameters on a regional basis and over the entire lungs. Hyperpolarized nuclei MRI has opened up new territories for quantitative assessment of lung function and structure with an unprecedented spatial resolution and sensitivity. Over the past decade specific techniques have been developed or quantitative imaging of different aspects of pulmonary system, that broadly divide the field into five areas: lung microstructure, ventilation, oxygenation, perfusion, and gas exchange. Hyperpolarized gas diffusion-based techniques have been traditionally used as a robust method for probing structural changes in lung tissue at a microscopic level as induced by a variety of lung diseases. In parallel, parametric models of lung airways and alveoli have been developed for indirect assessment of airway geometry based on hyperpolarized gas MRI measurements. More recently, diffusion-based techniques have become the focus of development for coupled imaging of regional lung function and structure, most notably for real-time imaging of alveolar recruitment and dynamics. In the area of gas transport, recent development of extremely undersampled, high temporal resolution techniques has made it feasible to obtain whole lung images of dynamic gas flow into the airways. Along with recent breath-hold-based imaging of gas replacement, these two seemingly different approaches to gas distribution in the lungs have helped to develop a comprehensive understanding of pulmonary ventilation at an unprecedented fidelity. From a functional standpoint, regional partial pressure and gas uptake methods have become the cornerstone of functional imaging of lungs using this technology. There are however several challenges that still need to be overcome by researchers in this field in order to translate this rich body of imaging methodology into a wider-scale adoption for assessment of disease formation and progression, as well as for development of therapeutics.

Adaptive changes in brain function in response to pathological and physiological challenges: fMRI in rodents to assess plasticity in the CNS

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Functional recovery following traumatic injury to the CNS indicates the impressive capacity of the brain for reorganization. fMRI methods yield highly accurate information on brain areas activated in response to a specific task/ challenge and constitute an attractive tool to study such processes. Experiments in rodents are of interest as they potentially provide mechanistic information. We have applied fMRI to analyze the functional reorganization following focal CNS lesions in rats (focal cerebral ischemia[1] and spinal cord injury, SCI[2,3]). Both, local adaptations in the cortical somatosensory representations (SCI) and long range changes involving the contralateral hemisphere and subcortical relaying structures (cerebral ischemia) have been observed. fMRI measurements have been complemented by optical recordings using voltage sensitive dyes to monitor altered functional responses at a time scale of milliseconds.

For elucidation of mechanistic aspects underlying the processing of sensory input fMRI studies in (genetically engineered) mice are of particular interest. The principal challenge in mouse fMRI is sensitivity. Using low temperature MRI detector coils highly reproducible fMRI responses to sensory input have been obtained in anaesthetized mice. The method has been applied to study pain processing evoked by electrical, thermal and chemical stimulation. It has been shown that sensory processing is altered in mice lacking specific sodium channels. Moreover, interventions with local anaesthetics affected the fMRI signal in the brain, an effect that could be linked to cannabinoid receptors as demonstrated by experiments with corresponding knock-out animals.[4] These results highlight the potential of fMRI studies in using genetically engineered mice.

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EPR studies of the Quinone-Iron Complex Photosystem II

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Photosystem II (PSII) is able to use the energy of visible light to oxidize water and reduce plastoquinone, thereby supplying the electrons needed for photosynthesis. The electron acceptor side involves a pair of plastoquinones, Q_A and Q_B, working in series. These are symmetrically located on either side of a high-spin (S = 2) non-heme ferrous iron. The overall $Q_AFe^{II}Q_B$ structural motif is common to both PSII and purple bacterial reaction centers with the coordination sphere of the Fe^{II} consisting of 4 histines and a bidentate carboxyllate. In purple bacterial reaction centers the carboxyllate is a glutamate residue, while in PSII an exchangeable bicarbonate ion (HCO_3^-) plays this role. Given that CO_2 is not only the source of HCO_3^- in solution but also the ultimate electron acceptor in photosynthesis, a regulatory role for HCO_3^- is suspected. Recent EPR studies combined with molecular calculations have indicated that carbonate (CO_3^{2-}) rather than bicarbonate is present in the native functional form of the enzyme.[1] Here we report a study of PSII when inhibited by the binding of formate instead of bicarbonate/carbonate to the Fe^{II}. Two new signals were found and characterized: 1) the Q_B semiquinone anion antiferromagnetically coupled ($1cm^{-1}$) to the formate-bound Fe^{II}, and 2) the Q_A semiquinone coupled to the formate-bound Fe^{II} under conditions where electron transfer was blocked by having been turned-over in the presence of formate, i.e. a three-electron-reduced form of the quinone-iron complex. These studies provide insight for understanding 1) the proton-coupled electron transfer associated with Q_B reduction and 2) the potential regulation of electron transfer out of PSII.

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Controlled Self-Assembly of Nanoparticles: A General Template for Developing "Smart" MRI Contrast Agents

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Significant efforts have been dedicated to the development of "smart" magnetic resonance imaging (MRI) contrast agents. A common strategy involves a T_1 relaxivity change in response to the presence of the intended target. However, there is still an important need for a general molecular template within this class of MRI probes which through simple chemical modifications can generate unique probes specifically targeted to different molecular targets. Our strategy is based on a system we recently developed[1] based on the condensation reaction between two chemical groups: 1,2-aminothiol and 2-cyanobenzothiazole. We have demonstrated that this condensation chemistry can lead to the formation and assembly of nanoparticles *in vitro* and in living cells under the control of pH, disulfide reduction and/or enzymatic cleavage. For example, a small molecule probe Cys(SEt)-Lys(DOTA-Gd)-CBT, comprising these two chemical groups and a Gd^{3+} chelate, will condense to form oligomers upon disulfide reduction that generates the free 1,2-aminothiol. These oligomers will further aggregate and assemble into nanoparticles through hydrophobic interactions. The change of the chemical form of the probe from single monomers to assembled nanoparticles results in an increase in the T_1 relaxivity of Gd. A series of such compounds was prepared, and T_1 relaxivity vs field strength measurements were made between 0 and 3T. These measurements show that the as-formed Gd-nanoparticle exhibits an overall increased relaxivity in comparison to that of its monomeric precursor, as well as a distinct change in shape of the NMRD profile in the range 0.5T - 3T. For the initially synthesized probes, the T_1 relaxivity increase was 2.64 fold at ~21MHz (0.5T) and 2.14 fold at 64 MHz (1.5T). These probes are efficiently taken up by cells, and T_1 measurements of loaded cell pellets demonstrate good intracellular T_1 relaxivity. By varying reactive groups used to mask the presence of the free 1,2-aminothiol, a class of "smart" MRI contrast agents can be similarly developed to sense and image a variety of molecular targets.

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Rotating Micro-coils for High-Resolution Spectroscopy and MRI Microscopy

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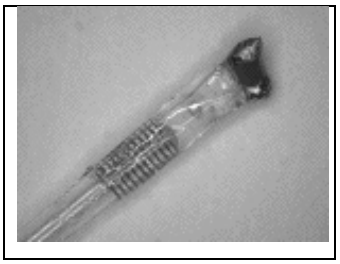
Microscopic samples, objects or subjects cannot be routinely analysed using magnetic resonance spectroscopy or imaging because of the low sensitivity of the technique and the inadequate size of commercial detectors. Micro-coils can be inductively coupled to most commercial probes and be used for static as well as solid samples since the coupling is wireless.[1] This approach was coined Magic Angle Coil Spinning or MACS. Spinning the coil together with the sample eliminates most of susceptibility-related line-broadening and leads to line-widths sufficient resolution to allow for quantification of metabolites using HRMAS spectroscopy.[2]



We are presenting results from spectroscopic applications of MACS in liquids, biological samples, solids and we propose the extension of this approach to ultra high-resolution microscopy imaging[3]. A detailed numerical analysis of eddy current effects will also be shown.[4]

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Introducing new variables to MAS NMR

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Recent progress in spinning technology has opened up new experiment categories in NMR. Extended range of the rotation speeds allows to control spin-diffusion and probe molecular dimensions via timing the magnetization transport distance. Reduced mechanical angular momenta and enhanced bearing stiffness provide for a fast flip of the spinning angle, which can be used for temporary, suitably scaled restoration of anisotropic spin interactions. Measurements of bulk and selective long range spin distances can be optimized and become thus more reliable.

We present hardware and pilot experiments towards structural biology, based on availability of new mechanical options of MAS NMR.

PELDOR on DNA: Orientations, Dynamics, Bending, Non-Covalent Labelling and Protein Binding

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Pulsed Electron Electron Double Resonance (PELDOR) has been established as a very precise and reliable method to measure nanometer distances between spin centres. Recently, we have shown that PELDOR enables also determining angles between spin labels.[1] To overcome the challenging label synthesis, the next generation of labels makes use of non-covalent binding via hydrogen bridges and stacking interactions. We can show that the specificity and strength of binding is large enough to yield high-quality PELDOR data with modulation. This non-covalent labelling concept proves also successful for the study of protein binding. For example, the DNA bending of the Lac operator DNA upon binding of the Lac repressor protein is clearly resolved in the PELDOR data. In a further class of DNA binding proteins, the helicases, we can resolve its switching between different conformational states induced by binding of ATP, ADP, and DNA.

Thus, PELDOR does not only provide mere distances but access to orientations and changes between conformational states of large protein complexes difficult to access otherwise.

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Encapsulated Xenon as an NMR Sensor for Biomedical Applications

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Conventional magnetic resonance imaging (MRI) suffers from intrinsic low sensitivity that makes it difficult to detect molecules other than water. The increasing interest in disease detection based on related biomarkers on the molecular level requires alternative detection methods that can sense such markers in nanomolar concentrations. MRI with hyperpolarized xenon is a promising approach since the ^{129}Xe NMR signal is extremely sensitive to its molecular environment.

When encapsulated in molecular cages, xenon can be used as a contrast agent in solution state NMR and can be functionalized to form so-called biosensors for various target molecules[1]. Indirect detection through the Hyper-CEST method allows for further sensitivity enhancement while preserving the ability to selectively encode information from different biosensors. Some recent developments like NMR thermometry with encapsulated xenon and selective detection of different micro-environments will be presented.

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Sparse and Bayesian Magnetic Resonance Techniques: Application to Transient and Flowing Systems

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Magnetic Resonance (MR) imaging is increasingly being used to study flowing systems in the field of chemical engineering. Understanding single and multi-phase flows in both vessels and porous materials are topics of substantial interest, since these systems are difficult to study using conventional optical or tomographic techniques. Despite the opportunity to study these using MR techniques, multi-phase flows typically provide significant challenges due to their transient nature or low signal-to-noise. This presentation shows how prior knowledge can be used to overcome some of these problems and how increasing prior can reduce acquisition times further. These approaches are based around sparse sampling strategies and we will demonstrate how they can be applied to the imaging of flowing systems. In particular we are exploiting recent developments in Compressed Sensing[1] to achieve higher temporal/spatial resolution imaging. Where there is greater prior knowledge of the system, we are using a Bayesian design of experiments such that only a very small proportion (~1%) of data points need to be acquired, compared to a fully sampled image, to extract the required information.

Two case studies will be discussed to illustrate these approaches:

Velocity imaging of gas and liquid flow in porous media: Using non-uniform Fourier techniques and Compressed Sensing reconstruction we can achieve an order of magnitude reduction in the acquisition time of velocity images.[2]

Gas-liquid bubbly flows: Both non-uniform Fourier strategies and Bayesian experimental approaches have been used to study bubble sizing. Compared with conventional MR methods, the Bayesian approach achieves a reduction of data acquired by two orders of magnitude and can be applied to characterise systems of much higher gas voidage (>30%) compared to conventional methods (<3%).

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Cells, Drugs and NMR

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Post-translational protein phosphorylation endows the eukaryotic proteome with the ability to establish, store and relay information via multiple cellular signaling pathways. Aberrant kinase activities, in turn, are implicated in a number of human diseases, including cancer.[1] It is hence not surprising that quantitative methods for annotating cellular kinase activities are of great pharmacological importance. To date, protein kinases constitute the second largest group of drug targets and several kinase inhibitors are presently tested in late stage clinical trials.[2] Based on a previously established rationale,[3] we present a high-resolution NMR approach to simultaneously and quantitatively profile multiple cellular kinase activities in real-time, and under native *in vivo* conditions.

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Free-Electron Laser-Based Pulsed EPR at 240 GHz and Beyond

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Like NMR, pulsed EPR becomes more powerful at high fields and frequencies. The spectral and orientation resolution, sensitivity, polarization, and time resolution improve dramatically. The highest-field commercial NMR magnets push the Larmor precession frequency for spin ½ electrons above 500 GHz. However, at frequencies above 100 GHz, it is extremely difficult to generate a programmable sequence of phase-coherent pulses with the high peak powers and nanosecond durations needed to realize the potential of pulsed EPR at high magnetic fields. The UC Santa Barbara Free-Electron Lasers (FELs), which generate high-power pulses across the frequency band of interest, are now being used to drive the world's first FEL-based pulsed EPR spectrometer, which operates at 240 GHz. This talk will focus on the design, operation, scientific goals, and future prospects for FEL-based pulsed EPR spectrometers. In particular, this talk will describe UCSB's FELs, which are unusual in that they are powered by an electrostatic rather than a radio-frequency accelerator;[1] locking the FEL frequency to a microwave source;[2] ultrafast light-activated switches for turning THz beams on and off; the current performance of the instrument; and planned experiments in solids and measurements of the functional dynamics of light-activated proteins. New accelerator technologies promise transformative improvements in the performance of electrostatic accelerator-based FELs, and hence pulsed EPR spectrometers based on such FELs.

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A Large Intrinsically Disordered Region in SKIP and its Disorder-Order Transition Induced by PPIL1 Binding Revealed by NMR

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Human ski-interaction protein (SKIP) and peptidyl-prolyl isomerase-like protein 1 (PPIL1) are the essential component of RNP core of activated spliceosome B* and C. They belong to Prp19-related factors. Prior activation of spliceosome, SKIP recruits PPIL1 into spliceosome B*. Here, we report that a highly flexible region of SKIP (SKIPN, residues 59-129 of SKIP) is an intrinsically disordered protein fragment. Upon binding to PPIL1, SKIPN undergoes a disorder-order transition. We found that highly conserved PBF (residues 59-79 of SKIP) was sufficient to bind PPIL1. The complex structure of PBF:PPIL1 solved by NMR shows that PBF exhibits an ordered structure and complexes with PPIL1 through electrostatic and hydrophobic interactions. PPIL1 is a cyclophilin family protein. It recruited by SKIP into spliceosome by a region other than the active site. This enables the active site of PPIL1 remain open. Its disorder-order transition induced by PPIL1 binding may adapt the requirement of large structural rearrangement occured in the activation of spliceosome.[1]

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Structural basis of the interaction between chemokines and their G-protein-coupled receptors

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The chemokine stromal cell-derived factor-1 (SDF-1/CXCL12) and its G-protein-coupled receptor (GPCR) CXCR4 play fundamental roles in many physiological processes, and CXCR4 is a drug target for various diseases such as cancer metastasis and human immunodeficiency virus, type 1, infection. However, almost no structural information about the SDF-1-CXCR4 interaction is available, mainly because of the difficulties in expression, purification of CXCR4 and the crystallization of the complex. In this study, an extensive investigation of the preparation of CXCR4 and optimization of the experimental conditions enables NMR analyses of the interaction between the full-length CXCR4 and SDF-1. We demonstrated that the binding of an extended surface on the SDF-1 beta-sheet, 50-s loop, and N-loop to the CXCR4 extracellular region and that of the SDF-1 N terminus to the CXCR4 transmembrane region, which is critical for G-protein signaling, take place independently by methyl-utilizing transferred cross-saturation experiments along with the usage of the CXCR4-selective antagonist AMD3100. Furthermore, based upon the data, we conclude that the highly dynamic SDF-1 N terminus in the 1st step bound state plays a crucial role in efficiently searching the deeply buried binding pocket in the CXCR4 transmembrane region by the "fly-casting" mechanism. Our methodology would be applicable to other GPCR-ligand systems, for which the structural studies are still challenging.

Structures, functions and stability of proteins in mammalian cells investigated by in-cell NMR spectroscopy

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We have established a method for measurement of high resolution two-dimensional NMR spectra of proteins in human cells. ^{15}N-enriched proteins were delivered to HeLa cells by the action of cell-penetrating peptide (CPP) fused to the proteins with co-treatement with pyrenbutyrate. Sufficient amount of ^{15}N-labeled proteins were transduced to intra-cellular space for NMR measurement, but NMR measurement and fluore-microscopy observation indicated that detachment of CPP moiety from the proteins are further required for obtaining well-resolved cross peaks in the spectra and smooth distribution of the proteins in cells. The in-cell NMR spectroscopy potentially provides information about conformation, chemical structure, interaction and folding stability of proteins in cells. Several examples will be presented.

Very high sensitivity, orientation dependent, long-range distance measurements in biomolecules using PELDOR at 94 GHz

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The last ten years has seen the rapid emergence of Pulse Electron Double Resonance (PELDOR) techniques for the measurement of long range distance measurements and distance distributions in biomolecules using site-directed spin labelling methodologies with nitroxides. At conventional X-band (10 GHz) frequencies such measurements typically require averaging times of 12 h for spin concentrations of 100 micro-molar when distances up to 8 nm but more typically 5 nm can be measured. Such measurements have been used to characterise conformational changes, protein folding, protein or DNA or RNA interactions, and tertiary structure in large biomolecular complexes. In this paper we demonstrate PELDOR measurements with sub-micro-molar sensitivity using a new 94 GHz spectrometer operating at 1 kW power levels.[1] The spectrometer also incorporates fast averaging (up to 80 kHz at high power), easy sample handling, low deadtime, 1GHz instantaneous bandwidth whilst operating at sufficiently high frequencies that the orientation dependent g-anisotropy of the nitroxide becomes fully resolved. This permits a set of measurements that also allow the relative orientation and orientation distribution of the spin labels to be accurately characterised. The instrument also allows considerable flexibility in the specification of pulse sequences and we show that composite pulses hold considerable promise in improving sensitivity for PELDOR measurements. Measurements are also demonstrated on fully deuterated proteins, which also offer significant gains in sensitivity through dramatically increased phase memory times of the nitroxides. The sensitivity gains possible through these instrumental and methodological advances open up many new potential opportunities for biomolecular characterisation using pulsed EPR and some of these will be outlined in the talk.

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Nanoporous Solids: Gas storage, Host-Guest Interactions and Dynamics

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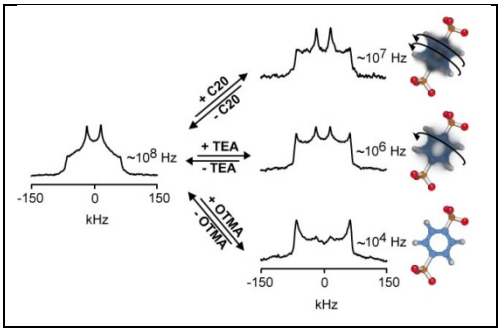
The combination of 2D $^{1}H-^{13}C$ and $^{1}H-^{29}Si$ solid state NMR and 1D/2D hyperpolarized ^{129}Xe NMR, was used to investigate the structure and the properties of hybrid mesoporous organosilicas and metallorganic frameworks. These materials containing open nanochannels of cross-sections varying from 1 to 5 nm and remarkable surface areas present high crystalline order in their channel walls. The arrangement of the hybrid structures, the host-guest interfaces and the exchange times of absorbed/free gases could be determined. Moreover, variable-temperature solid-state ^{2}H spin-echo NMR spectra were performed on $[D_4]$*p*-phenylenesilica to determine the reorientation rate and the mechanism of motion of *p*-phenylene rings in the porous material both empty and filled with guests. The line-shape analysis indicates a rapid two-site 180° flip reorientation of *p*-phenylene moieties about their *para*-axis and a first example of extremely fast motion regime in crystalline porous materials. We were able to fine tune the collective dynamics of the entire population of rotors by the active use of included molecules, thus enabling the external regulation of the motional regime through weak intermolecular interactions.[1-3]



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Integration of Nontargeted and Targeted Screening by NMR

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NMR based mixture analysis is finding more and more applications, driven by metabolic profiling and fingerprinting. Toxicity screening of drug candidates was a first successful example, which has rapidly spread into clinical screening, raw material screening and food analysis. NMR now is one of the two major analytical technologies besides Mass Spectrometry. With the introduction of digital receiver systems and optimized pulse sequences for water suppression, NMR has reached a degree of reproducibility unmatched by other analytical tools. This allows fully automated measurement and data analysis in rapid screening mode. Based on its unmatched reproducibility, it is possible to visualize smallest changes in multiple metabolite concentrations simultaneously, defining the strength of NMR as a multimarker analytical tool.

In food quality control as well as in newborn screening untargeted screening allows to detect all types of deviations, even those, previously unknown. Spectral NMR data acquired for nontargeted screening (statistical analysis) can at the same time be used for targeted analysis, meaning the quantification of a set of compounds. In the quality control of fruit juices such a system is already available under full automation from measurement to final report.[1]

The same technical procedure can be applied to baby milkpowder quality control (e.g. Melamin), heparin quality screening, inborn error screening and human plasma analysis. These examples are explained together with further applications in food quality control.

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Spin-qubits for quantum information processing

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Processing of digital information has progressed at an enormous speed over the last decades and thus become an indispensable resource. Still, for some computational problems, no efficient algorithms are known for today's computers. If quantum mechanical systems are used instead of classical ones, some of these problems become solvable, with an exponential speedup over classical computers.[1] Many different physical systems are being considered for implementing quantum algorithms, but so far, spins have proved most successful for storing and processing digital information. This feat may be tracked to two different causes:

- Spins are the only naturally occurring 2-level systems and therefore ideal qubits.
- Magnetic resonance, in particular NMR, has developed a wide range of sophisticated techniques for coherent control of quantum systems.

Quantum mechanical systems with many degrees of freedom are still difficult to control with sufficient precision to implement large, general-purpose quantum information processors. Nevertheless, it has become possible to implement many different quantum algorithms in systems consisting of a small number of spins (=qubits), and thereby test fundamental concepts and to develop new techniques for optimizing control. An area of applications that has proved particularly stimulating for physical research is that of quantum simulations[2]: quantum mechanical systems can be used for efficiently simulating different quantum systems. Such “special-purpose” quantum computers have been implemented with several thousand qubits, and the simulations running on them have shed some light on phenomena that are hard to investigate with alternative techniques.

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Probing novel order and dynamics in strongly correlated electron systems by NMR

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In this talk I will discuss the following three topics, where NMR is used to identify order parameters of novel quantum phases or to probe novel dynamics in superconductors and other strongly correlated electron systems. **[1]** Some of the recently discovered iron-pnictide compounds are known to become superconducting not only by carrier doping but also by applying pressure, providing opportunity to study quantum phase transitions without being affected by disorder. We have developed a pressure cell[1] providing hydrostatic pressure environment up to 10 GPa over a large volume of 7 mm^3. By ^{75}As NMR, we find a novel hybrid state in $SrFe_2As_2$ near 5 GPa, where the superconducting and antiferromagnetic phases coexist in spatially distinct regions but appear below a common transition temperature T_C=30 K.[2] **[2]** Rare earth or actinide elements in highly symmetric crystal fields often show ordering of high-order electro-magnetic multipoles, such as electric quadrupole or magnetic octupole moments. NMR is a powerful local probe to detect such an ordering. NMR detection of antiferro-octupole order was first demonstrated in CeB_6.[3,4] In a more recent example of $PrFe_4P_{12}$, we proved by ^{31}P NMR that the order parameter is electric and totally symmetric, i.e. does not break any point- group symmetry at the Pr sites, hence involves hexadecapole or even higher order multipoles.[5] **[3]** NMR spin-lattice relaxation in metals is usually caused by magnetic hyperfine interaction with conduction electrons. The β-pyrochlore oxide $KOsO_6$ provides a rare example, where NMR provides information on lattice dynamics.[6] We found that the relaxation at the K sites is dominantly caused by anharmonic low-frequency phonons called "rattling", which are strongly coupled to the superconducting transition, stimulating the subsequent theoretical development.[7]

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A Few Steps towards the Implementation of Molecular Spin Quantum Computers: Pulse-Based Electron Magnetic Resonance Spin Technology

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The past decade has witnessed that quantum computers (QCs) and quantum information processing systems (QIPSs) have been rapidly emerging in pure and applied sciences. Chemical applications of quantum computing to quantum chemistry are now the focus of current topics in the fields.[1-3] A photon-qubit based QIPS was practically utilized in Swiss Federal Election in October, 2007. Implementation of scalable qubits is the most intractable issue to be solved for any physical systems pursuing realistic practical QCs/QIPSs. Thus, the implementation of scalable matter qubits is now a materials challenge for scientists from the experimental side. Among matter qubits, molecular electron spin-qubits are the latest arrival, but can afford promise in implementing scalable QCs/QIPSs,[2] as relevant to an electron spin-qubit version of Lloyd model.[4-6] In this presentation, we illustrate how to design and implement electron spin-qubits and nuclear spin-qubits in organic-based molecular frames. They are all synthetic qubits as well defined in terms of matter spin-qubits in ensemble. The synthetic qubits allow us to generate quantum entanglements between the electron spin and proton nuclear spins. We have shown that both pulsed Electron-Nuclear-DOuble Resonance (ENDOR) and Electron-Electron DOuble Resonance (ELDOR) techniques serve as the most useful spin manipulation technology in implementing QCs/QIPSs.

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Residual Dipolar Couplings (RDCs) as restraints in Organic Structure Determination

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Residual Dipolar Couplings (RDCs) are becoming increasingly important not only in biomacromolecular NMR but also in the determination of configuration and conformation of small organic compounds.[1] Especially in cases of conformational averaging RDCs can provide complementary information to NOEs and *J*-couplings in organic structure determination. To come to broad applicability, methods need to be developed to also include conformational flexibility into RDC analyses. These will be discussed in detail.[2]

Furthermore the use of local alignment tensors (one alignment tensor for each stereogenic center) for the determination of relative configurations will be presented.[3]

With these methods in hand we have also investigated catalytically active species and could show that important insights towards reactivity and selectivity could be gained from the solution structure and dynamics of the catalytically active species.[4]

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Trapping and Magnetic Manipulation of the Spin Isomers of $H_2@C_{60}$

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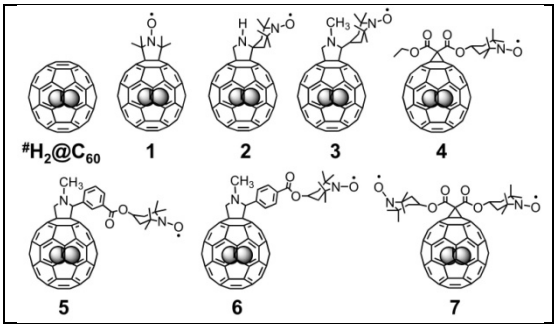
The two spin isomers of elemental hydrogen, pH_2 (↑↓) and oH_2 (↑↑) have been quantitatively incarcerated in C_{60} to form the supramolecular complexes $pH_2@C_{60}$ and $oH_2@C_{60}$, respectively.[1] The spin chemistry of the incarcerated H_2 has been investigated through relaxation and relaxivity measurements.[2] In addition, the mechanism of the interconversion of $pH_2@C_{60}$ and $oH_2@C_{60}$ catalyzed by nitroxides have been investigated.[3] The synthesis of a magnetic switch for spin interconversion will be described.[4] In addition, an investigation of the distance dependence of the nitroxide catalyzed relaxivity and spin conversion of compounds **1-7** will be reported.[5]



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Transient protein-protein interactions studied by NMR

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Many protein complexes are weak and short-lived, because their biological function requires fast turn-over. Some electron transfer complexes are among the most transient of complexes, with lifetimes as low as 1 ms and dissociation constants in the micromolar range.

We use paramagnetic NMR methods to characterize the structure and dynamics of these complexes. A paramagnetic center[1] (Figure) is engineered specifically on one of the two proteins By using such tags, we have recently determined the structures of the 152 kDa complex of nitrite reductase and pseudoazurin,[2] the 64 kDa complex of adrenodoxin reductase and adrenodoxin and the 46 kDa complex of cytochrome *c* (Cc) and cytochrome *c* peroxidase (CcP).[3] In the latter case we were able to describe not only the specific state, but also the dynamic encounter state that precedes the formation of the specific state and represents 30% of the entire complex. It was demonstrated that the sampling by Cc in the encounter state is limited to 15% of the CcP surface, in an area surrounding the specific binding site, an observation in line with theoretical predictions. It shows that the initial steps of complex formation are essential for rapid and successful formation of the active complex.[4]

Lanthanide containing probe CLaNP-5 is attached to a protein (Paz) via two S-S bridges[1]

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Human Imaging with ever increasing magnetic fields and strange RF behavior

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In the last two decades, we have explored ever increasing magnetic fields for use in magnetic resonance imaging (MRI) and spectroscopy (MRS) in pursuit of extracting unique physiological information in humans, going first to 4 Tesla, and subsequently to 7 and 9.4T. A plethora of early experiments, particularly at 7T, demonstrated superior sensitivity and accuracy of functional brain imaging (fMRI) signals, detection of increased number of metabolites in spectroscopy, and improvements in several contrast mechanisms for anatomical imaging (e.g.[1]). In fMRI, these gains have ultimately resulted in unique applications such as robust functional mapping of elementary computational units in the human brain,[2-4] These applications had to deal with complexities arising from damped traveling wave behavior[5,6] of 300 MHz RF, the 7T proton frequency, in the human body. These were managed through multichannel transmit capability on the transmit side, while on the receive side, they lead to significant gains in spatial encoding using parallel imaging. With these engineering and methodological solutions, human imaging at 7T has been feasible not only in the human head but also in the human torso,[7-9] where the challenges have been considered insurmountable until our recent work.

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High-Field EPR Studies of Mn(II) Binding in Biological Systems

Sun Un

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Manganese is an essential metal ion for many organisms. It plays an important role in a number of important biological processes such as photosynthetic oxygen production, as well as controlling the deleterious effects of oxygen. The predominant oxidation state of manganese in organisms is Mn(II). Using high-field electron paramagnetic resonance spectroscopy (HFEPR), we have been studying how the ligand environment around an Mn(II) ion influences its chemistry and function. Studies on proteins and synthetic complexes have shown that the ligand environment can cause large shifts in redox potentials and modify protein function. Recently, we have moved beyond isolated proteins and have exploited the highly sensitive nature of the Mn(II) hyperfine and zero-field interactions to examine the Mn(II) distribution in whole seeds. Preliminary results and their implications to manganese transport and homeostasis will be discussed.

Portrayal of complex dynamic properties of defensins by NMR: multiple motions associated with membrane interaction

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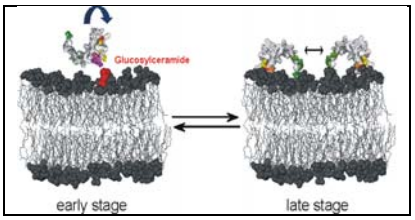
Proteins exist in solution as equilibrium among ensemble of conformations. These dynamic properties are essential for biological function. Our group studies the dynamic properties of plant defensins and their interactions with membrane models. Some important structural features can be identified by comparing the plant defensins: a) high primary structure diversity despite the same global fold, which comprises three antiparallel β sheets, one α–helix and four disulfide bridges; b) no amino acid signature that enables the assignment of their diverse activity, so that the antimicrobial activity cannot be easily predicted. Measurements of relaxation rates revealed that several regions of defensins are dynamic on different timescales and account for conformational fluctuations, despite stabilization by four disulfide bonds. We also followed protein dynamic properties through the events of membrane recognition and insertion. Our results show that the dynamic properties of defensins are directly linked to their function.



Acknowledgments: National Institute for Science and Technology in Structure Biology and Bioimaging, CNPq, CAPES, FAPERJ, PRONEX.

A combined EPR and DFT approach to tackle chiral catalysis

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The outcome of an enantioselectively catalyzed reaction is often governed by very subtle stereochemical and electronic effects. Here, we show how information about these catalytic mechanisms can be gained from a combination of DFT and detailed multi-frequency EPR techniques. Using the example of different chiral Cu(II) and Co(II) Schiff base catalysts, it will be shown how advanced pulsed EPR techniques give a unique insight in different activations processes of these molecules. Furthermore, binding of different enantiomeric ligands to the chiral transition-metal containing molecules results in detectable differences in their EPR characteristics that can be interpreted in terms of stereoselective and electronic effects via DFT modelling.

Triplet state EPR Spectroscopy of Bioluminescent Proteins

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Bioluminescence is one of Nature's most remarkable and most visible reactions catalyzed by enzymes. Many people have been fascinated by the phenomenon of lights from fireflies switching on and off on a warm summer day at sunset, or by beetles, fish, glow worms or even bacteria that can produce light in a variety of colours ranging from blue to red. One of the first and also one of the most striking reported observations of bioluminescence was made by Sir Robert Boyle as early as 1668 that the emission of light is not accompanied by an increase in temperature.[1,2] Since then, many bioluminescent organisms have been found, and owing to the wide diversity of bioluminescence in nature, at least 30 different luminescent enzymes have been discovered.[3] Still, progress after the Second World War was slow.[4] This situation improved when cloning techniques became available and the first bioluminescent enzyme from the American firefly *Photinus pyralis* was cloned.[4,5] Shortly after, other organisms in many laboratories started to light up, and nowadays, the bioluminescent enzyme of fireflies is used in a great number of applications for imaging in the fields of medical and biological sciences. At the electronic level, detailed information of the mechanism of bioluminescence has up to now only scarcely been obtained. In this project, we aim to better understand the high efficiency of bioluminescence by studying paramagnetic reaction intermediates with magnetic resonance techniques. For these purposes, study of the metastable photoexcited triplet states of the pigment molecules involved in the bioluminescent reaction will reveal critical information about the electronic structure. It will provide a detailed picture of the mechanism by which bacteria produce light and allow a critical analysis of the structure-function relationship in these biologically fascinating systems.

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Frequency Domain Magnetic Resonance in Molecular Magnetism

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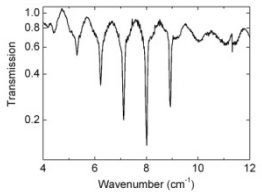
We will present an overview of results that we have obtained with the Frequency Domain Magnetic Resonance (FDMR) technique in the past years. We have used this method for the study of both mononuclear transition metal complexes, and of polynuclear clusters (molecular nanomagnets). The main application of FDMR is the study of zero-field splitting in highly anisotropic molecular materials. We will describe the measurement principle and compare FDMR with other techniques to obtain similar information. Examples that will be shown include the magnetic anisotropy in Mn_6 single-molecule magnets,[1] magnetization dynamics in Mn_{12},[2] and terahertz magneto-optical effects in Mn_{12}.[3]



FDMR spectra of Mn_6

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RNA-binding peptidomimetics repress HIV viral replication by specifically inhibiting transcriptional activation

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The interaction between the human immunodeficiency virus (HIV-1) transactivator protein Tat and its response element TAR plays an essential role in viral replication by controlling transcriptional elongation and contributing to reverse transcription. Many previous attempts to inhibit this interaction have failed to yield molecules with sufficient potency and specificity to warrant pharmaceutical development. Using a structure-based approach, we have identified conformationally constrained cyclic peptide structural mimics of Tat that are sub-nM inhibitors of the Tat-TAR interaction. These peptides are potent inhibitors of viral replication with no cytotoxicity and efficient cell penetration. They specifically inhibit TAR-dependent reverse transcription and activation of transcription in cells and repress replication of a wide variety of viral strains representing all the major HIV clades in primary human lymphocytes. The potency and selectivity observed for this family of peptides is unprecedented among Tat inhibitors and suggest that these compounds may be widely useful for the pharmacological inhibition of other protein-RNA interactions. Using NMR, we have established a structural rationale for their activity and are using this information to optimize their potency.

In search of line narrowing, extended spin memory, and enhanced polarisation: through the looking glass of NMR

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In order to reach beyond the current molecular size and timescale limits of structural and dynamic studies, NMR requires enhanced resolution, sensitivity, and spin-order lifetimes.[1] To improve the resolution of liquid-state measurements, we have used long-lived coherences[2] (LLC's) to obtain narrow and intense signals upon Fourier transform. Homogeneous broadening may be considerably reduced by exploiting the long life-times T_{LLC} associated with these coherences, which consist of superpositions of quantum states with different symmetry. The effect was illustrated by proton nuclear magnetic resonance spectroscopy of proteins in isotropic solution, where the slow oscillatory decays of LLC's yield spectra with considerably improved resolution compared to classical coherences, which decay with the spin-spin relaxation time constant, T_2. If external contributions to relaxation may be neglected, the gain in resolution obtained for a pair of coupled homonuclear spins agrees with the calculated ratio of transverse relaxation times, i.e. $T_{LLC}/T_2 = 3$ in small molecules and up to $T_{LLC}/T_2 = 9$ in macromolecules or viscous media. This opens the way for studies of biomolecules one order of magnitude larger than the current threshold. Dynamic nuclear polarization (DNP) has been employed to considerably enhance the sensitivity of NMR measurements. Long-lived magnetisation states allow us to preserve enhanced polarisation and improve resolution for sensitive proton spins. The use of long-lived coherences should facilitate NMR experiments in viscous environments such as the inside the cell.

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Characterization Ground and Excited States of Membrane Proteins by Hybrid Solution and Solid-State NMR methods

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Membrane proteins exist in an ensemble of conformational states. While membrane protein structural characterization is often focused on the ensemble averaged conformation detected by NMR, their function is carried out by energetically excited states. Using a combination of solution and solid-state NMR techniques (hybrid method), it is possible to characterize both ground and excited states of membrane proteins. This approach is demonstrated for phospholamban, a membrane protein inhibitor of the sarcoplasmic reticulum Ca-ATPase (SERCA), which is phosphorylated by protein kinase A. I will show how the excited states of phospholamban play a major role in the recognition and regulation of SERCA and protein kinase A.

Advances in the characterization of free energy landscapes of proteins by NMR spectroscopy

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It is increasingly clear that the dynamics of proteins play a key role in many of their biological functions, including ligand binding and enzyme catalysis. It is therefore of very great importance to be able to characterize such dynamics at a high level of detail in order to better understand the mechanisms by which proteins perform their activities. I will describe recent advances in the development of procedures to include NMR information about dynamics in the process of protein structure determination, and that provide ensembles of conformations that represent with accuracy the free energy landscapes of proteins.

Invisible states in paramagnetic copper proteins

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NMR of oxidized copper proteins has been largely overlooked, mostly due to the slow electron relaxation times of Cu2+ ion which induce extremely fast relaxation rates in nearby nuclei, rendering them undetectable. It has been shown, however, that these unfavorable electron relaxation features are restricted to T2 copper sites, since T1, T3 and CuA centers display faster electron relaxation rates which make them amenable to NMR studies. In all these cases, the fast electron relaxation stems from to the availability of low-lying excited electronic states which is due to the particular electronic structure of these centers. These features are strongly related to the physiological requirements of these copper centers to perform efficient electron transfer or oxidation chemistry.

The binuclear copper sites CuA and T3 display particularly fast electron relaxation rates which are due to low-lying excited states that can be populated at room temperature and contribute to the reactivity of the metal site. Other magnetic techniques, such as EPR, ENDOR and MCD, normally recorded at cryogenic temperatures, are able to monitor exclusively the ground state. NMR in solution, instead can shed light on the availability of these invisible electronic states. We have studied different mutants of a native CuA site in which small perturbations are able to tune the energy gap between the ground state and the invisible excited state without perturbing the electronic structure of each of them, thus providing a mechanism to regulate the electronic structure of the metal site at room temperature.We have also studied a multicopper oxidase, Fet3 from yeast, in which signals from the T1, T2 and T3 centers could be identified and assigned to each metal site. The temperature dependence of the hyperfine shifts reveals the accesibility of the invisible electronic states in the T3 site of this oxidase, which differ from the description for homologous T3 centers present in other enzymes, again suggesting a role of these excited states in regulating the chemistry of the metal binding site.[1,2]

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Protein Structure and Dynamics of the Nitrophorins from a New World Blood-Sucking Insect

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The ferriheme proteins from the salivary glands of the blood-sucking insect *Rhodnius prolixus*, which is native to the Amazon River basin, are ~20 kDa 8-stranded β-barrel proteins of the lipocalin family, with the ferriheme inside. NO is bound to the Fe^{III} of the NPs in the salivary glands, which allows it to be stored for long periods of time at pH 5-6. Upon injection of the bright red saliva into the tissues of a victim, NO dissociates upon dilution and pH rise to ~7.35. It can then pass through cell walls to reach the capillaries and cause vasodilation. The A-B and G-H loops (Fig. 1) of the β-barrel of the NPs are closed at low pH with NO bound, and more open at high pH, thus suggesting that the rate of NO release may depend on the dynamics of these loops. Structure determination of apo-NP2 by NMR shows that the structure is similar to that of the holoprotein. Thus hemin is not required for protein folding. But for hemin to enter the β-barrel, both calyx and hemin must distort. The N-terminal residue stabilizes the closed loop form at low pH. At pH 5.0 all four NPs of the adult insect have similar NO release rates (k_d ~0.02-0.03s^{-1}), but at pH 7.5 the four proteins have k_d = 1.1-1.6 s^{-1} (NP1,4) or 0.09 s^{-1} (NP2,3). These k_ds vary significantly depending on whether the protein has its native N-terminus (black circle) (K1, D1, D1, A1 for NP1-4, respectively) or contains the M0 that results from expression initiation.[2] Only recombinant NP4 has its native A1 N-terminal residue. We have engineered the other three NPs for soluble expression via export of the protein to the *E. coli* periplasm. A signal sequence is cleaved to provide the native N-terminus. We are investigating the dynamics of apo- and holo-NP2, and in future NP1 and NP4, over multiple timescales using standard multidimensional NMR methods.

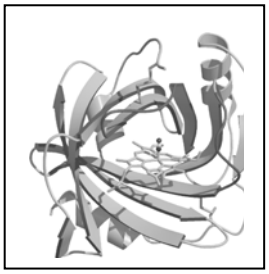

Fig. 1. Structure of NP4-NO,[1] with AB and GH loops (dark curved lines to right and left of opening to heme distal pocket) and N-terminus (circle). The heme is ruffled. Proximal His57 is not shown.

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Metabonomics our wormy world

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Metabonomics is the science that studies dynamic alterations of metabolites in a cell, organ or entire organism.[1,2] Since the birth of metabonomics, there have been many successful applications of metabonomics across diverse research fields with two main purposes: exploring the potential of metabonomics as a tool for the disease diagnosis and further understanding the etiology of a particular intervention or disease process. The first application of the NMR-based metabonomics in studying the human response to parasitic infection started in 2002. Since then, the metabolic responses of laboratory animal hosts to several parasitic infections have been investigated including *S. mansoni*,[3] *S. japonicum*,[4] and soil-transmitted nematodes, *Necator americanus*[5] and protozoans, i.e. *Trypanosoma brucei brucei*[6] and *Plasmodium berghei*.[7] Here, I summarize the knowledge gained from the metabolic investigations of animal host responses to these parasitic infections. Roles and future research direction of NMR-based metabonomic strategy in parasitic infection are discussed.

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Extending T_1 and T_2 relaxation times to improve contrast and sensitivity

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NMR and MRI are extremely powerful techniques with a mature theoretical framework and a broad range of applications. However, this maturity implies that the fundamental limitations are well understood. For example, T_1 and T_2 contrast in tissue often only weakly correlates with metabolically significant characteristics (such as malignancy), and so much of the apparent contrast in application such as breast imaging is useless. As another example, hyperpolarization of carbon or nitrogen in small molecules-is limited in its clinical relevance by short T_1 relaxation times. Can "homogeneous" relaxation times be lengthened by pulse sequences? The traditional answer is no; because the distinction between homogeneous and inhomogeneous broadening (the characteristic timescales of resonance frequency fluctuations) is usually quite clear in NMR. When such distinctions are not clearcut, relaxation times are amenable to pulse sequence manipulation-as recognized in a theoretical quantum computing paper[1] which showed that unequally spaced echo pulses can outperform a CPMG sequence in refocusing coherences. While that paper (and subsequent experimental work) were restricted to microwave spectroscopy of ions in Penning traps, we recently showed[2] similar improvements are possible in MRI. Thus, optimized multiple echo sequences can enhance signal (by as much as 70% in some of our experiments) and image contrast. We will also discuss the uses of pulse sequences and chemical manipulations to increase effective T_1 times of hyperpolarized reagents by storing and retrieving populations from singlet states.[3]

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Progress in interrogating quadrupolar nuclei via solid-state NMR spectroscopy

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Over the past few years we have devoted considerable time and effort investigating the potential of little-studied and considered difficult quadrupolar nuclei such as $^{69/71}$Ga, ^{75}As, $^{113/115}$In, and $^{121/123}$Sb via solid-state NMR. While NMR studies of such isotopes remain challenging, the use of moderate to high magnetic field strengths together with various signal enhancement techniques makes these studies feasible and is leading to interesting applications in inorganic and organometallic chemistry. In this talk, I will provide a brief tour of the NMR periodic table as it pertains to non-integer spin quadrupolar nuclei, focusing on the importance of using high magnetic field strengths. Recent results from our laboratory will be presented, including $^{69/71}$Ga and ^{115}In NMR studies of Lewis-acid – Lewis-base adducts such as X_3Ga-PR_3 and X_3In-PR_3 where X = halide and R = aryl. These studies yielded electric-field gradient tensors at gallium and indium as well as information about the spin-spin coupling tensors, ^{1}J($^{69/71}$Ga, ^{31}P) and ^{1}J(^{115}In, ^{31}P). Another example will highlight results of solid-state NMR studies of materials that are known to exhibit colossal thermal framework expansion such as $Ag_3Co(CN)_6$ [1] and $In[Au(CN)_2]_3$.[2] One of the questions to be addressed is how are the unusual thermal properties of these materials reflected by the NMR parameters?

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Differential dynamics of bound ligands in membrane targets

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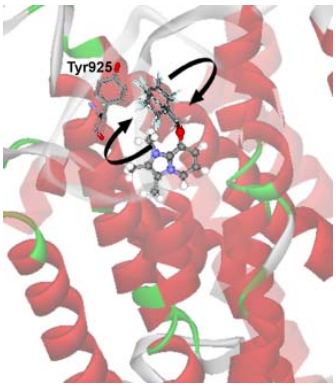

The interactions between macromolecules and small molecules take place on a wide range of timescales. Probing their structure and dynamics is a major challenge, especially for membrane targets, and such information is required to supplement rigid atom detail and functional description, where available. It is now possible to resolve local dynamics within a membrane bound protein at near physiological conditions in natural membrane fragments or in reconstituted complexes, using solid state NMR approaches. This information is obtained by isotopically (^{2}H, ^{13}C, ^{19}F, ^{15}N, ^{17}O) labeling selective parts of either a ligand, or the protein understudy, and observing the nucleus in non-crystalline, macromolecular complexes.

Ligands with complex structure have differential mobility at their binding sites. Substituted imidazole pyridines, for example, which inhibit the H^{+}/K^{+}-ATPase and have therapeutic use, are constrained in the imidazole moiety, but show significant flexibility at the pyridine group (see figure). It is this group which has a direct interaction with an aromatic (Phe198) residue, with the potential for π-electron sharing. Similarly, the steroid moiety of ouabain undergoes motions which are similar to those of the protein, but the rhamnose undergoes a high degree of flexibility at fast rates of motions whilst interacting with Tyr198. The quaternary ammonium group of acetylcholine, undergoes both kinds of interaction which are driven by thermal fluctuations and may be functionally significant.

These apparently generic differential dynamics will be discussed in functional terms, and suggestions about how such large complexes are controlled, made from these observations.

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Atomic-Resolution Spin Mapping and Magnetometry at the Atomic Level

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Spin-Polarized Scanning Tunneling Microscopy (SP-STM) provides new insight into spin structures at a length scale and a sensitivity level which are inaccessible by other magnetic-sensitive measurement techniques. The combination of atomic resolution in direct space, single spin sensitivity, and high energy resolution nowadays offers unique possibilities for probing spin-dependent states and interactions in natural or artificially created nanostructures. Moreover, spin-state manipulation based on spin-current induced switching and spin-state read-out by SP-STM methods offers another novel exciting research direction. Ultimately, a new type of magnetic recording technology might be developed based on spin-state writing and read-out rather than using magnetic stray fields. While the detection of magnetic stray fields becomes more and more difficult as the magnetic bit size is further reduced, the concept of spin manipulation and spin-state determination has already been demonstrated down to the atomic level using SP-STM based techniques. Besides spin-resolved studies of nanometer-scale structures, the magnetism of individual atoms on surfaces has become a focus of research in recent years. The ultimate goal has been the combination of spin-resolved imaging with atomic resolution and magnetometry at the single-atom level in order to probe spin states and magnetic interactions of individual adatoms and nanostructures at solid surfaces in a most direct way. This challenging goal has recently been achieved by operating a SP-STM system at temperatures below 1 Kelvin and in external magnetic fields up to several Tesla. The new method of single-atom magnetometry with an unprecedented degree of magnetization measurement sensitivity is applicable to metallic as well as to semiconducting and molecular systems. The combination of single-atom manipulation techniques and single-atom magnetometry allows unprecedented insight into the magnetic properties of artificially created nanostructures. For magnetic insulators, e.g. oxides, another experimental method, i.e. Magnetic Exchange Force Microscopy (MExFM), has been developed, combining atomic-resolution spin mapping and single-atom sensitivity independent of the sample's electrical conductivity. MExFM provides an additional powerful tool to investigate different types of spin-spin interactions based on direct-, super-, or RKKY-type exchange down to the atomic level.

Generating complex spin quantum states from single electrons and nuclear spins

Philipp Neumann, Johannes Beck, F. Reinhard, Fedor Jelezko and Joerg Wrachtrup

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Single spins in diamond allow for an unprecedented access to single electron and nuclear spins states (1). Hence specific spin states which are of use in quantum information processing or magnetomery can be created. Methods to generate those quantum states deviate from classical approaches based on mere unitary evolution of the spin system by adaptive feedback algorithms. Here the spin system is measured in a specific quantum state (2) and depending on the outcome of that state the system is driven towards specific target states. The talk will describe how to conduct such experiments and will exemplify their power by demonstrating squeezed spin states or entanglement purified two spin states.

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Magnetic Resonance Imaging and Magnetic Molecular Imaging with Atomic Magnetometers

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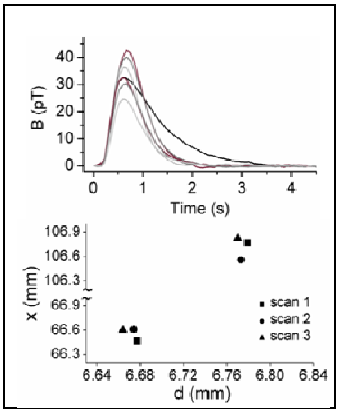
Atomic magnetometry is the most sensitive technique to directly detect magnetic field. It relies on the magneto-optical rotation of the polarization of a laser beam induced by optically-pumped atoms. With a sensitivity in femtotesla range, atomic magnetometers are capable of detecting nuclear magnetic resonance and magnetic particles in the absence of a strong magnetic field. This capability brings three advantages compared to conventional inductive detection: improved penetration in metal, enhanced T_1 contrast, and direct detection of magnetic particles for molecular imaging.

We will first present a cesium atomic magnetometer with 150 fT/Hz[1,2] dc sensitivity and operating temperature of 37 ˚C. Its compact design facilitates applications in MRI and magnetic molecular imaging. Then we will show applications to demonstrate the three advantages of this detection method. For MRI application, we investigated flow in porous metallic materials and compared it with porous plastic materials (top panel). For enhanced contrast, we discovered a gadolinium-based contrast agent that is capable of pH sensing. For molecular imaging with magnetic nanoparticles, we developed a scanning imaging scheme to simultaneously obtain the quantity and location of the particles (bottom panel).



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5 Special Sessions

5.1 CASD-NMR

Special Sessions

Protein structure determination from NMR chemical shifts

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Although still in its infancy, the strategy of determining the structures of proteins from NMR chemical shifts is rapidly gaining momentum, both in terms of range of applications and in terms of accuracy. In this lecture, we will review the most recent results of the CASD exercise, with particular emphasis on the comparison between chemical shift based and NOE based approaches.

Blind-test evaluation of automated protein structure determination by NMR and new developments in CYANA

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The CASD-NMR (Critical Assessment of automated Structure Determination of proteins from NMR data) project aims at evaluating the reliability of present methods for automated protein structure determination by NMR. In a first assessment data sets consisting of the sequence, chemical shift assignments, and unassigned NOESY peak lists were received for 10 proteins. The structures of these proteins were not publicly known when seven different structure calculation methods, one of them CYANA, were applied to the data sets. The resulting structures were evaluated with common structure validation tools and compared to the original structures that afterwards became available from the PDB. The outcome of this first CASD-NMR blind test assessment indicates that currently available software tools are capable of producing in an unsupervised manner structures that are close to the reference structure. In the case of CYANA, the mean backbone RMSD to the reference structure was about 1 Å. The agreement with the data is a good indicator of a correct structure, whereas stereochemical quality indicators are not particularly informative in this respect.

In a second part, new developments in automated spectra analysis and structure calculation with the CYANA software package will be presented. Topics may include: (*a*) Protein structure determination using exclusively NOESY data for obtaining the chemical shift assignments and conformational restraints with the FLYA algorithm. (*b*) A new algorithm implemented in CYANA for automated backbone and side-chain chemical shift assignments based on evolutionary and local optimization. (*c*) Extension of the automated NOESY assignment algorithm of CYANA to solid-state NMR spectra. (*d*) Structure calculation of molecular systems with arbitrary symmetries, including tetrameric and amyloidic structures. (*e*) CYANA structure calculation with automated residue library generation for almost all of the ~10'000 different molecules in the PDB Chemical Component Dictionary. (*f*) Simultaneous representation of NMR structures as a bundle and a single torsion-angle-space-regularized mean structure.

CASD-NMR and liquid- and solid-state NMR experiment-driven modeling of macromolecular systems with UNIO

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First, we will present the novel UNIO protocol that enables us to perform highly to fully automated solution NMR structure determination of proteins. UNIO represents the result of more than 10 years of basic research in the field of protein NMR structure determination. The entire UNIO protocol ranging from backbone resonance assignment, side-chain assignment, NOE assignment to 3D protein structure has already been successfully applied to more than 20 de novo NMR structures with a molecular weight up to 24kDa.

Second, we will summarize the performance of UNIO in the NMR community-wide CASD initiative ("Critical assessment of automated structure determination of proteins from NMR data"). Notably UNIO flawlessly determined all protein structures in this blind testing software competition starting either from raw or interactively edited peak lists or directly from the FID data provided.

Third, we will introduce novel data analysis models for automated signal identification and cross peak assignment of 2D ^{13}C-^{13}C PDSD-type solid-state experiments. Novel concepts for robust discrimination between NMR signals and spectral artifacts and for reliable conversion of PDSD signal intensities into upper distance restraints will be described that allow us to determine high-resolution structures of the two microcrystalline proteins GB1 and Ubiquitin. The determined solid-state NMR structures compare favorably to the X-ray or solution NMR structures both in terms of accuracy and precision of the atomic coordinates.

ARIA, Bayesian structure calculation and CASD

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For NMR structure refinement, Bayesian analysis suggests optimal choices for potential shapes and weights to include experimental data.[1,2] We recently showed that Bayesian potential shape and weight improve the structure quality and reduce bias.[3] In this paper, we compare calculations with the Bayesian restraint potential extensively with a standard calculation for more than 300 proteins structures, in terms of similarity to homologous X-ray crystal structures, the distribution of the structures around their average, and independent validation criteria.

The improvement obtained with the Bayesian potential and weighting in a large number of structures suggests that this way of calculating structures should also be advantageous in the context of automated structure calculations with ARIA.[4] We present experiences with the new approach with the CASD targets.

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New Tools for NMR Structure Validation. Application to the CASD-NMR Structures

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For NMR-derived biomolecular structures, proper assessment of structural quality has proven particularly resistant to reduction into a single relevant parameter.[1] We have developed a program suite called CING (Common Interface for NMR Structure Generation, http://nmr.cmbi.ru.nl/cing) which aims to provide for an integrated, residue-based approach for structure validation. CING integrates the results of a large number of external programs, such as PROCHECK_NMR, WHATIF, Wattos and SHIFTX, with its own internal routines to generate a comprehensive, Web-2.0, interactive validation report. The report particularly emphasizes the relation between experimental data and structural results.

We will present new structural analysis tools implemented within the CING framework. The CING program is also continuously used to analyze the experimental datasets generated by the NMR Restraints Grid (NRG) repository at the BMRB.[2] We now have generated >5000 CING validation reports (http://nmr.cmbi.ru.nl/NRG-CING/) and a first analysis of these results will be presented. The CING program was also used to asses the quality of the automated-structure calculation tests, CASD-NMR.[3] Based upon the results of 81 submissions for 10 targets, we evaluated the state-of-the-art of fully automated NMR structure calculation.

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5.2 Excerpts from BioNMR in Europe

Special Sessions

Structural studies of prion fibrils by solid-state NMR spectroscopy

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Prions are infectious, self-propagating polymers of otherwise soluble, host-encoded proteins. The structural basis of prion infectivity remains largely elusive today; this issue is a technical challenge as prion oligomers are heterogeneous high molecular weight particles that are neither suitable for protein crystallography nor classical NMR studies. Moreover, it has long been believed that misfolded proteins like prions and amyloids give poorly resolved NMR spectra. We show that the spectra of full-length prion fibrils from Ure2p and HET-s interestingly lead to very highly resolved solid-state NMR spectra, at least for parts of the proteins. Ure2p and HET-s are of similar architecture, with a compactly folded globular domain and an in solution flexible prion domain. Comparing the NMR spectra of the full-length proteins reveals however surprising features and stresses the structural diversity underlying prion propagation suggesting that no unique mode exists for the assembly of these proteins into fibrils.[1,2] For Ure2p, the globular part in particular shows almost perfect structural order. This is not the case for HET-s, where the globular part in the full-length fibrils shows features reminiscent of a molten globule, with the associated loss of tertiary structure. Interestingly, sequential resonance assignments of the 32.8 kDa Ure2pCtd using optimized 3D experiments[3] reveal a conformational order in the fibrils superior to the one observed in the crystals, and illustrate in a site-resolved manner the near to perfect conservation of the structure of the isolated C-ter domain in crystals.

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Dynamics of ubiquitination complexes

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The ubiquitination pathway has emerged as a major signalling pathway in eukaryotic cells with a role in proteasomal protein degradation, signal transduction, transcription and DNA repair. The E2 enzymes are the key enzymes in these pathways. More than 30 E2 structures are available, and all share a topologically conserved α/β-fold core domain of ~150 residues. Every E2 can bind three other proteins: the E1 ubiquitin activating enzyme, a cognate E3 ligase enzyme, and activated ubiquitin non-covalently and covalently via a labile thioester linkage. Using a combination of NMR, crystallography, computational techniques and different biochemical and biophysical methods, we study E2-E3 ubiquitination complexes involved in transcription and DNA repair,[1] such as the CCR4-NOT complex,[2-4] complexes with the proto-oncogen c-Cbl,[5] and more recently the Rad6/Rad18 complex.

The human E2 Rad6 and the RING E3 ligase Rad18 are involved in the monoubiquitination of PCNA in response to DNA damage and play a crucial role in translesion DNA synthesis. Using a combination of NMR and crystallography we analyzed the structures and the complex protein interaction network of Rad6, of the Rad18 RING and the second R6 binding domains, and of non-covalently and covalently attached ubiquitin. We discuss the implications of the various interactions for PCNA ubiquitination.

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Progress in ^{13}C direct detection for biomolecular NMR

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The growing interest in direct detection of heteronuclei for biomolecular NMR applications stems from the intrinsically different properties of heteronuclear spins compared to protons that can provide alternative spectroscopic solutions when protons find limitations. In this frame a set of exclusively heteronuclear NMR experiments based on direct ^{13}C detection, have recently been proposed to study proteins[1-4]. Common and complementary aspects with solid state NMR create fertile grounds for improved methods. A series of examples in which ^{13}C direct detection NMR experiments are used to obtain additional, in some cases unique, information to that available through ^{1}H detected NMR experiments will be presented. Thanks to the development of new experimental schemes as well as improved hardware, carried out in close collaboration with Bruker, ^{13}C direct detection now provides a new tool that can be generally applied for biomolecular NMR applications.

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Protein structures by solid-state NMR: Recent progress

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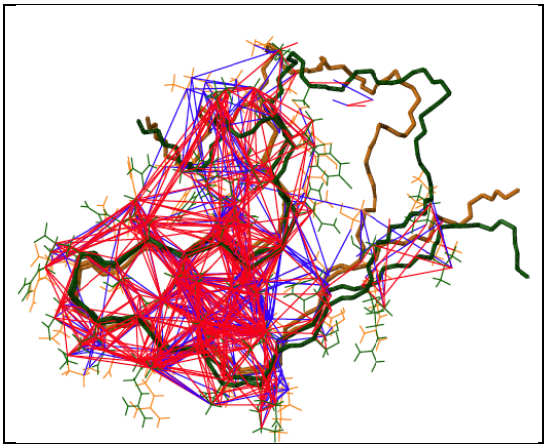
Structure determination from solid-state NMR spectra is still a challenge. While a handful of proteins have indeed be solved atomic resolution, structure determination of completely unknown proteins and of larger proteins (>150 residues) is still difficult and prone to error.

The talk will describe some of the problems and pitfalls and sketch or describe some recipes that promise to advance the field in the future.

These include better and more efficient pulse sequences, enhanced spectral resolution, higher-dimensional spectroscopy, and improved computational processes to arrive at the correct structure form a set of given solid-state spectra.

The principles will be illustrated using microcrystalline proteins as well as fibrils.



Multiple Signal Integration by the Intrinsically Disordered Unique Domain of human c-Src: An NMR view

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c-Src is the leading member of the SKF family of non-receptor protein kinases that play a central role in many signaling pathways. The family contains at least 9 members that display large sequence and structural similarity in the SH3, SH2 and kinase domains but not in the intrinsically disordered N-terminal Unique domain.[1] The regulation of c-Src activity by interaction of SH3 and SH2 domains with polyproline and phosphorylated motifs is well known. However, no functional role had been assigned so far to the Unique domain. Following our previous work in c-Src,[2,3] we shall present recent NMR results of the interactions of the Unique domain of c-Src with the SH3 domain and its modulation and we shall show that both the Unique and SH3 domains contain previously uncharacterized lipid binding regions that are selective for specific phosphoinositides in a way that is modulated by the mutual interaction between the folded and unfolded domains.

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Control of periplasmic interdomain thiol:disulfide exchange in the transmembrane oxidoreductase DsbD

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The bacterial protein DsbD transfers reductant, required for essential pathways, from the cytoplasm to the oxidizing periplasm. DsbD comprises a transmembrane domain (tmDsbD) flanked by two globular periplasmic domains; each contains a cysteine pair involved in a disulfide cascade. The C-terminal domain of DsbD (cDsbD) has a thioredoxin fold. The two cysteines in the characteristic CXXC motif reduce the disulfide bond of the N-terminal domain (nDsbD) which in turn reduces the disulfide in various periplasmic partners. Knowledge of cysteine pK_a values is important in understanding the reactivity of these residues. pK_a values have been determined, using 2D NMR, for the N-terminal cysteine of the CXXC motif, C461, as well as for other active-site residues. It is demonstrated using site-directed mutagenesis that the negative charges of the side chains of D455 and E468 in the active site contribute to the unusually high pK_a, 10.5, of C461. The pK_a value is higher than expected from knowledge of the reduction potential of cDsbD; this makes C461, in isolated cDsbD, a poor nucleophile and implies relative unreactivity towards the target disulfide in nDsbD. NMR studies of an nDsbD-^{15}N-cDsbD covalent complex allowed specific changes in the active site of cDsbD due to contact with its physiological partner to be examined. A substantial increase in the pK_a of D455 occurred in this complex; a consequential decrease in the pK_a of C461 would explain how the disulfide-exchange reaction in the nDsbD-cDsbD complex is initiated. This modulation of pK_a values is critical for the specificity and function of cDsbD. Isolated cDsbD is a poor nucleophile, allowing it to avoid non-specific reoxidation, however, in complex with nDsbD, the nucleophilicity of cDsbD increases permitting reductant transfer. In recent studies the interactions of nDsbD and cDsbD have been shown to be oxidation-state dependent such that the functionally-relevant complex $nDsbD_{ox}$-$cDsbD_{red}$, has a significantly higher affinity than the product complex, $nDsbD_{red}$-$cDsbD_{ox}$. The principles established have wider significance for the understanding of processes mediated by thioredoxin-like proteins which are critical in both prokaryotes and eukaryotes.

Riboswitch-RNAs in transcriptional regulation and RNA thermometers in translational regulation studied by NMR spectroscopy

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Riboswitch-RNA**:** Our group has investigated the structure and dynamics of full length purine-sensing riboswitch-RNAs located in the 5'-untranslated region of mRNA. Formation of these alternate conformations constitutes the regulation mechanism on the transcriptional level in bacteria. By NMR spectroscopy, we investigated the full-length guanine riboswitch and RNAs of different lengths representing different transcriptional intermediates based on the presence of potential transcriptional pause-sites interspersed in the sequence that allow for kinetic control.

RNA-Thermometers**:** In prokaryotes, RNA thermometers regulate a number of heat shock and virulence genes. They repress translation initiation by base pairing to the Shine-Dalgarno sequence at low temperatures. We investigated the thermodynamic stability of the temperature labile hairpin 2 of the Salmonella fourU RNA thermometer over a broad temperature range and determined free energy, enthalpy and entropy values for the base-pair opening of individual nucleobases by measuring the temperature dependence of the imino proton exchange rates via NMR spectroscopy.

High-dimensionality experiments and assignment strategies for partially disordered proteins with highly repetitive sequences

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NMR represents the ultimate tool for studies of unstructured or partially disordered proteins at the atomic resolution. In principle, intrinsically disordered proteins can be assigned using a standard set of triple-resonance NMR experiments applied to ^{13}C,^{15}N-labelled samples. However, combination of the structural disorder with a high incidence of sequential repeats often results in spectra with severely overlapped peaks, impossible to assign by the traditional approach. The lecture will present a strategy that combines several NMR techniques to significantly shorten time needed for thorough description of unstructured or partially disordered proteins. As an inherently insensitive spectroscopic technique, NMR struggles with long measurement times when high dimensionality spectra are acquired. We show that exploitation of non-uniform sampling in indirectly detected dimensions in combination with the optimization of transverse relaxation brings substantial resolution advantages while keeping the acquisition time of high quality 5D spectra at the level of a conventional 3D experiment. In addition, it will be shown that the direct carbon detection becomes preferable due to higher dispersion of carbonyl resonances. Two novel 5D experiments, based on different correlation schemes with distinct areas of applications, allowing complete assignment of the disordered protein from a single NMR spectrum will be discussed. The power of the presented approach is documented on a case study of a tricky sample, the delta subunit of RNA polymerase unique for gram-positive bacteria (δ). The 20 kDa protein contains a disordered C-terminal region of 81 amino acids with a highly repetitive sequence. An importance of better understanding of the δ subunit function together with a great stability and solubility of the sample make this protein an attractive target for NMR investigation. Using a combination of traditional and non-traditional approaches, the structure of the well-folded domain was solved and chemical shift mapping of the disordered region was performed.

5.3 NMR and Cultural Heritage

Special Sessions

Mobile NMR and Cultural Heritage

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Mobile NMR concerns NMR investigations with equipment that can be moved to the object. Today such equipment is available for relaxometry, imaging, and spectroscopy with closed as well with open magnets.[1] Closed magnets accommodate the object under study inside, while with open magnets, the object is positioned outside in the stray field of the magnet. Mobile stray-field NMR with open magnets is particularly suited for analysis of objects of cultural heritage, a concept pursued with great enthusiasm by the late Annalaura Segre. Much of the technological evolution of the NMR-MOUSE has in fact been stimulated by work in the field of cultural heritage.[2]

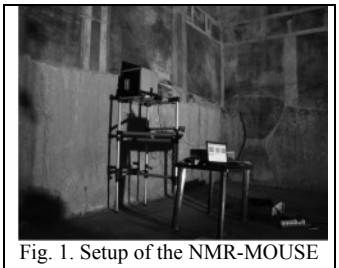

Fig. 1. Setup of the NMR-MOUSE measuring a fresco painting

The state of the art of mobile NMR will be presented and the measurement procedures for unilateral stray-field NMR be summarized. The use of NMR for characterization of material properties and heterogeneity will be illustrated with measurements on mummies, easel paintings, wall paintings, and violins.[3,4]

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Nuclear Magnetic Resonance in Cultural Heritage

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This lecture is dedicated to the memory of our beloved teacher and friend Annalaura Segre most distinguished scientist in NMR. She was a pioneer in applying NMR in many different research fields, among these polymers, food science and cultural heritage.

Standard NMR methods are particularly suitable for studying materials of interest for cultural heritage and these methods are generally considered as non-destructive because the sample can be recovered after performing the analysis. Cellulose-based materials, clays, tuffs, hard stones may be studied and characterized by solid state NMR. However, when studying rare and precious and/or unmovable objects belonging to the cultural heritage, the sampling of even a small amount of material is forbidden and often the analysis must be performed *in situ*. The sampling can be avoided using a mobile NMR instrumentation. Unilateral NMR is portable and its use is fully non-invasive, allowing the measurement of some NMR parameters which are important to establish the state of degradation of the investigated object. The sensor can be positioned near intact objects in different positions. The main advantage of the unilateral NMR technique is that it is not only portable but can also be performed directly on large objects such as *frescoes*, monuments and in general any building fully preserving the integrity and the dimension of the object under investigation. With unilateral NMR it is possible to evaluate the state of degradation of cellulose-based materials to evaluate the performances of consolidation and cleaning treatments on wall paintings, to monitor the detachment of the painted layer from its support, the plaster, to quantitatively map the dampness in a wall painting and to evaluate the performances of protective and/or consolidating treatments on porous materials. The most recent development of mobile NMR allows the obtainment of depth profiles with microscopic resolution. For instance, it is possible to carefully evaluate the penetration depth of a protective treatment as a function of the time of application.

Unilateral NMR as a tool to characterize deterioration processes and follow up conservation treatments in works of art

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A novel application of NMR to study a deterioration process and to follow up a conservation treatment is presented. The technique allows to differentiate and characterize micrometer-thick oil stains of different iodine indexes applied on paper by means of their transverse relaxation decay. Measurements were performed with a portable unilateral NMR system, the NMR MOUSE® (MObile Universal Surface Explorer), which allows the in-situ, non-invasive measurement of spin relaxation and diffusion parameters and to generate depth profiles with a spatial resolution of less than 10 µm. A correlation between $T_{2\text{eff}}$ of the stain and the degree of cross-linking of the oil is observed. This information is crucial when choosing an appropriate conservation treatment to remove the stain. It is demonstrated that it is possible to discriminate the oil groups independently of the paper substrates and of the accelerated aging methods used. The technique is also demonstrated to be useful to non-invasively follow up the progress of conservation treatments frequently used in paper conservation. It is expected that unilateral NMR in combination with multivariate data analysis will fill a gap within the set of high-spatial-resolution techniques currently available for the non-invasive analysis of materials in works of art.

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Advantages and Pitfalls of Magnetic Resonance for Fluids in Porous Media Applied to Cultural Heritage

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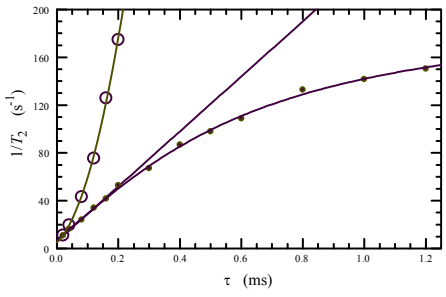
Magnetic Resonance for fluids in Porous Media (MRPM)[1] has origins in the petroleum industry in the fifties, when tools and theories were developed to detect and interpret proton signals from water and oil in reservoir rocks.[2] Not only were surface effects on relaxation times discovered, but also the first tools were developed for *ex situ* signal detection, now widely exploited.[3] In the last 20 years MRPM has been extensively used to study porous media, and, starting from the end of the nineties,[4] it has been applied to the Cultural Heritage for laboratory and *in situ* studies. Beside advantages, MRPM presents pitfalls of which one must be aware for good data collection and interpretation. As an example, the figure[5] shows $1/T_2$ from CPMG data *vs* half echo time τ, with only pore-scale local field gradients (dots), and in the presence of a substantial large-scale field gradient (open circles)[6] for water saturating a porcelain sample. But, what is a pitfall can become a source of information about porous media and the fluids inside.



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Characterization of binders in ancient and modern paintings by NMR-MOUSE

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For the effective study and characterisation of an artist's painting technique, there are three main critical questions to which analytical techniques can find an answer. The first concerns the pigments employed, the second, the stratigraphy which constitutes the painting and the third regards the utilized binder. Many of the pigments commonly adopted, especially in ancient paintings, are inorganic compounds. In which case the NMR technique can specifically evaluate the interaction between the pigment and the binder, whilst other techniques are much more specific for the identification. Instead the NMR-Mouse, as already demonstrated,[1-2] is a powerful tool for the non-invasive study of the stratigraphy of paintings. The last question is probably the most complex to answer using only non-invasive techniques. Therefore a multi-technique approach is mandatory. The aim of this research is to evaluate the contribution that NMR- MOUSE can give in the determination of binders in ancient and modern paintings. The study commenced with the characterization by T1 and T2 of binders commonly employed in ancient paintings, such as tempera and oil. Then the formation of degrade products, namely carboxylates, was simulated by the interaction between oil and lead-based pigments at certain conditions of humidity and temperature. Finally attention was focused on different classes of modern synthetic binders, in particular acrylic, alkyd and PVA in order to find a discriminant parameter to be used on the study of actual paintings.

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Portable NMR in cultural heritage: the contribution of Annalaura Segre

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Annalaura Segre actively carried out research regarding the application of magnetic resonance to the field of cultural heritage. As she had always loved and appreciated art in its many forms she was able to cultivate her two great passions working in the cultural heritage discipline, her interest for art and magnetic resonance. Her experience in macromolecular chemistry naturally brought her towards the characterization of cellulose materials, paper and textiles, of conservation and reinforcement by polymers that with such enthusiasm she actively contributed and participated in CNR projects relating to the field of cultural heritage. She coined the idea and contributed to the creation of a unilateral NMR instrument specific for in-situ, non-invasive analyses for porous materials of cultural heritage interest. This instrumentation optimized as part of the European project Eureka, has permitted the insertion of magnetic resonance as a method for on-site investigation and monitoring and for conservation in cultural heritage. She was particularly involved in numerous in-situ examinations such as those conducted on the frescos of the Criptoportico in the Domus Aurea (Rome), of the Vasari house (Florence) and of the San Clemente church (Rome).

6 Young Investigator Awards

6.1 ISMAR Young Investigator Award Finalists

Sponsored by Magritek

Eduard Y. Chekmenev (**P282**)

Automated Parahydrogen-Induced Polarizer (PHIP) Employing Low Field NMR Spectrometer, Tunable RF Circuit and *in situ* Detection

Institute of Imaging Science, Vanderbilt University, 1161 21st Avenue South, 37232, Nashville, USA

Daniela Delli Castelli (**P285**)

Magnetically oriented nanovesicles as MRI CEST agents

Department of Chemistry I.F.M. and Molecular Imaging Center, University of Torino, Torino, Italy

Christofer Lendel (**P138**)

Structure based drug design for intrinsically unstructured proteins

Dept. of Chemistry, University of Cambridge, UK, Lensfield road, Cambridge CB2 1EW, UK, bDept. of Molecular Biology, SLU, Box 590, 751 24 Uppsala, Sweden

Andi Mainz (**P150**)

The bigger the better: Large protein complexes investigated in solution by MAS NMR

Department NMR-supported Structural Biology, Leibniz-Institut fuer molekulare Pharmakologie (FMP), Robert-Roessle-Str. 10, 13125, Berlin, Germany

6.2 Wiley Prizes

Magnetic Resonance in Chemistry Awards

Mathilde Giffard (**P580**)

Effect of RF phase shift on the Third Spin Assisted Recoupling in Solid-state NMR

Laboratoire de Chimie Inorganique et Biologique, UMR-E3 (CEA/UJF), FRE3200 (CEA/CNRS), INAC, CEA, 38054, Grenoble, France,

Alexej Jerschow (**P601**)

Cutoff-free Traveling Wave NMR

Chemistry Department, New York University, 100 Washington Square E, New York, NY 10012

Meike Roth (**P654**)

Constant ^{1}H and ^{13}C signal enhancement in NMR using hollow fiber membranes and parahydrogen.

Max Planck Institute for Polymer Research, Ackermannweg 10, 55128 Mainz, Germany

7 Posters

The posters with an asterisk have been selected for oral presentation

All posters will be displayed for the entire duration of the conference. Posters should be mounted before the first poster session and removed at the end of the conference at the latest. Posters should be mounted on the poster panel marked with the corrisponding poster number as reported in this book.

Three poster sessions will be held during the conference. Presenting Authors should attend their poster(s) during one of the sessions according to the scheme below.

There are three poster sessions:

Monday poster session: Posters numbered 3n-2 (e.g. 1, 4, 7...)

Tuesday poster session: Posters numbered 3n-1 (e.g. 2, 5, 8...)

Thursday poster session: Posters numbered 3n (e.g. 3, 6, 9...)

7.1 Biological Systems

Posters

P1

Structural rearrangements upon assembly of Cu_A centers

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Cytochrome *c* oxidase (COX) is a terminal oxidase present in all aerobic organisms, which shuttles electrons from cytochrome *c* to oxygen using the released energy to pump protons across the membrane and thus contribute to its potential[1]. It is embedded in the mitochondrial inner membrane of eukaryotic cells and in the cytoplasmic membrane of prokaryotes.[1] The function of COX depends critically on the correct disposition of two copper centers (Cu_A and Cu_B) and two heme moieties (*b*/*a* and a_3), hence the assembly of these cofactors is assisted by (co)chaperone proteins.[2,3] Mutations on genes coding for these proteins lead to several fatal disorders in humans, related to inefficient energy production by mitochondria.[2] We have previously shown that a thiol:disulfide oxidoreductase and a Cu(I) metallochaperone are required for *in vitro* maturation of the Cu_A site in *Thermus thermophilus* ba_3 oxidase (*Tt*Cu_A).[3] During that work we gathered evidence that the structure and dynamics of the apoprotein are perturbed compared to the copper-bound form, in line with the fact that the copper center is embedded inside the holoprotein but the ligand loops must be somewhat exposed for metallation to occur in the apoprotein. Our current goal is to study the solution structure and dynamics of apo*Tt*CuA relative to the holoprotein in the reduced state. All the backbone and most of the side chain resonances have been completely assigned for 118 out of 126 residues. Broadening is observed for most resonances assigned to nuclei in two ligand loops, while residues lacking resonance assignments are located in these loops. These observations indicate enhanced dynamics in these regions compared to the holoprotein, which is highly rigid along the entire sequence. These studies will be complemented aiming to describe the rearrangements that occur in the apoprotein upon copper insertion at atomic level and to provide a qualitative assessment of the contribution of metal binding to protein folding and stability.

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P2

Structural state of the antimicrobial peptide PGLa in the membrane-bound functionally synergistic complex with magainin 2 - a solid state ^{19}F-NMR study

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The cytolytic action of many antimicrobial peptides is attributed to the pore-mediated permeabilization of the target cell membrane. Structural state of the membrane-associated peptide – conformation, alignment and dynamic behavior – explicitly reflects underlying molecular mechanisms of the process and is therefore essential for the understanding. Structural characterization of the membrane-active peptide in its native-like lipid bilayer bound condition can be readily determined using solid state NMR (ssNMR).

Herein we apply ss-^{19}F-NMR to one of the very few known native synergistic antimicrobial peptide pairs – PGLa/magainin 2 (MAG). PGLa was previously characterized in great details and is known to assume different alignment states. One of them - membrane immersed I-state - is compatible with a functionally relevant pore structure. To date two factors have been demonstrated to induce I-state, namely gel phase state of the lipid bilayer and presence of MAG. Whereas the influence of lipid polymorphism was investigated systematically on stand-alone PGLa by ss-^{19}F-NMR, alignment of PGLa in presence of MAG was investigated solely at ambient temperature (ss-^{2}H-NMR). Both situations are combined in the present study. We demonstrate herein that PGLa in presence of MAG stays helical at all conditions, but membrane fluidity exhibits significant influence on the dynamics and alignment of the peptide. In gel phase lipid bilayers we observe a complex situation with two populations of PGLa molecules. Around the gel-to-fluid phase transition PGLa adopts a rigid membrane-inserted alignment, confirming the ss-^{2}H-NMR data. In the fluid phase lipid bilayers, we found PGLa strongly inclined and rotationally mobile – in a different way compared to PGLa only. Compared with previous data on PGLa in lipid bilayers of variable composition, these results provide valuable clues on the functional synergism of PGLa and MAG in native biological membranes. At the same time this study unravels important experimental aspects thus further developing ss-^{19}F-NMR methodology in application to the membrane-active peptides.

P3

NMR study of RNA binding domain of human CPEB in complex with cytoplasmic polyadenylation element

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Cytoplasmic polyadenylation is one of the mechanisms of controlling mRNA translation and is regulated by CPEB, a highly conserved sequence specific RNA binding protein that binds to cytoplasmic polyadenylation element (CPE) present in 3'-UTR's of mRNA's. By regulating mRNA translation, CPEB influences gametogenesis, early development, synaptic plasticity and cellular senescence.[1] Human CPEB has a carboxy terminal RNA binding domain, which is comprised of two RNA recognition motifs (RRM) and a Zinc binding domain. This work aims at determining the structure of RNA binding domain of human CPEB in complex with the target RNA sequence (CPE, 5' UUUUUAU 3'). Structure of the complex would help us to understand the way the RRM's co-operate to achieve the desired degree of specificity and affinity for the target RNA and also to identify new potential RNA targets which can then be validated by *in vitro* and *in vivo* biochemical assays. Following a modular approach for NMR studies, protein constructs corresponding to different lengths of RNA binding domain were cloned and screened for soluble protein expression using different fusion protein tags. The purification protocol was optimized and conditions were screened for obtaining higher protein concentrations required for NMR experiments. The 15N HSQC spectrum of RRM12 and zinc binding domain shows that the proteins are well folded. In accordance with the biochemical data,[2] protein-RNA titrations indicate that RRM12 binds the consensus CPE RNA sequence. Backbone assignments of protein in free form as well as in complex were made using TROSY based NMR experiments on deuterated protein samples. Side chain assignment of protein in complex is in progress. Preliminary structural data for Zinc binding domain will be presented as well.

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P4

Targeting Bacterial Membranes: NMR Characterization of Substrate Recognition and Binding Requirements of D-arabinose 5P Isomerase, a key enzyme in the biosynthesis of LPS

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Arabinose 5-phosphate isomerase (API) catalyzes the interconversion of D-ribulose 5-phosphate (Ru5P) and D-arabinose 5-phosphate (A5P). It is the first enzyme in the biosynthesis of 3-deoxy-D-*manno*-octulosonate (KDO), a sugar moiety located in the lipopolysaccharide (LPS) layer of most Gram-negative bacteria.[1] Since the LPS layer is an essential outer membrane glycolipid located on the cellular surface of virtually all Gram-negative bacteria, all the enzymes implicated in its metabolism represent a potential target for the development of new antibiotic drugs. The characterization of their catalytic mechanisms and the processes of substrate recognition and binding plays a fundamental role for the rational design of new LPS biosynthesis inhibitors. As API represents a logical control point of KDO synthesis, we studed its enzymatic activity and identified structural requirements for substrate/product recognition, useful for drawing the structure of potential inhibitors. In particular, we characterized *E. coli* and *P. aeruginosa* API binding to Ru5P, A5P and other monosaccharides analogues, by NMR spectroscopy.[2, 3] On the basis of data collected, that will be presented in this communication, we are now designing and synthesizing new potential API inhibitors.

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P5
Dynamic Nuclear Polarization of Deuterated Proteins

Ümit Akbey[a], W. Trent Franks[a], Arne Linden[a], Sascha Lange[a], Robert G. Griffin[b], Barth-Jan van Rossum[a] and Hartmut Oschkinat[a]

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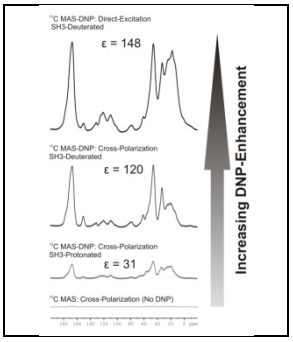
More efficient dynamic nuclear polarization (DNP) for biological solid-state NMR applications is presented. We demonstrate that protein deuteration[1,2] is a new means of increasing the efficiency of DNP enhancement in MAS NMR spectroscopy. We investigated several ratios of protonation/deuteration by using samples of SH3 protein which are fully deuterated at all exchangeable and non-exchangeable sites and then recrystallized in appropriate H2O/D2O buffers to tune the proton ratio at the exchangeable sites. An enhancement of 120 is obtained and the efficiency of the DNP enhancement increases up to a factor of ~3.9 compared to the protonated protein for 13C cross-polarization (CP) MAS NMR experiments. Moreover, by using direct 13C excitation on the deuterated sample, the enhancement is increased by a factor of ~4.8 compared to the 13C CPMAS experiment on the fully protonated SH3. The maximum enhancement of ε≈148 is observed in a ^{13}C MAS spectrum of SH3 with 50 % exchangeable proton content by using zirconium rotor. By taking into account the ~20 % increase in enhancement by using sapphire rotors, higher 13C DNP enhancement of ε≈180 can be expected. Moreover, by using the deuterated SH3 protein, it is possible to increase the temperatures at which DNP experiments yield still considerable enhancements.



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P6
Application of EPR to probe circadian rhythms of brain oxidation

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Circadian rhythms of physiological and behavioral parameters such as body temperature, sleep-wake cycle, heart-rate, blood pressure, and host-immune response are fundamental factors in health and disease states in most organisms. Increasing evidence indicate that aging is characterized by a progressive deterioration of the circadian timekeeping. On the other hand, direct and indirect evidences in literature link aging and associated pathologies with disturbances in cellular redox homeostasis which is believed to follow periodic daily rhythms. For example, circadian or diurnal variations in pro- and anti-oxidant molecules and enzymes and in markers of oxidative stress such as malonedialdehyde are reported. Nothing is known however about the source(s) of free radicals that trigger, or otherwise are modulating, those periodic variations in brain oxidants. In this presentation, we introduce direct evidence that brain levels of reactive oxygen species (ROS) follow daily rhythms. Employing powerful biophysical techniques including spin-trapping/labeling electron paramagnetic resonance (EPR) and electrochemical detections, we have been able to detect daily rhythms of substrate-specific oxygen consumptions by mitochondria and by NADPH oxidase (NOX) enzyme in parallel with ROS production in the same specimen. These observations triggered the following questions: why would evolution maintain daily rhythms of what is perceived as harmful chemical species? Do ROS merely constitute byproducts of other rhythmically regulated parameters? Here we suggest that circadian ROS oscillation orchestrates the interplay between metabolism and sleep-wake cycles, core body temperature, blood pressure, inflammatory cytokines, memory consolidation, etc.

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P7

Dynamics and Structural Characterization of Trx1 D24n and Trx2 D25N mutants by Nuclear Magnetic Resonance

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Thioredoxins are small proteins and their function as disulfide oxido-reductases, through the reversible oxidation of two cysteine residues, present in a conserved active site. The *Saccharomyces cerevisiae* contains two different cytoplasmic isoforms: Trx1 and Trx2. Despite the many redundant functions of yeast cytoplasmic thioredoxins, it has been shown that they specifically interact with different cellular targets. To establish a structure-function relationship between these proteins, we have determined the three-dimensional structure of Trx1 and 2 in solution by NMR. Comparison of Trx1 with the solution structure of Trx2 shows that they mainly differ in the active site. The backbone dynamics of both reduced and oxidized forms of Trx1 and Trx2 have been characterized. The reduced and oxidized forms of Trx1 and Trx2 exhibit similar dynamics behavior on the pico- to nanosecond time scale. The residues D24 (Trx1) and D25 (Trx2), act as an important proton acceptor, essential for the catalytic reduction mechanism. The residue D24 is internalized just below the handle of the active site, containing the conserved cysteine residues. D25 is less exposed because Trx2 contains a phenylalanine residue capping it (tyrosine in Trx1). According to the dynamics for native thioredoxins in different oxidation states, we observe that this residue undergoes one of the most important dynamic differences between the reduced and oxidized forms. In the oxidized state this residue displays motions in millisecond timescale. To study the function of this residue we construct two mutants, Trx1D24N and Trx2 D25N.

We acquired relaxation dispersion for the native protein and the mutants which indicates that Trx´s displays motions that are partially quenched in the D24/25N mutants. These motions seem to be essential for the catalytic mechanism of thioredoxins. We will also show molecular dynamics simulations depicting discrete conformationl states of Trxs.

Acknowledgments: CNPq, FAPERJ, CAPES, INBEB – National Institute for Structural Biology and Bioimage.

P8

Metabolic fate of [U-^{13}C]glucose and [3-^{13}C]lactate in hearts submitted to ischemia/reperfusion: role of cardioplegic solutions and gender

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In transplantation procedures, the organ is stored in cold cardioplegic solutions (ischemia) and finally transplanted. During heart preservation the energy metabolism is readapted according to substrate availability and the recovery of the organ during the re-establishment of the blood flow (reperfusion) is related with the energetic state.

We aimed to disclose the variation in substrate preference related with gender and the use of different cardioplegic solutions in the ischemia period. For that we used male (M) and female (F) Wistar rats. The M and F groups were then divided in 2 subgroups: perfusion control (Ctrl_P) and ischemia/reperfusion (I/R). Hearts from Ctrl_P group were only subjected to 30' perfusion. I/R group was divided in three subgroups, depending on the cardioplegic solution used, and subjected to 4 or 6 hours ischemia followed by 30' perfusion. The hearts were perfused with KH containing [U-^{13}C]glucose and [3-^{13}C]lactate. After perfusion, the hearts were freeze-clamped and metabolites were extracted for further ^{13}C NMR analysis in a 500 MHz Varian spectrometer.

In Ctrl_P conditions, there were no differences according to gender nor lactate origin from unlabeled sources, [U-^{13}C]glucose or [3-^{13}C]lactate. After 6h preservation in Cs, hearts from males prefered [U-^{13}C]glucose while hearts from females prefered [3-^{13}C]lactate. After 6h preservation in HBS, hearts from males prefered unlabeled substrates while hearts from females prefered [U-^{13}C]glucose. Alanine index (S/D) decreased in hearts from females preserved in Cs. Lactate and glutamate indexes will be presented. The amino acids content will be also discussed.

We conclude that substrate preference is different according to gender and the cardioplegic solution used in the ischemic period.

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P9

Structure and dynamics of HasB, a specific TonB like protein, and its interaction with HasR, a heme / hemophore transporter

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The active transport across the outer membrane of Gram-negative bacteria, via TonB-dependent transporters (TBDT), depends on the energy transduced by the inner membrane TonB protein complex. Despite several available data, the molecular mechanism of TonB-dependent active transport is still poorly understood.

In heme uptake, the TonB-like protein HasB is responsible for the energy transfer to the transporter HasR, which internalises heme as an iron source. HasB is composed of three distinct domains, one of them is the C-terminal periplasmic globular domain (CTD). To play its role, the CTD should interact with the periplasmic surface of the transporter. A small unstructured region of the transporter, called the TonB box, is known to participate in the interaction. Both HasB and TonB are able to transduce energy to HasR, however HasB is specific for this transporter. To define the basis of this specificity we solved the 3D solution structure of the periplasmic CTD of HasB ($HasB_{CTD}$) by NMR. The structure presents an N-terminal tail, a globular folded region and a short unstructured C-terminal sequence. Some important differences could be pointed out between $HasB_{CTD}$ and TonB structures. The HasB fold revealed a new structural class of TonB-like proteins. To investigate if these differences could explain the specificity of HasB for HasR, we studied the interaction of HasB with a peptide representing the TonB box of HasR by NMR and ITC and used the acquired information to construct a model of the complex $HasB_{CTD}$-TonB box.

P10

Multidisciplinary approach to the characterization of farmed fish: the case of Gilthead Seabream

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The complete characterization of fish muscle is gaining great significance in the rapidly growing field of aquaculture. Different rearing systems have been so far exploited in lagoons, in land-based units and in sea cages, adopting extensive to intensive farming systems. Among farmed fish species, Gilthead Seabream (*Sparus Aurata*) is a very suitable species for extensive aquaculture, especially in the Mediterranean. Within this context, both the farmers and the consumers have increased interest toward a certification of the quality of farmed fish. On one hand, the investigation on the effects of feeding and rearing system on the composition of fish allows to control and predict quality, on the other it allows the strict control of the production performance.

In our laboratory, different complementary techniques are being optimized with the aim of providing a thorough characterization of the composition of farmed fish, and to analyze factors which potentially influence fish health and growth. First of all, a detailed protocol has been set up for sampling the fish and for lipid extraction. The results of lipid analysis by two complementary techniques such as NMR and GC can be compared. Gel-based proteomic analysis reveals typical features due to fish diet, such as oxidative stress derived by the action of peroxydated fatty acids, and up- or down-regulation of metabolic pathways. NMR is a powerful technique as it allows a nondestructive analysis of the extracted oils and of the polar metabolites, is rapid and allows metabolomic analysis on a large number of samples, provides a simultaneous snapshot of the total omposition of the fish. As far as TAGs and PL are concerned, NMR provides information on the positional distribution of fatty acids. We show that NMR-based metabolomic analysis is able to recognize molecular patterns which characterize wild and farmed Gilthead seabream and fish from different regions (rearing systems) in Sardinia. Moreover, NMR can discriminate between healthy and stressed fish.

P11

Probing light-induced changes in blue-light photoreceptors with laser-polarized ^{129}Xe

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Recent work has shown the utility of laser-polarized ^{129}Xe NMR in studies of biomolecular structures and interactions, most notably in studies of its interactions with protein surfaces and cavities.[1-2] Due to its extremely large chemical shift range, xenon is an effective reporter of even small changes in its local magnetic environment. ^{129}Xe NMR spectra exhibit dramatic chemical shifts due to both specific binding with protein cavities as well as nonspecific binding with protein surfaces. Hyperpolarized (HP) ^{129}Xe offers NMR signal enhancements of several orders of magnitude compared to thermal polarization.[3] Under conditions of continuously flowing HP ^{129}Xe, studies of the ^{129}Xe chemical shift due to Xe/protein interactions offer opportunities to understand conformational changes and interactions in proteins due to changes in external conditions.[4]

We intend to apply these methods to study light-induced changes in blue-light photoreceptors.[5] We will report on our efforts to understand the effect of light on light sensitive proteins.

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P12

NMR Studies of Tip5-RNA Interactions Involved in Gene Inactivation

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In metabolically active mammalian cells a significant part of the tandemly repeated rRNA genes (rDNA) is epigenetically silenced. The key mediator for maintaining this heterochromatic state is the recently described nucleolar remodelling complex (NoRC) with Tip5 as its largest subunit. The evolutionary conserved TAM domain of Tip5 shows sequence homology to MBD domains of methyl-CpG-binding proteins. However, in contrast to MBD domains, Tip5 TAM domain specifically binds to a phylogenetically conserved RNA hairpin (pRNA), derived from small noncoding RNA, produced from PolI promoters in the intergenic spacer (IGS) that separate rDNA repeats and matching the rDNA promoter sequence. Interaction of Tip5 with pRNA was shown to be required for nucleolar localization of NoRC and heterochromatin formation in the rDNA regions.[1]

Here, we present a study of Tip5-pRNA interaction by NMR spectroscopy in combination with biochemical techniques to gain insight into the molecular functions of Tip5 and its RNA binding. Our results demonstrate that while the free TAM-domain resembles the MBD domain,[2] it is extended with additional features at both termini. The most prominent of those is the additional antiparallel twisted β-sheet that may provide a platform for nucleic acid binding. Structural studies and mutational analysis of pRNA recognition by Tip5 is on-going.

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P13

NMR studies reveal the role of biomembranes in modulating ligand binding and release by intracellular bile acid binding proteins

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Bile acid molecules are transferred vectorially between basolateral and apical membranes of hepatocytes and enterocytes in the context of the enterohepatic circulation, a process regulating whole body lipid homeostasis.[1] This work addresses the role of the cytosolic lipid binding proteins in the intracellular transfer of bile acids between different membrane compartments.[2] We present nuclear magnetic resonance (NMR) data describing the ternary system composed of the bile acid binding protein, bile acids, and membrane mimetic systems, such as anionic liposomes. We provide evidence that the investigated liver bile acid binding protein undergoes association with the anionic membrane and binding-induced partial unfolding. The addition of the physiological ligand to the protein-liposome mixture is capable of modulating this interaction, shifting the equilibrium towards the free folded holo protein. An ensemble of NMR titration experiments, based on nitrogen-15 protein and ligand observation, confirm that the membrane and the ligand establish competing binding equilibria, modulating the cytoplasmic permeability of bile acids. These results support a mechanism of ligand binding and release controlled by the onset of a bile salt concentration gradient within the polarized cell. The location of a specific protein region interacting with liposomes is highlighted.

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P14

Chemical engineering of artificial lipid vesicles and bilayer membrane in toxicological studying: theory to experiment

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Venoms produced by snakes of the family Viperidae contain proteins that interfere with the coagulation cascade, the normal haemostatic system and tissue repair, and human envenomations are often characterized by clotting disorders, hypofibrinogenemia and local tissue necrosis.

Studies on the interaction of snake venom and organized lipid interfaces have been conducted using a variety of systems, including BLMs, SUVs and LUVs. Giant unilamellar vesicles (GUVs) with a mean diameter of 30 μm have a minimum curvature and mimic cell membranes in this respect. GUVs were formed from the total lipid fraction from bovine brain by the electroformation method. *Vipera lebetina obtusa* venom was added to the sample chamber before the vesicles were formed. The membrane fluorescence probes, ANS and pyrene, were used to assess the state of the membrane and specifically mark the phospholipid domains. Fluorescent spectra were acquired on a *Varian* fluoremeter instrument.

ANS and pyrene allows us to quantify the fluidity changes in the membrane by measuring of the fluorescence intensity. The presence of viper venom in GUVs media reveals a noticable decreasing of membrane fluidity compare the control, while the binding of fluorophores with GUVs modified by venom lead to appearance of channel activity. It was recognized early that the vipers venom components preferred an organized lipid substrate near the lipid's phase transition and were particularly active against micellar lipids. These studies also emphasize the importance of a membrane surface curvature for its interaction with enzymatic components of venom.

P15
Quadruplex folding of G-rich DNA studied by DEER

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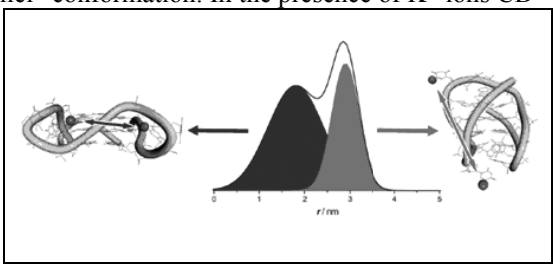
Telomeric quadruplex sequences have attracted much attention over the past 15 years since a biological function of these unusual folds is anticipated. Although it has been an important quest to decipher the physiologically relevant quadruplex topologies, the exact structures contributing to the mixtures present in potassium-rich solutions are still discussed controversially. Here we present a Double Electron-Electron Resonance (DEER) study of folding of human telomeric oligonucleotides. The model sequence of human telomeric DNA d-[A(GGGTTA)$_3$GGG] was synthesized with spin labels at positions 5 and 11. This sequence is known to adopt different G-quadruplexes depending on alkali ions present. In the presence of Na^+ it is, found with CD and confirmed with NMR, a "parallel" conformation. In the presence of K^+-ions CD-spectra can be interpreted either as a mixture of two conformation or as a single "mixed" – parallel/antiparallel – conformation. The X-ray structure was solved for an "antiparallel" one. By measuring spin-spin coupling in double spin labeled sequences the distance distributions between two spin labels were extracted. The results for the Na^+-probe were in good agreement with the NMR structure. The found distances for the K^+-sample were 1.8+/-0.2 nm and 3.0+/-0.1 nm and were pronounced evidence of coexisting of two conformations in approximately 1:1 ratio.[1]



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P16
Exploring Hyperpolarized Bioactive Compounds & Drugs via Heteronuclear MRI

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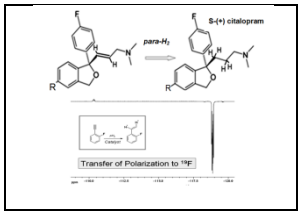
^{13}C-MRI/MRS-investigations[1] of the function or metabolisms of biologically active compounds (BACs) requires signal enhancement, e.g. hyperpolarization obtained as ParaHydrogen Induced Polarization (PHIP) based on the PASADENA effect.[2] *In situ* prahydrogenation of unsaturated precursors containing double or triple bonds[3] provide access to various ^{13}C-hyperpolarized BACs: The cardioselective beta$_1$-receptor blocker beta blocker esmolol inhibits the actions of naturally occurring epinephrine and norepinephrine with rapid onset and short duration *(halflife ~ 9 min.)*. BACs containing *carboxylate* groups like the angio-tensin converting enzyme (ACE) inhibitors *(enalaprilat or similar ones)* may be ^{13}C- or ^{19}F-hyperpolarized accordingly. Furthermore, antidepressant medications, in particular selective seratonin reuptake inhibitors (SSRI) such as citalopram (Celexa) or fluoxetine (Prozac), can be ^{13}C- or ^{19}F-hyperpolarized and investigated. Wide-spread mood disorders causing clinical depression affect ~ 15% of the population and rank as the leading cause of disability in North America, where almost 20 million people take Prozac. - Citalopram exists as 2 enantiomers. Studying the fate of the antidepressant *S*-(+) enantiomer requires its generation in ^{13}C- or ^{19}F-hyperpolarized form[4-6] from unsaturated precursors (Fig. 1). Transferring the initial ^{1}H-hyperpolarization to ^{13}C- or ^{19}F-nuclei that exhibit sufficiently long T_1 relaxation times succeeds at low magnetic fields (ALTADENA effect).[5] The 100% abundance of ^{19}F-nuclei and its higher magnetic moment compensate for their short T_1 times in phenyl groups relative to quarternary ^{13}C.[5]



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P17

NMR Structural Study of hnRNP A1 in complex with RNA. Comparison between solution structure and crystal structure

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Human hnRNP A1 (A1) is a versatile nucleic acid binding protein that is involved in many aspects of nucleic acid processing such as pre-mRNA alternative splicing, telomere biogenesis and microRNA maturation. So far, the only structural data that shed light on the molecular basis of nucleic acid recognition come from the crystal structure of the two-RRM domain of A1 (UP1) complexed with a ss telomeric DNA.[1] In this structure, two symmetry-related molecules of UP1 interact to form a dimer that bind two strands of DNA in a anti-parallel manner. This peculiar organization could be only relevant for binding to telomeric DNA repeats that hold short linkage sequence. This could prevent the binding of the two RRMs of A1 within the same nucleic acid strand. An attractive hypothesis would be that two binding sequences separated by a longer linker could be bound in a anti-parallel manner, leading to a looping of the RNA, similarly to PTB.

In this context, we investigate by NMR the solution structure of A1 bound to a single stranded RNA containing two binding sites for each RRM of A1 separated by a 6 nt linker. We have NMR indications that in solution UP1 is monomeric and that each RRM is bound to both ends of this RNA target. To solve the structure of this relatively large protein/RNA complex, we chose to use a modular approach. We thus solved the structures of each individual RRM bound to a shorter RNA with a very high precision. These structures have shown interesting features when compared to the available crystal structure. The main differences are located in loops whose structure is distored by crystal packing interactions. These regions are important for the recognition of the nucleotides flanking the AG central core motif. These structures of the isolated RRMs in complex with RNA together with ITC measurements have shown a degenerate sequence specificity of each RRM regarding its RNA target. Indeed, both RRM have a preference for the UAGG motif, but whereas RRM1 can tolerate only a C at the first position (YAGG motif), RRM2 is more tolerant and can accomodate any nucleotide at the first and the fourth position (NAGN motif). We will exploit this specificity to improve the NMR line width of the large complex, a crucial condition in order to solve the structure of this large protein/RNA complex.

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P18

Spectroscopic characterization of caveolar structures

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Caveolae are a region of cell membrane characterized by a different lipidic and proteic composition compared to normal membrane. They appear as flask-shaped invaginations and are present in many tissues such as pulmonary, cardiac, vascular, muscular and adipose and perform several tasks. One of these is to be an anchor surface for some proteins involved in cell signalling. Their main difference from other membrane domains with similar tasks, the lipid rafts, is the presence of caveolin, an oligomeric protein specific of this system. This protein is deemed to be responsible for many functions performed by caveolae and its presence is thought to be the driving-force behind the segregation of cholesterol and lipid species between the outer and inner leaflet of the bilayer. NMR and X-ray are the election spectroscopies for the analysis of molecular structures at atomic scale. Both techniques were instrumental for conformational studies of several proteins and were also successfully employed to study artificial, membrane-like aggregates.

The novelty of this study is the application of these techniques to study a phospholipid bilayer characterized by peculiar morphology and a specific proteic component without disrupting its supramolecular organization to separate the lipidic components from the proteins. Caveolae from different tissues are compared and quantitative differences are observed regarding the internal ratio of the main lipidic species. Their distribution between the inner and the outer leaflets of the bilayer is also investigated and it is observed that caveolae form various tissues show a different lipid composition for each leaflet.[1]

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P19

Progress in the structure determination of two helical human membrane proteins

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About a quarter of all proteins in mammalian organisms are integral membrane proteins. They play a crucial role in many cellular processes. However, only ~240 folds of integral membrane proteins and a few structures of human membrane proteins have been solved so far. This is due to bottlenecks both in protein preparation and structure determination. We are very interested in the investigation of two helical human membrane proteins, the human transmembrane protein 141 (hTM141) and the human voltage gated proton channel (Hv1[1] or VSDO[2]). While the function of hTM141 is unknown, Hv1 plays an important role in the human innate immune system. Its predicted structure differs considerably from other cation channels. In order to gain insight in the function of these two proteins we want to investigate both membrane proteins by liquid-state nuclear magnetic resonance (NMR) spectroscopy. The production of hTM141 was done with the Cell-Free expression system.[3] It is a 108 residue protein and exhibits an excellent [^{15}N, ^{1}H]-TROSY spectrum. Therefore, sequential assignment using triple resonance experiments, collection of upper limit distance restraints by NOESY experiments and the use of paramagnetic spin labels enabled the determination of the backbone structure. Hv1 was expressed in functional form in *E. coli*. It is a 273 residue protein, which dimerises, and exhibits a partially overlapped [^{15}N, ^{1}H]-TROSY spectrum. Since the transmembrane domain of Hv1 is monomeric and still functional[4], this domain will be investigated first. The assignment of the NMR resonances is in progress. The goal is to understand the voltage-sensing and the proton permeation pathway of this unique channel.

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P20 (*)

Solid-state NMR spectroscopy of oriented membrane polypeptides at 100 K with signal enhancement by Dynamic Nuclear Polarization

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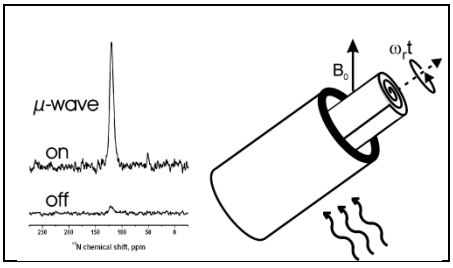
Solid-state NMR spectroscopy provides a powerful tool for the structural investigation of membrane peptides and proteins in their native lipid environment. Whereas solid-state NMR spectroscopy has been used to study the structure, dynamics and topology of these polypeptides it has been difficult to develop its full potential due to the inherently low sensitivity of NMR spectroscopy. This problem is particular pronounced for static oriented samples where the inherently dynamic properties and the mosaic spread of polypeptides in lipid environments cause additional line broadening

Oriented membrane samples encompassing the bi-radical bTbK and a transmembrane peptide carrying a single ^{15}N label have been prepared on polymer sheets with sample geometries that fit into a 3.2 mm MAS rotor. Irradiating these samples with μ-waves resulted in Dynamic Nuclear Polarization and a concomitant 18-fold signal enhancement, which considerably shortened the NMR acquisition times.[1] Furthermore, the side band patterns of magic angle oriented sample spinning (MAOSS) solid-state NMR spectra at 1kHz MAS are indicative that the lipids and peptides form well-oriented bilayers at 100 K despite the small i.d. of the rotor and the presence of bi-radicals.[1] The DNP signal enhancement opens up interesting possibilities for multidimensional solid-state NMR investigation of oriented membrane polypeptides.



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P21
STD NMR Analysis of the Binding of Human CD4 with Glycopeptides

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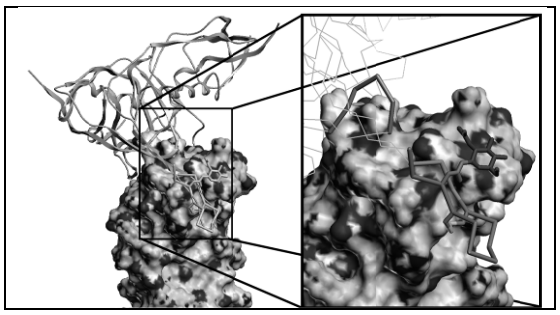
The first step in the infection of human cells with HIV is the interaction between the human protein CD4 and the viral envelope glycoprotein gp120.[1] The X-ray structure of the gp120/CD4 complex suggests that contacts between the carbohydrate attached to Asn^{197} of gp120 might assist its binding to CD4 (cf. Figure).[2] Here we report experimental data that support the positive role of glycans of gp120 at Asn^{197} on binding of gp120 to CD4 by analyzing the interaction of different gp120 based peptides and glycopeptides with CD4.



We find that glycopeptides bind up to a factor of four more strongly to CD4 than the parent peptides. Analysis of the binding epitope obtained by STD NMR[3] shows that the sugar interacts mainly in the region of C2 with the CD4 protein.

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P22
Structure of the Cytosolic Portion of Motor Protein Prestin

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Prestin is the motor protein responsible for the somatic electromotility of cochlear outer hair cells[1] and is essential for normal hearing sensitivity and frequency selectivity of mammals. Prestin is a member of mammalian solute linked carrier 26 (SLC26) anion exchangers, a family of membrane proteins capable of transporting a wide variety of monovalent and divalent anions. SLC26 transporters play important roles in normal human physiology in different tissues and many of them are involved in genetic diseases. SLC26 and the related SulP transporters carry a hydrophobic membrane core and a C-terminal cytosolic portion that is essential in plasma membrane targeting and protein function. This C-terminal portion is mainly composed of a STAS domain, whose name (Sulfate Transporter and Anti-Sigma factor antagonist) is due to a remote but significant sequence similarity with bacterial ASA (Anti-Sigma factor Antagonist) proteins.[2] We have recently solved the crystal structure at 1.57 Å resolution of the cytosolic portion of prestin,[3] the first structure of a SulP transporter STAS domain. Here we present its characterization in solution by heteronuclear, multidimensional NMR spectroscopy. Prestin STAS significantly deviates from the related bacterial ASA proteins, especially in the N-terminal region that, whereas previously considered merely a generic linker between the domain and the last transmembrane helix, is actually fully part of the domain.

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P23

NMR Studies of the extracellular domain of a prokaryotic ligand-Gated Ion Channel (LGIC)

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Pentameric ligand-gated ion channels of the Cys-loop family are of special importance for the rapid chemo-electrical signal transduction at synapses,[1–3] but the mechanisms of ion permeation and gating of these membrane proteins remain elusive. Recently the X-ray structures of two prokaryotic homologues of the nicotinic acetylcholine receptor (nAChR), the best studied member of the LGIC family, have been determined: 1) the bacterial *Gloeobacter violaceus* pentameric ligand-gated ion channel homologue 4 (GLIC; 2.9 Å) in an open conformation[2] and 2) a homologue from the bacterium *Erwinia chrysanthemi* (ELIC; 3.3 Å) in a closed conformation.[3]

The 200-residue extracellular domain of GLIC, which is found to be a monomer in solution, was cloned and expressed in high yields in *E. coli.* The ^{1}H-^{15}N HSQC exhibits signal dispersion typical for polypeptides with mainly beta structure. $^{13}C/^{15}N$ labeled GLIC is now studied using heteronuclear multidimensional NMR spectroscopy and more than 40% of the backbone $^{13}C/^{15}N$ nuclei have already been assigned. Additionally, protein deuteration from 60 to 100% is expected to increase the spectral sensitivity and resolution and allow the complete resonance assignment of the protein and the extraction of NOE constraints (Chasapis C.T. *et al.* work in progress).

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P24

Paramagnetic perturbation profiles of protein surface accessibility and hydration

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The way proteins, together with all other molecular ingredients of life, interact with one another is encoded in their surface accessibility, a dynamic parameter which is difficult to analyse. In any case, the accessibility of protein surfaces at atomic resolution needs to be investigated in detail in order to understand the mechanisms of the molecular interactions. To help understanding how particular surface regions of complex molecules can be preferential targets for binding ligand and/or water molecules, we present paramagnetic attenuation profiles of NOESY and ePHOGSY signals of BS RNAse induced by the presence of TEMPOL. The obtained results confirm how this approach yields powerful information on protein surface dynamics and protein-solvent interaction. The strong attenuations of NOESY signals observed for Gln11 an His119 indicate that the P1 moiety of the enzyme active site results among the most TEMPOL accessible regions of BS RNAse. On the other side, the scarcely attenuated ePHOGSY signals of Asp83 and Ile81 suggest some protection from the access of the soluble spin-label in the B1 moiety of the enzyme active site. This hindrance to TEMPOL access to protein active sites, not observed before,[1] in BS RNAse is consistent with the presence of resident water molecules having catalytic activity.

Paramagnetic attenuation profiles obtained for BS RNAse have been compared with X ray diffraction data obtained for bovine pancreatic RNAase A, an enzyme highly homologous to BS RNAase, with Multiple Solvent Crystal Structure.[2] The total agreement between Xray and NMR data is impressive, revealing that valuable information from of paramagnetic attenuations of ePHOGSY signals can delineate not only buried water molecules, but also the ones which are protected in strong surface hydration sites waiting for their catalytic action.

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P25

Structural Impact of Pseudophosphorylation of 441-residue tau

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Alzheimer's disease is characterized by abnormal protein deposits in the brain, such as extracellular amyloid plaques and intracellular neurofibrillary tangles (NFTs). The tangles are made of a protein called tau comprising 441 residues in its longest isoform. Binding and release of tau from microtubules is controlled via phophorylation. Hyperphosphorylation plays a critical role in AD and tau's aggregation into NFTs. Here we replaced several serine and threonine residues in 441-residue tau with glutamic acid to mimic homogenous phosphorylation at specific sites. The combined mutations S199E+S202E+T205E+T212E+S214E+ S396E+S404E in the recognition sites of the AT8, AT100 and PHF1 antibodies do not induce formation of rigid secondary structure. Instead pseudophosphorylation leads to an opening of the protein conformation. Especially the repeat regions containing two aggregation-prone hexapeptides are less protected, making these regions more susceptible to inter-residual contacts and aggregation.

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P26

Dimeric Structure of ING4 and Bivalent Recognition of histone H3 trimethylated at K4 studied by NMR

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The INhibitor of Growth (ING) family of tumor suppressors regulates the transcriptional state of chromatin by recruiting remodeling complexes to sites with histone H3 trimethylated at K4 (H3K4me3). This histone modification is recognized by the Plant HomeoDomain (PHD) present at the C-terminus of the five ING proteins.[1,2,3] ING4 facilitates histone H3 acetylation by the HBO1 complex. ING4 forms homodimers through its N-terminal domain, which folds independently into an elongated coiled-coil structure[4]. The central region of ING4 is disordered and flexible, and does not directly interact with p53 or does it with very low affinity, in contrast to previous findings.

NMR analysis of the full-length protein reveals that the two PHD fingers of the dimer are chemically equivalent and independent of the rest of the molecule, and bind H3K4me3 with the same binding site and affinity as the isolated PHD finger. Therefore, the ING4 dimer has two identical and independent binding sites for H3K4me3 tails which, in the context of the chromatin, could belong to the same or to different nucleosomes. These results show that ING4 is a bivalent reader of the chromatin H3K4me3 modification and suggest a mechanism for enhanced targeting of HBO1 complex to specific chromatin sites. The same mechanism could be common in other ING-containing remodeling complexes.

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P27

Chemical shift mapping of large proteins without the need of deuteration

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The characterization of ligand protein interactions is an important theme in drug discovery programs. There are various techniques available to detect the binding of a ligand to a protein and to determine its affinity. One of the most robust methods is the observation of chemical shift changes of resonances of the receptor protein upon binding of a ligand. Chemical shift mapping can be performed preferably by measuring 2D NMR spectra of ^{15}N and/or ^{13}C labeled proteins or measuring 1D spectra of unlabeled proteins. Many important drug targets, however, are larger than 25 kDa. For large proteins, single resonances are broad and spare and therefore selective labeling and in particular deuteration in combination with TROSY detection is required to achieve the resolution and signal sensitivity required for protein-observed chemical shift mapping. The same holds true for protein-protein complexes. It should be noted, that many drug targets can only be expressed in *Baculovirus* infected insect cells (*BV*), where deuteration is not feasible.

In the poster we present ^{13}C methionine labeling of large proteins as an elegant workaround. It is convincingly shown for several drug targets with a size up to 45 kDa that high resolution spectra can be obtained for the ^{13}C methionine methyl resonance because of its fortunate relaxation properties. This becomes obvious in the examples shown of *BV* and *E. coli* expressed proteins, where chemical shift mapping and affinities (K_d's) could be obtained for ligands with fast-exchange as well as slow-exchange binding kinetics. Application of spin labels and NOEs complement chemical shift changes for the validation of ligand binding docking models. An analysis of about 3000 proteins in our protein structure database that consist exclusively of drug targets reveals that all proteins in this database have at least one methionine. In 2/3 of these proteins, at least one methionine methyl is present in or close to the ligand binding site. The expectation that the chemical shift of at least one methionine methyl changes upon ligand binding is therefore realistic. Apart from the selective labeling in *BV*, our experience of selective labeling of methionine in *E. coli* and in cell-free systems shows that the label is not distributed to other amino acids (almost no scrambling). Extension to other expression systems seems straightforward.

P28

Specific helix-helix interactions of the ErbB transmembrane domains

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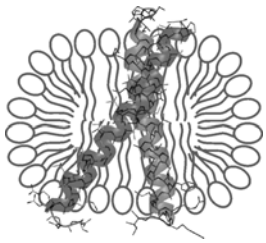
The epidermal growth factor receptor family, also know as ErbB or HER, of receptor tyrosine kinases mediates a variety of cellular responses in normal biological processes and in pathological states of multicellular organisms. During signal transduction across plasma membrane, four human ErbB1-4 members are activated by proper ligand-induced homo- and heterodimerization or by reorientation of monomers in preformed receptor dimers upon ligand binding. The single-span transmembrane (TM) domains of ErbB revealed ability to homo- and heterodimerize in the absence of extracellular ligand-binding and cytoplasmic kinase domains. Using heteronuclear NMR spectroscopy we investigated spatial structures and internal dynamics of the homo- and heterodimers formed by TM domains of ErbB1, ErbB2 and ErbB4 receptors inside the supramolecular complexes mimicking membrane environment, such as detergent micelles and lipid bicelles. We characterized monomer-dimer transitions, described atomistic picture of the intra- and intermolecular interactions, and analyzed diverse TM helix-helix packing interfaces, providing the evidence that ErbB TM domains can associate in a different manner. The obtained homo- and heterodimeric conformations of ErbB2tm, ErbB4tm and ErbB1tm/ErbB2tm employing N-terminal dimerization motifs are believed to support the active configuration of receptor juxtamembrane and kinase domains, while the described ErbB1tm homodimer via C-terminal motif presumably corresponds to the receptor inactive state, that appear to favor the so-called "rotation-coupled" activation mechanism of ErbB receptor signaling. The found capability for multiple polar interactions along with hydrogen bonding between ErbB1tm and ErbB2tm correlates with that this heterodimer is strongest in vitro and that ErbB1/ErbB2 has high mitogenic activity and oncogenic potential in vivo.



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P29

TPPP/P25: A New Unstructured Protein With Gtpase Actvity

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Tubulin Polymerization Promoting Protein (TPPP of 25kDa) is a highly dynamic protein for which the supreme target is the microtubule system. In normal human brain TPPP is expressed predominantly in oligodendrocyte; and in pathological inclusions TPPP co-accumulates with α-synuclein in both glial and neuronal cells leading to synucleinopathies. Multinuclear NMR investigations reveal two types of peaks: the intense peaks show low signal dispersion and are in accordance with a disordered protein structure; while the low intensity peaks are dispersed and belong to the ordered part. SCS data obtained from the assignment of the 3D measurements (HNCA, (H)CC(CO)NH, TOCSY-HSQC, NOESY-HSQC) for the intense signals of TPPP prove that both the *C*- and *N*-terminal parts are 'disordered'. GTP binding of TPPP was monitored by HSQC measurements. Presumed binding positions can be the Rossmann fold sequence and Switch II region, both situated at the *C*-terminal part; however, GTP binding occurs in the 'core' region. The specific hydrolytic activity of TPPP was followed by ^{31}P NMR measurements. Real time kinetic analysis showed that GTP hydrolysis leads to the formation of GMP and free phosphate, whilst GDP concentration is maintained at steady-state condition. GDP alone doesn't have hydrolytic activity in the presence of TPPP. This specific GTPase activity of TPPP is comparable with that of the non-activated small G protein, suggesting its involvement in multiple physiological processes in addition to the regulation of GTP-mediated microtubule assembly.

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P30

NMR study of Gd-based nanoparticles to tag boron compounds in boron neutron capture therapy (BNCT)

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Boron neutron capture therapy (BNCT) has proved to be very promising for certain kind of tumours like spread liver metastases. On the other hand Magnetic Resonance Imaging (MRI) is a powerful non-destructive and non-invasive technique that allows visualisation of nuclide's distribution in tissues and organs in vivo. The optimisation of the BNCT relies on the knowledge of the distribution and concentration of boron in the tumour tissue compared to the healthy tissue.

We aim at synthesising new organic complexes containing a magnetic moment (Gd-based molecular nanomagnets) which would serve the double purpose of acting as BNCT agents, and at the same time act as contrast agents (CA) to detect the molecule in the tissue via proton MRI. We also explore the possibility of monitoring the concentration of the BNCT agent directly via proton and boron nuclear magnetic resonance (NMR) relaxation.

We present preliminary NMR measurements of spin-spin and spin-lattice relaxation rates in the liver tissue of laboratory animals on both protons and on 10B nuclei to establish if the relaxation rate is significantly different for nuclei in the tumoral tissue vs. the ones in the healthy tissue. From 10B NMR we were able to evaluate in rat liver tissues a 10B amount less than 2 ppm. In tumoral liver tissues, values up to 11 ppm were obtained. The detected concentrations are in good agreement with the data obtained by α-spectrometry.

We have measured the relaxivity of protons in the range 10 kHz-65 MHz for a newly sinthesized Gd-tagged BNCT agent in the liquid used as culture for the tumoral cells and in water. Such relaxivity is considerably higher than the one of commercially available contrast agents of similar molecular weight.

P31

NMR study of the interaction between the *Escherichia coli* peptidyl-tRNA hydrolase and an analog of its substrate

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It accidentally happens that an elongating peptidyl-tRNA dissociates from the ribosome during translation. The accumulation of inactive peptidyl-tRNAs being toxic for the cell, they need to be discharged. This is realized by peptidyl-tRNA hydrolases that cleaves the ester bond between the C-terminal end of the peptide and the ribose at the 3' extremity of the tRNA. Peptidyl-tRNA hydrolase 1 (Pth1) is found in all bacterial genomes and was shown essential in *E. coli*, *B. subtilis* and *M. tuberculosis*. In the opposite, based on the study of *S. cerevisiae* mutants it seems that Pths are dispensable in eukaryotic cells, making Pth1 an attractive target for antibiotherapy.

Using an ^{15}N-^{13}C-labelled sample, we assigned the resonances frequencies of all *E.coli* Pth1 backbone atoms and verified the compatibility of its structure with that previously resolved by X-ray diffraction.[1] We studied, by chemical shift mapping, its interactions with a non-hydrolysable analog of the substrate and built, using the HADDOCK software, structural models of the corresponding complex. This analysis allowed us to extend the Pth1 functional models previously proposed.[1-3]

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P32

NMR insights into the inhibition of peptidoglycan L,D-transpeptidation

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Peptidoglycan is a major structural component of the bacterial cell envelope and is the target of the two main classes of drugs available to treat severe infections due to Gram-positive bacteria, the β-lactams and the glycopeptides. In the late years an increasing number of patient's infections with multi-drug resistant strains has nevertheless been reported and a search to identify the resistance mechanisms has been started. Bypass of the penicillin-binding proteins (PBPs) by L,D-transpeptidases (LDts), that catalyze the formation of 3→3 crosslinks instead of the classical 4→3 peptide crosslinks in the peptidoglycan structure has been identified as one of them.[1] As the latter proteins have also been identified in non-replicating forms of pathogenic *Mycobacterium tuberculosis,*[2] they are becoming an increasing center of interest.

The present work focuses on the identification of key molecular determinants for this new class of active-site cysteine transpeptidase and on its comparison with the active-site serine PBP-transpeptidases. The NMR solution structures of the free form and a carbapenem-bound form of the 20 kDa L,D-transpeptidase from *Bacillus subtilis* will be presented. The role of a catalytic triad will be discussed.

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P33

EPR Double Electron Electron Resonance measurements of a Cytochrome P450 and Ferredoxin complex to determine the docked structure

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Cytochrome P450 is an important enzyme for catalytic hydroxylation using molecular oxygen which ultilises electron transfer from ferredoxin to P450.[1] Although crystallographic data are available for the individual proteins, structures of the docked conformations, in which electron transfer occurs, are not. This work aims to provide the first experimental data on P450 recognition. Ferredoxin contains a reduced Fe_2S_2 centre in which the two irons are antiferromagnetically coupled to give an S=1/2 ground state. In order to provide the correct g-matrix orientation for the iron-sulphur cluster density functional calculations have been performed as well as comparison with similar systems where the orientation is known. The heme group in P450 was reduced and capped with CO to give EPR silent low-spin Fe(II). The P450 protein sequence is then selectively mutated to contain additional cysteines and spin labelled at these positions using the nitroxide spin label MTSL. Double Electron Electron Resonance (DEER) measurements utilising the through space dipolar interaction were made between the Fe_2S_2 centre and the $NO^{\bullet}$ at 5 different pump positions in order to give orientationally selective spectra.

The data were analyzed by least-squares fitting to a grid of simulated DEER spectra to give the separation distance and relative spin-spin orientation.[2] From the fitted data, protein structures and positions of the $NO^{\bullet}$ calculated from a rotamer library,[3] the relative positions of the two spin labels were found and used to form possible docked models of the two proteins that will be refined using molecular dynamics simulations to provide the final structure of the docked system.

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P34

A prion oligomer that can destroy membranes

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The HET-s protein from the filamentous fungi *Podospora anserina* is a functional prion involved in a limited cell death reaction termed heterokaryon incompatibility. It is observed between two fungi of which one is carrying HET-s in the prion state and the other one a monomeric HET-S protein. Our aim is to get a detailed insight into this prion-induced toxicity mechanism. Previous data showed, that the N-terminal domain of HET-S does exert a prion inhibitory effect, i.e. it can inhibit HET-s and self-fibrilization, and might function as a fungal cell death inducing domain.[1] However, about the prion-initiated incompatibility reaction little is known. We show in liposomes, that the interaction of the HET-s fibril with HET-S, results in the expulsion of a hydrophobic domain, which targets the HET-S/HET-s complex to the membrane and subsequently perforates it. In the absence of liposomes the interaction of HET-S and HET-s leads to precipitation of the BigMESs (Big Membrane Entering Ss) complex, an event that could also explain the inhibition of fibril growth by HET-S. Proteinase K digestion of liposome inserted HET-S restricts the liposome inserted fragment to the first 34 residues of the N-terminal region of HET-S. Further, mutations in this domain abolish the toxicity of HET-S in vivo and switch HET-S and HET-s phenotypes.[2] Kinetic analysis of liposome leakage assays revealed that BigMESs formation requires equal amounts of HET-s and HET-S monomers. We used ssNMR to show that HET-S and HET-s do interact through their PFD and conclude that HET-s fibrils trigger the conformational change of HET-S. A toxic BigMESs complex is then formed that can disrupt membranes.

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P35

High-Resolution NMR Studies of Prokaryotic Toxin-Antitoxin Systems Reveal Intricate Regulatory Networks

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Prokaryotic toxin-antitoxin (TA) systems are regulatory genetic elements combining a stable toxin and a specific, labile antitoxin.[1] Each toxin is capable of interfering with an essential cellular function, leading to a reduction in metabolic activity which has been associated with the response of bacterial cells to stress conditions. This response is of great medical interest in the case of the formation of persister cells which survive antibiotic therapy. The antitoxins are typically two-domain proteins, consisting of a well-structured DNA-binding domain and a highly dynamic toxin-binding domain. The latter domain is generally disordered in its free state, only adopting a specific conformation when it binds to its toxin partner. The DNA binding activity of the antitoxin has a central role in the autoregulation of the TA system.

Two representative TA systems were studied using high-resolution NMR methods. The first comprises the antitoxin CcdA and the toxin CcdB, which poisons the topoisomerase gyrase, thus inhibiting DNA replication and transcription. Simultaneous binding and folding of the CcdA neutralisation domain triggers an allosteric change in CcdB, releasing it from the target.[2] The second prototypical system consists of the antitoxin MazE and the toxin MazF. The latter is a ribonuclease which degrades the majority of messenger RNA molecules in the cell, leading to a global shutdown of protein biosynthesis.[3] Sequence-specific resonance assignments and structure calculations were completed for MazE from *Escherichia coli* and CcdB from *Vibrio fisheri*. The resulting structures are being used as a foundation for an in-depth study of the antitoxin-DNA and toxin-antitoxin interactions, leading to a complete description of the intricate regulatory networks in TA systems.

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P36

Nuclear magnetic resonance applied in structural studies of secretory chaperones and protein-ligand interactions

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The Orange agricultural production suffers from several diseases and the Citric Canker is among them. This disease is caused by the phytopathogen *Xanthomonas axonopodis* pv. *citri* (*Xac*), a Gram negative bacterium whose pathogenicity factors are not well understood. With the aim to comprehend the disease pathways in *Xac*, Type III (T3SS) and Type IV Secretion Systems (T4SS), especially the secretion chaperones belonging to these, were the objects of our studies. Upon cloning, expression and purification procedure standardizations, for NMR assays, the target protein (XAC1990) was isotope labeled with ^{15}N using $^{15}NH_4Cl$ in minimum medium. After isolation and purification, mainly by chromatographic methods, such as column gel filtration, the labeled protein was analyzed by ^{1}H NMR and is encountered folded. The second part of our investigations consisted in interaction studies among *Xac's* proteins and probable ligands and/or inhibitors, once these are crucial in any biochemical process. These were also monitored by ^{1}H-NMR and STD-NMR (Saturation Transfer Difference) assays. Our studies also embody NMR analysis of Orange Heat Shock Protein, Hsp90, with geldanamycin (Figure 1) this protein inhibitor.

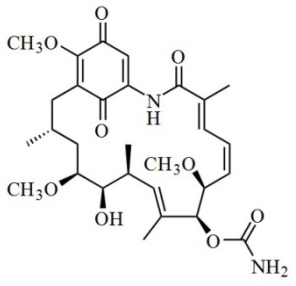

Figure 1 - Geldanamycin

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P37

Unfolding States of Integral Membrane Proteins by GPS NMR: TFE-induced disintegration of KcsA reconstituted in micelles

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Membrane proteins are vital for biological function, and the mechanism of their action and their role in biology critically depends on how they interact with membranes, their folding properties, and their topology under given conditions. Because of its impact in molecular medicine, the membrane folding problem has become a central point in many investigations.[1] The experimental conditions to unfold helical membrane proteins are crucial and the difficult handling of membrane proteins represents a challenge as it applies classical and new techniques to investigate the folding and unfolding of membrane proteins.[2] The following study takes advantage of a new technology called GPS-NMR[3] (Global Protein Structure - NMR). This technology combines the ability of NMR spectroscopy to simultaneously record signals from the individual hydrogen atoms (1H) and the capacity of Multivariate Analysis to reduce data complexity. By GPS-NMR, we have studied the main steps involved in the unfolding of KcsA under influence of TFE. Because of the clear influence of the environment on membrane protein folding and topology, GPS-NMR may serve as a useful way to map membrane protein conformations in a high-throughput fashion. Our study shed new light to the highly complex TFE-DDM-KcsA and describes step by step the overall changes happening in solution to the micelles and to the protein during the unfolding process.

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P38

Identification of Protein - Nanoparticle Interaction Site

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Nanoparticles (NP) are materials characterized by dimensions smaller than 100 nm. Due to their small size they have properties that can be quite different from the bulk form of the same material. These particles are small enough to enter almost all areas of the body, including cells and organelles and their use is constantly increasing in biomedicine. At the moment there is a strong interest in using NP as drug delivery systems and there are already several diagnostics tools in biomedicine that make use of different kinds of NP. At the moment not much is known about the interaction of nanoscale objects with biological systems and their potential toxicity.

We show that it is possible to identify the protein-nanoparticle interaction site at amino acid scale in solution. Using NMR, chemical shift perturbation analysis, and dynamic light scattering we have identified a specific domain of human ubiquitin that interacts with gold nanoparticles. This method allows a detailed structural analysis of proteins absorbed onto surfaces of nanoparticles in physiological conditions and it will provide much needed experimental data for understanding the interaction of proteins with nanoscale particles.

P39

New insights into FGFR-HS-FGF interactions. Towards the elucidation of the ternary complex in solution by using NMR spectroscopy

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The fibroblast growth factors (FGFs) constitute a family of more than twenty signalling polypeptides which are involved in a variety of biological processes including cell proliferation, differentiation and angiogenesis. FGF biological functions are triggered by binding of the polypeptide to specific transmembrane tyrosine kinase receptors (FGFRs) at the cell surface. In addition, FGFs strongly bind to glycosaminoglycans (GAGs) of the type of heparin and heparan sulphate (HS). Sulfated GAGs seem also essential for the appropriate assemblage of an effective signalling complex between FGF and FGFRs. Crystal structure data of FGF1-GAG-FGFR2[1] and FGF2-GAG-FGFR1[2] complexes reveal striking discrepancies in the proposed topologies of the ternary complex. Since the contacts detected by X-ray crystallography may be due to crystal packing forces, it is important to validate the presence of these interactions in solution. In this context, A. Kochoyan et al[3] have studied the interaction between FGFR Ig2 module and FGF in solution by NMR, showing that in the absence of heparin, FGFR Ig2 can bind to FGF not only via the primary site (present in both models), but also via the secondary site (present only in the symmetric model described by Schlessinger *et al.*). Following this approach, we present our data on FGFR Ig2-HS interactions and FGFR Ig2-HS-FGF1 interactions by NMR to gain insight into the role of HS oligosaccharides in the architecture of the ternary complex in solution.

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P40

Hunting down the squid - towards understanding Legionella pneumophila SKP

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The objective of this study is to investigate the Seventeen Kilodalton Protein (SKP) of the bacterium Legionella pneumophila using various biochemical and biophysical methods, such as NMR (both in solution and solid-state), X-ray crystallography, crosslinking studies, dynamics experiments etc. Most important is the elucidation of the structure of SKP, but also to clarify its working mechanism and how it interacts with other proteins and membrane lipids (especially lipopolysaccharides).

The protein SKP is believed to take part in the process where outer membrane proteins (OMPs) are inserted into the outer membrane of the L pneumophila cells, and the bacterium is believed to be pathogenic due to some of its OMPs. If SKP could be more readily studied, new drugs to counter the diseases it is causing could possibly be developed.

Also, SKP has several other intriguing properties. One notable is the ability to prevent other proteins from aggregating in solution which can be very usefull when overproducing other proteins This since SKP then can be coexpressed to surpress problems with aggregation and inclusion body formation.

SKP has a very high expression level in the E coli rosetta system used previously, so that approximately 4 g SKP can be produced per litre cell culture. The growth of the bacteria usually reaches an optical density of more than 11, which is unusually high for batch cultures (i.e. no fermentor is used). Hence, lots of analyses can be performed from just one round of cell culture. SKP is produced as inclusion bodies and therefore requires refolding after purification. However, this process is straightforward and simple, and the purification itself is also easy. After cell harvest the cells are disrupted with 8 M urea and the solution is centrifuged to get rid of the cellular debris. After this, the remaining solution is run through an IMAC column, resulting in a protein purity of >99%. Following this, the protein is refolded and concentrated to suitable level for the various studies.

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Study of the 37-nt hairpin model RNA of human HAR1F RNA by NMR

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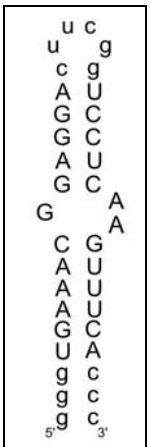
The 118-nt long human accelerated region 1 of humans (hHAR1F RNA) has 18 substitutions in comparison to the homologous sequence from the chimpanzee (cHAR1F). The function of this non-coding RNA in development of human consciousness is still unknown. Two different cloverleaf-like secondary structure models have been offered for the full length hHAR1F RNA.[1,2] It was proposed that the rapid evolutionary changes in humans enabled the hHAR1F RNA to function without the need to change its conformation.

We designed a 37-nt hairpin model RNA which mimics helix 1 of the full length hHAR1F RNA. The 37-nt ^{15}N-labeled RNA was transcribed *in vitro* with T7 RNA polymerase. The resonance assignment was performed using ^{15}N-HSQC, HNN-COSY and NOESY spectra. The 37-nt RNA imino proton signal pattern in the NOESY spectrum was comparable to the imino proton signal pattern of the full length hHAR1F RNA construct. We studied the dynamic properties of the 37-nt RNA construct with the help of ^{15}N-relaxation NMR measurements. First we checked for the signal broadening at different temperatures and the presence or disappearance of the signals due to the relaxation. Then we estimated the fast internal motions in the 37-nt RNA construct by the measurement of longitudinal and transverse relaxation rates, along with heteronuclear NOEs.



37-nt RNA

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P42

Modeling the Properties and Function of the enzymic cofactor Pyridoxal 5'-phosphate (PLP) dependent enzymes byNMR

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In order to extend the knowledge of the transimination mechanism of pyridoxal 5'-phosphate PLP-dependent enzymes, the properties and function of the enzymic cofactor PLP have been modelled. The properties considered in this work are the chemical, the protonation states, the pK_a values and the tautomeric equilibrium constants of PLP species.

The potential model intermediates of the transimination have been characterized by ^{13}C and ^{15}N liquid state NMR of products stemming from the spontaneous reaction of ^{13}C-4', 5'-PLP with $^{15}N_2$-diaminopropane and ^{15}N-α or ε-L-lysine. Around pH 7, all major PLP species are present in similar amounts which is associated to a subtle balance of the acid-base properties of the functional groups. For the first time, fast tautomerism in the intramolecular OHN hydrogen bond of PLP Schiff bases is observed in aqueous solution as deduced from the ^{15}N chemical shifts. The tautomeric equilibrium involves two forms of the PLP Schiff base: the enolimine form where the proton is located on the phenol group and the more reactive iminophenoxide where the proton is located on the nitrogen. The same tautomerism is noticed in PLP Schiff bases with ^{15}N-ε-poly-L-lysine in the solid state. It is observed that water molecules have influence on the tautomeric equilibrium of the Schiff bases leading to more iminophenoxide form.

The modeling of properties and functions of the enzymic cofactor PLP by NMR demonstrates the critical importance of the Schiff base tautomerism for the activation of an internal Schiff base, which triggers transimination. This is well illustrated by the ^{15}N chemical shift of the ^{15}N-PLP bound to alanine racemase lyophilized showing a high field shift of the ^{15}N NMR signal upon water addition in the gas phase. Transimination was found to be more favorable to proceed *via* microsolvation of the chiff base than *via* the geminal diamine pathway. This microsolvation leads to hydrolysis of the PLP Schiff base liberating the aldehyde form of PLP which condenses with another amino group thus performing the transimination reaction.

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P43

Entropic paradox in the protein-ligand complex observed by time-resolved EPR

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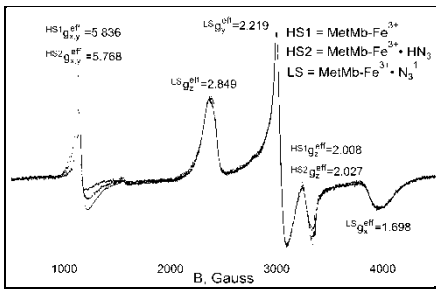

An early intermediate state of the reaction between the 15mM horse heart metmyoglobin (MetMb-Fe^{3+}) and 2M azide was obtained by microsecond freeze-hyperquenching at pH=4.7. The apparent sample ageing time was 20-30μs. X-band cw EPR at 14K showed rapid binding of azide to the haem iron during the 1-3-min-long heat-cool cycles performed in the increasing 5K steps between the temperatures of 160-180K. In the same range, the reaction rates at fixed temperatures were at least 10-50-fold slower. Could the collective motions of highly viscous liquid water steer the protein conformational changes during crystallization of cubic ice at ~170K? Judging the relatively high activation energy for metmyoglobin-azide binding (15.8 kcal mol^{-1}) and low heat of ice crystallization (-0.31 kcal mol^{-1}), azide binding is expected to be both enthalpically and entropically slow.

Incubation of low-spin haem iron – azide complex at 220K leads to the formation of the high-spin species with more than 2-fold broader EPR line at $g^{eff}_{x,y}$ compared to the free MetMb-Fe^{3+} and g^{eff}_{z} shifted from 2.008 to 2.027. These species can be attributed to the metmyoglobin-hydrazoic acid complex MetMbFe^{3+}-HN_3.

Sample preparation. Metmyoglobin-azide binding was triggered by a submicrosecond mixing, resulting in a 18-20-μm-diameter liquid jet with nozzle velocity of 250 m s^{-1}. The jet was sprayed in supercooled liquid methane rotating at 250 rpm at the temperature of 77K. The resulting freeze-quenched metastable glassy reaction mixture contained >65% of non-reacted metmyoglobin as detected by X-band EPR and low-temperature optical spectroscopy. X-ray diffraction analysis showed that the sample phase consisted of >90% vitrified water. After warming to ~190K formation of cubic ice was observed.

P44

EPR investigation of *Origanum vulgare* essential oil effect on *Listeria monocytogenes* membrane

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Today, different strategies are applied in order to control pathogens in food, and particular interest has been focused on the application of essential oils (EOs).[1] Among pathogens, special attention has to be paid to *Listeria monocytogenes*. Although EOs antimicrobial action is established, their mechanism of action has not been completely explained in detail,[1] even if the bacterial membrane seems to be one target. Membrane bilayer modifications can be investigated by EPR spectroscopy using nitroxide spin-label, whose signal enables one to evaluate changes on micro-environmental order and fluidity, therefore the aim of our work was to study the effect of oregano essential oil on *L. monocytogenes* membrane.

The EPR approach has been largely and successfully used in the study of biological membranes for decades but, at best of our knowledge, was neither applied to studies on this topic nor on living prokaryotic cells, for that reason measurements required a preliminary optimization of experimental conditions. Analyses of experimental spectra by computer simulation (eprsim 4.99)[2] showed that EPR signal in the bacterial membrane consist of three components, in agreement with the coexistence of different lateral lipid domains. EO treatments revealed that, after exposure to concentrations up to 0.50%, the cells membrane fluidity was changed and its order increased, suggesting a cell reaction to the stressing event. When *L. monocytogenes* was exposed to higher concentrations, membrane order parameters slightly returned to the values of untreated cells. On the contrary, when the cells were exposed to EO in presence of sodium azide, which impairs energy metabolism so that the cells do not have the energy to react, the membrane fluidity was progressively enhanced, even at the lowest EO concentration (0.25%).[3] Our rationalization coming from the analysis of EPR results were supported by microbiological analyses. In conclusion the combined approach including EPR and microbiological analyses provided relevant information on membrane modification and cell response to essential oils.

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P45

Open-access software and Databases to help metabolites recognition in Metabonomics: the user's point of view

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Nowadays, metabolomics is underpinned by a number of freely and commercially available databases and automated approaches for metabolite identification and chemical structure elucidation. We have built a CCPN metabolomics project containing around 80 standard compounds.[1] Standards have been chosen between metabolites present in the common biofluids' and common urine's library of the open-access software MetaboMiner.[2] 2D 1H-13C HSQC, 1H-1H TOCSY and 1D 1H spectra of standard compounds have been free obtained from public database.[3] With the presented CCPN metabolomics project, spectra acquired on an unknown mixture can be easily compared, superimposed and assigned on the basis of the standard compounds spectra available in the project. Moreover, any other standard compounds required by users can be easily implemented in the same project and used for assignment purposes. The project enables (a) to have an overview of the similarity and differences between spectra coming from different samples; (b) to have an overview of the number of unassigned peaks; (c) to have detailed lists of the unambiguous and ambiguous assigned peaks. These peak lists could also be quality checked, exported or screened against other public databases, or could be the starting point for de novo metabolite identification. In conclusion, here we present a protocol based on open-access databases and software, which we believe could help in the management of complex mixtures of spectra, and in the identification of metabolites on the basis of 2D and 1D -NMR spectra.

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P46

pH-Gating of the KcsA Potassium Channel in its Cytoplasmic Domain

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The KcsA potassium channel adopts a closed state at pH 7 and an open state at pH 4. Its pH-sensor is assumed to lie between its second transmembrane helix and its cytoplasmic C-terminal domain (CTD), a 40-residue long segment (121-160) which behaves as an amphiphilic helix.[1] The crystal structure of full-length KcsA shows CTD to form a four-fold symmetrical helical bundle, but fails to detect pH-related effects in this domain.[2] Contrary to these findings, peptides corresponding to the CTD domain have exhibit pH-dependent sedimentation behavior.[3] The main goal of our study, therefore, is to unequivocally establish the role of CTD in pH-gating of the KcsA channel.

We employ a dual approach to address this question, examining pH-dependent behavior of CTD as an independent domain and in the context of full-length KcsA. Backbone chemical shifts and $R_{1\rho}$ rates measured for U-^{13}C,^{15}N-CTD34, a peptide corresponding to residues 127-160, determine it to be helical and monomeric at pH 4. However, at pH 7 and 298 K CTD34 exhibits additional well-dispersed and fast-relaxing resonances, suggesting partial formation of a tetramer. We have also compared the dynamics of the CTD segment in micelle-solubilized full-length KcsA at various pH values. Typical CTD ^{15}N $R_{1\rho}$ rates measured for a U-^{2}H,^{13}C,^{15}N-KcsA sample at 323 K are 9-12 s^{-1} at pH 4, indicating monomeric behavior, but these significantly increase to 20-30 s^{-1} at pH 7, indicating tetrameric behavior. We conclude that (i) the last 35 amino acids of CTD possess an inherent capability of pH-induced oligomerization, independently of the consensus 'pH-switch', and (ii) CTD loses its helical bundle conformation upon exposure to acidic pH.

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P47

Structural Characterization of C-Terminal Zinc Finger Domain of XAF1 Protein

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The human XAF1 protein is a putative tumor suppressor protein, which was demonstrated to antagonize the caspase-inhibition activity of XIAP and thereby promoting apoptosis. The XAF1 protein presents at low or undetectable levels in different cancer cell lines and human cancer specimens. The study of domain architecture of XAF1 protein revealed that the full length protein (M1-S301) contain four structural domains namely NT (M1-G106), Znf-A1 (G106-R143), Znf-A2 (I144-R215) and Znf-B2 (G256-K293). However, the relationship between XAF1 with pro-apoptotic function is largely unknown.

The structure determination using NMR spectroscopy indicated that the Znf-B2 domain adopted a classic ββ-α C_2H_2 zinc finger topology with a notable amphiphatic surface charge distribution. Structural comparative analysis suggested that the Znf-B2 domain is probably belonged to be a Protein-protein interaction (PPIs)-class C_2H_2 zinc finger.

GST-pull down study probed the direct *in-vitro* interaction between all the XIAP domains and CT (G106-S301) region of XAF1, in which Znf-B2 domain displayed differential preference towards particular XIAP domains. The binding interfaces of the zinc finger domain were mapped by NMR Chemical Shift Perturbation method.

P48

Probing Collagen Matrices by Solid-State NMR

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Collagen is the most abundant structural protein in the human body, and a major component of the extracellular matrix. However, our understanding of collagen structure is derived mainly from model peptides that can be synthesised, crystallised and analysed by X-ray diffraction. Much is still not understood on the fibrillar level structure, which exhibits disorder and complex cross links.

Previous attempts to grow collagen matrix in cell cultures have been followed with solid-state NMR. While upstream biochemical markers indicate that the cell had attempted to synthesise collagen, and binding stains show the presence of long molecules being produced, solid-state NMR indicates that the necessary post-translational modification of proline have not always been carried out. An NMR toolkit for collagen structure that can directly pick out key motifs of collagen, such as triple helical units and fibrillar packing, will allow us to characterise such synthetic attempts with even greater precision.

To increase the information on native collagen structure available from NMR, we are developing in vitro and in vivo techniques for isotopic labelling. To understand the data from these complex systems, we are undertaking detailed structural studies on model, isotope labelled, synthetic collagen fragments, so that we can identify spectroscopic fingerprints of collagen structural motifs using a toolkit of key NMR experiments.

To this end, we have synthesised 15N labelled model collagen peptides and have initiated 13C CSA, 15N CSA, and 13C{15N} REDOR experiments. These are being analysed to provide constraints on the triple helical geometry.

P49

Structural studies of SpaI: The protein conferring autoimmunity against the lantibiotic subtilin in *Bacillus subtilis*

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The heavy indiscriminatory use of many antibiotics in the past lead to an emerging resistance even against 'last resort' drugs such as vancomycin. Thus, there is an urgent need for structurally novel antimicrobial agents. Lantibiotics are small ribosomally synthesized peptide antibiotics with posttranslational modified amino acids like lanthionines and dehydrated amino acids. *Bacillus subtilis* ATCC 6633 produces the lantibiotic subtilin which damages the membrane of gram-positive bacteria through pore formation. Thus, the producer strain must protect itself against subtilin by expressing four self-immunity proteins called SpaIEFG.

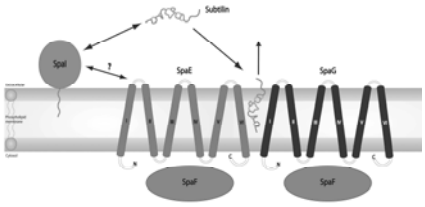

Figure 1: Proposed interactions of Subtilin with autoimmunity proteins SpaIEFG modified after 1,2

SpaI is a 17 kDa lipoprotein which is attached to the outside of the cytoplasmic membrane. It specifically interacts with subtilin and can protect the membrane from subtilin insertion or prevents subtilin oligomerization prior to pore formation. Interestingly SpaI shows only little sequence homology to the related NisI which protects *Lactococcus lactis* from nisin and cannot protect *B. subtilis* from nisin.[1] The structure of SpaI is not known yet and a BLAST search did not identify homologous proteins with a known structure.

Here we present solution NMR data on a 15 kDa C-teminal fragment (lipid free) of SpaI. The secondary structure derived from chemical shift data indicates a predominant β-sheet structure.

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P50

Structural study of the hnRNP C RRM in complex with its RNA target

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HnRNP C is an abundant RNA-binding protein shuttling between the nucleus and the cytoplasm, involved in various aspects of posttranscriptional gene regulation such as nascent RNA packaging, alternative splicing and control of IRES-mediated translation. The protein exists in the cell as a stable tetramer. Each monomer contains one *RNA recognition motif* (RRM) known to specifically recognize tracts of five or more uridines.[1] At the molecular level, little is known about the mechanism of interaction of hnRNP C with its different RNA targets. We therefore used the NMR spectroscopy to investigate the structural features of the recognition between the RRM of hnRNP C and a poly-U oligomer.

Our results suggest that the RRM of hnRNP C binds up to five consecutive uridines with an affinity in the low micromolar range. Despite having tested RNA oligomers with different poly-U lengths, we systematically observed precipitation or register exchange of the bound RNA, resulting in broad NMR signals and ambiguous NOEs. With the sequence AUUUUUC we obtained finally two RNA conformers in a slow exchange. For the structure determination, we included only the intermolecular NOEs (170 in total) corresponding to the major RNA conformer. Our preliminary structure shows that the RRM accommodates three uridines on the β-sheet in a canonical interaction mediated by aromatic ring stacking. Important roles are played by both N and C termini which become structured upon binding and form a tight network of interactions around the three uridines, allowing for a specific recognition. In addition, the loops β_1 - α_1 and β_2 - β_3, assisted by charged residues in the C terminus, bind two more nucleotides unspecifically. This is in contrast with the known preference of hnRNP C for longer U-tracts. The observed sliding of the RNA on the protein surface could be the mechanism allowing the protein to select longer uridine stretches despite using a limited number of specific sites.

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P51

NMR to study the interaction and the dynamic properties of cL-BABPs binding with two bile acids

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Understanding the mechanism regulating the interactions of intracellular carriers with bile acids is a key step to provide a model for the transfer of BAs from cytoplasm to the nucleus and can be used to inspire the design of therapeutic agents in the treatment of metabolic disorders, such as obesity, type 2 diabetes, hyperlipidaemia and atherosclerosis.

To achieve a detailed molecular and dynamical description of the binding mechanism driving to the formation of the ternary complex of cytosolic Liver Bile Acid Binding Protein (L-BABPs) with two bile acid (BA) molecules, spectroscopic methods together with kinetic and thermodynamic analysis have been applied and implemented. In particular structural, dynamical and interaction properties of two forms of chicken L-BABP (cL-BABP), differing by the presence/absence of a naturally occurring disulphide bridge, have been investigated by nuclear magnetic resonance (NMR) approaches.[1] NMR lineshape analysis as a function of ligand concentration was chosen as an appropriate tool to investigate the complex interaction mechanism within the cL-BABP/BA system.[3] Particularly, the combination of lineshape and relaxation dispersion experiments[2] has been useful to define a multi-step binding mechanism and to provide an estimate of the kinetics involved, allowing the correlation between protein dynamics and function.

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P52

Application of the Protein Meta-Structure to Weak Binder Screening by NMR for Fragment Based Drug Discovery

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The fragment based drug discovery approach recently emerged as a powerful tool in rational drug design.[1] The fundamental idea is to screen fragment libraries for small, typically weak, binders, which can then evolve to larger, and more affine compounds either by merging small fragments together or by decorating the original small ligand. The crucial point of this approach is the design of the fragment library, which is often tedious and rather subjective.

The recently introduced protein meta-structure concept[2] can considerably speed up the screening procedure by dramatically narrowing the choice of fragments and obviates the usage of large fragment libraries. Indeed, within the protein meta-structure framework, putative ligands can be easily predicted only based on the primary sequence (in contrast with the protein-structure similarity clustering). This approach called protein meta-structure similarity clustering allows the identification of possible target optimized ligand scaffolds independent of the subjective choice of a fragment library. Additionally, these ligands already meet the essential requirement for pharmacophores as they are predicted from databases of potent drugs. Therefore, the PMSSC approach can be readily used to design target oriented fragment library.

In order to demonstrate the validity of this approach, we chose, as a model system, the lipocalin Q83, which ligand binding properties have been extensively investigated in our team. We successfully identified a scaffold fragment that we were able to rationally decorate using only commercial compounds. The obtained ligands bind to Q83 with affinities ranging from mM to nM with increasing molecular complexity. In addition, we provide protein dynamics data showing the importance of dynamic consideration in drug design approaches.

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P53 (*)

A Tyrosyl-Dimanganese Coupled Spin System in Ribonucleotide Reductase of *C. ammoniagenes*: A Multifrequency EPR and X-ray Crystallography Study

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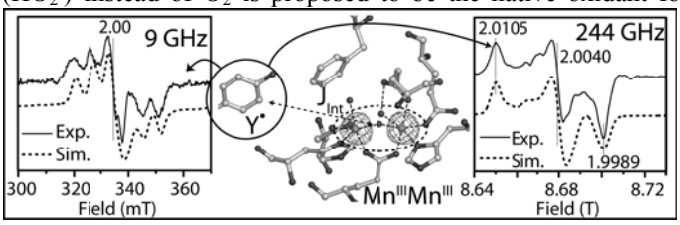
The crystal structure of the native R2F subunit of the ribonucleotide reductase (RNR) of *Corynebabacterium ammoniagenes*, with a resolution of 1.36 Å, demonstrates that the metal site contains an oxo/hydroxo-bridged manganese dimer, located near a tyrosine residue.[1,2] The manganese dimer resembles the di-iron metalloradical cofactor of class I RNR isolated from *Escherichia coli.*[3] Multi-frequency EPR measurements at X-band, Q-band and 244 GHz of the highly active *C. ammoniagenes* R2F subunit show that the metal site contains a ferromagnetically exchange-coupled $Mn^{III}Mn^{III}$ dimer weakly coupled to a tyrosyl radical. Geometrical information was determined from EPR data and insights obtained concerning the mechanism of tyrosine oxidation. H_2O_2 (HO_2^-) instead of O_2 is proposed to be the native oxidant for metalloradical cofactor generation ($Mn^{III}Mn^{III}Y^{\bullet}$). Changes in the ligand sphere of both metals during assembly directs the complex formation of the metalloradical cofactor. The proposed mechanism disfavors alternate reaction pathways such as H_2O_2 dismutation, thus distinguishing it from the manganese catalase,[4] which is a structural analogue.



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P54

Direct detection of nucleotides binding to L-histidine chromatography supports by STD-NMR spectroscopy

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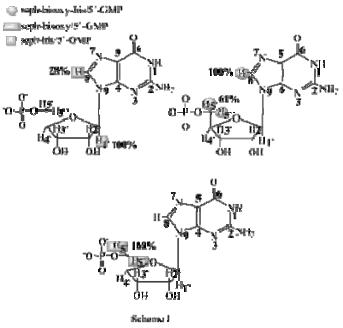
The recent application of amino acids supports in affinity chromatography for the separation of plasmid DNA isoforms takes advantage of the naturally occurring interactions between nucleotides and amino acid.[1] These interactions are highly specific; however, the molecular basis for this specificity is not well understood.[2] In this study, we report a direct application of saturation transfer difference (STD) NMR spectroscopy to identify the binding epitope of 5'(3´)-nucleotides, with atomic resolution, to three chromatographic supports: seph-bisoxy-his, seph-hist and seph-bisoxy. The specific interactions of histidine and bisoxyran spacer immobilized onto sepharose were analysed to help us understand the absorption mechanism between 5'(3´)-nucleotides and these supports. The NMR samples were prepared in 10% D_2O/90% potassium phosphate buffer using molar excess of 5'(3´)-nucleotides over the chromatographic support concentration at pH 8.0. For all experiments, the on- and off-resonance frequency was therefore set to 993 and 21600 ppm, respectively, with saturation time 2.0 s. Scheme 1 shows the relative STD effects for 5´-GMP bound to the three supports, for comparison. This scheme illustrates that the proton $H_{1'}$ of ribose, the proton H_8 of purine and the protons $H_{5'}$ and $H_{5''}$ give higher STD signals with seph-hist, seph-bisoxy-his and seph-bisoxy, respectively. Also, it is clear that the bisoxyran spacer influences the binding interaction with 5´-GMP.



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Structural characterization of a cyclic-nucleotide regulated K^+ channel using solid-state NMR spectroscopy

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Ion channels regulated by cyclic nucleotides (CNG) control the membrane potential of various cell types in neuronal systems. Although a considerable amount of information is available regarding the physiological role of these channels that open upon binding cyclic nucleotides (cNMPs), the molecular mechanism of activation remains poorly understood. Here we report an initial structural characterization of a bacterial CNG channel (mlCNG[1]) in lipid bilayers using solid-state NMR spectroscopy under magic angle spinning (ssNMR) to comprehend the molecular mechanism of activation. Previous crystallography studies revealed structural details of the trans-membrane segments of this channel but were unable to resolve the ligand binding domain.[2]

Using a set of (^{15}N, ^{13}C) and (^{13}C,^{13}C) correlation experiments, we have investigated uniformly labeled variants of the full length (355 amino acids), membrane-embedded mlCNG channel as well as of the isolated binding domain. Comparison of these data sets and our earlier work on a chimeric ion channel[3] suggests a tight association of lipids, that are known to influence MP function, to the channel. Moreover, we report resonance assignments obtained in both transmembrane and ligand-binding domains that provide the spectrosopic basis to further dissect the conformational landscape of the membrane-embedded mlCNG channel during the gating cycle.

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P56

NMR investigation of BMP-Chitlac, a chitosan derivative for osteoregenerative applications

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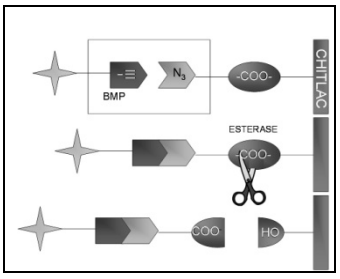
In this work we report the NMR characterization of a chitosan derivative for applications in the field of osteoregenerative medicine. The polymer was functionalized through the introduction of lactose (Chitlac) to enhance solubility and promote the interaction with cells,[1] and a peptide belonging to the BMP family, to induce new bone formation.[2] The structure and the dynamics of Chitlac were explored by NOE effect and relaxation measurements, showing that the lactose moiety does not give significant interactions with the chitosan chain, but, rather, is projected toward the surrounding environment, and is thus available for lectin receptors exposed on cell surface.



Functionalization with osteoregenerative BMP peptide was obtained as follows. BMP peptide was derivatized with pentynoic acid to give rise to a reactant for the click chemistry with the suitably modified polymer. In the linker an ester bond was introduced, so that the compound is susceptible to the hydrolytic action of esterases. In this way it was possible to obtain a BMP-delivery system, potentially able to control over time the local concentration of the peptide.

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Acknowledgements: We thank Bruker Biospin s.r.l. for the NMR instrumentation in CBM laboratories.

P57

Insights into the enzymatic mechanism of phosphoryl transfer

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β-Phosphoglucomutase (βPGM) catalyses the interchange between βGlucose-1-Phosphate and βGlucose-6-Phosphate; it is a phosphoryl transfer enzyme. The planar geometry and charge of the transferring phosphate in the transition state of the reaction[1] is mimicked by metal fluorides,[2] which therefore form transition state analogue (TSA) complexes with a variety of phosphoryl transfer enzymes. Enzyme-catalysed phosphoryl transfer displays the largest rate acceleration by enzymes found so far when compared to the uncatalysed reaction in solution,[3] and as such it is an excellent candidate to study the basis of the catalytic proficiency of enzymes. The use of various NMR techniques to analyse TSA complexes of βPGM allows for evaluation of the existing theories of enzyme catalytic ability, e.g., electrostatic pre-organisation, substrate destabilisation, & entropy. Particularly of interest is the role of protein dynamics found in the TSA complex whose rate correlates with the catalytic turnover rate. Relaxation dispersion and ^{19}F exchange spectroscopy allow a concerted motion throughout the molecule to be characterised, and its role in catalysis speculated.

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P58

Resolution enhancement for PELDOR distance measurements in phospholipid membranes

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Pulsed electron-electron double resonance (PELDOR) spectroscopy is increasingly applied to spin-labeled membrane proteins. However, after reconstitution into liposomes spin labels often exhibit a much faster transversal relaxation (T_m) than in detergent micelles, thus limiting the method in lipid bilayers. In this study, the main reasons for enhanced transversal relaxation in phospholipid membranes were investigated systematically using spin-labeled derivatives of stearic acid, phosphatidylcholine, as well as spin-labeled derivatives of the peptide Gramicidin A under the conditions typically employed for PELDOR distance measurements. Our results clearly show that the dephasing due to instantaneous diffusion that depends on dipolar interaction among electron spins is an important contributor to the fast echo decay in cases of high local concentrations of spin labels in membranes. The main difference between spin labels in detergent micelles and membranes is their local concentration. Consequently, avoiding spin clustering and suppressing instantaneous diffusion is the key step for maximizing the PELDOR sensitivity in lipid membranes. Even though proton spin diffusion is an important relaxation mechanism, only in samples of low local concentration deuteration of acyl chains and buffer significantly prolongs T_m and values of up to 5 µs have been achieved here. Furthermore, our study revealed that membrane composition and labeling position in the membrane can also affect T_m, either by promoting the segregation of spin-labeled species or by altering their exposure to matrix protons. Effects of other experimental parameters like temperature (< 50K), presence of oxygen, and cryoprotectant type are negligible under our experimental conditions.

P59

Intrinsic Order and Disorder in the Cell Death-inducing Protein Harakiri

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Bcl-2 family members are key mediators in programmed cell death. They share up to four regions of sequence homology known as BH domains (BH1-BH4). Members solely showing the BH3 domain belong to the BH3-only subfamily that includes cell death-inducing proteins predicted to be intrinsically unstructured and to contain a C-terminal transmembrane domain. As these features pose significant challenges for structural studies, the operating mode of BH3-only proteins is poorly understood. The BH3 region is mainly random coil in isolation although it forms an α-helix when complexed to prosurvival partners, suggesting a mechanism of coupled folding and binding. Despite their important role in apoptosis, structural information at the atomic level on isolated BH3-only proteins is lacking. To improve our knowledge on their function we report here structural studies by NMR and circular dichroism of human Harakiri, a BH3-only protein that localizes in membranes and binds to prosurvival Bcl-2 members.

These studies were performed with synthetic fragments that together encompass the full-length protein (91 residues). The fragments comprise the BH3 domain in the N-terminal region and the C-terminal transmembrane domain (residues 61-91). The N-terminal region is largely disordered, however low populated α-helical conformation is observed. The three-dimensional structure determined by enhancing its population in alcohol-water mixture closely resembles other BH3 domains bound to prosurvival partners. This result suggests that intrinsic structure propensity in the disordered protein is very relevant in the mechanism of coupled folding and binding. In contrast, the transmembrane domain that is highly insoluble in aqueous milieu forms a monomeric α-helix in micelles at ~100% population. The structure is reported here revealing features that explain its function as membrane anchor. Taken together, the data on the N-terminal and transmembrane regions are used to propose a tentative model of how Harakiri works.

P60

NMR Studies of the G Protein-Coupled Receptor CXCR1 and its interactions with Interleukin-8

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G Protein-Coupled Receptors (GPCRs) constitute one of the largest and most diverse families of membrane proteins. They are among the most targeted families of drug receptors, and already more than half of the drugs currently used interact with GPCRs. With more than 350 residues and 7 trans-membrane helices these proteins are challenging targets for NMR spectroscopy, especially in their native functional environment of phospholipid bilayers. We are developing and applying NMR methods for studying the structure, dynamics, and interactions of GPCRs using CXCR1 as a principal example. Binding of the small chemokine ligand interleukin-8 (IL-8) to CXCR1 induces activation and cell migration of polymorphonuclear neutrophil. Defects in signal transduction result in inflammatory disorders such as allergies, rheumatoid arthritis, and contribute to cancer growth and metastasis.

Progress towards determining the three-dimensional structure of CXCR1 alone and complexed with IL-8 in phospholipid bilayers will be described. Isotopically labeled pure, monomeric full-length CXCR1 and a wide variety of truncated CXCR1 constructs are reconstituted micelles, bicelles, and bilayers to enable the full range of solution NMR and solid-state NMR methods to be applied. Local motions are limited to the residues near the N- and C- termini, however, the entire protein undergoes rapid rotational diffusion about the bilayer normal at 30°C. Isotopically labeled CXCR1 give high-resolution solid-state NMR spectra in magnetically aligned bilayers that provide input for structure calculations, comparisons among constructs, and information about interactions with IL-8.

P61

Phosphorylation of S776 and 14-3-3 binding modulate ataxin-1 interaction with splicing factors

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Ataxin-1 (Atx1), a member of the polyglutamine (polyQ) expanded protein family, is responsible for spinocerebellar ataxia type 1, a genetically inherited human neurodegenerative disease.[1] Requirements for developing the disease are polyQ expansion, nuclear localization and phosphorylation of S776.[2]

Using a combination of bioinformatics, cell and structural biology approaches, we have identified a UHM ligand motif (ULM), present in proteins associated with splicing, in the C-terminus of Atx1, and shown that Atx1 interacts with and influences the function of the splicing factor U2AF65 via this motif.[3] ULM comprises S776 of Atx1 and overlaps with a nuclear localization signal and a 14-3-3 binding motif.

We demonstrate that phosphorylation of S776 provides the molecular switch which discriminates between 14-3-3 and components of the spliceosome, and regulates the development of the disease. We also show that an S776D Atx1 mutant previously designed to mimic phosphorylation is unsuitable for this aim because of the different chemical properties of the two groups.

Our results indicate that Atx1 takes part in a complex network of interactions with splicing factors and suggest that development of the pathology is the consequence of a competition of aggregation with native interactions. Studies of the interactions formed by non-expanded Atx1 thus provide valuable hints for understanding both the function of the non-pathologic protein and the causes of the disease.

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P62

Structural insights into the intracellular surface of vasopressin receptors

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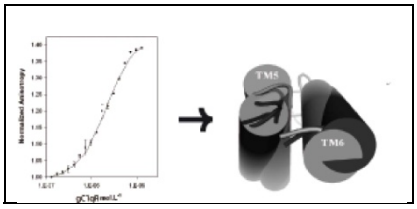
G-protein coupled receptors (GPCRs) are cell membrane proteins that share a common architecture of seven transmembrane helices and transduce the presence of various extracellular signals to intracellular signalling cascades. Only a few structures of GPCRs have been solved thus far. In almost structures, the electronic density of the i3 intracellular loop, whose involvement is crucial for the recruiting of the G protein effector, was missing. No structure of a complex between a GPCR and a partner protein has been elucidated to date. The V1 and V2 receptors are GPCRs binding to the extracellular vasopressin peptide. We show here how structural insights into the structure of the cellular interface of the vasopressin receptors and their complexes with partner proteins can be elicited using a variety of biophysical approaches, including limited proteolysis, fluorescence and NMR spectroscopy.[1,2,4] In particular, we have resolved the structure of the V2 i3 loop, isolated and in complex with the gC1qR protein and shown how the interaction induces the reorientation of transmembrane helices, illustrating the phenomena of signal transduction across the membrane.



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P63

Orientation of the Central Domains of KSRP and its Implications for the Interaction with the RNA Targets

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KSRP is a multi-domain RNA binding protein that recruits the exosome-containing mRNA degradation complex to mRNAs coding for cellular proliferation and inflammatory response factors. The selectivity of this mRNA degradation mechanism relies on KSRP recognition of AU-rich elements in the mRNA 3'UTR, that is mediated by KSRP's KH domains. Our structural analysis shows that the inter-domain linker orients the two central KH domains of KSRP – and their RNA binding surfaces – creating a two-domain unit. We also show that this inter-domain arrangement is important to the interaction with KSRP's RNA targets.[1]

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P64

A combined molecular dynamics and liquid state NMR study of the incorporation of the antimicrobial peptide alamethicin into membranes

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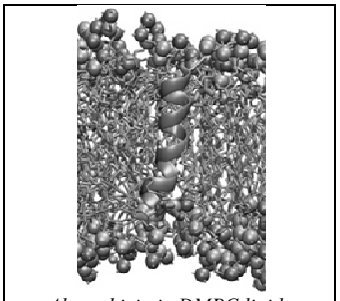
Antimicrobial peptides as alamethicin (20 amino acids, from the fungus *Trichoderma viride*) are of interest as potential substitutes of small molecule antibiotics. There are many models proposed for their antibiotic mechanism in nature, but there is experimental evidence necessary to reduce the number of hypotheses. We have studied alamethicin in a solution of DHPC/DMPC bicelles, mimicking the membrane environment but allowing to benefit from the resolution of liquid state NMR. Peptide-lipid NOE as well as paramagnetic relaxation enhancement (PRE, measuring the immersion depth of a hydrogen) indicate a transmembrane orientation of the peptide, but not all data could be explained by a rigid model. Therefore, we compared the NMR results with an all-atom MD simulation of an ensemble of alamethicin peptides in a DMPC bilayer: peptide-lipid NOE can be imitated by the number of contacts of a peptide hydrogen to a lipid group over the time of simulation. The contacts were weighted with r^{-6} in order to account for the distance dependence of the NOE. Similarly, the PRE can be compared with the number of contacts of a hydrogen atom to water molecules. In the simulation, the peptides show a high mobility within the membrane environment. The number of contacts not only correspond overall well to the PRE and NOE data, but also allowed to explain the initially ambiguous observations[1].


Alamethicin in DMPC lipids

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P65

Mobility and Topology of VPU and CD4 Proteins by Solid State NMR Spectroscopy

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The viral protein VPU of HIV-1 directly interacts with the human T-cell coreceptor CD4 and subsequently induces the degradation of this protein.[1,2] Towards the study of this interaction on a residue-specific level, shorter constructs of these proteins comprising the cytoplasmic domains with and without the transmembrane part, have been expressed and studied in the presence of detergents.[3,4]

In this contribution, we present the results of initial solid-state MAS NMR studies on liposome-reconstituted constructs of both membrane proteins. Sequential assignments were obtained using standard methods. Utilizing double-quantum buildup characteristics at different temperatures, we could identify different dynamics of transmembrane and cytoplasmic domains, and the overall topology with respect to the membrane was probed utilizing well-established experiments[5].

Initial results suggest that the transmembrane helices of both proteins are rather rigid, whereas the cytoplasmic domains are flexible.

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P66

Exploring Copper(II)Rusticyanin by ^{13}C NMR

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Slow copper(II) electronic relaxation makes extremely difficult the characterization by NMR of the active site of copper proteins in its oxidized state. This is specially true for the ^{1}H nucleus where signals can be even unobservable. In copper type 1 centers nucleus relaxation becomes faster and, consequently, proton signals, although broad, can be then observed. In these systems exchange spectroscopy has permitted nuclei assignments.[1] ^{13}C, with a gyromagnetic ratio significantly lower than that of the ^{1}H, is an excellent candidate for sounding out the vicinity of paramagnetic centers. Some of us have also been successful in applying exchange spectroscopy with ^{13}C in the CuA center of Cytochrome c oxidase.[2] In this last system, magnetic coupling between the two existing copper ions (formally with a redox state +1.5 each) allows copper ions relax faster, giving relatively narrow ^{13}C signals that facilitate the assignment.

One further step is the assignment of ^{13}C signals in mononuclear copper(II) systems (i.e. with a net unpaired electron). Here, we present such development. Rusticyanin is a blue copper protein present in *Acidithiobacillus ferrooxidans*. We assigned the ^{1}H NMR signals corresponding to the ligands of the copper(II) ion in the past.[1b] Now, we extend the assignment to ^{13}C nuclei by using the so-called blind noe irradiation, i.e., saturating signals without observing them. We have been able of localizing carbon nuclei corresponding to the equatorial cysteine and histidine ligands, as well to the methyl carbon of the axial methionine. The complete heteronuclei assignment will allow us to improve the knowledge of the unpaired spin delocalization onto the ligands and, hence, of how copper(II) interacts with them.

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P67
NMR investigation of a group II intron ribozyme

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NMR is a powerful technique to gain structural and (thermo)dynamic information as described in numerous reviews published in the past years dealing with the use of NMR spectroscopy in nucleic acids research.[1] In contrast to proteins, the chemical shift dispersion in the spectra of nucleic acids is reduced and the resonances suffer from strong overlap. This made it difficult for a long time to solve nucleic acids structure at a high level of precision. The introduction of isotopic labelling techniques, associated with the use of 2D and 3D NMR experiments, improved the situation substantially.

In RNA research, information about 3D packing and architecture in solution is strongly desirable. In this project, we intend to solve the NMR structure of the so called κ-ζ region of the Sc.ai5γ group IIB intron ribozyme, from yeast mitochondria. Group II intron ribozymes are naturally occurring catalytic RNAs, found in organellar genes of plants, fungi, bacteria and lower eukaryotes, able to undergo the self splicing, likewise the eukaryotic spliceosome.[2] In general, despite its importance, very little information is known on the 3D structure of these molecules so far.[3] Biochemical studies with the Sc.ai5γ ribozyme showed that the most crucial atoms responsible for folding and catalysis are located within the κ-ζ region, a small fraction of domain 1.[4] This special RNA fragment is 45 nucleotides long and comprises an unusual three way junction with two flexible tetraloops and one internal loop. Structural modifications as well as the different NMR experiments used to gain insights into the structure of this RNA molecule will be presented.

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P68
Is human Liver FABP a good carrier for MRI contrast agents? An NMR study

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Human Liver Fatty Acid Binding Protein (hL-FABP) is an abundant protein in the liver cytoplasm able to carry a variety of endogenous ligands, making it probably the most versatile chaperone in terms of its ligand repertoire. This raises the possibility that hL-FABP may play a role in the intracellular transport of exogenous molecules, like lipophilic drugs. Lipid-conjugated gadolinium chelates display promising features for the development of hepatospecific contrast agents for MRI. For this reason we tested the ability of hL-FABP to interact with a series of compounds carrying two different Gd(III) chelating moieties, DTPA and AAZTA[1] using relaxivity measurements. These findings allowed us to select two complexes, Gd(III)-DTPA-bile acid[2] and Gd(III)-AAZTA-C_{17} for further detailed characterization. A number of NMR experiments have been performed using both diamagnetic Y(III) and paramagnetic Gd(III) complexes to feature the binding with hL-FABP. The characteristic of the paramagnetic ions to alter the NMR relaxation properties of atoms located nearby, has been exploited to localize the position of the Gd(III) ion, while NMR titration experiments have been performed to localize the molecules in the cavity. Moreover, since Gd(III)-AAZTA-C_{17} is able to form micelles,[1] we carried out NMR experiments to characterize hl-FABP/micelle adduct.

The results of relaxometry and NMR experiments allowed us to hypothesize the occurrence of intracellular associations between a novel class of lipophilic potential contrast agents and a highly abundant liver protein. This interaction enhances the relaxivity of the compounds *in vitro*, and will provide an increased contrasting power *in vivo*.

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P69

Pulse EPR Reveals the Coordination Sphere of Cu(II) in the 1-16 Amyloid beta peptide

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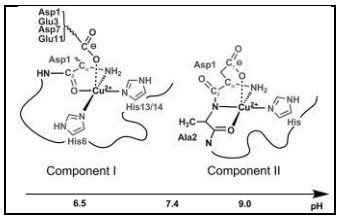
There is a broad interest in metal-induced peptide/protein aggregation, which is a common feature of several degenerative diseases (NDs). In particular, Cu^{II} seems to play an important role as it binds to NDs peptides/proteins and modulate their aggregation behavior. Knowledge on the Cu^{II} coordination to these peptides/proteins are pivotal to understand its role, in particular since small changes in coordination may have important impact on the aggregation or on the generation of reactive oxygen species, another key feature of Cu^{II} in NDs.

The system Cu^{II}-amyloid β (Cu^{II}-Aβ), involved in Alzheimer's disease, has been extensively studied. However, the unambiguous identification of the Cu^{II} ligands has remained difficult and no real consensus emerged in the literature.[1] In almost all studies, a mixture of species was present at the pH at which the experiments were performed.

We have used a wide range of EPR methods to study the Cu^{II} binding to Aβ. This, combined with specific isotopic labelling of amino acids and the use of selected pHs to study one complex at a time, has provided direct evidence for each ligand on Cu^{II}-Aβ and allowed us to propose a novel model for the Cu-coordination to Aβ at physiological pH.[2] In addition, NMR data have provided insights into the dynamics of the system.[3]



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P70 (*)

Analysis of the ATPase cycle of a 200kDa molecular chaperone by NMR

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The Hsp90 chaperone assists the folding of a specific subset of regulatory proteins, many of which are oncogenic kinases. Hsp90's activity is controlled by ATP and various co-factors, the mechanism of which is elusive. We used NMR spectroscopy to reveal molecular insights into the Hsp0- mechanisms. To overcome the NMR size limitation for 200 kDa Hsp90 complexes, we used specific isoleucine labeling combined with ^{1}H-^{13}C-Methyl-TROSY. We resolved 92% of Hsp90's isoleucines and assigned the signals by mapping the individual domains to the full length human Hsp90. We titrated Hsp90 with nucleotides, co-factors and Hsp90 targeting anti cancer drugs. We mapped the binding site of co-chaperone p23 and we obtained molecular insights into the p23-Hsp90 interaction. We also studied the nucleotide bound and drug bound states of full length Hsp90, which allow us to obtain a dynamic picture of the ATP cycle. We anticipate that our approach has significant impact on future studies of Hsp90 with co-factors, inhibitors and substrates, but also on studying large proteins by NMR.

P71 (*)

Anatomy of a minimalistic riboswitch: Highly modular structure and ligand binding by conformational capture by the 27nt neomycin sensing regulatory RNA element

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The engineered 27 nt neomycin sensing riboswitch (Fig. 1A) represents the smallest functional riboswitch reported so far.[1] Here, we have identified structural determinants for gene regulatory activity of this minimal gene regulatory element from a combination of high resolution NMR spectroscopy and other biophysical methods.

The gene regulatory activity of the neomycin sensing riboswitch is attributed to a sterical interference of the highly stabilized RNA:aminoglycoside complex with the translation initiation process. Therefore, an extraordinarily stable RNA-ligand complex (Fig. 1B) constituting the regulatory 'off' state combined with a largely destabilized free 'on' state likely forms the basis of riboswitch activity (Fig. 1C).[2] Our detailed analysis allows deriving structural determinants for both states, such as destabilizing spacer elements and factors for cooperative stabilization, and thus establishes structure based parameters for riboswitch activity.

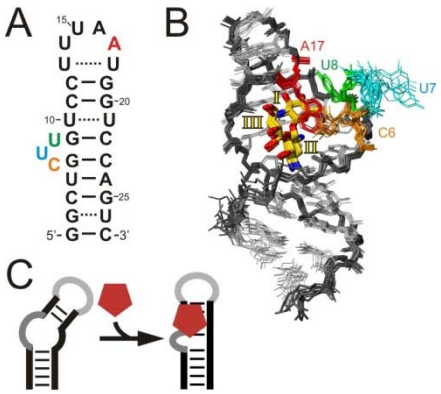

Figure 1

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P72

Functional Genomics of *Caenorhabditis elegans* by Whole organism NMR Spectroscopy in Applications to Ageing and Toxicology

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Caenorhabditis elegans is used to monitor a wide range of biological processes and to characterize genetic mutations responsible for human diseases. We have developed a robust protocol based on ^{1}H HR-MAS NMR spectroscopy of intact *C. elegans* worms to investigate the metabolic signature induced by genetic mutations, and demonstrated its use as a molecular phenotyping device for functional genomics at the system level. Here we focus on the metabonomic characterization of two different *C. elegans* mutants to obtain insight into the metabolic mechanisms ageing and toxicological processes.

We derived the metabolic signature of ageing in a *C. elegans* mutant that mimics caloric restriction (CR), a well-known process responsible for increase in lifespan in various organisms. We compared metabolic profiles obtained by ^{1}H HR-MAS NMR spectroscopy for wild type (N2) nematodes and CR mutants, young adults and 7-day old adult worms. We used supervised statistical analyses, such as OPLS, coupled to statistical recoupling of variables to derive significant metabolic discriminations. The metabolic signature of both ageing and CR in intact *C. elegans* is found to share similarities with signatures previously described from the plasma of non-human primates. Furthermore, we find that the difference between the metabolic profiles of wild-type worms and CR mutants increases with age. 7-day old CR mutants appear metabolically younger than their wild type counterparts.

We also illustrate the characterization of the metabolic signature of mutants of the aryl hydrocarbon receptor (AhR) which plays a central role in xenobiotic-induced toxicity and carcinogenesis.

P73

Protein-Ligand Affinity Measurements by Ligand Detected One-Dimensional NMR

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A method is described to determine affinities of two ligands binding to the same site of a protein, based on mutual titration and observation of binding from ligand detected NMR. Example of application to binding of two fragments to beta-secretase is shown.

P74

Side-Chain Dynamics Revealed by Methyl CH RDCs

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The RDC-based model-free analysis (MFA)[1] was applied to methyl group RDCs measured in ubiquitin dispersed in 13 different alignment media in order to characterise dynamics in the ns-µs time range.[2] The order parameter (S^2) results cover a wide range of mobility with correlation to residue type, distance to backbone and solvent exposure and bring evidence to the existence of fluctuations contributing as much additional mobility as those already present in the faster ps-ns time scale measured from relaxation data. Of the available dynamic ensembles of ubiquitin, the broadest one, namely the EROS ensemble,[2] fits the collection of methyl group order parameters presented here best. Finally, the MFA-derived averaged spherical harmonics were used to perform highly-parameterized rotameric searches of the side chains conformation and find expanded rotamer distributions with excellent fit to our data. These rotamer distributions suggest the presence of concerted motions along the side chains.

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P75

Supramolecular NMR: Cyclodextrins/Captopril Nanoparticles and Self-Assembly

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Cyclodextrins (CD) have been studied extensively as host molecules in supramolecular chemistry due to increasing interest in optimizing the efficacy of drugs activities. Understanding supramolecular topology in these complexes is fundamental for understanding the drug inclusion, as well, for controlling the host/guest systems. Herein, nanoparticles of inclusion complexes between captopril, an angiostensin converting enzyme inhibitor, and α-CD, β-CD and 2-hydroxypropyl-β-CD (HP-β-CD) were investigated using NMR and compared to self-assembly of these species. To understand the supramolecular topology of nanoparticles, ^{1}H and ^{13}C NMR experiments were performed in a Varian INOVA 500 MHz spectrometer. The complexation induced chemical shifts (CICS),[1] ROESY and DOSY data were obtained and confirmed the bioactivity results, as the nanoparticle named FC004 showed strongest interaction between captopril and α-CD. The NMR data suggested that the terminal alkyl-thiol portion of the captopril is included in the α-CD cavity, while for β-CD and HP-β-CD complexes the terminal thiol moiety may be exposed to solvent. The ROESY experiments also confirmed the captopril inclusion in CD for the FC004 formulation and CICS analysis. DOSY experiments also provided valuable information on molecular organization of the complexes. We have compared the obtained NMR results with captopril and CD self-assembly in the same proportions as used, and observed that the nanoparticles preparations gave much better results, which are very promissory in achieving a new way for captopril administration and provide better life conditions for high pressure suffering people, who presents around 15% of world's population.

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P76

Efficiency of a delayed treatment of neurointoxication evaluated by ^{1}H HRMAS NMR metabolic profile of mouse brain

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Introduction: Severe intoxications with organophosphorus compounds lead to a status epilepticus with related neurological lesions in surviving animals. There is actually no delayed treatment after at least 45 minutes of seizure.

Purpose: In this work we have evaluated the ability of ketamine (KET) associated with atropine sulphate (AS), to reduce the metabolic disorders induced by soman intoxication in mouse brain.[1]

Methods: The AS-KET treatment was given 1 h or 2 h after soman intoxication and compared to the very early treatment with the benzodiazepine midazolam (1 min after soman). Mice were sacrificed at 4 h, 24 h, 48 h, 72 h and 7 days after intoxication. Piriform cortex and cerebellum were rapidly sampled and immediately stored in liquid nitrogen.

^{1}H HRMAS NMR spectra were acquired at 400MHz with a CPMG pulse sequence (TE=30 ms), at a 4 KHz spin rate. 17 metabolites were quantified with the QUEST procedure of the jMRUI sofware (www.mrui.uab.es/mrui/).

Results/discussion: In piriform cortex, 11 among the 17 quantified metabolites significantly varied after intoxication,[1] mainly lactate (energetic metabolism disruption), myo-inositol (major brain osmolyte linked to brain oedema), N-acetyl-aspartate (a marker of neuronal suffering or death), glutamine (ammonium toxicity?) and glycerophosphocholine (inflammatory response, phospholipase A_2?). AS-KET administration 1 h after intoxication considerably reduces the metabolic disruption induced by soman, while AS-KET 2 hours after soman was poorly efficient.

Conclusions: The association of ketamine and atropine sulfate given until one hour after severe soman intoxication could be a valuable delayed neuroprotective treatment.

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P77

Mapping of epitopes of anti–IL-17A antibodies by NMR

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In contemporary drug research, therapeutic antibodies are playing an increasingly important role. Precise knowledge of the antibody-target interaction is of high value for understanding of the mode of action and for intellectual property issues. IL-17A is an important inflammatory cytokine involved in autoimmune pathology. AIN457, a human high-affinity antibody against IL-17A, has been shown to be effective against psoriasis, rheumatoid arthritis and uveitis and is now in clinical trials. Here, NMR methodology was applied to efficiently obtain this crucial information on IL-17A–antibody complexes, including the IL-17A–AIN457 complex.

Despite intense efforts over the past 5 years, the binding epitope of AIN457 on IL-17A could not be determined precisely, and no crystal structures of the complex were available. In the present studies, we characterized the binding stoichiometry and the epitope of different anti-IL-17A antibodies by NMR. Backbone resonance assignment of the 33 kDa IL-17A homodimer was the basis for characterization of the binding mode and the epitope at amino acid resolution of IL-17A in complexes with Fab, Fv and scFv fragments of AIN457. The obtained binding site is in agreement with data from orthogonal methods, *i.e.* H/D Exchange MS and mutagenesis combined with SPR. Finally, titration of IL-17A with the AIN457- Fab fragment was monitored by TROSY spectra, which showed that two Fab fragments can bind per IL-17A dimer. Using this information on the correct stoichiometry of IL-17A–antibody complexes, new crystallisation trials were set up. This strategy finally resulted in the crystal structure determination of an IL-17A–antibody complex at atomic resolution. The results presented here illustrate the excellent interplay of NMR and X-ray in structural biology, particularly in the context of modern drug discovery.

P78

Structure of an elongated thrombin-binding aptamer and its influence on the activity

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The DNA aptamer HD1 was discovered in 1992 by Bock et al. as anticoagulant targetting thrombin and was intensively studied in the following years.[1] The structure of the 15 nucleotide minimal motif was solved in the following years and consists of two G-Quartets which are linked by two TT loops and one TGT loop.[2]

To turn off the activity of the thrombin-binding aptamer extensions were attached to the 3'- or 5'-ends including light activatable "cages". The site of the extensions had a surprising effect on the activity: while 5'-extensions decreased the activity, 3'-extensions caused an increase.[3] Both effects did not depend on any specific type of extension. To understand these observations, we performed a NMR structural investigation of modified thrombin-binding aptamers with four adenosin nucleotides attached to the 3'- or 5'-termini.

While the less active 5'-elongated aptamer displays a rather undefined ensemble of conformations, the 3'-extended aptamer consists of one major conformation at low temperatures (278K) and a nearly equalized equilibrium with a second conformation at 298K. Here we will present the results of the structural investigation and discuss the influence on the activity.

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P79

Characterisation of NS5A(191 – 369) of Hepatitis C Virus by NMR Spectroscopy

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Nonstructural protein 5A (NS5A) of hepatitis C virus (HCV) plays an important role in viral replication, interferon resistance and apoptosis regulation. The protein consists of three domains. A well characterised domain 1 (D1), an instrinsically unfolded domain 2 (D2) and a less conserved domain 3 (D3). D1 is membrane anchored by a N-terminal amphipatic helix and comprises a structural scaffold with a zinc-binding domain, which is essential for RNA replication.

D2 interacts with NS5B as part of the HCV replicase complex and with a series of host cell proteins. It is therefore involved in several biologic regulations. Beside the interferon sensitivity determining region, the protein kinase RNA-activated binding domain and a potential Bcl2 homology region 2, a polyproline rich motif interacts with SH3-domains of several kinases of the Src family.

NMR-studies showed that D2 is instrinsically unfolded and undergoes local conformational changes upon binding to an interaction partner.[1,2] The low chemical shift dispersion observed for such a natively disordered protein presents a challenge for NMR spectroscopy. To achieve sequential resonance assignment for the D2 comprising fragment NS5A(191–369) we use high resolution 3D correlation experiments combined with advanced NMR techniques for fast data acquisition. The polypeptide is further characterised by backbone ^{15}N relaxation data which allows to detect segments within the protein sequence that show increased propensity of forming secondary and tertiary structure.

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P80

Evaluation of AChE Reactivators as Defense Agents against Organophosphorus Neurotoxic Compounds using Kinetics Studies by NMR

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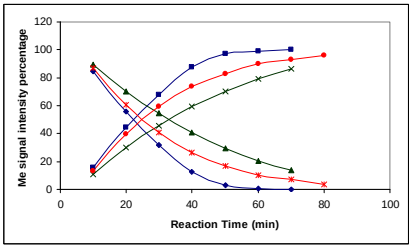
The use of organophosphorus compounds (OPs) as chemical warfare agents and pesticides leads to worldwide 700.000 to 1.000.000 intoxications every year. The enzyme acetylcholinesterase (AChE) is the main target of OPs, which phosphilate the Ser203 residue at its active site, interrupting the hydrolysis of the neurotransmitter acetylcholine (ACh), leading to cholinergic syndrome[1]. The compounds actually used as enzyme reactivators are cationic oximes, which usually possess low membrane and hemato-encephalic barrier permeation. Molecular modelling and dynamics studies showed that neutral oximes have potential to function as AChE reactivators[2]. We have used NMR methods for the evaluation of the capacity of neutral oximes, which display better membrane permeation, for the reactivation of AChE inhibited with the paraoxon. The NMR monitored kinetics of the paraoxon inhibited AChE reactivation showed that some neutral oximes have potential to be used as antidotes for OPs intoxication. These studies also showed that neutral oximes are also competitive reversible inhibitors of AChE. The enzyme-ligand interactions were determined by T_1 and DOSY and the enzyme kinetics by monitoring the time dependence of the ^1H signal intensity of the methyl groups of ACh (1.96 ppm, decreasing) and acetate (1.72 ppm, increasing), as shown in the figure for the inhibition of AChE with 2-thiophenylaldoxime (green) and 4-methoxyphenylaldoxime (red).



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P81
Non-alcoholic Fatty Liver Disease: a HR-MAS analysis

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Non-alcoholic fatty liver disease (NAFLD) is a common cause of chronic liver disease. The NAFLD includes non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH). The mechanisms of NAFLD to NASH transition remain to be clarified. NAFLD appears to originate from the dysregulation of hepatic lipid metabolism as a part of the metabolic syndrome accompanied by visceral obesity dyslipidemia, atherosclerosis, and insulin resistance. High Resolution Magic Angle Spinning (HR-MAS) NMR is a useful tool for the metabolic characterization of intact tissues and can be used to support the clinical diagnosis. The aim of this study is to characterized the NAFLD and NASH metabolism using HR-MAS NMR Spectroscopy, and to evaluate the possible transition from NAFLD to NASH. Liver needle biopsies were collected for the HR-MAS and histological analyses. Preliminary HR-MAS NMR results show a higher amount of lipids in the biopsies from patients with 30-50% of steatosis (fig. 1, spectra 1, 2), whereas lipids and of small metabolites are present when the liver is affected from a market fibrosis (fig.1, spectrum 3).

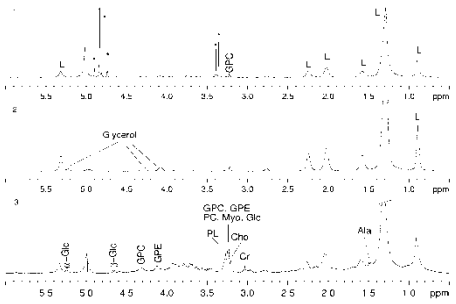

Figure 1: *Ex vivo* HR-MAS ^{1}H NMR spectra of steatotic liver biopsies. Major metabolites and lipids are labeled.

P82
CCPN – NMR analysis software and software pipelines

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The CCPN project was set up to serve as a nucleus for collaborative, open software development within macromolecular NMR. The basis of our work is the CCPN data standard for NMR and structural biology, supported by extensive subroutine libraries, with tools for producing and maintaining both.[1] Based on these standards we have developed the CcpNmr suite for visualisation, analysis and data extraction[2] (Analysis), conversion to and from external data formats[2] (FormatConverter), and deposition to the BioMagResBank and PDB[3] (ECI). We have also worked extensively with other developers of NMR software to produce an integrated software pipeline covering all stages from data acquisition, through assignment analysis, structure generation and validation to deposition.

The poster presents the newest developments of the CCPN software, including the end-of-project release of the Extend-NMR software pipeline with its joint interface, the CcpNmr ECI deposition tool, and the latest release of CcpNmr Analysis,with improvements in documentation, support for solid state NMR, protein labelling schemes, automatic assignment, and more.

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P83

NMR Studies Reveal Distinct Modes of Retinol Binding and Releaseby its Two Primary Cellular Carriers

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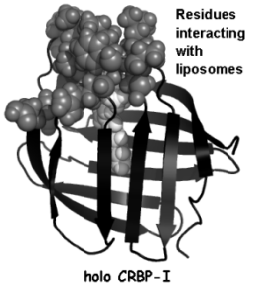
Vitamin A is an essential precursor in the biosynthesis of critical metabolites, through which it exerts multiple biological effects. Many insights into the retinoid metabolism have come from studies of their plasma and cytoplasmic carriers. The two primary cellular retinol-binding proteins are CRBP-I and CRBP-II, that play central roles in the maintenance of vitamin A homeostasis by directing it to the proper enzymes, either for storage as retinyl esters or for oxidation to retinaldehyde and retinoic acid. CRBP-I shows wide tissue expression, while CRBP-II is present only in the enterocytes. The study of proteins dynamics in the *μs-ms* timescale combined with line-shape analysis of ^{15}N-HSQC spectra recorded during a retinol titration provided new insights on the mode of ligand binding to CRBP-I.[1]

In the present study we have applied the same approach to CRBP-II. The data indicate the existence of low-populated ″active″ conformers in the apo protein which are able to sequester retinol from solution,[2] very differently to CRBP-I.[1]

Considering that both homologs deliver retinol to several membrane-associated enzymes, we also started to address the mechanism of ligand release by performing NMR experiments in the presence of membrane mimetic systems. Again the results suggest a different behaviour of holo CRBP-I with respect to holo CRBP-II, as will be discussed.

All these peculiar differences might help to account for the different tissue-specific expression patterns and distinct functional roles of CRBP-I and CRBP-II.



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P84

Use of NMR to study the interaction of heme with Human SOUL

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Human SOUL (hSOUL) is a 23 kDa heme-binding protein initially identified in the retina and pineal gland of chicken. SOUL is thought to be involved in photoreceptive functions, act as a circadian clock, or in the transport of heme to other heme proteins. Recent studies suggest that hSOUL is also involved in necrotic cell death by inducing mitochondrial membrane permeability.[1]

It has been suggested that hSOUL becomes hexameric upon heme binding with a single His residue involved as an axial ligand for Fe(III).[2] We have used NMR to study protein structure alterations upon heme binding which shows no evidence for a hexameric form. ^{1}H,^{15}N-HSQC experiments allowed us to follow chemical shift alterations upon heme titration and relaxation data has been used to identify dynamic alterations. Triple resonance spectra have been acquired for ^{13}C/^{15}N and ^{2}H/ ^{13}C/^{15}N labelled hSOUL samples in order to perform spectral assignment and identify the amino acids involved in these interactions.

hSOUL has the ability to bind several protoporphyrins and intrinsic tryptophan fluorescence has been used to determine dissociation constants for hemin and protoporphyrin IX binding to hSOUL. Results have been compared with p22HBP (27% sequence identity) the other member of the SOUL/HBP family of proteins. The p22HBP structure has been solved by NMR[3] and preliminary crystallographic data has already been obtained for hSOUL.[4]

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P85

Structural Characterization of a Domain Swapping Converted into a Fibrilar Architecture

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Amyloidosis is a clinical disorder caused by extracellular deposition of proteins that are normally soluble in their native conformation, but suffer conformational modifications resulting in insoluble and abnormal fibrils that impair organ function. Despite the many challenges that have been overcome, in this field, many questions remain unanswered and more improvements need to be made. So far, it still unknown which forces drives different primary structures into the misfolding pathway. The limitations of many biophysical and biochemical approaches to study fibril formation have slowed the advance in the understanding of how soluble proteins undergo conformational changes that result in aggregation.

In this work we followed some structural features belonging to Stefin B fibril by solid-state NMR. Stefin B is a domain swapping protein that is implicated in various pathologies of the brain. Its fibrils are composed of rigid and mobile parts that allowed us to select the region of the fibril to be analyzed based on mobility. ^{13}C-^{13}C correlations NMR spectroscopy were derived either from proton-driven spin diffusion (PDSD) or total through-bond correlation spectroscopy (TOBSY) under magic-angle spinning (MAS). The PDSD showed broad lines and few peaks suggesting high fibril mobility or high amount of peaks overlapped. In fact, the TOBSY and INEPT experiments showed several and well resolved signals reinforcing high fibril mobility. The ^{13}C-^{15}N correlations spectra were obtained either in the presence of cross-polarization or INEPT. The proposed assignment has suggesting the presence of a flexible N-terminal.

Despite of many results had been obtained more information concerning Stefin B structure seems to be necessary to try to solve the challenge about a runaway fibril structure.

P86

Exploring new possibilities in high field NMR combining 900MHz and a sample changer

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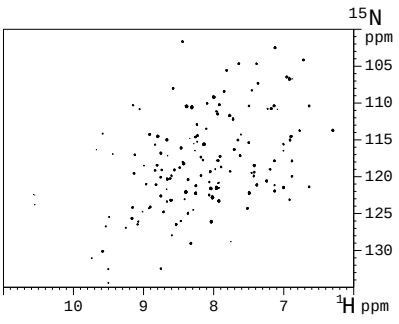
With the arrival of the Sample Jet, the newly installed 900MHz Bruker Avance III system at Lille1 University offers exciting new opportunities for future research. The combination of high field (900MHz), high sensitivity and resolution (using a TCI cryo probe) and high throughput (Sample Jet with temperature control) allows to study systems with NMR that were not considered to be feasible before. In addition, it is possible to work with small volumes (50µl in 1.7mm tubes) and thereby allowing NMR research on samples with limited availability (or high price).

As a proof of principle, samples of Cyclophilin A have been measured under different conditions to show the power of the system.



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P87

SSNMR study of intact E. *faecium* sacculi. Effects of the antibiotic resistance mechanism on the structure and dynamics of the cell wall

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E. *faecium* is a non pathogenic bacteria that develops a similar resistance mechanism to β-lactam antibiotics as M. *tuberculosis*. As for it's highly pathogenic counterpart, the mechanism relies on the selection of an alternative protein, the L,D-transpeptidase (Ldt_{fm}), involved in the synthesis of the bacterial cell wall. As a result, some of the structural features of the peptidoglycan cell wall are different in the resistant strains.

We are applying Solid State NMR on intact cell wall sacculi in order to characterize these differences in terms of molecular structure and dynamics.[1] The physical properties of the peptidoglycan material (size, flexibility, heterogeneity) make it a difficult system to tackle by standard NMR methods. Nevertheless we show here that a combination of liquid and solid state NMR pulse sequences allows the observation of structural differences depending of the active transpeptidation protein. Several methods are evaluated (T1 measurements, LGCP, R-PDLF) allowing a semi-quantitative characterization of the dynamics of the bacterial cell wall elements (glycan strands, peptide bridges, Teichoic acids). A comparison is presented for peptidoglycan originating from E. *faecium* bacteria grown under different antibiotic stress media.

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P88

Solid-state NMR investigations on membrane peptides from Hepatitis C virus

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Hepatitis C virus (HCV) genome encodes a 3000 residue polyprotein precursor at the endoplasmic reticulum membrane, resulting in at least 10 mature proteins, all associated to the membrane. Membrane proteins constitute a challenging class of proteins for their heterologous expression, purification and sample optimization. Solid-state NMR is well suited to structural and dynamical investigation at an atomic level of proteins that are insoluble and/or difficult to crystallize and provides ways to study membrane proteins in their lipids environment, thus in their fully functional state. Membrane peptides of HCV reconstituted in lipid bilayers are serving as models, as phospholipids often play a crucial role in their stabilization and folding. We started the optimization of sample preparation and experimental parameters on chemically synthesized peptides with uniformly [^{13}C, ^{15}N] labeled alanines, glycines and valines. Among them, the 31-residue N-terminal anchor of the NS5A protein, an in plane amphipatic α-helix, constitutes an attractive target for antiviral intervention since it is essential for HCV replication. We present here the sample preparation for solid-state NMR as well as the first 1D and 2D spectra of the synthetic peptide taken at 500MHz. 2D carbon-carbon DARR cross-signals of the peptide could be obtained, showing good linewidths of 0.8 ppm and enabling the resonance assignment of the labeled residues. Besides, we show the overexpression and purification protocols that have ben optimizede to enable full labeling of HCV membrane peptides, as well as first solution spectra of NS5A-Nter overexpressed in bacteria. NS5A-Nter is ready to serve as a model membrane peptide to probe peptide-lipids interactions.

P89

Solution structure of the protective D1 domain of *Streptococcus pneumoniae* RrgB pilus subunit

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Streptococcus pneumoniae, like many others Gram-positive bacteria, assembles long filamentous pili on their surface through which they adhere to host cells. Pneumococcal pilus-1 is composed of three subunits (RrgA, RrgB and RrgC); RrgB is the backbone component and the other two are the ancillary proteins.[1] Recently, the crystal structure of the D2-D4 domains of RrgB was solved at 1.6Å resolution. Moreover the three dimensional structure at 18Å resolution of the *whole* native pilus was obtained using a combination of electron microscopy (EM) and single-particle image reconstruction method. The rigid body fitting of the RrgBD2-D4 X-ray coordinates into the electron density map indicates that the pilus shaft is made exclusively by several copies of the RrgB protein arranged in a head-to-tail organization.[2] In order to define the structure and assembly mechanism of the pneumococcal pilus in this study we determined the solution structure of *S. pneumoniae* RrgB D1 domain by NMR spectroscopy. The D1 structure shows a common immunoglobulin-like (IgG-like) β sandwich fold. This work provides new information to understand the assembly mechanisms of the pilus and of how the ancillary proteins RrgA and RrgC are incorporated into the pilus backbone.

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P90

Structure determination of KSRP-RNA complexes

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K-Homology Splicing Regulator Protein (KSRP) is a multi-domain protein involved in important cellular processes, such as mRNA localization, splicing, and mRNA decay. Recent work has established the role of KSRP in promoting the degradation of AU-rich element (ARE)-containing mRNAs, making this protein a good model system for ARE-mediated mRNA decay (AMD) studies at the molecular level.

KSRP contains four K-Homology (KH) domains responsible for recognizing the target ARE sequences. The KSRP-RNA interaction recruits the degradation machinery promoting the mRNA decay. The malfunction of this mechanism has been related to inflammatory diseases and abnormal cell proliferation leading to cancer.

To characterize the molecular features of the functional mechanism of KSRP we have determined the structures of the isolated KH domains and studied their stability, dynamics, and relationship. We have performed NMR and CD-monitored RNA binding assays with several oligos spanning overlapping regions of the TNFa ARE core, the best known physiological target of KSRP, and evaluated the binding affinities and specificities of the transient KH-RNA complexes. The four domains have different sequence specificities and in particular, KH3 recognizes short G-rich stretches with high specificity and affinity. Work in our group revealed that KH3 recognizes the 5' GGG triplet in the terminal loop of pre-let7 miRNA. In order to understand the role of KH3 in the recognition of G-RNA stretches the solution structure of two complexes KH3-AGGGU and KH3-UGGGU are solved. KH3 interacts with the RNAs using the canonical hydrophobic RNA binding surface of KH domains. The interactions are primarily hydrophobic and the RNA adopts an extended single-stranded conformation.

P91

Solution structure and membrane-binding of the toxin Fst of the *par* toxin-antitoxin system

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Toxin-antitoxin systems, also known as addiction systems, are highly common in plasmids and bacterial chromosomes. By providing the host cell continuously with a toxic protein and an ongoing degraded antitoxin, these systems ensure that cells that have lost the plasmid do not survive.[1]

The *par* toxin-antitoxin system guarantees stable inheritance of the plasmid pAD1 in its native host *Enterococcus faecalis*.[2] It codes for the toxin Fst and a small antisense RNA which inhibits translation of toxin mRNA and is the only known antisense regulated toxin-antitoxin system in gram-positive bacteria. This study presents the structure of the *par* toxin Fst, the first atomic resolution structure of a component of an antisense regulated toxin-antitoxin system.[3] The mode of membrane binding is determined by relaxation enhancements in a paramagnetic environment by addition of Gd(DTPA-BMA). The determination of PRE values allows positioning of peptides in membrane mimicking micelles.[4]
Fst binds in a transmembrane orientation with the C-terminus likely pointing towards the cytosol. It forms a membrane-binding α-helix in the N-terminal part and contains an intrinsically disordered region near the C-terminus.

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P92

Magic angle spinning NMR studies of class-I and class-II intact filamentous bacteriophage viruses

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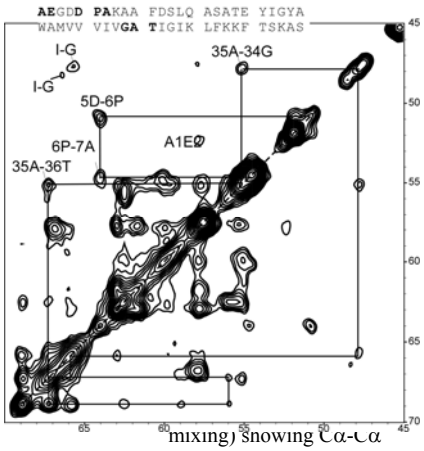
Filamentous bacteriophages are viruses that infect specific bacterial hosts. They all have a rod-like shape and are comprised of a circular ssDNA, wrapped by several thousands of copies of a major coat protein, each made of approximately 50 amino acids. The phages are divided into two structural groups according to their capsid symmetry. Pf1 bacteriophage (Class-II) and fd (Class-I) have been studied extensively by fiber diffraction and static solid-state NMR of aligned samples, and by cryo-EM (fd). Our magic-angle spinning NMR studies on infectious, wild-type, intact viruses reveal new information on these systems. For Pf1, which undergoes a structural phase transition at ~10°C, the residues driving the transition are identified and associated with the hydrophobic surface connecting the capsid proteins. These results were based on careful analysis of chemical shift changes between the two forms of the virus. For the fd phage we show that the capsid is highly symmetric in the precipitated and unaligned form (other studies by fiber diffraction, static ssnmr and cryoEM required a Y21M mutation to obtain structural homogeneity). Despite the highly condensed character of helical coats, we manage to identify and to site-specifically assign many amino acids in the sequence using multi-dimensional NMR experiments, and correlate them to secondary structure elements.



mixing) showing Cα-Cα contacts in fd phage

Acknowledgments: Ann McDermott (CU, NY), Loren Day (PHRI), Omry morag & Gili Abramov (TAU).

P93

Interaction Studies and Structure Determination of the Pin1 WW Domain and phosphorylated Ligands by ITC and NMR

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The prolyl cis/trans isomerase Pin1 catalyses the cis to trans interconversion of prolines located directly downstream of phosphorylated serines or threonines. Recently it was shown that Pin1 decreases Smad3 protein levels in a PPIase dependent manner.[1] The receptor regulated Smad3 is a TGFß signal mediator that consists of two globular MH1 and MH2 domains connected by a linker. The Smad3 linker contains four serine/threonine phosphorylation sites followed by proline, suggesting that cis/trans isomerisation of the peptide bond could regulate the interaction to other proteins. We expressed and purified the Pin1 WW domain as a GST-fusion protein and synthesized four peptides (7-10 aa) containing either one or two of the four Smad3 pS/pTP motifs by Fmoc-solid phase peptide synthesis. We identified one phosphorylation site as a preferred binding motif for the Pin1 WW domain by ITC and NMR titration experiments. In order to optimize the conditions for NMR experiments we measured binding affinities between the Pin1 WW domain and pSmad3 at different pHs by ITC and observed an increased binding affinity by changing to higher pHs. We finally solved the complex structure of the Pin1 WW domain bound to the pSmad3 ligand by NMR and identified a N- to C-terminus orientation of the bound peptide, typical for group IV of WW domains.

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P94

Structural Analysis by EPR and Site-Directed Spin Labeling of the Role of the Flexible Chaperon Protein NarJ in the Biogenesis of Nitrate Reductase

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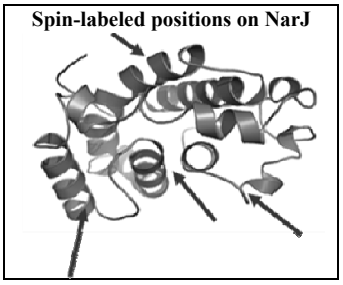
Nitrate reductase A from *Escherichia coli* is a respiratory enzyme induced upon growing in anerobiosis and in the presence of nitrate. This membrane-bound complex is composed of three subunits containing eight metal cofactors: the catalytic Mo-cofactor, five FeS clusters and two b-type hemes. The biogenesis of this Mo-enzyme is a complex process in which the role of the chaperon protein NarJ appears to be essential:[1] this 236 aminoacids protein was shown to trigger the sequential insertion within the catalytic subunit NarG[2, 3] of both the Mo-cofactor and of a FeS center with S=3/2 spin state.



To understand the molecular basis of this biogenesis process, the interaction site of NarJ with model peptides mimicking the N-terminal region of NarG were investigated by EPR and site-directed spin labeling. Our EPR results and spin-spin interaction analysis reveal the flexibility of NarJ and demonstrate that important structural transitions undergone by the chaperone protein are involved in the recognition process.

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P95

Structural investigations on the 40.5 kDa Ure2 prion: sequential assignments of its globular domain by solid-state NMR

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A subset of neurodegenerative illnesses is intimately linked to the misfolding and subsequent aggregation of an infectious protein: the Prion.

The yeast Ure2 prion is a two-domain protein. The structure of the globular C-terminal domain (94-354) was solved by X-ray crystallography.[1] The N-terminal (1-93) domain is essential for prion propagation. Structural investigations on Ure2p fibrils by solid-state NMR are challenging considering the protein size (354 residues). We present here solid-state NMR experiments on full-length Ure2p yeast prion fibrils, which demonstrate that fibrils formed under near-physiological conditions have a mostly well-ordered and well-defined atomic structure leading to highly resolved NMR spectra.[2] Data presented here include 2D and 3D solid-state NMR experiments of the isolated prion and globular domains, as well as on the native-like full-length Ure2p fibrils. Using a recently developed optimized set of 3D solid-state NMR experiments[3] we achieved the *de novo* sequential assignment of 74% of the 30 kDa C-terminal domain, which is the largest monomer assigned by solid-state NMR today.

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P96

Determination of Ile and Leu side-chain conformations in ground and excited states of proteins from chemical shifts

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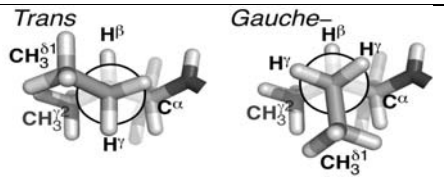
Although it has become clear that backbone chemical shifts are strongly correlated with backbone conformation it is still not straight forward to derive the side-chain conformations from chemical shifts. Here we establish simple relationships between side-chain chemical shifts and side-chain rotamer conformations for isoleucine and leucine residues, thereby extending the utility of chemical shifts as probes of structure and dynamics to also include side-chains.



The two most populated χ2 conformations of the isoleucine side-chain in solution: *Trans*, 66%; *Gauche*–, 30%. One γ-gauche effect is present in *Trans*, $C^{\delta1}$-$C^{\gamma2}$, while two γ-gauche effects are present in the *Gauche*–, $C^{\delta1}$-$C^{\gamma2}$ and $C^{\delta1}$-C^{α}, which is responsible for the chemical shift difference of ~6 ppm between $\delta_{trans}(^{13}C^{\delta1})$ and $\delta_{gauche-}(^{13}C^{\delta1})$.

Of interest for relating side-chain chemical shifts to structure and dynamics is the γ-gauche effect. Here the chemical shift of an aliphatic carbon is strongly influenced by its orientation relative to γ-substituents. We show that the side-chain χ2 rotamer conformation of Ile residues can be derived from the $^{13}C^{\delta1}$ chemical shift and that the population of the *Gauche*– conformation is given by the simple relation, $p_{Gauche-}(Ile)=[14.8ppm - \delta(^{13}C^{\delta1})]/5.5ppm$. Similarly, the conformational sampling of Leu side-chains can be derived from the chemical shifts of the $^{13}C^{\delta1}$ and $^{13}C^{\delta2}$ nuclei. Chemical shift measurements are very sensitive and the relationship between shift and structure that is derived allow determination of side-chain conformational sampling in very challenging systems, such as large protein complexes (> 1MDa) and excited states of proteins. As an example, we derive the conformational sampling of Ile and Leu of a protein folding intermediate.

P97

Direct assignment of EPR spectra to structurally defined iron-sulfur clusters in complex I by double electron-electron resonance

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The In oxidative phosphorylation, complex I (NADH:quinone oxidoreductase) couples electron transfer to proton translocation across an energy-transducing membrane. Complex I contains a flavin mononucleotide to oxidize NADH, and an unusually long series of iron-sulfur (FeS) clusters, in several subunits, to transfer the electrons to quinone. Understanding coupled electron transfer in complex I requires a detailed knowledge of the properties of individual clusters and of the cluster ensemble, and so it requires the correlation of spectroscopic and structural data: this has proved a challenging task. EPR studies on complex I from *Bos taurus* have established that EPR signals N1b, N2 and N3 arise, respectively, from the 2Fe cluster in the 75 kDa subunit, and from 4Fe clusters in the PSST and 51 kDa subunits (positions 2, 7 and 1 along the seven-cluster chain extending from the flavin). The other clusters have either evaded detection or definitive signal assignments have not been established. Here, we combine double electron-electron resonance (DEER) spectroscopy on *B. taurus* complex I with the structure of the hydrophilic domain of *Thermus thermophilus* complex I. By considering the magnetic moments of the clusters and the orientation selectivity of the DEER experiment explicitly, signal N4 is assigned to the first 4Fe cluster in the TYKY subunit (position 5), and N5 to the all-cysteine ligated 4Fe cluster in the 75 kDa subunit (position 3). The implications of our assignment for the mechanisms of electron transfer and energy transduction by complex I are discussed.

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P98

A charge-sensitive loop in the catalytic domain of FKBP38 modulates binding to Bcl-2

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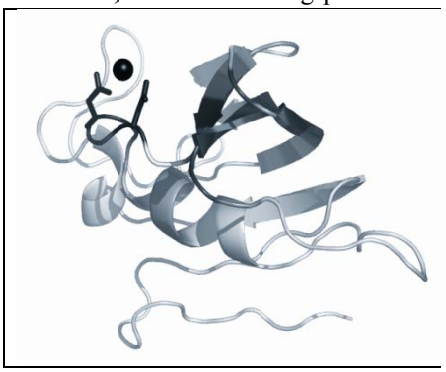
We identified for the first time a low-affinity binding site for cations in an FKBP domain. This electrostatic interaction at the catalytic domain of FKBP38 influences the interaction with and regulation of Bcl-2, a known binding partner of FKBP38. Mainly responsible for this charge sensitivity are the acidic side-chains of Asp92 and Asp94 within the α5-β1 loop of the FKBP domain as identified by chemical shift perturbation mapping. Determination of the binding constants for several cations (K^+, Mg^{2+}, Ca^{2+}, La^{3+}) indicated an influence of both ion net charge and ion radius, presenting Ca^{2+} as the most favoured binding partner of physiological relevance. Titration with Tb^{3+} revealed the position of the bound ion via pseudocontact shift data derived from $^1H/^{15}N$-HSQC spectra. The affinity of the CaM/FKBP38$^{35\text{-}153}$/Bcl-2 complex was attenuated both by addition of Ca^{2+} and by deletion of the cation-binding aspartates, as implemented in an FKBP38$^{35\text{-}153}$ D92N/D94N double mutant. Hence, the charge-sensitive loop in the catalytic domain of FKBP38 is apparently involved in the regulation of the protein function via electrostatic association to ligand targets such as other proteins or salt ions.



P99

A tri-fold magnetic resonance approach reveals the consequences of nucleotide binding to a multidrug resistance ABC transporter

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The multidrug ATP Binding Cassette (ABC) transporter LmrA is an integral membrane protein and a functional homologue of human P-glycoprotein involved in resistance against anti-cancer drugs in chemotherapy. LmrA forms a homodimer with the typical ABC transporter architecture of two nucleotide binding domains (NBDs) and two transmembrane domains (TMDs). It utilizes ATP binding or hydrolysis at its NBDs to drive the extrusion of toxic hydrophobic compounds via its TMDs. Combined, NMR and EPR present excellent tools to investigate all aspects associated with membrane transporter functionality, such as dynamics or structural effects. With a tri-fold magnetic resonance approach including solid-state NMR, solution NMR as well as pulsed EPR, a comprehensive picture of the functional dynamics of LmrA was developed. LmrA displays high flexibility in the apo state as shown by EPR. Our data indicate that the TMDs and NBDs undergo concerted domain movements upon nucleotide binding and that the TMDs adopt a favorable conformation with a significantly reduced degree of flexibility. A possible explanation for this observation was obtained from solution NMR on the isolated NBD: peptides emulating the coupling helices of LmrA were found to bind only in the presence of nucleotides. Distance constraints acquired from pulsed EPR measurements paint a dynamic picture of LmrA in agreement with the conformational changes expected from current structures. Nucleotide binding was investigated by ssNMR in more detail. In summary, our findings are in agreement with an alternating site mechanism with nucleotide induced interaction between the NBD and the coupling helices. Nucleotide binding is therefore sufficient to initiate the structural changes in the TMD leading to substrate extrusion.

P100

Molecular Interactions of GIP Incretin Hormone with its N-terminal Domain of GIP Receptor

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Diabetes is a major threat to the global community. In this regard, incretin hormones play an important role for secreting insulin for the beta-cell. One of the hormones, glucose-dependent insulinotropic polypeptide (GIP) is a gastrointestinal hormone that stimulates insulin secretion by interacting with a G-protein coupled receptor located in pancreatic β-cell. Due to its glucose lowering and insulinotropic properties, GIP is considered as a potential target for treating type 2 diabetes. In our laboratory, we identified the solution structures of GIP in various solution conditions including membrane mimicking (micellular and bicellular) media using NMR spectroscopy and computational modelling techniques. In order to exploit the potential of GIP for diabetes therapy, our research focus on understanding the GIP hormone-receptor interactions. In this work using NMR based docking approach we have determined the likely docking position of the hormone with its receptor binding region and revealed a likely interaction of GIP amino acid side chains with specific residues on the extra cellular domain from the GIP receptor. These results provide a basic understanding of the interaction mechanism of GIP with its receptor that can be useful for studying the development of peptide or non-peptide drugs for treating type 2 diabetes and other related disorders.

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P101

Understanding how FLIPs inhibit death receptor-mediated apoptosis

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Upon stimulation, death receptors such as Fas/CD95 recruit the adaptor protein FADD and procaspase-8 into the death-inducing signalling complex (DISC). Assembly of the DISC promotes the dimerisation and activation of procaspase-8 via an induced proximity mechanism. This process can be inhibited by a family of cellular and viral proteins known as FLIPs. cFLIP exists as long ($cFLIP_L$) and short ($cFLIP_S$ and $cFLIP_R$) splice variants, all capable of protecting cells from apoptosis by blocking procaspase-8 activation at the DISC. Several herpesviruses and poxviruses also express FLIPs to suppress apoptosis and promote their survival in host cells. The hallmark of FLIPs is the presence of tandem death effector domains (DEDs) that interact with the complementary DED of FADD and prodomain of caspase-8 to hinder caspase recruitment and activation. However, the underlying mechanisms remain unclear. At present structural information on the assembly and regulation of the DISC is relatively limited and DED complexes have remained elusive. To further characterise the molecular basis of FLIP-mediated inhibition of apoptosis, we have optimised the production of FADD for structural studies and successfully formed a stable FADD-FLIP complex. Here we present a detailed structural and biochemical analysis of the 44kDa FADD-FLIP complex. Our results offer new insights into the mechanism by which FLIPs subvert death receptor signalling.

Acknowledgments: We thank the Queensland NMR Network for access to excellent NMR facilities. This work was supported by the Australian National Health and Medical Research Council (NHMRC) from a project grant [351503] and RD Wright Biomedical Career Development Award [401748] to JMH.

P102

Possible involvement of superoxide and dioxygen with cryptochrome in avian magnetoreception[1]

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Many species of migratory bird have been shown to use the Earth's magnetic field for orientation although the biophysical mechanism of this sense remains unknown.[2] One of the two prevailing theories suggested to explain the phenomenon involves a magnetically sensitive radical pair reaction in the retina of the birds eye.[3] Recent studies have shown that migratory birds are disorientated by radio-frequency fields applied with frequency (~1.3 MHz) corresponding to the Zeeman splitting of an electron in the Earth's magnetic field (~ 47 μT).[4] It has been argued that in order for such a "Zeeman resonance" to appear, one radical of the pair must have no significant hyperfine interactions.[4] As superoxide and dioxygen fulfil this requirement, they have been suggested as a possible partner to the flavin radical in a magnetically sensitive radical pair formed in the photo-active protein cryptochrome.[4,5]

We examine the chemistry of these paramagnetic species and propose a viable reaction scheme. Superoxide and dioxygen have strong zero-field splittings due to spin-orbit coupling and dipolar interaction, respectively, and we demonstrate that both interactions could act as a source of magnetic anisotropy, and hence provide directional information to the bird. These interactions also cause the effective *g*-value of the radical to deviate strongly from 2.0 and are expected to induce extremely fast spin relaxation. As a result, neither offers a credible explanation of the *in vivo* EPR signals.

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P103

Two aromatic residues in yeast V-ATPase may be crucial for proton translocation

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V-type ATPases are ATP-dependent proton pumps which exist in endomembranes of all eukaryotic cells, as well as in the plasma membrane of specialised cells. They are involved in various processes, such as receptor-mediated endocytosis, intracellular trafficking and protein degradation. In humans, they participate in bone remodelling, osteoporosis, urinary acidification and maturation of sperm cells.[1] We have studied the seventh transmembrane helix (TM7) of subunit a of yeast V-ATPase in a membrane-mimetic environment by paramagnetic NMR spectroscopy. TM7 contains an Arg residue previously shown to be essential for proton translocation.[2] Surprisingly, we found this residue to be located inside of the membrane.[3] Also, the essential Arg is in close contact with two nearby aromatic residues. In contrast, a transmembrane peptide (WALPR) with a central Arg residue lacking nearby aromatic amino acids could be shown to reside outside of the membrane. Homology searches show aromatic residues near the essential Arg residue to be a highly conserved motive among V-ATPases of different organisms. Extensive future investigation remains to be carried out. Still, we propose a tentative mechanism for the involvement of the essential Arg residue in proton translocation, involving π-cation interactions with the aromatic residues.

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P104

The PsbQ protein from Photosystem II – The NMR point of view

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PsbQ is the smallest extrinsic protein (16 kDa) located on the lumenal surface of Photosystem II (PSII) in higher plants, cyanobacteria and green algae.[1] Its solution structure and interactions are the subjects of this research effort.

The high-resolution crystal structure[2] of PsbQ from PSII of *Spinacea oleracea* indicates two different structure domains: the C-terminal region (residues 45-149) folded as a four up-down α-helix bundle and the flexible N-terminal region (residues 1-44) with two parallel β-strands. In the crystal structure the loop formed by residues 14-33 („missing link") is highly disordered. It is supposed that PsbQ interacts via this N-terminal region with other proteins involved in binding to PSII.[2, 3] We initially focused on the NMR assignment as a prerequisite for further interaction studies by NMR.

We present the assignments of the PsbQ protein with complete backbone and nearly complete side-chain assignments of the missing link. Twelve of the 13 proline residues of PsbQ (including the very challenging polyproline stretches) could be assigned using ^{13}C-detected experiments. Additionally, titration experiments monitored by chemical shift, T_1 and T_2 relaxation provide insights into the interactions of PsbQ with calcium.

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P105
NMR of Bioactive Saccharides: Comparison of DFT and Experimental Data

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Theoretical DFT calculations, using B3LYP functional and the triple-ζ basis set, were aimed at analysis of both counter-ions and solvent upon the structure heparin oligosaccharides. NMR chemical shieldings and scalar spin-spin coupling constants were computed from the optimized structures of heparin disaccharides and tetrasaccharides. The analysis of the data showed the influence of counter-ions (Na^+, Ca^{2+}) upon conformations of IdoA and the glycosidic linkages in oligosaccharides. Electrostatic interactions among Na^+ ions and the negatively charged sulfates and carboxylates were found different in $^1C_4$ and $^2S_0$ forms of the IdoA residues. Such differences in positions of counter-ions and the differences in electrostatic interactions could explain in part the stabilization of various IdoA conformers. Three-bond proton-proton ($^3J_{H-H}$) and one-bond proton-carbon spin-spin coupling constants were also calculated from the optimized molecular geometries.

P106
NMR structural study of exopolysaccharide produced by bacterium isolated from cloud water

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Besides an importance of microbial exopolysaccharides (EPS) for their different applications in biotechnologies, their precise role in EPS-producing bacteria depends on the microorganism natural environment. Most of functions ascribed to EPS are of a protective nature - a protection against desiccation and predation by protozoas. Fructans were shown to protect microbial cells against abiotic and biotic stress, such as desiccation, freezing, antibiotics or toxic compounds.

Incubation of *Bacillus* sp. 3B6 on sucrose afforded complex mixture of metabolites. NMR structural analysis showed that this bacterium is able to transform sucrose into fructan EPS - levan, composed of 2,6-linked β-fructofuranoses. This result might have some implications considering the environment: first, production of levan could be an efficient way for this strain to survive in clouds which represent an extreme environment for living cells. This could explain why *Bacillus* genus is often present as cultivable strains in atmospheric water. Second, it was found that EPS could be an important factor controlling the formation of cloud droplets by changing the "wettability" of biological particles. Indeed the presence of lipopolysaccharide or polysaccharidic and proteic structures was shown to enhance the hydrophilicity of the cell surface. It can have a great importance concerning the cloud condensation nuclei (CCN) properties of strains in the atmosphere. Our results highly suggest that *Bacillus* sp. 3B6, which was isolated directly from cloud water collected in free troposphere of Puy de Dôme in France, could present the same properties in the atmosphere.

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P107

A dumbbell double nicked duplex dodecamer DNA with PEG_6 tether

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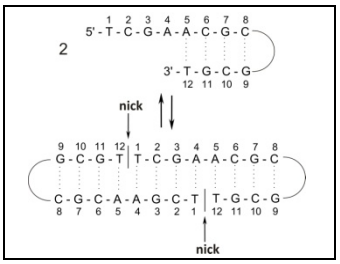
We have recently shown that nicked decamer DNA duplex with PEG_6 tether is a useful model for studying the binary complex with topotecan (topo I poison) in solution.[1] Here we present NMR characteristics of the hairpin motif and optimized conditions under which a dumbbell form, as a model to study the interactions with topo II poisons, can be observed in solution.



A classical approach to increase the dumbbell population by increase the buffer concentration does not work sufficiently at low solute concentration in a present case. We have therefore studied the concentration dependence of a solute, solvent and ionic strength influence on the position of the equilibrium in order to find conditions which would promote the dumbbell form at moderate concentration of the solute, and allow observation of sharp signals allowing to run NMR experiments based on scalar coupling. The addition of 15 vol. % of methanol also changes the equilibrium of both species, in favour of a dumbbell form. With addition of 15% of $(CD_3)_2SO$ or CD_3CN there is only one set of signals assigned to hairpin motif. At 2 mM solute, 25/200 mM ($NaCl/K_3PO_4$) buffer and 15% of methanol at 0 deg only the dumbbell form is observed. Computed structure based on NOESY and TOCSY spectra is presented.

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P108

Solution of spatial structure of PH and C2 domains from oncoprotein BCR/ABL by heteronuclear NMR. The first step to development of alternative medicaion for the treatment of chronic myeloid leukemia

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Philadelphia (Ph) chromosome – reciprocal translocation between chromosomes 9 and 22 that leads to the fusion of the 5′ region of the bcr gene to the 3′ region of the abl gene. BCR-ABL fusion protein have constitutively active ABL tyrosine kinase activity. Bcr-ABL-induced signaling is known to activate Ras-dependent signaling, phosphatidylinositol-3-kinase/Akt, and Jak/STAT pathway. The modern approaches in treatment of chronic myelogenous leukemia patients are mainly focused on inhibition of Abl-kinase domain. Unfortunately, many resistant clones of Bcr-Abl gene are appeared under the treatment. The PH domain consists around 120 amino acids, specifically binds to PtdIns(3)P, PtdIns(4)P and PtdIns(5)P phosphoinositides in biological membranes as a number of other proteins involved in vital cellular processes.[1] C2 domain binds with the components of the mammalian endosomal sorting complex required for transport (ESCRT) and regulates protein sorting process during endosomal trafficking.[2] We choose PH and C2 domains of BCR protein as a target for find a new tangets to inhibit this oncoprotein. Now, we plan to solve 3D structure of both, PH and C2 domains, from Bcr/Abl protein by multidimensional NMR spectroscopy. Unfortunately, limited solubility recombinant proteins and low stability stop our work. At the moment, looking for optimal conditions for NMR sample (pH, buffers, salt, etc) using microdrop screening method.[3]

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P109

In-Cell NMR studies for molecular interactions and folding stability of proteins inside human cells

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In-cell NMR is an isotope-aided multi-dimensional NMR technique that enables observations of conformations and functions of proteins in living cells. However, application of in-cell NMR has been limited to *E. coli* or *Xenopus laevis* oocytes. For wider application, we have established a method to obtain high-resolution multi-dimensional heteronuclear NMR spectra of proteins inside living human cells. Proteins were delivered to the cytosol by the pyrenebutyrate-mediated action of cell-penetrating peptides (CPPs) linked covalently to the proteins. The proteins were subsequently released from CPPs by endogenous enzymatic activity or autonomous reductive cleavage.

In this presentation, we will demonstrate three applications of our in-"human cell" NMR. First, we detect protein-protein interaction of ubiquitin with endogenous binding partners. Intriguingly, the interaction was observed exclusively inside cells, but not in cell lysates. Second, we will demonstrate protein-drug interactions of FKBP12 with extracellularly administrated immunosuppuressants. Finally, we evaluate the folding stability of ubiquitin inside living cells by performing a hydrogen exchange experiment coupled with in-cell NMR spectroscopy.

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P110

NMR insight into the supramolecular structure of daunorubicin loaded poly(butylcyanoacrylate) nanoparticles

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Nuclear magnetic resonance (NMR) spectroscopy has been employed for structural characterization of daunorubicin-loaded poly(butylcyanoacrylate) nanoparticles. Measurements of the nuclear relaxation times (T_1) and application of diffusion ordered spectroscopy (DOSY) obtained through pulsed field gradient (PFG) NMR experiments have been performed to determine the supramolecular structure of the drug-polymer conjugates and to clarify the mechanisms of drug immobilization in the polymer matrix. The results confirm the coexistence of three different types of daunorubicin inclusion into the poly(butylcyanoacrylate) nanoparticles: (i) association with the polymer chain by H-bonds and/or dipole-charge interactions; (ii) physical entrapment in the polymer matrix; and (iii) adsorption on the surface of the nanoparticles. Due to the presence of these different modes of inclusion, the nanoparticulate drug delivery system has the potential for sustained delivery/release of daunorubicin *in vitro*. The present study shows how a synergistic combination of NMR spectroscopic techniques can be used to characterise the structural behaviour of complex nano-scaled intermolecular aggregates.

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P111
The photo-CIDNP effect in ^{15}N-labelled plant photosystem I and II

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Photo-chemically induced dynamic nuclear polarization (photo-CIDNP) can be observed by magic-angle spinning (MAS) NMR in frozen photosynthetic reaction centres (RCs), giving rise to a 10.000 fold increase of NMR signals.[1] This makes it a unique tool to directly access the heart of large photosynthetic protein complexes, where primary charge separation occurs. Although photo-CIDNP MAS NMR was successfully used to explore the electronic structure of bacterial RCs,[2] its application to plant RCs is limited.[3,4] Plant RCs are of particular challenge due to the difficulty of incorporating (specific) isotope labels.

Here we present data obtained from RCs of the small water plant *Spirodela Oligorrhiza* (duckweed).[5] We have successfully introduced ^{15}N isotope labels into the plant RCs and, by combining the resulting enhancement with the power of photo-CIDNP, we were able to straightforwardly determine the number of cofactors involved in primary charge separation in plant photosystem I and II. This information made it possible to come to a final assignment of the signals appearing in complex spectra obtained directly from ^{13}C-labeled entire duckweed plants.

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P112
Ensemble Descriptions of Intrinsically Disordered Proteins from NMR Data

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Over the last decade it has become increasingly clear that a large fraction (up to 40%) of the proteins encoded by the human genome are intrinsically disordered. Intrinsically disordered proteins (IDPs) remain functional despite a lack of a well-defined structure. The classical structure-function paradigm breaks down for this class of proteins and new methods are required to provide essential insight into the relationship between primary sequence and molecular function.

The high intrinsic flexibility of IDPs makes ensemble descriptions particularly appropriate to describe the conformational equilibrium. We have developed an approach, ASTEROIDS, that selects representative structural ensembles of IDPs that reproduce NMR data within experimental error. We have applied ASTEROIDS in conjunction with RDCs, demonstrating site-specific mapping of conformational space in urea-denatured ubiquitin. We have also incorporated the use of MTSL-induced paramagnetic relaxation enhancements in α-synuclein, taking into account the mobility of the spin label for each conformer of the ensemble, as well as possible distortions of the RDC-baseline from significantly populated long-range contacts in the disordered chain. Finally, we have shown that in combination with ^{13}C and ^{15}N chemical shifts, ASTEROIDS identifies entire secondary structural elements and their associated populations, as well as characterizing the subtle detail of local conformational sampling in disordered proteins. This raises the exciting prospect of probing the conformational behavior of IDPs under conditions where additional parameters cannot be easily measured, but where chemical shifts are still readily available, for example in crowded or cellular environments.

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P113
Mechanism for recognition of polyubiquitin chains

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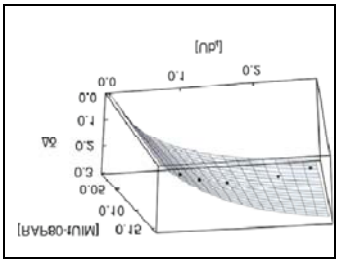
RAP80 plays a key role in signal transduction in the DNA damage response by recruiting proteins to DNA damage foci by binding K63-polyubiquitin chains with two tandem ubiquitin (Ub) interacting motifs (tUIM). We used NMR spectroscopy to characterize the binding of RAP80-tUIM to mono Ub and extended polyUb chains. It is generally recognized that the typically weak interaction between Ub and various recognition motifs is intensified using themes such as tandem recognition motifs and Ub polymerization to achieve biological relevance. However, it remains an intricate problem to develop a detailed molecular mechanism to describe the process that leads to amplification of the Ub signal. RAP80-tUIM employs multivalent interactions with ubiquitin chains, characterized by intrinsically weak binding and fast off-rates, that achieve enhanced affinity in comparison to mono Ub interactions alone. These weak interactions with fast kinetics may be an important factor underlying the transient nature of protein-protein interactions that comprise DNA damage foci.



P114
Modern NMR approach to the structure elucidation of bacterial polysaccharides

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Polysaccharides of bacterial cell walls play important role in pathogenesis of diseases, immune response and bacterial resistance.

NMR is powerful method for the investigation of bacterial polysaccharides. A combination of 1D, 1D-selective and gradient-enhanced 2D experiments allows to perform complete resonance assignment in ^{1}H, ^{13}C and ^{31}P spectra with further analysis of resonance peaks and structure elucidation of polysaccharide repeating unit, even for non-labeled (^{13}C) samples. The diversity of monosaccharide residuals and strong overlap of spectral lines in specific regions, on the other hand, makes difficulties for peak recognition. 1D-selective (selTOCSY, selROESY) and 2D combined experiments (HSQC-TOCSY, HSQC-NOESY) helps in spectra assignment.

We present our research and structure of new polysaccharides for several series of bacteria, including Azospirillum, Rahnella, Mesorhizobiums, Bifidobacterium and Providencia species. Several unusual substituents in the repeating unit were revealed, with complete identification of their structure: (2R,4R)- and (2S,4R)-2,4-dihydropentanoic acids, 2-acetamido-2-deoxy-4-O-methyl-D-glucopyranose and 3-methyl-D-Rhamnose.

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P115
Measurement of Dynamics of Aromatic Rings in Proteins by Solution-State NMR Spectroscopy

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Not only three-dimensional structures but also dynamics have essential roles in the functions of proteins. Nuclear magnetic resonance (NMR) spectroscopy has emerged as one of the most powerful tools to investigate protein dynamics at atomic resolution. Although the increasing number of studies has addressed the dynamics of proteins using NMR, few have employed aromatic side chains as spin probes. The aromatic rings constitute important parts of proteins, such as the cores of the molecules and the binding sites with their ligands. Major difficulties of NMR experiments on the aromatic residues reside in the sizable homonuclear C–C scalar couplings in the rings, which cannot be decoupled by simple pulse techniques, and their ring flipping motions, which can potentially manifest as exchange phenomena and complicate quantification of experimental data. Here we show a method to examine the dynamics of the aromatic rings in proteins on the picosecond to nanosecond time scale by solution-state NMR. The approach is based on a ^{13}C-labeling scheme that accomplishes production of $^{1}J_{CC}$ coupling-free aromatic rings and the longitudinal and transverse ^{1}H–^{13}C dipolar/^{13}C chemical shift anisotropy (CSA) cross-correlated relaxation rates, η_{Cz} and η_{Cxy}. Unlike the transverse relaxation rate, R_2, the η_{Cxy} rate is unaffected by exchange phenomena that occur on the microsecond-millisecond time scale, such as the aromatic ring flipping in the slowest limit. By using the two relaxation rates, dynamic parameters of aromatic rings are reliably extracted employing the Lipari-Szabo model-free formalism. The results show that buried and partially exposed aromatic rings have significantly different motional properties, and that the ring flipping motions of the latter, like those of the former, still take place at a time scale slower than the overall rotational correlation time of the molecule. The new methodology will enable us to analyze the flexibility of the aromatic side chains thereby allowing us to gain further insight into protein dynamics.

Acknowledgments: S.K. thanks a fellowship from the Japan Society for the Promotion of Science.

P116
Small-Molecule Inhibitors of PDZ-mediated Protein-protein Interactions

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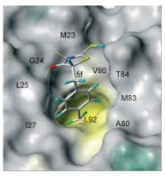
Protein-protein interactions (PPIs) are central to many biological processes which represents a large and important class of targets for therapeutics. PDZ (Post-synaptic density-95, Drosophila discs large, Zonula occludens -1) domains are protein-protein recognition modules that play a central role in organizing diverse cell signaling assemblies. PDZ binding specificity involves the recognition of the carboxyl-terminus of various proteins, belonging to receptor and ion channel families. Antagonizing PDZ-mediated interactions may allow for the treatment of several human disorders such as neuropathic pain, congenital diseases, psychiatric disorders, and cancers. NMR spectroscopy based screening methods are presented to identify small-molecules that bind to a protein target. The NMR techniques are based on the observation of chemical shift perturbations in two-dimensional (2D) ^{1}H-^{15}N HSQC and (1D) ^{1}H NMR line-broadening experiments. Here we report small-molecules for Shank3 PDZ, Af6 PDZ, Syn PDZ, Dvl PDZ and PSD95 PDZ with drug-like binding affinity.



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P117
Light by Spin Centers. A Magnetic Resonance Study of Bioluminescence in Bacteria

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Bioluminescence is the emission of visible light by a living organism catalyzed by enzymes and makes a great visual impact of one of nature's most remarkable reactions. It was discovered that the production of light is not accompanied by heat (Boyle, 1668), which was the first indication of the high efficiency with which light is produced. The emission of colored light by marine bacteria is generated by the oxidation of $FMNH_2$ and a long-chain aliphatic aldehyde, catalyzed by the luciferase.[1] Once $FMNH_2$ binds to the enzyme, it is protected from auto-oxidation. In the presence of oxygen, a flavin-hydroperoxide is formed that is still bound to luciferase. The reaction is proposed to continue *via* several radical intermediate states, in which the long-chain aldehyde participates.[2] The crystal structures of bacterial luciferases have become available only recently. The protein consists of a heterodimer of which the alpha and beta subunits have a slightly different molecular weight.[3]

Cells from *Vibrio fischeri* are grown under aerobic conditions and the luciferase is purified *via* FPLC using DEAE Sepharose. A light emitting reaction caused with decanal and reduced flavin gave evidence for the activity of the examined luciferase. UV-VIS spectroscopy of purified protein furthermore showed an absorption maximum at 411 nm and is a strong indication of the luciferase-bound flavin. The electronic structure of the reaction intermediates are investigated by electron paramagnetic resonance (EPR) spectroscopy. Recent cloning experiment provide the opportunity of heterologous expression of the bacterial luciferase and therefore a more efficient purification procedure.

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P118
NMR characterization of the regulation of microtubule dynamics by EB1

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End-binding 1 (EB1) is a member of plus-end-tracking proteins (+TIPs) that bind to microtubule (MT) plus-end and regulate MT dynamics. EB1 binds to MT with its N-terminal CH domain. Cryo-EM study suggested that Mal3p, the fission yeast EB1 homolog, specifically bound to MT A-lattice and thus promoted its polymerization.[1] Further, EB1 is thought to lead MTs to cell peripherals by interactions between its C-terminal domain and other +TIPs, APC and CLIPs, and cytoskeletal proteins that locate cell membrane.

We have attempted to reveal the molecular basis of MT-regulating mechanisms by EB1 using NMR spectroscopy. Chemical shift perturbation experiments of EB1 C-terminal domain with APC C-terminal region revealed that APC bound to EB1 via I2805 and P2806. This observation was consistent with previous report.[2] Moreover, we found that EB1 bound to APC by the same binding interface for dynactin subunit $p150^{glued}$. These results suggest the possibility that APC could compete $p150^{glued}$ by interacting with EB1. In addition, chemical shift perturbation experiments of CH domain with taxol-stabilized tubulin or colchicine-depolymerized tubulin, we found that CH domain bound to only polymerized tubulins specifically in solution. To identify the binding interface of CH domain for MT, we are trying to apply the transferred-cross saturation experiment[3] to this system at present. Taken together, we will discuss molecular basis of MT-regulating mechanism of MT dynamics by EB1.

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P119

A systematic approach to NMR-based structural glycobiology

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Recent advances in structural glycomics have enabled us to collect information on glycoforms, i.e. sequences, linkage, positions and profiling of carbohydrate moieties of glycoproteins in systematic manners. The central issue in the next stage of structural glycobiology is to provide structural basis of the biological functions of the individual glycoforms of glycoproteins and glycolipids. Although NMR spectroscopy has great potential to provide atomic-level information on these glycoconjugates, the carbohydrate NMR analyses are frequently hampered by the low sensitivity, the severe spectral overlapping, and the insufficiency of conformational restraints. In view of this situation, we have been developing a systematic method for the elucidation of the underlying mechanisms of the glycan functions by combined use of high-field NMR spectroscopy and stable isotope labeling techniques of glycoconjugates along with sugar library constructed based on our home-made HPLC database 'GALAXY' (http://www.glycoanalysis.info/).[1]

In this presentation, we will illustrate several examples of applications of our stable-isotope-assisted NMR approach to characterize structures, dynamics, and interactions of oligosaccharides, glycoproteins, and glycolipids of clinical interest.

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P120

The SAGA of zinc-fingers goes on...

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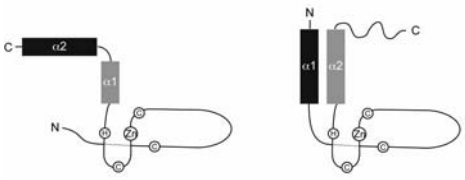
SAGA is a large co-activator complex involved in chromatin remodelling, which harbours both histone acetylation and deubiquitination activities. Two subunits of the SAGA deubiquitination module contain atypical zinc-fingers sequences characterised by a long sequence insertion between the first and second zinc coordinating residues. Biochemical experiments revealed the ability of one of these domains (ATXN7) to bind the H2A/H2B histone dimer, whereas the other one (ATXN7L3) is lacking this property. Comparison of the solution structures of the two zinc-finger domains revealed a novel, common zinc-finger motif at the heart of two distinct folds, providing a molecular basis for the observed functional differences. Chemical shift titration and saturation transfer experiments between zinc-fingers and highly purified nucleosomes or histone H2A/H2B dimers provided further insights into this interaction.



ATXN7 ATXN7L3

P121

Solid-state NMR structural studies of disease related membrane proteins

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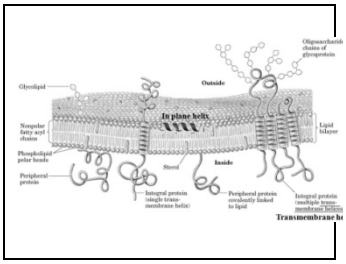
Membrane proteins, constituting nearly 30% of eukaryotic genomes, play central roles in cellular transport processes, intercellular signaling, and growth regulation. However, of the more than 28,000 high resolution protein structures known, only some 25 unique families of membrane proteins are represented. This inequality is accounted for by two bottlenecks in membrane protein structural studies: high-yield of integral membrane protein production and membrane bound structure which is difficult to study by using X ray crystallography and conventional solution NMR spectroscopy techniques. Solid-state NMR experiments on lipid bilayer samples are especially valuable for membrane proteins. Here we will present the optimized results of large scale growth and purification to get disease related membrane proteins like obesity related human melanocortin 4 receptor TM, dementia related Amyloid AβTM, and signal transduction related Syndecan 4, and antimicrobacterial peptide of bovine milk, Lactophoricin. And we will also present the solid-state NMR spectra of mechanically aligned planar lipid bilayer samples and magnetically oriented bicelle samples of disease related membrane proteins.



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P122

Dimerization of the Oncogenic Transcription Factor Myc

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The structural motif of the C- terminal domain of Myc is a DNA-binding and dimerization basic/helix-loop-helix/leucine-zipper motif (bHLHZip). The C-terminal domain of homodimeric v-Myc was already observed by Frykberg et al. 1987[1] postulating that the bHLHZip domain does not exist as a random coil in solution, but rather exhibits α-helical regions. Max is the main binding partner for C-terminal Myc, which also forms a bHLHZip motif on its C-terminal.

With X-Ray crystallography the heterodimeric structure of the Myc/Max/DNA complex is still ambiguous.[2,3] We analyze the transient complex formation characteristics of Myc with Max and its chemical mechanism and elementary steps, aiming to prove their antiparallel orientation as it could be proven for other dimers with bHLH motifs before[4] and to show apo-states of Myc. The orientation of Myc with respect to Max was analyzed by NMR relaxation measurements. The relaxation features of Myc give overall insight into the dimerization behavior of Myc.

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P123

NMR resonance assignments of NarE, a putative ADP-ribosylating toxin from *Neisseria meningitidis*

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NarE is a 16 kDa protein identified from *Neisseria meningitidis*, one of the bacterial pathogens responsible for meningitis. NarE belongs to the ADP-ribosyltransferase family and catalyses the transfer of ADP-ribose moieties to arginine residues in target protein acceptors. Many pathogenic bacteria utilize ADP-ribosylating toxins to modify and alter essential functions of eukaryotic cells. NarE was proposed to bind iron through a Fe-S center which is supposed to be implied in catalysis. We have produced and purified uniformly labeled ^{15}N- and $^{15}N/^{13}C$- NarE and assigned backbone and side-chains resonances using multidimensional heteronuclear NMR spectroscopy. These assignments provide the starting point for the three-dimensional structure determination of NarE and the characterizing of the role of the Fe-S center in the catalytic mechanism.

P124 (*)

Atomic Resolution NMR Structure of a Transient and Low Populated Protein Folding Intermediate

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Proteins can sample rare conformational states that are critical for biological function but that are seldom detected directly because of their low occupancies and short lifetimes. Direct detection of on-pathway folding intermediates and elucidation of their structures can be especially difficult. Here we use NMR spectroscopy to determine an atomic-resolution three-dimensional structure of a transient folding intermediate of a small protein module - the FF domain - with an equilibrium population of 2-3% and a millisecond lifetime. A general strategy is introduced for the structure determination of such excited states based on measurement of a nearly complete set of backbone chemical shifts along with residual dipolar couplings from NMR relaxation dispersion methods, thus opening a new direction in protein structural biology.

The structure of the FF domain folding intermediate is the first for a transient, metastable state - such states have long been thought to be ubiquitous along protein folding pathways. The intermediate is unexpectedly well structured and can be thought of as representing an alternative, less favorable fold of the FF domain. In this fold there is a significant number of non-native interactions that prevent the formation of the native structure in the C-terminal part of the protein, suggesting that non-native interactions along folding pathways are likely more common than previously thought. Our work establishes why a well-structured intermediate can be formed rapidly (μs timescale) and then rearrange slowly (ms timescale) to the native conformation. The determined structure also provides a simple way for designing a variant FF domain closely mimicking the intermediate with the native state selectively destabilized.

P125
STD NMR Studies of Substrate Specificity of Core Fucosyltransferase A

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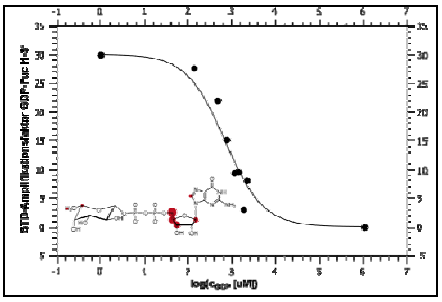
Fucosylated oligosaccharides play a crucial role in many physiological and patho-physiological processes. Recombinant core fucosyltransferase A (FUT-A) from honeybee (*apis mellifera*) was studied for donor substrate specificity by SPR and STD NMR techniques. Fragment screening by SPR revealed that the nucleoside part of the donor substrate GDP-Fucose contributes dominantly to binding affinity. These findings were confirmed by STD NMR ligand epitope mapping which showed the guanosine part binding closest to the enzyme surface in the enzyme-substrate complex.[1] Furthermore, according to SPR studies, the fucose part of GDP-Fuc has no effect on the dissociation constant of the enzyme substrate complex. However, competition of GDP-Fuc binding with GDP monitored by STD NMR allowed a more direct insight into the contribution of the fucose to binding affinity (cf. Fig). Data obtained from a competitive titration of GDP-Fuc against GDP revealed a K_i of 150 µM for GDP, taken K_D = 28 µM for GDP-Fuc as basis. Thus, the fucosyl residue improves binding by a factor of 5 equivalent to about 1 kcal/mol binding energy. Characterization of the binding epitope will be used for design of a selective inhibitor of FUT-A.



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P126
PELDOR Spectroscopy on Tertiary Folded RNA

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Pulsed electron electron double resonance (PELDOR) spectroscopy[1] is established as a powerful tool to measure long range distances (1.5–8 nm) in macromolecular systems such as polymers, oligonucleotides, and proteins, using the dipolar spin-spin interaction between two paramagnetic centers. We investigated the structure and conformational dynamics of different tertiary folded RNA molecules by means of site-directed spin labeling[2] and PELDOR spectroscopy.

Previous NMR/MD investigation on a 14-mer-cUUCGg-tetraloop[3] showed different dynamics of the two uridines in the loop region, however low temperature PELDOR experiments and CW EPR near room temperature do not support significant differences between this two uridines.

We studied conformational rearrangement of the neomycin-sensing riboswitch upon ligand binding.[4] PELDOR measurements at low temperature showed no distance alteration upon neomycin binding, which implies the existence of a prearranged tertiary structure without a significant global conformational change induced by ligand binding. Excellent agreement with structural models based on the NMR data of the ligand-bound state of the riboswitch was observed. The EPR measurements reveal the intrinsic propensity of the global riboswitch architecture toward its ligand-bound form.

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P127

Direct NMR Evidence for the Presence of Native and Non-Native Interactions in the Denatured States of Some Ultrafast Folders

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Deciphering the process of how nascent polypeptide chains attain a native fold only within a few tenths of a second in the crowded environment of a living cell has recently been acknowledged as a key element for better understanding how the formation of pathogenous protein aggregates occurs *in vivo*.[1] Residue-specific spectroscopic information on unfolded protein states, however, remains sparse due to their intrinsic conformational heterogeneity and dynamic nature.

Here, we present NMR data on the urea-denatured state of the ultrafast folding TC5b molecule, a small peptide exhibiting natively a globular fold and long-range interactions. By combining 2D NMR spectroscopy together with ^{15}N relaxation experiments on the unfolded state of TC5b and a structurally optimized point mutant, we were able to highlight the importance of both native *and* non-native interactions for ultrafast and productive refolding.[2,3] Among other things, NOE contacts between Trp and aliphatic amino acids exhibiting both native *and* non-native character were identified. Moreover, a mutationally induced enhancement of the nucleation site's hydrophobicity led to the detection of not only additional non-random interactions but also a concomitant acceleration of refolding rates. The results are complemented with ^{15}N $R_{1,2}$ and het. NOE measurements thus providing distance-independent sources of structural information.

Hence, our data productively contribute to the ongoing discussion of how only a few sequence determinants can direct the entire folding pathway of globular proteins starting from the very early stages of structure formation.[4]

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P128

The TPR2B domain of Sti1 is involved in blocking Hsp90 ATPase function

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Hsp90 is a molecular chaperone that activates a large number of client proteins. In particular maturation of the steroid hormone receptor substrates mediated by Hsp90 is strongly dependent on the function of the cochaperone Sti1/Hop. This adaptor molecule links the Hsp70 and Hsp90 chaperone systems by binding to both chaperones simultaneously thus allowing a substrate transfer from Hsp70 to Hp90.[1] Sti1/Hop contains 3 TPR (tetratricopeptide repeat) domains, which are generally known as peptide binding domains: TPR1 was shown to bind the Hsp70 C-terminal peptide whereas TPR2A binds the Hsp90 C-terminal peptide.[2] The function of the TPR2B domain however remains unclear.

Our data now show that TPR2B binds Hsp70 and Hsp90 C-terminal peptides with nearly similar affinity although NMR chemical shift perturbation patterns are different. Regarding its peptide binding ability this TPR domain can obviously not discriminate between Hsp70 and Hsp90. However we could identify an additional weak interaction of TPR2B with the Hsp90 middle domain by NMR that is independent from peptide binding. This indicates TPR2B playing a role in blocking Hsp90 ATP hydrolysis since Sti1/Hop was shown to be a strong inhibitor of Hsp90 ATPase activity.[3] A Sti1 fragment combining both the TPR2A and TPR2B domain displays increased binding to Hsp90 middle domain and maximum inhibition of Hsp90 ATP hydrolysis. We conclude that TPR2B together with TPR2A forms a joint binding site for Hsp90 middle domain independent from TPR peptide binding to block Hsp90 ATP hydrolysis.

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P129

Structure and dynamics of the HIV viral envelope gp41 fusion domain

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A homotrimeric construct encompassing residues 1-194 of the HIV viral coat protein gp41 is being investigated with TROSY-based NMR methods. Current work focuses on the structure and dynamics of the fusion domain of this protein. A comparison of amide chemical shifts with those of the isolated fusion peptide in SDS micelles[1] shows close agreement, indicating that the fusion domain in the intact trimer also adopts an alpha-helical structure when embedded in DDM micelles. The dynamics of the fusion domain has been studied by NMR relaxation measurements adapted for high molecular weight systems. We find the fusion domain to exhibit high amplitude motions with respect to the remainder of the protein.

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P130

Capillary Isotachophoresis hyphenated with slotted Microstrip Nuclear Magnetic Resonance Detection

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The high theoretical plate numbers and the flat flow profiles of capillary electrophoretic (CE) methods are optimal prerequisites for a successful hyphenation with NMR. Essential for hyphenation is a probe with a detection volume that matches the peak dimension of the CE separation. With their detection volumes being in the high nanoliter range, microprobes are well suited as detectors. In the common solenoidal microcoil designs, however, the sample capillary in electrophoretic separations has to be oriented perpendicular to the external magnetic field. Hence electrophoretic currents give rise to magnetic field gradients that cause substantial distortions in the NMR spectral linewidths:[1] the magnetic field in the capillary is a superposition of the external magnetic field and the magnetic field arising from the electrophoretic current and hence depends on the position of the spins in the capillary. The spectral resolution in the online detection mode is therefore drastically lower than in standard NMR measurements. A common workaround to avoid the detrimental line broadening effect is to sample an NMR spectrum in stopped-flow mode, at the expense of a considerable diffusion broadening of the CE peak profile. We here demonstrate that microprobes based on slotted microstrip[2,3] NMR detection allow high resolution NMR spectra to be obtained in online mode as well, as the sample tube can be oriented in parallel to the external magnetic field. The linewidth does not depend on the current, even at electrophoretic currents as high as 60 μA, that result in drastic linewidth enhancements of a factor 5 in solenoidal coil CE-NMR setups. The performance of the slotted microstrip detection is demonstrated for capillary isotachophoresis (ITP).

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P131
Red cell shape from NMR-^1H_2O *q*-space analysis

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Human red blood cells (RBCs) from healthy donors have the shape of biconcave discs, called discocytes. In some diseases, and under various metabolic conditions, RBCs adopt several possible shapes. These include cup-shaped cells called stomatocytes, and cells with ~20 membranous projections (spicules), called echinocytes. The *q*-space plots obtained experimentally using pulsed field-gradient spin echo nuclear magnetic resonance (PGSE-NMR) spectroscopy from water diffusing in RBCs of different morphologies have 'signature' features. To understand the forms of these plots, geometrical models of stomatocytes, echinocytes, and spherocytes, were taken as restricting boundaries for water diffusion in simulations using Monte-Carlo random walks.[1] For stomatocytes formed by treating RBCs with dithiothreitol the *q*-space plots had no diffraction features in contrast to those from a patient with hereditary stomatocytosis that showed distinct diffraction minima.[2] Numerical simulations of diffusion in stomatocytes indicated that diffraction minima are indeed expected, so it was concluded that the absence of diffraction minima in the dithiothreitol-treated cells was due to them not aligning with the external magnetic field unlike RBCs of normal discocyte shape.Spherocytes and echinocytes were prepared from RBCs by inhibiting glycolysis with NaF, and depleting the cells of Mg^{2+}, respectively. The experimental *q*-space plots from suspensions of RBCs from both treatments showed no diffraction minima. Monte-Carlo simulations, however, suggested that diffraction minima should be observed. Differential interference contrast (DIC) microscopy images of spherocyte and echinocyte suspensions showed them to be heterogeneous in cell shape and size. Therefore we concluded that the heterogeneous nature of the RBC suspensions resulted in the loss of the diffraction minima. These insights are relevant to interpreting *q*-space images of other cell-types and tissues, including clinical imaging.

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P132
Recognition Elements in 7SK RNA for HEXIM1 binding

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The 7SK RNA, an abundant snRNA, acts as a regulator of transcription by RNA polymerase II (RNApol II) by sequestering the positive transcription elongation factor b (P-TEFb) into a ribonucleoprotein complex that also contains the three nuclear proteins Hexim1, LaRP7 and MePCE.[1-3] The La-related protein LaRP7 and the methylphosphate capping enzyme MePCE act cooperatively to ensure the stability of 7SK and to promote the 7SK RNP assembly.[4] P-TEFb, formed by the kinase cyclin-dependent Cdk9 and the cyclin T1/T2, activates transcription by phosphorylating the C-terminal domain of RNApol II. For the activation of the transcription, P-TEFb is released from the 7SK RNP complex. The 7SK snRNA mediates the interaction of Hexim 1 with P-TEFb, enabling its inhibitory effect on the kinase activity of P-TEFb.

The mechanism of recognition between 7SK RNA and Hexim1 is not characterized at atomic level. The 5'-terminal hairpin of 7SK has been shown to support the Hexim1 binding.[5] In this work, we investigated how Hexim1 recognizes its target. We used Nuclear Magnetic Resonance in conjunction with biochemical techniques to define the elements in 7SK that are required for recognition by Hexim1. Our results clearly demonstrate that a motif located in the upper part of the hairpin appears to be essential for specific recognition.

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P133

Alteration of the axial coordination in the heme carrier HasA. Role of the ligands in the heme uptake and release process. The case of H32A and Y75A mutants

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Bacteria have developed different systems to take advantage of all the iron sources present in their hosts, including heme, free or bound to hemoproteins. One of them, the heme uptake system Has, has been identified in numerous pathogenic Gram-negative bacteria. The hemophore HasA from *Serratia marcescens*, was the first to be discovered and characterized. Heme binding is provided by His 32 and by Tyr 75. The heme iron is in the ferric state and presents a thermal high spin - low spin equilibrium in fast exchange on the NMR time scale. The spin state equilibrium is triggered by the hydrogen bond between its Oη and the Nδ1 of a nearby His 83.

To decipher the role of H32 and Y75 in heme uptake and release process, we perform a wide spectroscopic characterization of holoHasA-H32A and holoHasA-Y75A mutants. Electronic properties were addressed via absorption and Resonance Raman spectroscopies; EPR and paramagnetic NMR experiments were used to obtain information about spin states and Fe(III) coordination. Finally, chemical shift mapping and ^{1}H and ^{13}C direct detection experiments on WT, H32A and Y75A variants in both Fe(III) and Ga(III) derivatives provided information concerning global folds, pH dependent properties and iron coordination spheres. The whole body of experimental data will upgrade the existing picture on heme uptake and release processes.

P134

Measuring kinetics of conformational sampling in ubiquitin: Implications for protein-protein recognition

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The RDC enhanced NMR spectroscopy has recently detected intra-molecular protein dynamics in a previously hidden time window between a few ns and 50 μs (supra-τ_c) to play a major role for protein-protein recognition in a conformational selection scenario for ubiquitin.[1,2] Here we determine the mean lifetime of the different conformations in the ubiquitin solution ensemble to be about 1 μs at 309 K and approximately 50 μs at 265 K. This result is obtained by dielectric relaxation (DR) spectroscopy via a newly discovered mechanism of coupling conformational variation to the ion mobility. By considering RDC-derived ensembles and the time scale of inter-conversion between the different conformations as measured by DR spectroscopy, we are able to correctly predict NMR relaxation dispersion data of ubiquitin in super-cooled water, thus providing evidence for the 1 μs supra-τ_c motion with atomic resolution.

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P135
Advanced EPR Study on The Active Site of Ni-SOD

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Superoxide dismutases (SODs) protect cells against oxidative stress by disproportionating O_2^- to H_2O_2 and O_2. Recent finding of a Ni-containing SOD widened the diversity of SODs in terms of metal contents and SOD catalytic mechanisms, disclosing a new class among the redox-active Ni-containing enzymes. The coordination and geometrical structure of the site and the related electronic structure are the keys to understand the dismutase mechanism of the enzyme. We performed Q-band ^{14}N, $^{1/2}H$ CW and pulsed ENDOR and X-band ^{14}N ESEEM on the resting-state (Ni(III)) Ni-SOD extracted from *Streptomyces seoulensis*. In-depth analysis of the data obtained from the multi-frequency advanced-EPR techniques detailed the electronic structure of the active site of Ni-SOD. The analysis of the field dependent Q-band ^{14}N CW ENDOR yielded the nuclear hyperfine and quadrupole coupling tensors (**A** = [55 55 70.4] MHz, **P** = [0.4 0.6 -1.0] MHz) of the axial ^{14}N ligand ($N_{\square}$ of His-1 imidazole) of Ni(III). The tensors are coaxial with the **g**-tensor frame, implying the **g**-tensor direction is modulated by the imidazole plane. X-band ^{14}N ESEEM found a weakly-coupled ^{14}N nitrogen (**A** = [1.9 2.0 2.7] MHz) originating from $N_{\square}$ of His-1 imidazole. The nuclear quadrupole coupling constant of the nitrogen suggests that the hydrogen-bonding between $N_{\square}$-H and O_{Glu-17} present at the reduced state Ni-SOD is weakened or broken upon oxidizing the enzyme. Q-band ^1H CW ENDOR and pulsed ^2H Mims ENDOR observed the hyperfine coupling (**A** = [-3.8 -3.8 10.6] MHz) to the protons(s) of the coordinated His-1 amine, the equatorial ligand.

P136
NMR-based, Structural Insight into the Activation Process of a Molecular Chaperone Hsp33

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A molecular chaperone Hsp33 uses the oxidation state to modulate its activity, thereby protecting cells from severe oxidative stress. The expression of Hsp33 is regulated by heat at transcriptional level but post-translationally, it exhibits a holdase activity upon response to oxidative stress. Despite several crystal structures available, the redox-switch mechanism has been controversial. In this study, the structure of the reduced, inactive Hsp33 monomer was approached in solution by NMR spectroscopy. As a result, the secondary structure, global topology, and the interdomain interaction site of the inactive Hsp33 monomer were similar to those expected from the known crystal structures that showed an unexpectedly dimeric conformation. However, in NMR, three specific regions including beta-strands 1 and 10 and alpha-helix 1, as shown in the crystal structures, hardly exhibited NMR signals. In particular, beta-strand 1 in the crystal structures forms an antiparallel beta-sheet with the beta-strand 10 of which unfolding results in activation of the protein. Thus, we hypothesized that these regions are under dynamic conformational equilibrium, in real inactive state of Hsp33, in solution. In order to prove this hypothesis, a mutant forms lacking the beta-1 strand was produced. The results clearly indicated that the N-terminal deletions lead to constitutively active species. In addition, the dynamic properties in the alpha-helix 1 region might be involved in substrate binding.

P137

S-nitrosylation of C-terminal cysteine leads to structural changes and may regulates cell signaling processes of human *apo*-S100A1 protein

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S100A1 is a small EF-hand containing Ca^{2+}-binding protein, which exists as a homodimer. Despite strong efforts, the molecular mechanisms, through which S100A1 protein regulates cellular processes, are still not fully understood. Our previous studies demonstrate that S-nitrosylation of a C-terminal cysteine results in notable changes in three-dimensional structure of bovine S100A1 protein[1]. Estimation of changes induced by *S*-nitrosylation is important for understanding the mode of cellular signal transduction mediated by nitric oxide.

Here we present analysis of structural differences in human *apo*-S100A1 protein based on backbone chemical shifts perturbations which arise as a consequence of *S*-nitrosylation of the Cys85 thiol group. Our data indicate that hinge region (10 residues long sequence between two EF-hand motifs) as well as C-terminal parts of both subunits show noteworthy chemical shift alteration upon *S*-nitrosylation. This hydrophobic parts of human S100A1 structure are especially important for interaction with other biomolecules. The observed chemical shift perturbations strongly support our previous observations that *S*-nitrosylation of Cys85 causes structural changes of S100A1 protein and might control various cell signaling processes in which S100A1 protein is involved.

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P138

Structure based drug design for intrinsically unstructured proteins

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Increasing evidence accentuate the roles of intrinsically unstructured proteins in many devastating diseases. In particular, the self-assembly of such proteins is intimately linked with the molecular events leading to neuronal death in a range of neurodegenerative diseases. Drug development targeting this class of proteins is extremely challenging.[1] We present here a unique effort to rationalize this process. Our work involves development of new methods to describe the conformational space of unstructured proteins using NMR spectroscopy[2] and a critical assessment of NMR techniques for high-throughput drug screening for unstructured target proteins. Finally, we present a pioneering effort to use an NMR-derived ensemble structure of the intrinsically unstructured protein α-synuclein[3] to perform *in silico* screening of novel small molecule ligands. The misfolding and aggregation of this protein is a key event in the development of Parkinson's disease and our approach resulted in the identification of a drug-like small molecule which represents a new molecular scaffold that has not previously been reported to interact with α-synuclein or to modulate its self-assembly. Taken together, our results demonstrate that structure based drug design for intrinsically unstructured proteins is feasible.

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P139

NMR-based design and evaluation of novel inhibitors of the protein tyrosine phosphatase YopH

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We report on the use of a furanyl salicyl nitroxide derivative ("spin-labeled" compound), as a paramagnetic phosphotyrosine mimetic, to carry out a second-site screening by NMR techniques against the PTPase YopH from *Yersinia pestis*. By means of a fragment-based screening approach, we have identified several small molecules targeting YopH that bind at sites adjacent to the spin-labeled compound. These second-site binders have been then used to design and synthesize bidentate YopH inhibitors with sub-micromolar *in vitro* inhibition, selectivity against the human PTPase PTP1B, and cellular activity against *Y. Pseudotuberculosis.*[1]

We also describe the design and NMR-binding studies of novel cyclic peptides targeting the YopH N-terminal domain.[2] These studies may help in clarifying the structural determinants important for YopH inhibition and may pave the way to the design of even more active and drug-like compounds for the development of novel therapies against Yersiniae.

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P140

Using ligand-based NMR techniques to identify α-chymotrypsin inhibitors from a dynamic combinatorial library of boronate esters

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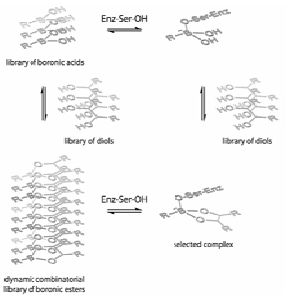
Dynamic combinatorial chemistry is a useful strategy in the area of drug discovery and inhibitor design.[1] It allows the generation of "dynamic combinatorial libraries" through reversible chemical interconversion of simple building blocks. Interactions inside the protein active site can hence be explored by the binding of these newly formed components, thus aiding the design and identification of lead compounds for drug discovery.



Boronic acids are well known inhibitors of serine proteases and serine β-lactamases.[2] It is also known that the addition of certain diols or sugars improves the inhibition of proteases by boronic acids.[3] The formation of boronate esters is reversible in aqueous solution under physiological pH conditions.[4] The reaction between boronic acids and diols can therefore be used to generate dynamic combinatorial libraries of potential inhibitors. Using α-chymotrypsin as a model system, we demonstrate the feasibility and address the challenges of studying such binding events using various NMR techniques such as direct observation using ^{11}B NMR, and indirect techniques such as waterLOGSY.

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P141

Measurement of changes in Calcium channel activity in rat neuronal tissue *in vivo* utilizing dynamic Manganese enhanced MRI (dMEMRI)

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The ability of Manganese (Mn^{2+}) to enter cells through Calcium channels (Ca^{2+}) make it a useful tool for functional as well as anatomical studies. By measuring changes of Mn^{2+} influx in tissue after Mn^{2+} and stimulant/inhibitor administration it should therefore also be possible to examine changes in Ca^{2+} channel activity *in vivo*. Previous studies observe for this purpose only the change in Mn^{2+} concentration in tissue. However, since agents acting on Ca^{2+} channel activity may also influence the overall Mn^{2+} concentration in blood, both the Mn^{2+} concentration in tissue and the Mn^{2+} concentration in blood have to be monitored in order to acquire full information about Mn^{2+} influx into tissue. In this study Mn^{2+} concentration in tissue and blood are measured using T_1-weighted dMEMRI over a 2 hr period after Mn^{2+} bolus injection. These concentration values are then evaluated using one of the two linear models, Logan plot[1] and Patlak plot.[2] The slope of Logan plot and Patlak plot, the so called total distribution volume (DVt) and the unidirectional influx constant (Ki) respectively, deliver information about Mn^{2+} uptake. Alteration of Ca^{2+} channel activity by stimulant/inhibitor administration should then be visible by observing a change in DVt and Ki. Measurements were performed for 15 rats at the anterior and posterior part of the pituitary gland (APit and PPit). An incomplete blood-brain-barrier (BBB) in that region enables almost unhindered Mn^{2+} influx. The two measured regions, APit and PPit, showed different Mn^{2+} uptake characteristics. While influx of Mn^{2+} into the PPit was reversible it showed to be irreversibly trapped in the APit during the course of the experiment. Application of the stimulant glutamate led to an increase in DVt of Mn^{2+} ions in the PPit and an increase of Ki in the APit. Application of the inhibiting agent verapamil led to a decrease in DVt in the PPit and a decrease of Ki in the APit.

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P142

Interaction study on human androgen receptor (AR) DNA-binding domain (DBD) and lysine-specific demethylase 1 (LSD1) SWIRM domain

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Prokaryotic and eukaryotic gene regulations are different. Prokaryotic gene regulation requires simply binding of regulatory proteins to help with or avoid forming transcription complex. For eukaryotes like humans, however, their regulation needs "chromatin remodeling" besides association of regulatory proteins, due to the need of opening up DNA-histone protein complex chromatin and unwinding DNA. Without remodeling, RNA polymerases responsible of transcription cannot get access and perform transcription.

Lysine-specific demethylase 1 is one of the chromatin remodeling enzymes. It can demethylate specifically the N-tail mono- or di-methylated K4 residue on histone H3 by oxidation.[1] LSD1 has three known domains: FAD-binding domain, demethylase domain and SWIRM domain. Till now, the exact function of SWIRM domain of LSD1 is still unclear, although its solution structure is solved and analyzed. In 2005, Metzger's group found that the LSD1 SWIRM domain could bind the N-terminus, the DNA-binding domain (DBD) and the ligand-binding domain (LBD) of androgen receptor (AR) by GST-pull down assay,[2] and showed that SWIRM domain has the strongest interaction among the domains of AR. This study is to investigate the interaction between ARDBD and LSD1 SWIRM domain by NMR techniques. We have carried out chemical shift perturbation titration of N-labeled ARDBD protein with unlabeled SW domain protein. This interaction shows a slow exchange of NMR titration profile. Interacting residues have been mapped onto the structure of ARDBD after backbone resonance assignments on a doubly labeled ARDBD protein sample.

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CopG repressor and CopG(A30E) defective mutant DNA binding studied by NMR

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Repressor CopG controls the replication of the promiscuous plasmid pMV158 by inhibiting the expression of the initiator of replication (repB) gene. Contacts of CopG span about 50 bp of the regulated promoter. The primary target of CopG is a 13-bp pseudo symmetric element (SE) that binds two dimers of CopG (each interacting with a half of the SE) and flanked by two additional outer binding sites (LA and RA). The current model for the whole DNA specifically contacted by CopG thus assumes the cooperative binding of four dimers of the protein[1]. Interdimer contacts induced by DNA binding play a main role on the activity of the CopG repressor as CopG mutants unable to form a number enough of those interprotein productive contacts loose the capability of the wild type protein to control the plasmid replication. In order to characterize the nature of such intermolecular contacts, we have determined the solution NMR structure of the native protein and of one defective mutant CopG(A30E) and studied their interaction with the cognate DNA.

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P144

Human RegIV Protein Adopts A Typical C-Type Lectin Fold But Binds Mannan With Two Calcium-Independent Site

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Human RegIV protein, which contains a calcium-dependent (C-type) lectin-like domain, is highly expressed in mucosa cells of the gastrointestinal tract during pathogen infection and carcinogenesis and may be applied in both diagnosis and treatment of gastric and colon cancers. However, the physiological function and possible carbohydrate ligands of this protein remain poorly characterized. Here, we provide evidence that, unlike other C-type lectins, human RegIV binds to polysaccharides, mannan and heparin in the absence of calcium. To elucidate the structural basis for carbohydrate recognition by NMR, we generated the mutant with Pro^{91} replaced by Ser (hRegIV-P91S) and showed that the structural property and carbohydrate binding ability of hRegIV-P91S are almost identical to those of wild-type protein. The solution structure of hRegIV-P91S was determined and showed that it adopts a typical fold of C-type lectin. Based on the chemical shift perturbations of amide resonances, two calcium-independent mannan-binding sites, which are mostly conserved in other mammalian RegIV proteins, were proposed. The second site is on the upper lobe of hRegIV-P91S, which is similar to the calcium-independent sugar-binding site on the carbohydrate recognition domain of Langerin. Interestingly, the first site, which is composed mainly of α2/β4 and β6/β7 loops, is adjacent to the conserved carbohydrate recognition site at position Ca-2 of typical C-type lectin. Moreover, model-free analysis of ^{15}N relaxation parameters and simplified CPMG relaxation dispersion experiments showed that a slow microsecond to millisecond time-scale backbone motion is involved in mannan binding by this site, suggesting a potential role for specific carbohydrate recognition. Our findings shed light on the sugar binding mode of Reg family proteins and we postulate that Reg family proteins evolved to bind sugar without calcium to keep the carbohydrate-recognition activity under low pH environments in the gastrointestinal tract.

P145

NMR studies of native and engineered forms of a Ring finger E3 ubiquitin ligase

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E3 ubiquitin ligases play a key role in the recognition of target proteins by catalyzing the covalent attachment of the ubiquitin and degradation by 26S proteasome.[1] Many known tumor suppressors or oncoproteins are RING type E3 ubiquitin ligases and among them the most well studied are the pairs of HDM2/HDMX, BRCA1/BARD1 RING fingers. Arkadia is a relatively new E3 ubiquitin ligase and comprise the first example of an E3 ligase that positively regulates TGF–β family signaling. Arkadia has been suggested to induce ubiquitin-dependent degradation of negative regulators of TGF–β signaling, through its C-terminal RING finger domain.[2] Based on our previous studies on HDM2 and HDMX RING fingers, the 68 a.a. Arkadia C-terminal polypeptide, including the RING finger, was cloned and expressed in its zinc-loaded form, as suggested by atomic absorption (two Zn(II)), and studied through multi-nuclear and multi-dimensional NMR Spectroscopy (BioMagResBank acces.no.. 15948).[3] Additionally, the 3D NMR solution structure of Arkadia RING finger was determined (PDB 2KIZ) and its interaction with the E2 UbcH5B enzyme was studies through titration experiments monitored by NMR The RING-E2 complex structure was also constructed through an NMR-driven docking protocol (using HADDOCK). Finally, engineered Arkadia forms are prepared bearing amino acid substitution inspired either by the atypical RING fingers sequences of HDM2 and HDMX or driven by identified cancer-related mutations observed in human tumors. Putting all these data together, new non-native RING finger forms were produced and currently are studied through multinuclear NMR

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P146

New Insights on the Protein-Ligand Interaction Differences between the Two Primary Cellular Retinol Carriers

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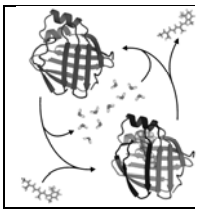

The cellular retinol-binding proteins types I and II (CRBP-I, CRBP-II) are the main retinol carriers in the cytosol. They exhibit distinct tissue distributions and play different roles in the maintenance of vitamin A homeostasis. Moreover, CRBP-I and CRBP-II feature a 100-fold difference in retinol affinity, despite the highly conserved three-dimensional protein fold and ligand-binding motif.[1-3]

Hydrogen/deuterium exchange data have revealed that many backbone amide protons exchange much faster in CRBP-II than in CRBP-I, both in the apo and holo form. This remarkable difference in intrinsic stability between the two homologs appears to modulate their binding properties: CRBP-I, as the stronger retinol-binder, displays a reduced backbone flexibility with respect to CRBP-II. A different pattern of potential salt-bridges on the protein surface and several key interactions inside the binding cavity have been identified and apparently provide an additional stabilization of the CRBP-I scaffold. Hence, a number of specific evolution-based sequence differences affect the internal protein dynamics to optimize each carrier for its particular physiological function.

Furthermore, by comparing 2D and 3D NMR spectra collected under a variety of solution conditions, it could be demonstrated that helix αII, which belongs to the characteristic helix-turn-helix motif in the ligand entry portal, exists in solution both in apo and holo CRBP-II, contrary to an earlier NMR report.[1] As a consequence, the previously proposed model[2] of retinol binding to CRBP-II needs to be revised.

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P147

The structure and dynamics of mouse α-Synuclein fibrils characterized by solid-state NMR spectroscopy

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Parkinson's disease (PD), the second most common neurodegenerative disorder, is caused by the loss of dopaminergic neurons in the substantia nigra region of the brain and is accompanied by the formation of Lewy bodies.[1] The major component of Lewy bodies is fibrillar α-synuclein (AS), a 140 residue-long cytoplasmic protein.[2] The primary sequence of mouse AS differs from human AS at seven positions. Like human AS, mouse AS has a "natively unfolded" structure in solution whereas at elevated concentrations mouse AS forms amyloid fibrils with predominant β-sheet secondary structure much more rapidly than its human counterpart.[3] The atomic-level structural details of the mouse AS fibrils are not yet well understood. Here, we present the characterization of structural and dynamic aspects of mouse AS fibrils using state-of-the-art solid-state NMR spectroscopy. We recorded a set of high resolution 2D and 3D NMR spectra on a uniformly $^{13}C/^{15}N$-labeled mouse AS fibril sample to obtain sequential resonance assignments. In the case of mouse AS which has a major straight morphology, residues from G41 to F94 were identified to be within the β–sheet rich fibril core by our current unambiguous resonance assignments. Major differences between mouse AS and human AS fibrils (B form)[4] are found for example around the mutation site S87N.

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P148

ATP Binding Induces Conformational Rearrangements of the Multidrug ABC Transporter LmrA: A PELDOR study

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The ATP-binding cassette (ABC) transporters are integral membrane proteins that couple hydrolysis of ATP to vectorial translocation of diverse substrates across cellular membranes. All ABC transporters share common structural architecture comprising two nucleotide binding domains (NBDs) where ATP is bound and hydrolyzed and two transmembrane domains (TMDs) which provide a translocation pathway for the substrate. LmrA is a homodimeric multidrug ABC transporter and a functional homologue of P-glycoprotein implicated in multi-drug resistance in treatment of cancer.[1] Here, we employ pulsed electron-electron double resonance (PELDOR) spectroscopy to gain insights into the functional mechanism and conformational dynamics of LmrA. For this purpose, several sites along the NBDs and helix six of the TMDs of LmrA have been selectively spin-labeled and the distance between them has been determined in five different intermediate states throughout the ATP hydrolysis cycle. The PELDOR data reveal that binding of ATP causes large changes in those distances and their distributions within both TMDs and NBDs implying that LmrA undergoes substantial conformational rearrangements during the catalytic cycle. Broad distributions of distances have been observed in the apo state pointing to a wide range of protein conformations. In contrast, narrow distance distributions have been observed in pre- and post-hydrolysis states (ATP, ADP•V_i and ADP) indicating that LmrA adopts a preferable conformation with a significantly reduced degree of flexibility upon nucleotide binding. Altogether, our findings reveal that nucleotide binding induces the structural changes in the TMDs leading to substrate extrusion, in agreement with the conclusions about communication between the NBDs and TMDs from structural studies of other ABC transporters.

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P149

Cell-Free Production of Membrane Proteins for NMR Studies

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The structural investigations of membrane proteins (MPs) require large-scale systems for their production. Cell-free (CF) expression systems are promising alternative to cell-based systems. In CF systems MPs can be produced in a soluble form by adding detergents, liposomes or lipid-protein nanodiscs (LPNs) directly into a reaction mixture (RM). The different approaches for CF production of helical MPs were studied using the transmembrane domain of human receptor tyrosine kinase ErbB3 (TM-ErbB3, 1TM, residues 632-675) and the isolated voltage-sensing domain of the K+ channel KvAP (VSD, 4TM, residues 1-148). Successful production of the TM-ErbB3 was achieved in three different ways: (1) in soluble form in presence of Brij detergents, (2) in soluble form in presence of LPNs, and (3) in the form of RM precipitate with subsequent refolding. In all cases high yields of the TM-ErbB3 (1.8-2.0 mg/ml of RM) were achieved. NMR analysis of selectively ^{15}N-Ile-labeled TM-ErbB3 obtained by different methods revealed concentration-dependent monomer to dimer transition, thus pointing to the correct folding of the protein. In contrast, the production of the VSD in soluble form in the presence of detergents or LPNs led to incorrectly folded domain. VSD conformation was monitored using a set of characteristic resonances in TROSY spectra of selectively ^{15}N-Ala-labeled protein. The successful refolding of the VSD with ~ 70% efficiency was achieved by solubilization of RM precipitate in urea/SDS mixture followed by detergent exchange to DPC on Ni^{2+}-NTA column. The final yield of refolded VSD was 0.6 mg/ml of RM. Obtained results indicate that CF production of polytopic helical MPs in soluble form does not always guarantee native folding of synthesized protein and in some cases additional refolding procedure is required.

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P150

The bigger the better: Large protein complexes investigated in solution by MAS NMR

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Various cellular processes like protein homoeostasis, gene transcription or cellular trafficking are controlled and regulated by large molecular machineries. The high molecular weight of these systems, and thus their slow reorientation in solution, imposes enhanced NMR relaxation properties and impedes the applicability of solution-state NMR.

Instead of accelerating the molecular tumbling for conventional solution-state NMR methods or completely abolishing molecular reorientation by crystallization or precipitation, we propose here to throttle the rotational diffusion of slow-tumbling macromolecules to push the system to the static limit. Magic-Angle-Spinning (MAS) NMR can then beneficially be applied in order to average anisotropic interactions and to accomplish adequate spectral resolution.

We applied the FROSTY approach (**F**reezing **R**otational diffusion **O**f protein **S**olutions at low **T**emperature and high viscosit**Y**) to human αB-Crystallin (αB) – a 20 kDa small heat-shock protein assembling into polydisperse 600 kDa particles. Comparison of spectra recorded on PEG-precipitated αB oligomers and those in solution revealed similar resolution and only marginal differences between the two sample preparations.[1] Furthermore, we were able to reproduce the feasibility of the approach on different constructs of the 20S proteasome of *Thermoplasma acidophilum* with molecular masses of 360 kDa ($\alpha_7\alpha_7$) and 670 kDa ($\alpha_7\beta_7\beta_7\alpha_7$), respectively. As the particle size increased, we observed improved resolution and signal intensities, revealing FROSTY MAS NMR spectroscopy as a suitable tool for the investigation of large protein complexes in solution.

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P151

The initial events of the unfolding of the human nephrocystin SH3 domain probed by hierarchical removal of NMR restraints

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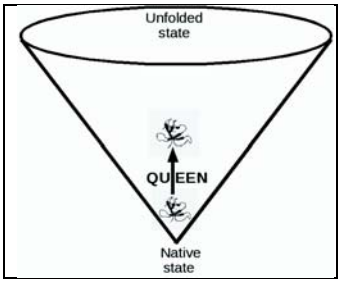
The stabilities of the wild-type and mutated nephrocystine SH3 domain were compared from molecular dynamics trajectories, using an original method based on the detection of the information hierarchy in NMR restraints. The trajectories in explicit solvent, started from two sets of NMR conformers, calculated with ARIA[1] from full and reduced sets of restraints, the reduced set being obtained by removing the most informative restraints detected by QUEEN[2]. The QUEEN information was shown to generalize the definition of the contact order, and to be closely related to the hierarchy of protein folding events. The use of a reduced set of restraints affect only marginally the global folding of NMR conformers, but induces nevertheless a destabilization of the protein structure along molecular dynamics trajectories and enhances significant differences between wild-type and mutant proteins. The destabilized structures display features similar to the observations previously made on other SH3 domains, for the initial events of unfolding process, and show also a destabilized water structure around the protein. The β-barrel shape, calculated using smooth constrained optimization, put in evidence a significant difference in structure stability, between wild-type and mutant proteins, in the case of the reduced restraints set. This geometric shape can thus be proposed as a general folding reaction coordinate for β-barrel structures.



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P152

Novel histone-binding ability of NuRD complex component CHD4

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Histone tails can have many different posttranslational modifications, such as lysine methylation or acetylation. Proteins that bind histone tails bearing specific patterns of modifications can affect chromatin packing, and hence transcriptional regulation. It is thought that the addition, recognition and removal of these modifications constitutes a 'histone code' that determines which parts of the genome are switched on and off at any given time in a given cell.

The nucleosome remodeling and histone deacetylase (NuRD) complex is a ~10-protein complex that has been shown to repress some target genes; however the molecular details by which this complex acts are not well understood. One of the core proteins is CHD4, a 218-kD protein that contains an ATP-binding helicase domain, two chromodomains and two PHD (plant homeodomain) fingers of unknown function. We have recently discovered that each of the PHDs of CHD4 has histone binding properties. Moreover, PHD2 exhibits unique binding preferences, in that it can simultaneously recognise the methylation state of two separate lysines on the N-terminal tail of histone H3. We have used NMR spectroscopy to determine the structure of PHD2 in complex with histone H3, revealing the molecular basis for this recognition. These data provide new insight into the mechanisms by which the NuRD complex regulates its target genes.

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Improved resolution in multidimensional Solid State NMR Spectra of proteins through 2D projections of 3D spectra

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Studies of biological macromolecules by NMR spectroscopy rely upon multidimensional, multinuclear experiments to separate, correlate and assign the large number of resonances present. Assignment of spin systems and related chemical connnectivities from 2D correlation maps can be challenging for molecules of large molecular weight.[2] 3D spectra offer bigger resolution at the price of a longer experimental time needed to sample systematically all of the grid points in the 3D frequency space. Instead of the customary step-wise exploration of the entire evolution space, multidimensional NMR spectroscopy can be speeded up appreciably by techniques based on minimal sampling, reducing the number of evolution periods examined independently.

This study will present the advantages of measuring 2D projections at a tilted angle $\pm\alpha$ of a higher-3D dimensional spectrum[3] in the field of Solid State NMR. Specifically, we will show the utility of this strategy in order to overcome the resolution limit of 2D biomolecular NMR spectra, providing a tool for sequential resonance assignment of microcrystalline proteins of large MW.

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P154

Pulsed Electron-Electron Double Resonance: Beyond Measuring Distances

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Pulsed Electron-Electron Double Resonance (PELDOR) Spectroscopy[1] has been widely applied utilizing stable nitroxide radicals in order to determine distances between unpaired electron spins.[2] Here, the advancement of X-band PELDOR spectroscopy (9 GHz, 0.3 T) on rigid nitroxide radicals beyond distance measurements towards unraveling an utmost of relevant information encoded in the experimental PELDOR data is described. In an interdisciplinary attempt between physical and organic chemistry, suitable polyradicals were synthesized, fully characterized and employed as PELDOR benchmark systems in proof of principle studies. The analysis lead to the extraction of the conformational flexibility of the studied compounds[3] as well as to the relative orientations of spin labels with respect to each other.[4] The latter was applied to short double helical deoxyribonucleic acids (DNAs).[5] In addition to the distance between the labels, r, the relative orientation of the spin labels described by the angle between the z-component of the ^{14}N-hyperfinecoupling tensor (A_{zz}) and r, φ, was unraveled. Moreover, using a multi-frequency approach including G-band (180 GHz, 6 T) PELDOR measurements to resolve the torsional motion, the conformational flexibility of such short double helical DNAs was additionally determined. As the introduced rigid nitroxide spin label is base paired to G these molecular cantilevers directly report on the dynamics of the studied DNAs and reflect a radial "breathing motion" of B-DNA.

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P155

C-terminal helix folding of PTB RRM1 upon binding of an IRES RNA stem-loop from Encephalomyocarditis virus and function implication for IRES activity

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The Polypyrimidine Tract Binding protein (PTB) is an important trans-acting factor required for translation of the genome of Encephalomyocarditis virus (EMCV) via internal ribosome entry site (IRES) mechanism. Among the four RNA recognition motif (RRM) domains of PTB, it has been shown recently that PTB RRM1 binds specifically the apical UCUUU pentaloop of the stem-loop F (SL F).[1] In order to understand the specificity of the loop interaction with PTB RRM1, we have solved the structure of this stem-loop F in complex with PTB RRM1 using NOE and RDC restraints. The structure reveals how the UCUU motif is specifically recognized via a set of hydrogen bonds. A lysine from loop 3 protrudes into the middle of the RNA pentaloop in order to neutralise the charges of the two phosphates. The loops 1, 3 and 5 at the edge of the β sheet and the conserved C and N termini of PTB RRM1 contact either the loop region or the RNA stem. The affinity of RRM1 PTB with the stem-loop increases by a factor of five compared to the one with the single stranded RNA. Unexpectedly, RNA binding induces the folding of a 14 amino acid C-terminal helix that packed on the side of the domain against β_2 strand and α_1 helix. In order to assess the biological relevance of this recognition, we performed bicistronic vector in vivo translation and band shift assays. These assays show how crucial is the interaction of the UCUUU loop with PTB RRM1 for proper binding of PTB on the whole IRES and the function of PTB in enhancing IRES-mediated translation. The simple mutation of UCUUU loop of SL F into the UUCG tetraloop almost abolish the IRES activity of EMCV and the only deletion of the C-terminal helix of RRM1 within the full length PTB protein decreases drastically both IRES-mediated translation and binding affinity. This work clearly demostrates that PTB RRM1 plays a central role for PTB function in promoting IRES-mediated translation of EMCV genome.

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P156

NMR Reveals Molecular Aspects of the Enzymatic Promiscuity

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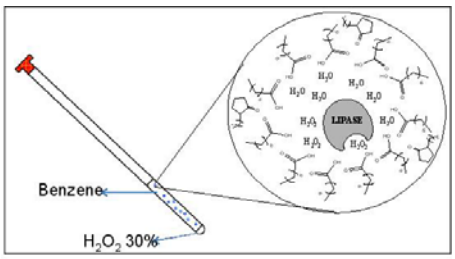
Enzyme promiscuity is mainly used to describe enzyme activities other than those for which an enzyme evolved and the d*egree of promiscuity* indicates how diverse is this promiscuous activity of and how different are the native and promiscuous functions. The native substrate may interact directly with active-site residues or through water molecules which play an important role in promiscuous interactions. Indeed, spatially defined active-site water molecules have catalytic powers that are comparable to amino acid residues. Searching more evidence for water-mediated promiscuity among the lipase superfamily our research group has optimized lipase mediated reactions and in most examples a radical change in the catalyzed reaction is caused by the polarity of the reaction medium. We were particularly interested in understanding the Bayer Villiger reaction which is mediated by C. cylindracea lipase providing no enantiomeric excess. The formation of octanoic acid inverse micelles in the benzene phase carrying the lipase and hydrogen peroxide was detected applying several NMR techniques [Diffusion experiments (DOSY), Relaxation (T1 and T2)]. The best answer was provided by ^{1}H NMR difference experiment using a two-phase (deuterobenzene–H_2O_2) and saturating the residual water signal in the deuterobenzene layer.



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P157

Switched angle spinning NMR: hardware and experiments for oriented membrane systems

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Isotropic-anisotropic correlation methods are a powerful means of obtaining structural information about strongly oriented systems, as they provide a link between easily assignable isotropic spectra and information-rich anisotropic data. Variable Angle Spinning (VAS) and Switched Angle Spinning (SAS) have a rich history in NMR of solids and liquid crystals, where they have been used by many groups to extract isotropic spectra without sacrificing the chemical shift anisotropy, dipolar couplings, or quadrupolar interactions. Recent work in our group has focused on optimizing SAS hardware and methods for measuring scaled dipolar couplings in oriented membrane systems. This method has the potential to be a powerful complement to existing static and MAS NMR techniques for membrane proteins, as it combines some of the advantages of both. Here we describe a prototype 500 MHz ^{1}H-^{13}C double-resonance SAS probe optimized for oriented membrane systems. It incorporates a transverse, high-homogeneity radiofrequency coil that moves with the sample in order to maximize the filling factor. The coil is connected to the static part of the probe using capacitive coupling, eliminating sliding or flexible contacts. Homogeneity and reduced sample heating are prioritized at the expense of RF field strength, however this is not a major liability since the systems under investigation are highly motionally averaged, reducing the need for high-power decoupling. Advantages and disadvantages of different coil choices for this application will be discussed. A pneumatic SAS system allows reproducible switching on a timescale of about 17 ms. We will present data from model systems in both thermotropic liquid crystals and stabilized DHPC/DMPC bicelles.

P158

NMR Studies on the Interactions between Model Membranes and selected Photosensitizers

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Photodynamic therapy (PDT) is a well known method for the treatment of several diseases and is currently applied in medical fields like oncology and dermatology. In this therapy, a key role is carried out by the photosensitizer (PS) which after uploading into the diseased cells can stimulate cell death if excited by light of a particular wavelength. In recent years, a huge amount of novel photosensitizers has been created. The major part of them has a common porphyrin or chlorin skeleton-based core. These PSs are usually discovered by "trial and error" procedure. In fact following a rational drug design is difficult, as the mechanisms behind PSs accumulation in tumour tissue and in particular the interactions between PSs and membrane are up to now still not clear.

In this study, interactions between a series of commercially available PSs and model membranes have been probed by NMR spectroscopy, which has proved to be a powerful and well-established method in this field[1,2]. A series of PSs with different chemical structures, and thus different physic-chemical properties, were used. Unilamellar vesicles consisting of dioleoyl-phosphatidyl-choline (DOPC), with an average radius of 30nm, were used as membrane models. Analyses of selected vesicle resonances permit to understand the membrane localization of the PS, and to obtain an approximate model of the diffusion of PS within the bilayer. Four main types of different interactions were found (called model-A1, A2, B1 and B2). Particular PSs assigned to a respective model of interaction show high similarity in their chemical structure, leading to the conclusion that there are specific correlations between PS structure and membrane interaction.

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P159

NMR Study of APOBEC1 Complementation Factor (ACF) RNA Recognition Motif in complex with RNA

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Apolipoprotein B (apoB) mRNA editing is a cytoplasmic event consisting of a single C to U substitution occuring at a specific position in the translated region. This modification encodes an early stop codon leading to the expression of an apoB isoform lacking the C-terminal half of the full length protein. Fine regulation of apoB mRNA editing is essential for the proper function of lipid metabolism in mammals.[1] Two main players are involved in this reaction: the cytosine deaminase APOBEC1 and the high affinity RNA binding protein ACF which is an adaptor between APOBEC1 and the RNA. ACF is an hnRNP protein containing three RNA Recognition Motifs (RRM) involved in the specific recognition of a RNA stretch surrounding the target cytosine.[2] The goal of this project is to obtain structural information about the RRMs domains of ACF in complex with RNA, in order to understand the determinant of the specificity of the editing reaction. Several constructs including one or two RRMs were cloned and expressed in E. Coli. 15N-HSQC overlays of the different constructs show that RRM1 and RRM2 interact together while RRM3 does not interact with the other RRMs. In order to determine RNA target sequences, we carried out RRM3 and RRM12 titrations using several RNA sequences deriving from the ACF binding region.. We could identify the CAGUA sequence as a good RNA target for RRM3. Complete resonances assignment of RRM3 and of CAGUA in complex was obtained using triple resonances NMR experiments. Chemical shift perturbation mapping and intermolecular noes indicate that the AGUA motif interacts with RRM3 residues lying on the beta sheet surface and at the C terminal end. The RNA cassette containing the ACF binding site has been shown to adopt a stem loop structure, the guanine of the CAGUA motif being localized in a bulge[1]. This study is a first step toward a better understanding of ACF-RNA complex formation.

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P160

NMR structural study of instant coffee arabinogalactan-protein oligosaccharides

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Coffee belongs to the most widely used beverage in the world. Usually it is prepared of two main varieties, *coffea arabica* or *robusta*, or their mixtures. Carbohydrate components - cellulose, (galacto)mannans and arabinogalactan-protein (AGP) - represent about 50% of dry weight of coffee beans, from which AGP, constitute about 17%. It is now well known that arabinogalactans from green coffee beans exist as an extremely heterogeneous mixture of arabinogalactan-proteins (AGPs) containing between 6 and 10% glucuronic acid and 0.4–1.9 % protein. This heterogeneity mostly resides in both, the degree of branching (Gal/Ara ratio) and monosaccharide composition of side chains. The result of this complexity is that different structural elements can be found depending on the adopted isolation and fractionation (if any) procedure. Carbohydrate part of the AGP molecule, is primarily O-linked to Hyp (but also Ser/Thr is possible), usually as type II arabino-3,6-galactans. Backbone, composed of 1,3-linked βGal, is highly substituted at O6 by 1,6-linked βGal side chains of different length branched at O3 by Ara*f*. Drastic industrial processing conditions during the instant coffee powder preparation cause its structural modifications including depolymerization of the main as well as side chains. We showed in previous study[1,2] that AGP from *Coffea arabica was* highly destructed: decreased molecular mass, very low content of Ara*f* substituents.

In the present study we will discuss structural features of oligosaccharides obtained by size exclusion chromatography after enzymatic and partial acidic hydrolysis of previously studied AGP.

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Acknowledgments: This project has been supported by the Illycaffè grant, the Slovak Grant Agency VEGA No. 2/0155/08 and 2/0116/10, APVV Grant No. 0030-08 and Slovak state program for infrastructure 2003SP200280203.

P161

Focused NMR Structural Proteomics on Hyperstable Proteome subsets

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In the interest of better understanding the factors that make some proteins more or less stable than others, we are conducting a systematic structural characterization of proteins from the extremophilic organism, *Sulfolobus solfataricus*, an archaea that grows optimally at 70 ºC. Previous work used a proteomics approach to identify a subset of the most thermostable proteins from this organism. (1) This subset contains about 300 proteins many of which are uncharacterized with unknown structure and function. In addition to being very stable, the majority of these proteins are also small and soluble, characteristics that render them ideal to structural characterization by NMR.

We have selected a small subset of this protein pool in order to set-up a pilot program for NMR based structural proteomics. Two of these targets, with 80 and 114 residues respectively, have been expressed and biophysically characterized: These were used for the implementation of the most efficient protocols for rapid acquisition, analysis and structure determination. Using double labelled samples and with the combination of rapid scanning, non-uniform sampling and simultaneous acquisition techniques, we were able to collect complete data sets for assignment and structure determination using less than 5 days of NMR time. Resonance assignment and initial structural studies have been carried out and will be presented here.

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P162

Solid-State NMR study of the Heptameric Oligomerization Domain of C4 Binding Protein, an adjuvant used for producing vaccines

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Adjuvants are commonly used in immunology research because most highly purified protein antigens are weakly immunogenic and produce unsatisfactory immune responses. Some proteins used as adjuvants can improve antigen presentation, and are suitable for the development of human vaccines, unlike many classical adjuvants. Fusion proteins comprising antigens of interest fused to the oligomerization domain of the human complement inhibitor C4-binding protein (C4bp) have been produced. These C4bp domains exhibit favourable physicochemical and immunological properties that make them suitable adjuvants for use with vaccines.

Here we present a structural study of IMX77, a homoheptamer of 65-residue chains comprising the oligomerization domain of the human C4bp alpha chain. Early biological studies have shown that this domain auto-assembles in aqueous solution into a stable coiled-coil alpha-helical heptameric structure stabilized by disulphide bonds. IMX77 is difficult to study by solution NMR due to its size; crystals of IMX77 were obtained, but were too small to diffract. We thus used solid-state NMR to study IMX77 samples fully labelled by 15N and 13C in crystals. The spectra exhibit nicely resolved and isolated cross signals, which demonstrates that the heptameric structure has a well-ordered and well-defined atomic structure. A set of NMR experiments (five 2D DARR with mixing times ranging from 10 to 200 ms and a 3D NCaCb) allowed us to assign more than 60% of the residues IMX77. Complete assignment and distance measurements should lead to the determination of the structure of monomeric IMX77, and heteronuclear correlation experiments on heterogeneously labeled 15N:13C samples, as used for example with PAIN, should allow us to reconstruct the full structure of the complete heptamer.

P163

Mapping cAMP Signalling in Eukaryotes by NMR: Dynamically-Driven Inter-Domain Allostery and Cyclic Nucleotide Selectivity

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The two major receptors for the universal second messenger cyclic AMP (cAMP) in mammals are protein kinase A (PKA) and the recently discovered Rap guanine-nucleotide-exchange protein directly activated by cAMP (EPAC). For both systems, the comparative analysis of the cAMP-binding domains (CBDs) in the apo, agonist and antagonist bound states has revealed that dynamics is a key determinant of the cAMP-dependent allosteric activation in eukaryotic signaling domains, whereby inhibitory interactions between regulatory and catalytic domains are weakened by increasing their entropic penalty.[1-7] cAMP is able to modulate this entropic penalty through long-range intra-molecular signaling pathways that relay the cAMP signal from the cyclic phosphate binding cassette to a distal helical bundle at the N-terminus of the CBD. This N-terminal helical bundle represents a distinctive feature of eukaryotic CBDs and is absent in the bacterial cAMP receptor, *i.e.* the catabolite activator protein. We will show how the cAMP-dependent changes in the dynamics of this N-terminal helical bundle control not only the activation within a single CBD, but also the inter-domain communication between the tandem CBDs of PKA as well as the cAMP *vs.* cGMP selectivity of EPAC.

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P164

Structure and function of Src-associated during mitosis, 68 kDa (Sam68)

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Sam68 (Src-associated in mitosis, 68 kDa) is a prototypical member of the STAR (signal transducer and activator of RNA) family of RNA-binding proteins and therefore involved in critical cellular processes. Sam68 has been shown to modulate alternative splicing of the pre-mRNAs of CD44 and Bcl-xL, which are linked to tumor progression and apoptosis. The so-called STAR domain consists of a KH domain, flanked by two domains, referred to as Qua1 and Qua2, respectively. Sam68 and other STAR proteins recognize bipartite RNA sequences and are thought to function as homodimers. Sam68 exhibits binding specificity for homopolymeric poly(U) RNA and specifically recognizes UAAA or UUUA sequences with high affinity However, the structural and functional roles of the self-association are not known.

Here, we present the solution structure of the Sam68 Qua1 homodimerization domain as well as the structural and biochemical characterization of the RNA-binding KH-Qua2 domain and the complete STAR domain comprising Qua1-KH-Qua2. NMR relaxation data and analytical ultracentrifugation are used to determine the dimerization state of Qua1 and of the STAR domain. These data clearly show that the STAR domain is a homodimer *in vitro*. The dimerization interface of the Qua1 domain was validated by residual dipolar couplings (RDCs) and paramagnetic relaxation enhancement (PRE). Mutational analysis of Sam68 *in vitro* and in a cell-based assay revealed that the Qua1 domain and residues within the dimerization interface as well as the KH-Qua2 domain are essential for alternative splicing of a CD44 minigene. Together, our results indicate that the Qua1 homodimerization domain is required for regulation of alternative splicing by Sam68.

P165

^{1}H NMR monitoring of urea of the children with type 1 diabetes. Differentiation and search for pathological stage markers

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The incidents of type 1 diabetes are mostly associated with the civilization development. Among the most probable reasons being attributed to the emergence of this sickness may be identified as: genetic background, diet, immunological respond for external stimuli. Recently has been published that it is conceivable that maternal diet or intestinal microbiota may influence on energy metabolism and immune system of the offsprings.[1]

In our studies we have monitored over 50 children with clinically confirmed type 1 diabetes who possessed the illness in different pathological stages. The 1H NMR spectra allow to assign in unambiguous way the level of the readily applied diagnostic species – glucose and ketone bodies. This methodology together with simply PCA statistical analysis enable to divide children into three specific group: with significant improvement of health, prognosis good and serious health condition. It became clear that the obtained results were in line with medical diagnosis. Furthermore, the remaining regions (with cut glucose and ketone bodies signals) of the spectra were also very useful and allowed to distinguish between the healthy (with significant improvement of health) and ill children. Moreover, an another diagnostic region of the spectra, which allocates the children to these two groups was found.

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P166

Molecular recognition of thiogalactosides by human galectin-1

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Glycosylsulfides and glycosyldisulfides constitute a new class of carbohydrate derivatives with interesting chemical and physical properties. They are glycomimetics with distinct structural and dynamic features relative to their parent *O*-glycosyl compounds. Synthetic methods have been described to access this type of molecules and some efforts to understand their molecular recognition properties versus sugar receptors have been reported.[1,2] In order to further explore the binding features of these analogues and to propose an explanation, in structural terms, of the recognition process, NMR and molecular modelling studies were performed. However, still there are not many conformational and interaction studies on these thioanalogues and herein we have performed a detailed NMR and MD analysis of the conformational behaviour of some of these molecules, and of their binding abilities towards human galectin-1, one of the most studied protein of this family, involved in a wide range of biological process, interacting in major signalling pathways.

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P167

Structural and functional studies on a new family of cytochromes c_7 from *Geobacter sulfurreducens*

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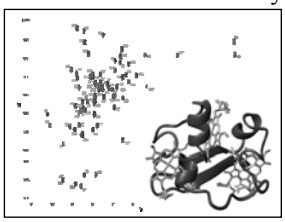
Geobacter sulfurreducens (*Gs*) is the number one microorganism for bioremediation and for the conversion of organic compounds to electricity.[1] This ability relies on an efficient delivery of cytoplasmic electrons to cell exterior. A family composed by five periplasmic cytochromes c_7 (PpcA-E) has a crucial role is this process.[2] These cytochromes were expressed and isotopically labeled in *E. coli*[3] and their structural features studied in solution. NMR spectroscopy was used to obtain the detailed thermodynamic characterization of the redox centers of *Gs* cytochromes c_7 (Gsc_7).[4] The results obtained show that despite the structural similarities among the Gsc_7, they are functionally diverse: PpcA and PpcD interact with redox partners involving e^-/H^+ transfer, which is not the case for PpcB and PpcE. This study constitutes a remarkable example of how structurally related proteins can be designed by Nature to perform quite different cellular functions.



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Structure and dynamics of delta RNA polymerase subunit from Bacillus subtilis

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Architecture of RNA polymerase (RNAP) from *B. subtilis* and other gram-positive bacteria differs from its analogue from gram-negative bacteria in the presence of two additional subunits - ω1 and δ. The δ subunit is important for virulence of pathogens such as *S. aureus*. Recent results indicated that the presence of delta subunit increases the transcription specificity and the efficiency of RNA synthesis. No structural information was available for the δ subunit due to the lack of sequence homology. As crystallization at structure genomics centers failed, we focused on the δ subunit in our NMR structural study. Because the C-terminal part of the δ subunit is unstructured and its peaks overlap as its sequence is highly repetitive, we first characterized the ordered N-terminal domain. Its structure was solved using a large set of high-quality NMR restraints, including 2341 NOE (544 long-range) and 384 RDC and ^{13}C CSA restraints from two aligning media (bacteriophage Pf1, 5% polyacrylamide gel). The calculations were run in CNS using a protocol modified in our lab to combine the SCULPTOR module with RECOORD scripts. Program CING was used to check the quality of the structures. The determined structure allowed us to identify unexpected structure homology of the N-terminal domain of δ subunit with several proteins from the Forkhead DNA/RNA-binding domain SCOP family and to propose residues interacting with the RNAP core. Relaxation dispersion revealed significant μs motions in the interaction surface, supporting the induced-fit model of binding. The partially disordered full-size protein was then completely assigned using 5D non-uniformly sampled spectra and results of a preliminary analysis of its structure and dynamics will be presented.

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Superoxide Reductase: Different Interaction Modes with its two Redox Partners

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Anaerobic organisms have molecular systems to quickly detoxify reactive oxygen species when transiently exposed to oxygen. One of these systems is superoxide reductase, which is able to reduce $O_2^{\cdot-}$ to H_2O_2 without production of molecular oxygen. In *Desulfovibrio gigas*, this metalloenzyme is a class II SOR, as it contains one Fe center coordinated to 4 histidinyl residues side chains and a cysteinyl sulphur. In order to complete the reaction, this enzyme requires an electron that is delivered either by rubredoxin or desulforedoxin.[1]

The interaction of rubredoxin and desulforedoxin with superoxide reductase was studied by 2D NMR and steady-state kinetics and a model structure of the electron transfer complexes was obtained by restrained docking, using BiGGER docking program.

Rubredoxin surface involved in the complex was identified by 2D NMR titration experiments, and comprises the solvent exposed hydrophobic residues in the vicinity of its metal center, which are surrounded by a slightly acidic patch. On the contrary, a complex between desulforedoxin and superoxide reductase could not be detected in a 2D NMR titration, due to the very short half-life of the complex in the NMR time scale. However, this protein was shown to be able to transfer electrons to superoxide reductase. NMR and steady-state kinetic competition assays show that these two electron donors must compete for the same site on the enzyme surface.

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P170

NMR Crystallography in an Enzyme Active Site: Characterizing the Chemical Structure of Catalytic Intermediates in Tryptophan Synthase

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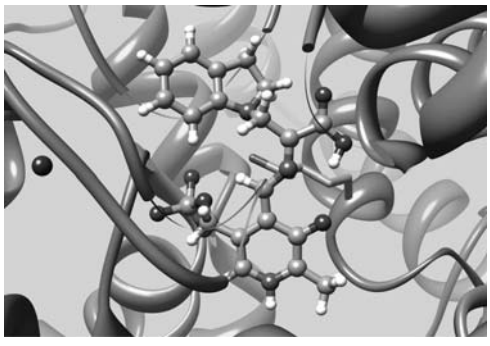

Chemical level details such as protonation and hybridization states are critical for understanding enzymatic mechanism and function. Even under moderately high resolution, these are difficult to determine from X-ray crystallography alone. The chemical shift, however, is an extremely sensitive probe of chemical environment and here we make use of a combined solid-state NMR, X-ray crystallographic, and *ab initio* approach to determine chemically-rich crystal structures for three enzymatic intermediates in the PLP-dependent enzyme, tryptophan synthase. In these experiments, the substrate ^{13}C and ^{15}N chemical shifts of the enzyme-bound species are measured in the crystalline-state under conditions of active catalysis. Chemically-rich structural models are then developed using a synergistic approach in which the structure of the substrate is freely optimized in the presence of active-site sidechain residues fixed at their crystallographically determined coordinates. Various models of charge and protonation state for the substrate and nearby catalytic residues can be distinguished by their calculated effect on the chemical shifts, allowing us to choose a single chemical species for each intermediate. Our models support the canonical protonation states proposed for two of the intermediates, but suggest that a third has a hydrogen shift that we believe has mechanistic implications.

P171

Cost-Effective and Comprehensive NMR Spectroscopy of Methyl Groups in Large Proteins

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Protein samples with ^{13}C isotopic enrichment of all methyl groups can be produced cost-effectively by bacterial expression using U-[^{1}H,^{13}C]-D-glucose and 100% D_2O. This approach ensures a high level of deuteration along all methyl-bearing amino acid side chains, facilitating the use of TOCSY transfer between side chain ^{13}C nuclei, and high levels of the favorable singly protonated methyl isotopomer for CHD_2-selective constant-time (CT) [^{1}H-^{13}C] HSQC detection (CT-CHD_2-[^{1}H-^{13}C]-HSQC). A 3D C-TOCSY-CHD_2-[^{1}H-^{13}C]-HSQC experiment was developed to match the measured Cα/Cβ frequency pairs with known backbone assignments, established from experiments collected on the same sample. Using only the 3D TOCSY experiment we were able to establish the sequence-specific assignment of 195 Ala, Val, Thr, Ile and Leu methyl groups (85%) for the 34 kDa periplasmic binding protein FepB. Due to the labeling with glucose, the stereospecific assignments are trivially obtained from a CT-CHD_2-[^{1}H-^{13}C]-HSQC experiment on a 10%- U-[^{1}H,^{13}C]-D-glucose based sample. The achieved level of completeness compares favorably with the expected ~60% complete assignment possible for I(δ_1)LV labeling, owing to the lack of methyl ^{13}C incorporation at Ala, Ile(γ_2) and Thr. Because of the favorable NMR spectroscopic properties of methyl groups, the strategy outlined here will also be sensitive for higher molecular weight systems, including oligomeric assemblies and solubilized membrane proteins.

Labeling with protonated glucose in heavy water has the added advantage that protein dynamics experiments can utilize both the CHD_2 and CH_2D isotopomer. This will be demonstrated with the design of a novel ^{1}H CPMG relaxation dispersion experiment to study microsecond time scale methyl group dynamics, which is facilitated by the fact that positions adjacent to methyl groups are scantilly protonated in these samples.

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P172

Molecular basis of methylated histone recognition by HP1 protein in the high molecular weight nucleosome system

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Epigenetic events, that provide heritable changes in phenotype without modification of the DNA sequence, play a critical role in gene regulation and cell differentiation.[1] Since they have a key role in carcinogenesis, a great effort is in progress to understand their molecular mechanisms, and the use of epigenetic targets is emerging as an effective approach to chemotherapy. One of the most studied and medically relevant epigenetic phenomena is histone tail modification.[2] Methylation of histones in the nucleosome governs the packaging of DNA in heterochromatin, a highly condensed and silent form of chromatin.[3] The key molecular event that triggers the heterochromatin assembly machinery is the recognition of the methylated K9 mark on histone 3 by Heterochromatin protein 1 (HP1).[4] To date, structural information has been obtained by using HP1 single domain and histone 3 mimic peptides.[5,6] However, we believe that the elucidation of structural and binding properties of this protein complex in the supra-molecular assembly is required to obtain new insights into the function of this key molecular recognition event. In this perspective we investigate by a combined biochemical and NMR-based approach, the structural and binding properties of the complex using the whole proteins: full-length HP1 and methylated histone 3 in the assembled nucleosome.

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P173

Probing 3D structure of a membrane-embedded potassium channel using solid-state NMR

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Solid-state NMR (ssNMR) has made significant progress to determine 3D molecular structures of globular and Amyloid proteins. In our contribution, we explore the use of ssNMR to determine 3D structures of the membrane-embedded potassium channel KcsA-Kv1.3. Previously, we reported ssNMR resonance assignments and characterized different functional states of KcsA-Kv1.3.[1,2] Using structural restraints derived from high-resolution CHHC[3] and CC correlation spectra along with the crystal structure of KcsA (PDB ID: 3EFF), we now generated a homology based ssNMR structure of the pore region of the KcsA-Kv1.3 channel. In addition, we produced channel variants using [2-Glycerol ^{13}C,^{15}N] and fractional deuteration. We show that both strategies facilitate the spectal analysis and enhance the prospects to structurally refine different functional states of the channel. Compared to the parent KcsA channel, our studies reveal distinct conformational changes which may account for the functioning of the protein in a lipid-like environment.

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P174

NMR structural characterization of the Ros protein prokaryotic zinc finger domain C27D mutant

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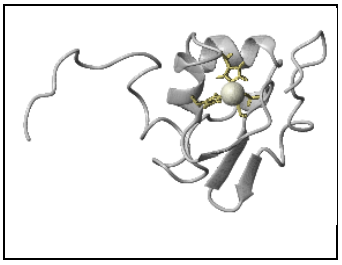
The first prokaryotic Cys_2His_2 zinc finger domain has been identified in the transcriptional regulator Ros from *Agrobacterium tumefaciens*.[1] Ros DNA-binding domain (Ros_{56-142}) was structurally characterized via NMR and it is observed that the prokaryotic Cys_2His_2 zinc-finger domain possesses a novel protein fold never found in literature.[3,4] The globular domain consists of 58 amino acids (from Pro9 to Tyr66), arranged in a ββββαα topology (Fig.) and it is stabilized by an extensive hydrophobic core.[2,3] The zinc ion is tetrahedrally coordinated by Cys-24, Cys-27, His-37 and His-42.[2] Here, we report the study of Ros_C27D, a point mutant of Ros_{56-142} in which the second coordinating cysteine is mutated to aspartic acid. Ros_C27D is still able to coordinate the zinc ion and to bind the DNA.[4] We demonstrate that in C27D mutant the zinc ion is tetrahedrally coordinated by Cys-24, Asp-27, His-37 and His-42,[4] forming a coordination sphere that has never been described in eukaryotic zinc finger domains but is naturally observed in many prokaryotic Ros homologues.[3,4] The structural and dynamic NMR characterization of Ros_C27D will therefore contribute to elucidate the extremely variable zinc coordination properties of this protein fold.



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P175 (*)

High-Resolution Structure Determination of a Low-Populated Folding Intermediate from NMR Relaxation Dispersion Experiments

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Understanding protein folding is important for protein structure prediction and design but also because of the critical role folding intermediates are suspected to play in amyloid fibril formation commonplace in neurodegenerative disorders. Unfortunately, experimental detection and characterization of intermediates is very difficult because they are both low and transiently populated. Our NMR relaxation dispersion (RD) studies have established that several fast-folding SH3 domain mutants fold through a low-populated on-pathway intermediate[1], which could not be detected with stopped-flow experiments. State-of-the-art CPMG RD experiments on suitably isotope-labeled samples allowed us to reconstruct the ^{15}N, ^{1}HN, ^{13}CO, ^{13}Cα, ^{1}Hα chemical shifts as well as 86 D_{NH} RDCs and 31 ^{13}CO RCSAs in two different alignment media for the 2% populated intermediate of the Fyn SH3 A39V/N53P/V55L with high precision. These experimental restraints were sufficient to calculate the high-resolution structure of the "invisible" intermediate by molecular dynamics with chemical shift restraints using the Camshift[2] approach, and the resulting topology could be cross-validated by H/D exchange. Formation of the 5-stranded SH3 fold proceeds hierarchically from the 3-stranded early transition state via a 4-stranded intermediate stabilized by several readily identifiable non-native long-range interactions but with a disordered C-terminus, thereby exposing a strand predicted to be highly aggregation-prone. Accordingly, a mutant designed to mimic the intermediate spontaneously formed fibrils that were clearly visible in negative stain electron micrographs, thus providing strong experimental evidence for the link between folding intermediates and amyloid fibril formation.

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P176

Solution NMR applied to the GNA1946 candidate antigen lipoprotein from Neisseria meningitidis

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GNA1946 (Genome-derived Neisseria Antigen 1946) is a highly conserved exposed outer membrane lipoprotein from *Neisseria meningitidis* bacteria of 287 amino acid length (31kDa). Although it has been predicted to be a periplasmic receptor in the D-methionine uptake ABC transporter. the recent structure of GNA1946 solved by X-Ray crystallography show it's specificity to L-methionine.[1] Understanding the behaviour and specificity of GNA1946 in aqueuos solution is highly relevant for the discovery of the antigenic determinants of the protein that will possibly lead to a more efficient vaccine development against virulent serogroup B strain of *N.meningitidis*. We produced uniformly ^{15}N/^{13}C-labelled GNA1946 and assigned the backbone and side-chains ^{1}H, ^{13}C and ^{15}N resonances using high-resolution NMR spectroscopy. In this work we report almost complete assignments of GNA1946 so as the backbone dynamics analysis describing the behavior of the protein in aqueous buffer solution.

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Acknowledgments: This work was supported by the Sixth Research Framework Programme of the European Community, FP6-STREP project ''BacAbs'', grant number LSHB-CT-2006-037325.

P177

Structural Basis of bZIP Transcription Factor Ribonuclease Activity

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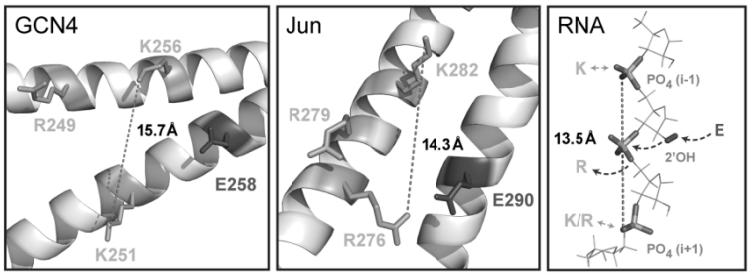
Basic-region leucine zipper (bZIP) proteins are one of the largest transcription factor families that regulate a wide range of cellular functions.[1] Owing to the stability of their coiled coil structure leucine zipper (LZ) domains of bZIP factors are widely employed as dimerization motifs in protein engineering studies.[2] In the course of one such study the ability of leucine zipper GCN4 (LZ_{GCN4}) to catalyze the hydrolysis of RNA was accidentally observed.[3]



Our new NMR and LC-MS data reveal the substrate specificity, catalytic conformations and RNA binding sites of the leucine zipper motifs of factors GCN4 and c-Jun. The topology of the RNA binding interfaces implies flexibility of the active site formulation, suggesting different bZIP motifs shall exhibit a varying degree of catalytic activity depending on the cellular context. Together with the data on reaction mechanism these results open a venue for *in vivo* investigation of biological role of bZIP ribonuclease activity.

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P178

Pressure/temperature-dependence of ubiquitin's hydrogen bond network

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The pressure-dependence of $^{3h}J_{NC'}$ scalar couplings through hydrogen bonds was measured by nuclear magnetic resonance (NMR) spectroscopy in ubiquitin as a model protein. Pressure series in the range from atmospheric pressure up to 2500 bar were recorded at different temperatures. The quantitative 'long-range'-HNCO experiment was used to detect changes in electronic orbital overlap between the hydrogen and acceptor nuclei in ubiquitin's H-bonds.

On average, these couplings are strengthened with increasing pressure. For some hydrogen bonds in the β-sheets the $^{3h}J_{NC'}$ couplings show a nonlinear pressure-dependence with initially increasing coupling constants that decrease above pressures of 1200 – 1500 bar. Together with H-bonds that are weakened already at low pressures, these residues mark the probably most pressure-labile regions of ubiquitin.

Only for the first β-hairpin and the α-helix, where the secondary structure is preserved at high pressures, a correlation between the pressure-dependent changes of the $^{3h}J_{NC'}$ coupling constants and the amide proton chemical shift changes is observed. This correlates with earlier findings that the N-terminal half of ubiquitin is more stable.

Comparison of pressure-induced changes in ubiquitin's hydrogen bond network with temperature-induced effects[1] shows that for most residues an increase in pressure corresponds to a decrease in temperature with respect to the effect on $^{3h}J_{NC'}$ coupling constants. Hydrogen bonds that do not follow the global trends mark points, where local unfolding is likely to be initiated.

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P179
NMR contribution to anti-apoptotic protein ligand development

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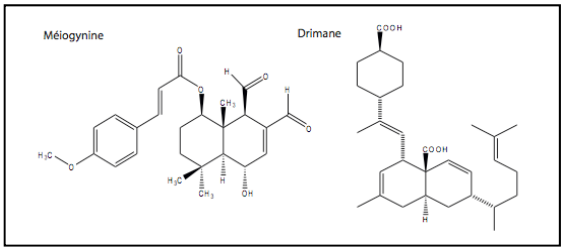
This project concerns the NMR study of the interactions between the anti-apoptotic proteins and two potential ligand candidates, the Meiogynine and the Drimane.[1]



These two terpenoids, identified from ICSN's chemical library screening against the Bcl-xL protein, have shown a significant inhibiting activity, thus opening promising perspectives for the treatment of cancer cells overexpressing anti-apoptotic proteins.[2] In fact, as the compounds are considerably smaller than the binding site, our objective is to introduce modifications (such as elongation of their structure, functionalization with hydrophilic groups etc.) that may improve their binding properties as well as their delivery and bioavailability. Following to the successful recombinant expression and purification, necessary to obtain labelled targets ($^{15}N/\ ^{13}C$), our preliminary NMR studies suggested a rather universal action of our ligands, capable to bind not only to Bcl-xL but also to the other major anti-apoptotic protein, Mcl-1. Titration experiments revealed significant disturbances of the HSQC protein spectra with the progressive disappearance of several H^N signals, confirming dissociation constants at the μM region for both targets. However, the intermediate chemical exchange observed, associated with the weak ligand solubility, poses severe difficulties for the structural elucidation of the complexes by classical NMR methods. In this work we'll present alternative approaches for the localisation of the ligands in the hydrophobic cleft of both target proteins.

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P180
NMR studies of the DNA Damage-Inducible UBL-UBA protein Ddi1

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The ubiquitin-proteasome pathway is the principal regulatory mechanism for the turnover of short-lived proteins in eukaryotes. It influences vital cellular events, including the cell cycle, malignant transformation, and responses to inflammation and immunity. Substrates for the ubiquitin-proteasome pathway are tagged with polyubiquitin chains and selectively driven to the proteasome for degradation.

DNA damage-inducible protein (Ddi1) has been identified as a ubiquitin receptor protein in *Saccharomyces cerevisiae*, although its precise role is yet to be unearthed. Three main domains can be identified in the Ddi1 gene: N-terminal ubiquitin-like domain (UBL), C-terminal ubiquitin-associated domain (UBA), and positioned in the middle aspartyl protease domain (RVP). As a member of the UBL-UBA protein family, Ddi1 is hypothesized to shuttle ubiquitinated substrates to the proteasome for degradation. Moreover, Ddi1 seems to be also involved in interactions with other shuttle proteins, although the scope and the roles of such interactions are not yet understood.

In order to shed light on the role of Ddi1 in the ubiquitin-proteasome pathway, we obtained complete NMR assignment (^{1}H, ^{15}N, ^{13}C) of the backbone and side chains of the UBL and UBA regions of Ddi1 and determined their three-dimensional structures in solution. Furthermore, we characterized using ^{15}N relaxation measurements the backbone dynamics of the UBL domain of Ddi1 and compare it with ubiquitin. Moreover, we examined whether the UBL and UBA regions of Ddi1 have the binding properties characteristic for the corresponding domains. For this purpose, we monitored by NMR interactions of Ddi1 domains with their respective potential binding partners from the ubiquitin-proteasome pathway. We expect that the outcomes of these studies will reveal details of Ddi1's function as a ubiquitin-shuttle protein and thus help understand the role of Ddi1 in the ubiquitin-proteasome system.

P181

New molecular and structural insights into protein-protein interaction in apoptosis

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Strong evidence points to dysfunctional apoptosis as a hallmark of cancer and other diseases. Thus, increasing research efforts are targeted at the detailed understanding of apoptotic mechanisms. Programmed cell death is mainly controlled by selective interactions between prosurvival and cell death-inducing members of the Bcl-2 family. Within this family, proteins share homology in short fragments called BH domains (BH1-BH4). In particular, the BH3-only subfamily (with homology solely in the BH3 region) includes intrinsically unstructured proapoptotic proteins known to bind to prosurvival members. Structural studies show that peptides comprising the BH3 domain of BH3-only proteins adopt alpha-helical structure when complexed to prosurvival Bcl-2 partners, whereas these peptides are random coil in isolation. However, the inner workings of this fine-tuned binding mechanism are still poorly understood and the role of some molecules whose activity has been reported as ambiguous remains to be clarified.

We report here on a novel interaction between the Bcl-2 member Diva (Boo), a controversial molecule categorized as both pro- and antiapoptotic, and the largely disordered BH3-only protein Harakiri. These studies have been performed with synthetic BH3 peptides derived from Harakiri using two specific techniques ELISA and NMR. We show that binding affinity increases with the length of Harakiri peptide fragment and provide the first experimental evidence indicating higher affinity of full-length BH3-only proteins than the corresponding BH3 peptides. Our results suggest that additional energetic factors besides those associated to specific intermolecular interactions influence the concomitant binding and folding process.

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DnaE intein structure from *Nostoc punctiforme* and design of new split inteins

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Protein splicing is a posttranslational modification where an intervening protein sequence, termed intein, is excised from a protein precursor.[1] The flanking sequences of the intein, termed N- and C-extein, are then ligated to form a mature polypeptide chain. Inteins have several possible biochemical applications, e.g. segmental isotope labeling, protein cyclization, and site-specific chemical modification. However, it remains elusive how the intein structure affects the protein splicing efficiency and requires further investigation to obtain full potential of inteins.

DnaE intein from *Nostoc punctiforme* (*Npu*) is a naturally split intein with a robust splicing activity.[2] To better understand the function and dynamic of inteins, we have performed a structural investigation of *Npu*DnaE intein.[3] For the structure determination of *Npu*DnaE intein an inactive single chain mutant (C1A) was constructed. The *Npu*DnaE intein structure assembles a horseshoe-like fold common in inteins. Effects of the C1A mutation were investigated by performing resonance assignment of *Npu*DnaE intein without the mutation and analyzing the difference in chemical shift. We determined ^{15}N T_1- and T_2- relaxation rates to investigate the dynamics of the intein. Analysis showed that new split sites could be introduced in flexible regions of the intein and a new functional split intein was designed that has a high potential in site-specific chemical modifications.

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NMR Structural Studies of Regulatory Protein-target Peptide-drug Complexes

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Our study is focused on the regulatory N-domain of cardiac troponin C (cNTnC) and its interaction with calmodulin antagonist, N-(6-aminohexyl)-5-chloro-1-naphthalenesulfinamide (W7), in the presence of cardiac troponin I (cTnI). We have previously determined the NMR solution structures of cNTnC•Ca^{2+} in complex with a 17-residue peptide representing the switch region of cardiac troponin I ($cTnI_{147-163}$),[1] with W7,[2] and with both $cTnI_{147-163}$ and W7.[3] Results show that electrostatic repelling between the positively charged primary amine at the end of polymethylene 'tail' of W7 and the basic R^{147} on the N-terminus of $cTnI_{147-163}$ plays an important role in interpretation of inhibitory effect of W7 in cardiac muscle contraction and offers an explanation for the ~13-fold affinity reduction of $cTnI_{147-163}$ for cNTnC•Ca^{2+} in the presence of W7. In this study we have concentrated on investigating the interaction of cNTnC with W7 and a longer version of the switch region of cTnI ($cTnI_{144-163}$). This 20-residue peptide possesses a three amino acids (RRV) N-terminal extension as compared to $cTnI_{147-163}$. This peptide demonstrates a ~10 fold higher affinity to cNTnC•Ca^{2+} than its shorter version[4] due to stronger electrostatic attraction between RRV and negatively charged residues in cNTnC located in the cNTnC:cTnI interface. We have made a stable cNTnC•Ca^{2+}•$cTnI_{144-167}$•W7 complex and we are in the process of determining the structure. We believe that comparison of this structure with previously obtained structures will generate insights into the features that are useful for the understanding the molecular mechanism underlying the cTnC-cTnI-W7 interaction and for the design of cTnC-specific cardiotonic drugs in the therapy of heart disease.

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P184

Assessment of Androgens and Estrogens as metabolic modulators in cultured Sertoli cells by ^{1}H-NMR spectroscopy

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Sertoli cells have been classified as the "nurse cells" within the seminiferous epithelium and their main function is to provide the adequate environment for germ cells development. They actively metabolize glucose and the majority of it is converted to lactate, which is preferentially used by developing germ cells, unable to use glucose for their energy metabolism. There is a growing awareness that androgens and estrogens have general metabolic roles that reach far beyond reproductive processes. So, the purpose of this study was to examine the effect of sex hormones on metabolite secretion or consumption in primary cultures of rat Sertoli cells using ^{1}H-NMR spectra analysis.

Sertoli cell-enriched cultures (>95%) were maintained in a defined medium for 50 hours (h). During this interval, glucose and pyruvate consumption, lactate and alanine secretion into the incubation medium were determined, by ^{1}H-NMR spectra analysis, in the presence or absence of either 100 nM 17β-estradiol (E2) or 100 mM dihydrotestosterone (DHT). Cells cultured in the absence (control) or presence of E2 consumed the same amount of glucose (29 ± 2 μmoles) at similar rates during the 50h treatment. After 25h treatment with DHT, glucose consumption and glucose consumption rate significantly increased. Control cells and cells treated with E2 secreted similar amount of lactate during the 50 h (18 ± 0,5 and 17 ± 0,5 mmoles, respectively) while cells treated with DHT secreted 15 ± 0,9 mmoles. As for glucose consumption, lactate production rate peaked after 25 h treatment with DHT. Pyruvate was entirely consumed in the first 5 h incubation for all conditions, and alanine production was significantly higher in E2-treated cells after 25 h treatment, which indicated a lower redox state/higher oxidative stress for the cells on those conditions when compared with cells of the other groups. This provides a first assessment on androgens and estrogens as metabolic modulators of Sertoli cells.

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Distinct ubiquitin binding modes exhibited by the SH3 domains of CD2AP. Molecular determinants and functional implications

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Ubiquitin is known to regulate a wide variety of cellular activities via mono- or poly- ubiquitination. Recently it was found that a subset of SH3 domains constitutes a new, distinct type of ubiquitin-binding domains.[1] We previously showed that the third SH3 domain (SH3-C) of CD2 associated protein (CD2AP) binds to ubiquitin,[2] but in an alternative orientation that does not coincide with the binding surface found for the Sla1 SH3-3 domain or the CIN85 SH3-C domain.[3] We present an RDC-based structural model of the complex between the first SH3 domain of CD2AP and ubiquitin. We show that the first and second SH3 domains of CD2AP bind ubiquitin in a similar orientation than Sla1 SH3-3 despite of their high sequence homology with the SH3-C domain of CD2AP. Both structures, in combination with a mutational analysis, have allowed us to decipher the determinants of the CD2AP SH3-C binding mode to ubiquitin. We have also shown that CD2AP SH3-C domain bind tighter to ubiquitin molecules with an extended C-terminus. We propose a different biological role for this interaction mode where the CD2AP SH3-C domain is able to interact with ubiquitin molecules covalently attached to CD2AP via its C-terminus, blocking this protein for further polyubiquitination as it has been observed previously,[4] thus changing the previously established mechanism of EGF-dependent CD2AP/CIN85 monoubiquitination.

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P186

Structural Study of the molecular recognition mechanism of P63 by ITCH-E3 Ligase

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Recently, it has been shown that Itch mediates the degradation of TAp63 and ΔNp63 proteins.[1] Itch E3–ligase is characterized by the presence of four WW domains that are important in the recognized process. Several signalling complexes, that these domains mediate, have been implicated in human diseases (Muscular Dystrophy, Alzheimer's Disease, Huntington Disease etc.). WW domains interact with short proline-rich sequences and on the basis of their ligand-binding specificity WW domains have been categorized into four groups. WW domains are highly compact protein-protein interaction modules, which fold into stable three-stranded antiparallel β-sheet structures, and are characterized by the presence of two conserved tryptophan residues spaced 20-22 amino acids apart. The four WW domains of Itch are considered belonging to the Group I, which binds polypeptides with PY motif characterized by a PPXY consensus sequence, where X can be any residue. It is probably that Itch-p63 interaction can be due to a direct interaction of Itch-WW2 domain with the PY motif of p63. Here, we report a characterization by fluorescence, CD and NMR spectroscopy of the structural features of the Itch-WW2 domain. Interaction studies *in vitro* between Itch-WW2 domain and pep63, which correspond to the fragment of the p63 protein including the PY motif, were performed. Moreover, the effects of a site specific mutation of p63, that has been reported in both Hay–Wells and Rapp–Hodgkin syndrome, were also evaluated both on the conformation of pep63 and on the WW-pep63 interaction.

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NMR-based interaction studies on the fibroblast growth factor-2 and a new non-peptidic thrombospondin-1 derived antiangiogenic compound

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Endogenous inhibitors of angiogenesis, such as thrombospondin-1 (TSP-1), are promising sources of therapeutic agents to treat angiogenesis-driven diseases, including cancer. TSP-1 regulates angiogenesis through different mechanisms, including binding and sequestration of the angiogenic fibroblast growth factor-2 (FGF-2), through a site located in the calcium binding type III repeats. A FGF-2 binding sequence of TSP-1 was identified in the 15-mer sequence DDDDDNDKIPDDRDN using a peptide array approach followed by binding assays with synthetic peptides and recombinant proteins.[1] The relevant residues and conformational determinants for the peptide-FGF-2 interaction were identified by STD nuclear magnetic resonance and molecular dynamics simulations. The information was translated into a pharmacophore model used to screen the NCI2003 small molecule databases, leading to the identification of three small molecules that bound FGF-2 with affinity in the submicromolar range. NMR titration experiments were carried out on the ^{15}N-labelled FGF-2 with the most active of the identified molecules. The analysis revealed a residue specific interaction in the fast exchange regime, whereas the FGF-2 structure remains overall unperturbed. The small molecule binds in a crucial position that provides specific contacts with FGF-2 that are essential for the binding to heparin and also potentially positioned to counteract the interaction with the FGF receptor kinases 1. The dynamics and the water bound molecules to the free FGF-2 protein were also characterized.

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P188

Side-chain dynamics of a mutant staphylococcal nuclease and the stabilizing effect of mannosylglycerate - a naturally occurring solute

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We have been investigating the effect of mannosylglycerate (MG), a charged stabilizing solute isolated from hyperthermophilic microorganisms,[1] on the dynamics of a Staphylococcal nuclease mutant (P117G, H124L, and S128A) using several NMR techniques. At 0.5 M concentration, MG increases the T_m of mSnase by 6.7°C - from 67.6°C to 74.3°C. The main objective of this work is to use NMR relaxation measurements to find out whether a relationship exists between protein stabilization and protein rigidification induced by compatible solutes. Our first studies focused on the protein backbone and its dynamics at different time scales.[2] Using techniques of ^{15}N relaxation, water-amide saturation transfer and hydrogen/deuterium exchange we showed that increasing amounts of MG resulted in the progressive reduction of the internal motions of the whole protein, in particular of those occurring at very high (us^{-1}) and very low frequencies (h^{-1}). Motions on the millisecond time-scale appeared to be little affected by the presence of MG, at least when compared to other solutes that do not stabilize the mSNase protein, such as glycerol and KCl which also served the purpose of viscosity and ionic strength controls, respectively. Moreover, it was observed that the effect of MG on the fast backbone motions correlates well with the added stability conferred to the mSNase. Since in the majority of the proteins, backbone motions are intrinsically small, we expanded our studies to the effect of MG on the dynamics of protein side chains. For this we have labelled methyl groups with ^{13}C isotopes and measured the relaxation rates of this nucleus thereby accessing the high frequency motions of the corresponding side chains. The results of this work will be presented during the symposium as well as other studies on the dynamics of mSNase.

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NMR structural characterization of the Ros protein prokaryotic zinc finger domain H42A mutant

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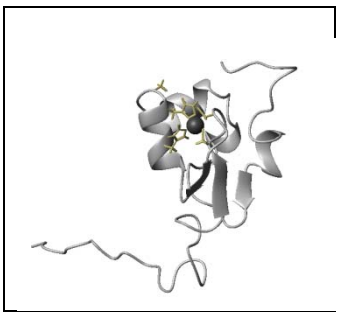
Ros protein from *A.tumefaciens* is the first prokaryotic classical zinc finger protein. Ros87, a mutant of Ros wild-type obtained by deletion of the first fifty-five amino acids, which is soluble and contains the zinc finger domain, is still able to bind DNA.[1] The NMR structure of Ros87[2] consists of a very well defined globular domain, in which the zinc ion is tetrahedrally coordinated by Cys-24 and Cys-27 and by His-37 and His-42, and two disordered tails at the N- and C-terminal region. Ros87 globular fold has ββββαα topology and it is stabilized by an extended hydrophobic core of 15 amino acid. These new features define a novel fold never found in literature. The mutant H42A of Ros87 (in which the second coordinating histidine is mutated to alanine) is still able to bind the specific DNA sequence. Moreover, HSQC-J18 experiment demonstrated that in H42A the zinc ion is tetrahedrally coordinated by Cys-24, Cys-27, His-37 and His-41.[1] We report the NMR structural characterization of H42A mutant (see fig.) to understand the structural variations caused by this mutation and a study of the protein folding behavior to determine the kinetics of secondary structure formation.



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P190

Protein Assisted Metal Reconstitution of a Biologically Unique Mo-Cu Cluster

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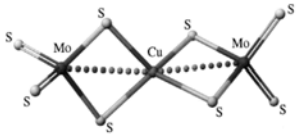
The ORange Protein (ORP) from *Desulfovibrio gigas* is an orange coloured protein that contains a mixed-metal sulphide cluster, of the type $[S_2MoS_2CuS_2MoS_2]^{3-}$, non-covalently bound to the polypeptide chain.[1,2] A blast search revealed that this protein has sequence homology of around 30 to 50 % with conserved proteins from eubacteria and hyperthermophilic archaea with unknown function. They all contain a conserved domain common to the nitrogenase accessory factors (NifB C-terminal domain, NifX and NafY). The ORP was produced for NMR studies by heterologous expression in *E. coli* as the apo-form.[3] Reconstitution of the metal cluster in the apo-ORP was studied either by addition of the pre-synthesized cluster or by its synthesis in the presence of the apo-protein, upon addition of copper sulphate and thiomolybdate or thiotungstate. NMR studies clearly show that there is a protein assisted metal reconstitution of the heterometallic cluster. The over-all solution structures of the apo and reconstituted ORP are similar, with a α/β motif, characteristic of the members of the ribonuclease H family. The two structures are similar and the mapping of the chemical shift differences between them was used to elucidate which region of the polypeptide chain is involved in the binding of the metal cluster. These results give insights into the metal binding mode of chaperons involved in the synthesis of the nitrogenase metal cofactor.



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Preliminary Structure of the 2-Domain Monothiol Grx3 from *Trypanosoma brucei*

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Glutaredoxins (Grxs) are general disulfide reductases important for the regulation of cellular redox homeostasis. Today, Grxs are recognized as versatile regulatory proteins with multiple functions in health and disease.[1] The recently discovered monothiolic Grxs have a single cysteine in the active site and their functional role is still not clear. Nevertheless, the extent of conservation of these proteins and the poor viability of some knock-outs, suggest a decisive importance in central processes within the cells, and a role which is not redundant with classical dithiolic Grxs. Two subclasses of these proteins exist, those with a single glutaredoxin domain[2] and those with a thioredoxin-like region followed by one or more glutaredoxin domains.[3] No structure of multidomain monothiol Grxs is currently available.

The challenge for control of reactive oxygen is greater for parasites since they are required to cope with the continuous production of ROS by the host immune system. Parasites have therefore developed unique antioxidant systems which may provide new drug targets. African trypanosomes have a thiol metabolism based on bis(glutathionyl)spermidine.[4] *T. brucei*, the causative agent of African sleeping sickness, encodes three genes for monothiol Grxs. Two of them are single-domain proteins, while Grx3 contains an additional N-terminal thioredoxin-like domain. We will present here a preliminary structural model of the multidomain *T. brucei* Grx3 based on homology, chemical shift and residual dipolar coupling data.

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P192

Optimized isotope incorporation with cell-free protein synthesis

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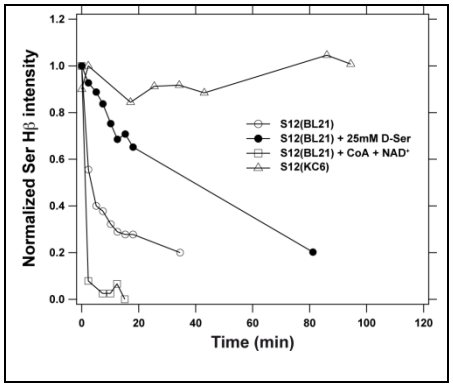
The current state of cell-free protein synthesis (CFPS) at the Swedish NMR Centre will be reported. We are using an *E. coli*-based batch system utilizing a crude extract (S12) containing the translation factors and necessary soluble enzymes. Specific expression is ensured by using constructs under T7 promotor control. So far the expression system has been used to produce ~100 different proteins, with more than 80% expressing at yields suitable for structural biology. General system optimization has been performed with experimental design principles and fluorescent proteins as reporters of expression yield. Extract activities metabolizing amino acids have been identified and inhibited, increasing synthesis yield up to threefold depending on expression target. Future strategies for improving the system for expression of difficult proteins will be outlined, including results on expression of membrane proteins, proteins with multiple disulfide bonds and viral proteins.



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Dynamics studies of Engrailed 2 homeoprotein

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Many proteins play a physiological role in living organisms while lacking well-defined regular secondary structure. Frequently, entire disordered molecules or longer unstructured fragments are essential in cellular signal transduction pathways. Interactions with different biological partners require fast conformational changes and the high flexibility.

Engrailed 2 is a transcription factor from the homeoprotein family. It is expressed in embryonic state as well as in dopaminergic neurons in adults. We are studying the construct of Engrailed 2 that comprises residues 146 to 259, corresponding to the homeodomain and a long disordered N-terminal extension.

A full assignment of backbone resonances has been performed in spite of the weak dispersion of signals. Minor resonances corresponding to the tryptophan residues from unfolded region have been identified, suggesting the presence of slow conformational exchange in this region.

Conventional nitrogen-15 relaxation rates (R1, R2 and heteronuclear nOe) have been measured. The analysis of nOe values confirmed the presence of a well structured homeodomain and a long disordered N-terminal tail. Higher nOe values have been measured for a hexapeptide in the middle of the N-terminal extension, which is a protein-protein interaction site. We estimated the exchange contribution to transverse R2 relaxation rates using longitudinal and transverse CSA/DD cross-correlated cross relaxation rates. Significant contributions of chemical exchange to transverse relaxation have been observed for this hexapeptide. This was confirmed by comparing R2 rates measured under CPMG and in a single-echo sequence. In addition, minor contributions of chemical exchange to R2 have been detected in loops, particularly between the first and the second α-helix. These results show the complex conformational equilibrium around this hexapeptide and show that transverse relaxation rates alone cannot be a good assessment of residual structure.

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NMR Structure and Ion Channel Activity of the p7 protein from Hepatitis C Virus

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The small membrane protein p7 of hepatitis C virus forms oligomers and exhibits ion channel activity essential for virus infectivity. These viroporin features render p7 an attractive target for antiviral drug development. In this study, p7 from strain HCV-J (genotype 1b) was chemically synthesized and purified for ion channel activity measurements and structure analyses. p7 forms cation-selective ion channels in planar lipid bilayers and at the single-channel level by the patch-clamp technique. Ion channel activity was shown to be inhibited by hexamethylene amiloride, but not by amantadine. Circular dichroism analyses revealed that the structure of p7 is mainly alpha-helical, irrespective of the membrane mimetic medium, e.g. lysolipids, detergents, organic solvent-water mixtures. The secondary structure elements of the monomeric form of p7 were determined by ^{1}H and ^{13}C NMR in trifluoroethanol-water mixtures. Molecular dynamics simulations in a model membrane were combined synergistically with structural data obtained from NMR experiments. This approach allowed us to determine the secondary structure elements of p7, which significantly differ from predictions, and to propose a three-dimensional model of the monomeric form of p7 associated to the phospholipid bilayer. These studies revealed the presence of a turn connecting an unexpected N-terminal alpha-helix to the first transmembrane helix TM1, and a long cytosolic loop bearing the dibasic motif and connecting TM1 to TM2. These results provide the first detailed experimental structural framework for a better understanding of p7 processing, oligomerization, and ion-channel gating mechanism.

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The fungus protein cerato platanin: structural characterization and the search for its biological function

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The understanding of plant disease resistance is of paramount importance to sustainable agriculture and human health. Plant defence mechanisms against virus and bacteria have been extensively studied, while little is known about plant-fungus interaction. Though fungi cause serious plant diseases of global relevance, the basis, at a molecular level, of their pathogenicity is still unclear.

Pazzagli and co-workers[1] have identified a 120 residues protein, named cerato-platanin (CP), expressed by the plane-tree pathogen *Ceratocystis platani* and that induces defence responses when applied to plane leaves: plasmolysis, cell death and production of phytoalexins.[2,3] The protein is the progenitor of the CP family.[4] The description of the protein 3D structure reveals a globular fold containing 2 α-helices, 6 β-strands forming a double-ψ β-barrel and long flexible loops (PDB 2KQA). As the proteins most structurally close to CP are proteins involved in polysaccharide recognition we explored the ability of CP to bind oligosaccharides.

The description of the structural and dynamic properties of CP, for which the real biological function is not known, will allow to understand its role both in fungus biology and in the host-fungus interaction: thus it will open the way to devise new and hopefully more effective and human-safe crop control methodologies.

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P196

Entropy and invariance in lipid membranes

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Structural studies of biological membranes are complicated due to the tremendous number of variables which include chemical compositions, preferential interactions, and molecular motions. Changes in lipid composition, for example addition of polyunsaturated lipid chains, ceramides, and cholesterol have been shown to alter substantially the activity of membrane receptors and of ion channels. To describe these effects, we employ statistical mechanics and thermodynamics. We analyze order parameter data from solid-state NMR measurements on lipid bilayers to describe how bilayer properties scale with temperature and with acyl chain length. We find an invariant description of acyl chain packing, which allows us to address the correspondence between changes in acyl length and changes in temperature. We present the functional form of this scaling relationship, the conclusions that can be drawn from such a study, and how this invariance is ultimately a step in determining the guiding principles of lipid organization in biological membranes.

P197

High resolution structure of a 2'-F/2'-OMe modified 42-nt dimeric siRNA construct

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RNA interference is triggered by short RNA duplexes, which can be used for the silencing of virtually any gene. Unfortunately, synthetic siRNAs consisting of solely standard nucleotides exhibit short half-life in serum due to the activity of endo- and exonucleases. A fully modified duplex, which is comprised of alternating 2'-F and 2'-OMe nucleotides exhibits several desirable pharmacokinetic properties, which results in a more than 500-fold increase in in vitro potency versus unmodified siRNA (1). To gain some insight into the structural features, which result in the high potency of the modified siRNA, we determined the 3D structure of the siRNA construct in solution.

Sufficient resolution of NMR signals was achieved on an 800 MHz NMR spectrometer which enabled sequential assignment of a 14 kDa dimer without isotope labeling. Structure calculations utilized a set of H-F RDC values under conditions of weak alignment achieved by Pf1 phages for all 21 2'-F modified nucleotides. The two 21-nt oligonucleotides efficiently hybridize thus forming an A-type double helix with 3' UU overhangs on both strands. The helical segment is completely complementary and exhibits 19 Watson-Crick base pairs. However, NMR data suggests that the stability of individual base pairs is not uniform through the whole length of the construct. The last three base pairs display somewhat different properties. Their imino protons are accessible to solvent exchange, which is an indication of base pair opening. Stabilization of these base pairs is achieved through favourable stacking interactions. The labile base pairs suggest which strand will serve as a guide strands and will thus control the incorporation of the siRNA duplex into the RISC complex.

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P198

Between high and low fields: DEER experiment on a nitroxide radical pair at Q-band frequency

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Measuring distance distributions between nitroxide radicals using Double Electron Electron Resonance (DEER) experiment is one of the standard experimental methods in modern structural biology. Routinely, such measurements are performed at low (standard) X-band frequencies (≈9.5 GHz). The reason for that is a favourable combination of the relatively narrow EPR spectrum of nitroxide at these frequencies with the measurement conditions (microwave power and resonator bandwidth) provided by commercial hardware. Thus, optimum excitation of the nitroxide spin pair is achieved, and the distance measurements are typically performed on the overnight time scale at X-band. Additionally, orientation selection effects in the spin-labelled proteins are negligible in most of the cases. At high fields, including and above W-band range (≥ 94 GHz), performance of the DEER experiment in both selective and non-selective regimes is still hindered by the lack of power of commercial spectrometers.

Here, we analyse performance of the DEER experiment at intermediate microwave frequencies in the Q-band (≈34.5 GHz) range. To enhance experimental flexibility we used a home-built spectrometer together with a custom probehead adapted for oversized samples. This allowed us to vary experimental conditions from the hard pulse excitation regime in order to achieve ultimate sensitivity of the measurement to the soft excitation regime aiming at better observation of orientation selection effects. Concentration sensitivity of the measurement is further improved by using 3-mm sample tubes possible in the oversized resonator. Performance is tested for shape-persistent biradicals as well as spin-labelled protein samples.

P199

Metabolic Studies of Bovine Urine and Blood Plasma using ^{1}H and ^{13}C-SSPF High Resolution NMR after Treatment with Ivermectin

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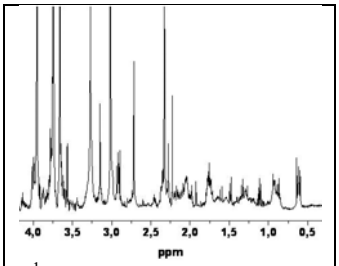
Bovine Metabolomics represents a novel tool for inspecting meat and dairy products quality and helping to detect animal diseases or disorders. In our studies, 24 Dutch Holstein female calves received intravenous Ivermectin 4% (dosage of 1 mL/50 Kg), from which urine and blood plasma were analysed before (blank), and 3 and 6 hours after treatment. Samples were analysed by using ^{1}H NMR PRESAT sequence, and ^{13}C SSFP (Steady-State Free Precession) sequence. The ^{13}C SSFP sequence leads to an average 3-fold increasing in the signal-to-noise ratio, when compared to standard ^{13}C sequences, avoiding the need for isotope labelling, and thus representing a pioneer technique for metabolic analysis. Metabolites were identified by using online databases, and chemometric studies were carried out to highlight the variations in both urine and plasma samples (both ^{1}H and ^{13}C-SSPF spectra). Several changes were observed in post-treatment samples, such as a considerable increasing in concentration of aminoacids and histamine (what clearly denotes an allergic reaction) and a decreasing of citrate levels. The information from these studies are the very first step for establishing a chemical fingerprint for ivermectin misuse and/or the non-observance of the drug withdrawal term and consequent contamination of milk and meat.



^{1}H-NMR Urine Spectrum

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P200

Towards Understanding the Folding Mechanism of a Four-Helix-Protein, ACBP the Cooperative Formation of Specific Tertiary Contacts in the Unfolded State

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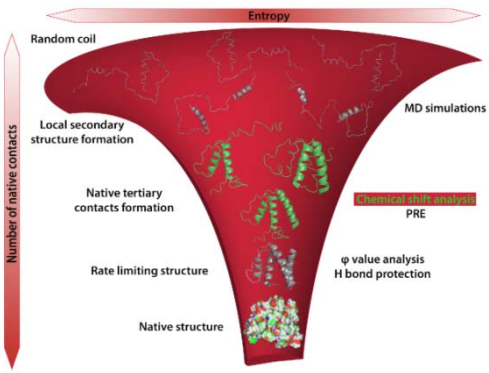
In studies of the ensembles of unfolded structures of a four helix bundle protein ACBP –acyl coenzyme A binding protein -we have detected the presence of potential precursors of native tertiary structures. These observations were based on the perturbation of NMR chemical shifts of the protein backbone atoms by single site mutations. Some mutations change the chemical shifts of residues remote from the site of mutation indicating the presence of an interaction between the mutated and the remote residues. This suggests that the formation of helix segments and helix-helix interactions is cooperative. We can begin to track down the folding mechanism of this protein using only experimental data by combining the information available for the rate limiting structure formation during the folding process with measurements of the site specific hydrogen bond formation in the burst phase, and with the existence prior to the folding reaction of tertiary structures in the ensemble of otherwise unfolded structures observed in the present study. We envisage that the detection of long range interactions in ensembles of unfolded proteins is not only restricted to ACBP. For many proteins it has been noticed that after the unfolding transition residual structure prevails which disappears only upon increase of the denaturant concentration as observed either by CD and/or NMR or other techniques. It is the ensemble of transient residual structure in the unfolded state, which has been characterized in case of ACBP, however, it seems most likely that many other unfolded proteins could be candidates for a similar study. The method is therefore seen to have a potential to become an important experimental tool for the advancement of our understanding of the protein folding problem.



P201
NMR in protein-ligand interaction studies

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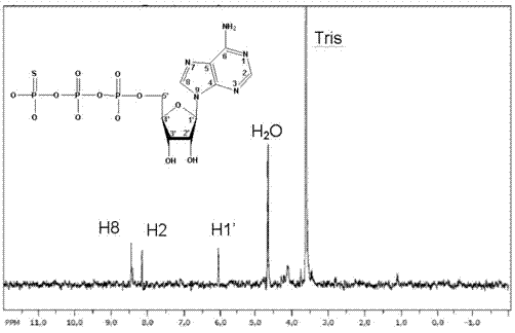
Studies related to intermolecular interactions especially among small ligands and macromolecular species are very important in biochemistry once these regulate great number of biochemical and physiologic processes, in which the complexes among macromolecule/ligands result in a biological feedback. These interactions can be followed by the STD – (STD-Saturation Transfer Difference) NMR techniques, that use low protein/ligand ratios (1:100). Herein, our investigations are concentrated on orange's Hsp90 (Heat Shock Protein of 90kDa),[1] chaperone responsible for the protein folding, that's probably involved in the infection process by the Citric canker agent *Xac* (*Xanthomonas axonopodis* pv. *citri*). It's known that this chaperone may interact with ATP, and this interaction was monitored by STD assays. We've also investigated the interactions among the Human Heat Shock Protein (Hsp100) and nucleotides, like ATP, ADP and ATPγS. The Hsp100 protein was used as a model for studying the interactions between orange's Hsp (Hsp90) and different drugs, including geldanamycin. The STD NMR spectra obtained for the Hsp100 and the ligands showed very similar profiles, and therefore, the spectrum obtained in Hsp100/ATPγS (shown on Figure) illustrates well all our measurements. Very interesting results were obtained in Hsp90 and drugs investigations all pointed specific protein-ligand complexation with specific Kd values.



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P202
Structural Studies of the Light Driven Enzyme NADPH: Protochlorophyllide Oxidoreductase

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The light driven enzyme Protochlorophyllide Oxidoreductase (POR) is responsible for catalysing the reduction of the $C_{17} – C_{18}$ double bond of the D ring of protochlorophyllide (Pchlide), in the presence of NADPH, forming chlorophyllide (Chlide).[1] The reduction of Pchlide involves a light-induced hydride transfer reaction from the *pro*-S face of nicotinamide adenine dinucleotide phosphate (NADPH), to the C_{17} position coupled to the addition of a proton to the C_{18} position forming Chlide.[2] The reaction catalysed by POR is a key step in chlorophyll biosynthesis and is essential in the development of chloroplasts.[3]

Due to the large size of POR (37 kDa) work has been conducted to optimise the conditions used to conduct the NMR, allowing all of the 322 signals to be resolved with sufficient intensity to confidently assign them. Chemical exchange broadening means that the improvements in relaxation rates to be gained from perdeuteration are offset by the sensitivity loses from the longer TROSY based pulse programmes. This coupled with the fact that spectra from this thermophilic enzyme can be acquired at 50 ºC means that optimal spectra are obtained at 600 MHz using a double labelled sample.

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P203

Pulsed ENDOR Spectroscopy of the S_2-State Multiline Signal of Photosystem II

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The oxygen evolving complex (OEC) in photosystem II (PSII) is the catalytic core where water splitting and oxygen evolution takes place. Water oxidation occurs during the five steps (S_{0-4}) of the redox-cycle (Kok-cycle).[1] The OEC consists of four Mn ions and one Ca ion connected by several μ-oxo bridges.[2] Due to radiation damage[3] and resolution limitations of the crystal structure[4] the bridging ligands between the metal ions and the protein ligands of the cluster have not been unequivocally identified yet. However, the knowledge of the electronic and geometric structure of the OEC as well as its ligand surrounding is the foundation for a mechanistic understanding of the OEC catalysed water splitting.

EPR spectroscopy is well suited to gain information on the electronic structure and subsequently on the geometry of radicals and paramagnetic centers. ^{55}Mn-ENDOR spectroscopy can directly provide information on the hyperfine couplings of the OEC. Here, we addressed two problems with this technique.

First, ^{55}Mn-ENDOR spectroscopy was applied to the S_2-state of the OEC in PSII single crystals. The analysis of the recorded spectra will be presented, allowing a tentative assignment of the Mn ion in the OEC carrying the largest hyperfine coupling, i. e. likely the oxidation state III, to two out of the four Mn positions in the structure.

The second question concerns the Ca ion in the OEC. Ca is a functional important part of the OEC as without Ca activity is lost. The only known functional substitute for Ca^{2+} is Sr^{2+}. To learn more about the role of Ca^{2+} we investigated the effects of Sr-substitution for Ca on the electronic structure of the OEC in the S_2-state by ^{55}Mn-ENDOR at 34 GHz.

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P204

Structural investigation of pro-apoptotic protein BNIP3

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Mitochondrial pro-apoptotic protein BNIP3 belongs to BH3-only subfamily of BCL-2 family. Cell death mediated by BNIP3 is independent of cytochrome c release and shows several characteristics of necrosis. BNIP3 plays important role in hypoxic cell death of normal and malignant cells. BNIP3 inserts into outer mitochondrial membrane by C-terminal transmembrane domain that is crucial for pro-apoptotic activity, mitochondrial localization, and homodimerization of the protein. BNIP3 unlike the other members of BH3-only subfamily does not require BH3 domain for its pro-apoptotic activity, whereas the its N-terminal region can be involved in interaction with BCL-2 family proteins. The deep analysis of BNIP3 activity is problematic without availability of raw material, containing this protein in big quantities. The BNIP3 gene was expressed in *Escherichia coli* directly or as fusion to 3′-end of SUMO or Mistic genes. The highest expression level was observed in the case of the SUMO fusion – more than 100 mg/L in rich autoinduction medium. The fusion protein contains two affinity tags – sequence of six histidins on N-terminus of SUMO and Streptag II on C-terminus of BNIP3 for efficient purification. Several milligrams of ^{15}N-labeled recombinant BNIP3 was isolated. The protein was solubilized in aqueous solution of DPC micelles and investigated by solution NMR spectroscopy. Acquired spectra represent dispersion of chemical shifts suited for predominantly alpha-helical protein that was confirmed by CD spectra.

P205

The dynamic hydrogen bonding network in the distal pocket of the nitrosyl complex of *Pseudomonas aeruginosa* cd_1 nitrite reductase

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P. aeruginosa cd_1 NiR contains a d_1-heme cofactor, found only in this class of enzymes, where the reduction of nitrite to NO occurs. The unique d_1-heme structure is possibly responsible for the fast NO dissociation rate from the ferrous form, not observed in standard *b*-type hemes.[1] The location of Tyr_{10}, His_{327} and His_{369} in the distal pocket, and the formation of H-bonds between these residues and the substrate (NO_2^-) and the product (NO) are strategic factors in catalysis. In this work we have demonstrated the value of high field ENDOR combined with DFT calculations[2] for the characterization of H-bonds and highlighted the major role of DFT in assigning and interpreting of ENDOR spectra. We have shown that the NO in the d_1-heme-NO complex of cd_1 NiR forms H-bonds with Tyr_{10} and His_{369} and by this stabilizes the NO complex. The second conserved histidine, His_{327}, appears to be less involved in NO stabilization by H-bonding. We have also observed a larger solvent accessibility to the distal pocket in the mutants compared to the WT. It is clear from this work that the H-bonding network within the active site is dynamic and that a change in one of the residues does affect the strength and position of the H-bonds formed by the other ones. In the Y10F mutant His_{369} moves closer to the NO and its hydrogen bond is shorter, whereas mutation of both distal histidines displaces Tyr_{10} removing its H-bond.

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P206

A Metalobonomic Study of Fatty Acids Influence in Hepatic Cell Growth

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In the recent years a big interest has been shown in the food science, with a particular interest in fatty acids. There is a very heated debate about the role of fatty acids in human nutrition, but the data is mostly empirical and subjective and no clear explanation of metabolic processes involved is given. To cast light on these issues we have studied the influence of different fatty acids on the metabolism of hepatic cells by analyzing their excreted metabolites. Liver cells grown in HepG2 growth medium enriched with small quantities of elaidic, oleic and stearic acid have been investigated by 1D NMR spectra of the medium. Based on the spectral regions that mostly show signals from excreted metabolites we can differentiate between cells grown in different cell growth media. Lactic acid is the metabolite that changes most between the four classes of growth medium. A difference in glucose levels is also observed. The difference between glucose levels and the changes in lactic acid show the influence of the fatty acids in metabolic processes.

P207

Interaction between amyloid beta peptide and an aggregation blocker IAPP mimicking peptide: Importance of histidine residues

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Amyloid beta peptide assembly into cytotoxic oligomeric and fibrillar aggregates is widely believed to be a main pathologic event in Alzheimer's disease (AD). Interfering with aggregation of Abeta is a major target in currently developing therapeutic strategies. Prior studies have shown that the double N-methylated analog of islet amyloid polypeptide (IAPP) IAPP-GI, which is a conformationally constrained IAPP analog mimicking non-amyloidogenic IAPP, is capable of blocking cytotoxic self-assembly of Abeta.[1] In contrast to native IAPP, IAPP-GI is soluble, non-amyloidogenic, and lacks, due to methylation of specific amide bond nitrogens, the ability to propagate the hydrogen-bond network of inter-molecular beta sheets. Here we investigate the interaction of IAPP-GI with Abeta40 using NMR spectroscopy. Chemical shift perturbation analysis after titration of Abeta40 with IAPP-GI showed fast-exchange of Abeta40 between bound and free forms, with the most significant perturbation observed around residues 11-20. In addition, water-amide proton exchange rates were lower for residues 15-20 and 28-38, suggesting that these residues are less accessible to chemical exchange with solvent in the bound form of Abeta40. In conclusion our data indicate that Abeta interacts with IAPP-GI predominantly in the two regions of the sequence which are converted into β-strands in amyloid fibrils, with the histidine residues at position 13 and 14 probably playing an important role in mediating intermolecular interactions.

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P208

Detecting the transition from normal to malignant phenotype in the brain of rats bearing implanted C6 gliomas by multinuclear HR MAS and genomic analysis

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We report a ^{1}H and ^{13}C HR-MAS NMR study of normal and diseased brain regions of rats bearing C6 gliomas implanted. The detection of selectively enriched metabolites through *ex vivo* HR-MAS spectroscopy and the correlations with the expression of the genes involved in the glycolytic metabolism are the aims of this work. C6 gliomas were induced in rats and tumour growth was evaluated *in vivo* using T_1 and T_2 weighted MRI. Three weeks after C6 implantation, rats were infused with [1-^{13}C] glucose and then cerebral metabolism was arrested. The fixed brain was removed from the skull and five biopsies were taken from different brain regions. The ^{1}H spectra show the increase in Lac and mobile lipids in the tumour biopsies (regions 3 and 4, fig. 1) and the ^{13}C spectra present a significant increase of (3-^{13}C) Lac and decrease of (4-^{13}C) Glu and (4-^{13}C) Gln, revealing a marked increase in glycolytic metabolism in the tumour (fig. 2). Then, we investigated the individual expression of specific genes coding for enzymes involved in the glycolytic pathway, to improve our understanding of the genetic basis of the metabolic profile observed by ^{13}C HR-MAS.

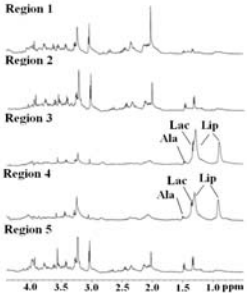

Figure 1. Representative CPMG *ex vivo* ^{1}H HR-MAS (TE:144ms) spectra.

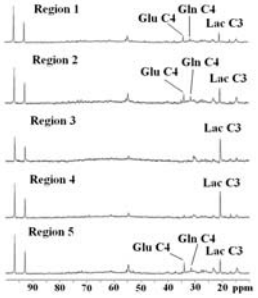

Figure 2. Representative ^{13}C *ex vivo* HR-MAS spectra.

P209

Analysis of the interaction of UIM domain STAM2 with Ubiquitin by NMR

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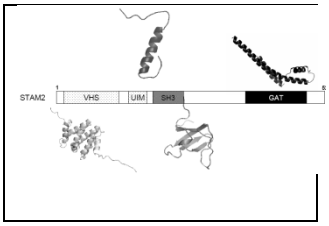

STAM2 is a human protein of the STAM (signal transducing adaptator molecule) family, involved in numerous cellular pathways as signal transduction, T-Cell development, lysosomal pathway,[1] as well as in the cell sorting ubiquitinated cargo. The cell sorting is possible because STAM2 contains two Ubiquitin Binding Domains (UBDs): modular protein domains that non-covalently bind to Ubiquitin, or ubiquitinated cargo, called: VHS and UIM (see Figure1).

Our NMR study focuses on the dynamical and structural characterization of the UIM domain in interaction with Ubiquitin. Two methods will be used: chemical shift perturbation (CSP) and spin relaxation. The CSP experiments on uniformly labeled ^{15}N UIM domain allowed us to map the interaction surface of UIM domain with Ubiquitin and to determine the dissociation constant (K_D) in presence of Ubiquitin. The same study has been done with labeled Ubiquitin in interaction with UIM. We observed that UIM domain, an amphiphilic helix, interacts on the hydrophobic patch of Ubiquitin. The spin relaxation data on UIM alone and UIM in interaction with Ubiquitin, indicates that UIM domain of STAM2 interacts with one Ubiquitin molecule.

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P210

Direct evidence of coexistence of horseshoe and extended helix conformations of membrane bound Alpha-Synuclein

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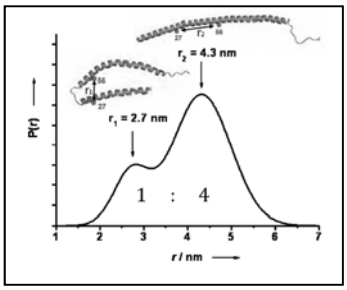
α-Synuclein (αS) is a 140 residue protein abundantly present in Lewy bodies characteristic of Parkinson disease. Although the exact function of αS has yet to be determined, membrane binding has been suggested to be important for its physiological role. While αS is natively unfolded in solution, upon binding to membranes it adopts an amphipathic, α-helical structure involving residues 1-100.



There is ongoing debate about the physiologically relevant conformation of membrane-bound αS with some reports favouring a horseshoe, others an extended helix structure.[1-4] Experimental data obtained by site-directed spin labelling in combination with pulsed electron paramagnetic resonance provide compelling evidence of the coexistence of the horseshoe structure and an extended helix of αS bound to a membrane surface, and potentially resolve the debate on the structure of membrane-bound αS.

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P211

Metabolic profiling of lung tumour tissues by High Resolution Magic Angle Spinning (HRMAS) NMR-metabonomics

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This work aims to evaluate the potential of NMR-metabonomics for discriminating between tumour and non-involved (control) pulmonary tissue as well as between different histological types. Paired tissue samples from 24 patients with primary lung cancer were directly analysed by ^{1}H HRMAS NMR (500 MHz) and the spectral profiles subjected to multivariate analysis, namely Principal Component Analysis (PCA) and Partial Least Squares Regression Discriminant Analysis (PLS-DA). Tumor and control tissues were clearly discriminated in the PLS-DA model with 95% sensitivity and 100% specificity. In agreement with previous work,[1] the metabolites giving rise to this separation were mainly lactate, glycerophosphocholine, phosphocholine, taurine, glutathione and uridine di/tri-phosphate (elevated in tumours), and glucose, phosphoethanolamine, acetate, lysine, methionine, glycine, *myo*- and *scyllo*-inositol (reduced in tumours compared to control tissues). Furthermore, PLS-DA of a sub-set of tumour samples allowed carcinoid tumours to be discriminated from adenocarcinomas and epidermoid carcinomas and suggested a trend for the metabolic differentiation between these latter classes.^{1}H HRMAS NMR was found to be suitable, in tandem with multivariate analysis, for characterizing the tumours both in terms of malignancy biomarkers and in respect to different histological types, suggesting that the metabolic profile may aid in the differential diagnosis of lung cancer.

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P212

Nuclear Magnetic Resonance applied in Adenosine Kinase inhibition

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The Adenosine Kinase (AK) is an important enzyme related to many important metabolic routes. The AK has two ligand sites; one is to ATP and the other to adenosine (ADO). Inhibitors of adenosine kinase elevate adenosine to levels that activate nearby adenosine receptors and produce a wide variety of therapeutically beneficial activities. For this reason, the investigations and development of compounds that can inhibit the AK are of great importance. The Nuclear Magnetic Resonance is a technique with great ability to characterize potential inhibitors, especially by ^{1}H-NMR, ^{13}C-NMR and 2D techniques, like HSQC, gHMBC, gCOSY, and also to evaluate the interactions among inhibitors/AK, by the utilization of STD (Saturation Transfer Difference, 1D and 2D) experiments. Different pharmacophore groups have been studied, but the 4-anilinoquinazolines are a class of compounds with special importance in this area. The 4-anilinoquinzolines mimic AK's natural substrates, and as our *in silico* studies have demonstrated, great affinities in binding to AK. The target compounds were obtained by three steps synthesis[1] with slight modifications in comparison to literature and two additional purification procedures; and characterized principally by NMR. For this purpose the compounds were solubilized in DMSO-d_6 and analyzed by INOVA Varian 500 MHz spectrometer. Our interaction studies were based on standard 1D and 2D STD-NMR procedures using 1:100 molar ratio between AK (cloned, expressed and purified by us), this enzyme substrates (ADO and ATPγS) and synthetized compounds. Our results showed that two of series of 30 inhibitors have strong interactions with AK, and hopefully, upon isotopic labeling of AK (pET28a-SUMO-Adk) further NMR investigations will indicate its binding site for 4-anilinoquinazoline inhibitors.

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P213

NMR – Derived Solution Structure and Calcium binding properties of partially folded EhCaM: a calmodulin like protein from Entamoeba histolytica

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The calcium binding proteins from *Entamoeba histolytica* belong to a superfamily of EF-hand proteins and play a vital role in the pathogenesis of this parasite, through various Ca^{2+}-signaling pathways. It has been identified to have 27 CaBPs in the genome of *E. histolytica* and one of them identified as EhCaM. This EhCaM is thought to be a calmodulin (CaM) like protein, from sequence homology studies. The presence of EhCaM in addition to other EhCaBPs, in the eukaryote *E. histolytica* is still under debate, though there is some biological evidence for the presence of CaM like proteins in this parasite. Further the immuno-fluorescence data suggests the localization of EhCaM in both cytoplasm and nucleus unlike other CaBPs.

In an attempt to understand the structural and functional similarity of EhCaM with CaM, the 3D high-resolution solution structure of EhCaM has been determined using NMR. NMR-derived structure of EhCaM revealed that, the protein is predominantly α-helical in conformation and possess converged N-terminal domain with the lowest RMSD (0.7 Å). On the other hand we noticed that the structure of its C-terminal domain could not be conserved revealing the EhCaM is partially folded. HSQC based NMR and ITC experiments showed that EhCaM sequentially binds two Ca^{2+} with a moderate affinity, both the binding sites are present in its N-terminal domain and no Ca^{2+}-binds to its C-terminal domain due to extensive mutations at the conserved Ca^{2+}-binding loops during the process of evolution. The structural features are further discussed in light of the ^{15}N-relaxation data.

P214

Interaction of STAT6$^{783-814}$ with NCoA1 PAS-B domain: elucidating the structural features of the complex by NMR

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Signal transducer and activator of transcription 6 (STAT6) regulates transcriptional activation in response to interleukin-4 (IL-4)-induced tyrosine phosphorylation by direct interaction with coactivators, an event which plays a very crucial role in several biologically important processes, including the activation of genes involved in immune and anti-inflammatory responses.[1] The CREB-binding protein and the nuclear coactivator 1 (NCoA1) bind independently to specific regions of STAT6 and act as coactivators. STAT6-NCoA1 interaction is mediated by a short region of the STAT6 transactivation domain that includes the motif LXXLL.[2] The crystal structure of STAT6 derived peptide (Leu^{794}-Gly^{814}) in complex with NCoA1 PAS-B domain$^{257-385}$ revealed that the leucine side-chains of the motif, are deeply embedded into the hydrophobic groove of the NCoA1 surface.[3] Recently, it has been demonstrated by a fluorescence polarization binding assay that additional residues (Leu^{794}, Pro^{797}, Thr^{798}), flanking the LXXLL motif in STAT6, may play an important role in stabilizing the protein binding to NCoA1.[4] In spite of this wealth of knowledge, the details of this strenghtened interaction are still poorly understood. In the current study, we have undertaken the structural characterization by NMR of STAT6$^{783-814}$-NCoA1 PAS-B domain complex to address the interaction mechanism in a more detailed level. The initial analysis of NMR data, including chemical shift mapping, mobility analysis and water amide proton exchange rates suggests that the interaction is stabilized by an extended region of STAT6. Upon completion of structural calculation, analysis of the three dimensional structure of the complex will shed further light on the structural rearrangements that characterize this protein-protein interaction.

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P215
Correlated Motion in the Protein Core: Implications for Protein Recognition

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The residual dipolar coupling (RDC) derived ubiquitin ensemble[1] covering a previously inaccessible supra-τ_c window between a few ns and 50 μs, provides evidence for molecular recognition governed by conformational selection. The ensemble also predicts a high amount of long-range correlated motion within a protein. This correlated motion is necessary to overcome the high entropic barrier imposed from a combinational standpoint, due to the probability of finding all residues simultaneously in the proper configuration for successful molecular recognition. The long-range correlated motion occurring in the supra-τ_c window can be studied by cross-correlated relaxation (CCR) rate measurements,[2-6] which cover the same time scale. However, observation of the long-range correlated motion has been hindered by the lack of experimental methodology. Here, we present a novel method to detect the long-range correlated motions using CCR between the methyl groups in the protein core. These results have implications not only for protein recognition, but may provide a method for following the trajectory of protein folding in regard to long-range correlated motions within the protein's core.

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P216
Linking function to dynamics in globular and unstructured proteins using NMR

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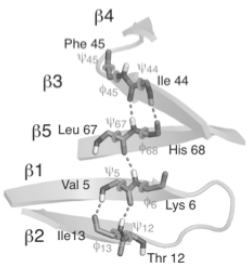
A detailed understanding of the biological function of macromolecules requires both knowledge of their 3D structure and their time-dependent fluctuations. Methods to determine the structure of proteins and nucleic acids are now well established and the static representations that they provide have contributed much to our understanding of protein stability and biological function. The determination of the structural fluctuations at atomic resolution is, by contrast, still in its infancy, particularly for motions taking place at biologically relevant time scales. Recently, however, newly developed methodologies that exploit the information contained in residual dipolar couplings (RDCs) have provided key insights into the link between the dynamics of macromolecules and their function. In this communication we will present the determination of native ensembles for globular and disordered proteins that explicitly represent their structural heterogeneity in the sub-ms time scale. The detailed descriptions of macromolecular dynamics that we have obtained have allowed us to characterize the transfer of structural information across a surface patch in ubiquitin involved in molecular recognition by the proteins that regulate protein degradation and the native residual contacts in chemically denatured ubiquitin that initiate the folding of this protein[1]



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P217

Atom By Atom Analysis Of The Ultrafast Folding α+β Protein GPW

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In contrast to the conventional view, in which single-domain proteins fold according to a two-state model with a large free energy barrier and no transient accumulation of intermediates, theory predicts the possibility of barrierless (downhill) protein folding.[1] Downhill protein folding is exciting from an experimental viewpoint because under these conditions it becomes possible to investigate the folding process at a full atomic level in equilibrium experiments using NMR methods.[2] This idea has been recently demonstrated experimentally in the small ultra-fast folding protein BBL.[3]

Here we extend this approach to gpW, another ultra-fast folding protein that appear to cross a marginal folding barrier according to a variety of equilibrium and kinetic tests.[4] gpW is a larger protein (62 aminoacids) with an α/β folding topology. The main goal of this analysis is to determine whether folding over marginal barriers is also accessible to the atom by atom equilibrium analysis of protein folding, thus extending its applicability to a wide range of proteins that fold in the sub-millisecond timescale. Particularly, we have followed the equilibrium thermal unfolding of GPW using multidimensional NMR to monitor the changes in ^{15}N, ^{13}C and ^{1}H chemical shifts in the 273-371 K temperature range. Our results confirm the presence of unfolding heterogeneity at the atomic level and enable a microscopic interpretation of structural events during the folding of GPW. Performing a residue pairwise comparison of atomic unfolding behaviors as described before,[3] we obtain a map of the critical network of interactions stabilizing GPW in its native structure that provides critical insights into the structural determinants and mechanism of folding in this protein.

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P218

NMR-based structural investigations of a minimalistic neomycin sensing riboswitch in complex with different aminoglycosids

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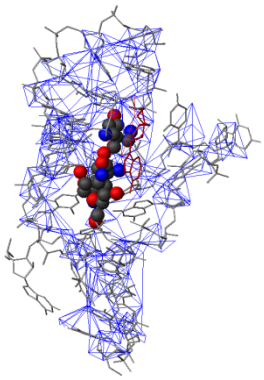
The 27nt engineered neomycin sensing riboswitch (N1) is the smallest functional riboswitch identified so far.[1] N1 represses gene expression upon binding of the aminoglycoside antibiotics neomycin B and ribostamycin. The NMR structure of the N1-ribostamycin-complex has already been solved and ligand binding was found to occur according to a conformational capture mechanism.[2] While the very closely related compounds paromomycin and tobramycin also bind to N1 with high affinity they are not able to inhibit gene expression in vivo. We used high resolution NMR structural studies to delineate structural differences of N1 in complex with regulatory active and inactive aminoglycosides (tobramycin and paromomacin) in order to identify the structural basis for the differences in their regulatory activities. These investigations will contribute to understanding the relationships between ligand binding and regulatory activity in vivo.



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P219

The HIV-1 gp120 Interaction with Nt-CCR5 – New Insights from NMR

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Human immunodeficiency virus type 1 (HIV-1) is the retrovirus that causes the *acquired immunodeficiency syndrome* (AIDS). HIV infection in humans is a pandemic affecting millions of people around the world. HIV-1entry into target cells is mediated by the successive interaction of the viral envelope glycoprotein gp120 with the cellular receptor CD4 and with a G protein-coupled chemokine co-receptor, mainly CCR5 or CXCR4. Interaction of CCR5 with the HIV-1 gp120-CD4 complex involves the amino-terminal domain of the CCR5 receptor (Nt-CCR5) and requires posttranslational sulfation of its tyrosine residues.

We studied a 27-residue peptide corresponding in sequence to Nt-CCR5 (residues 1-27) and containing two sulfated tyrosine residues at positions Y10, Y14, in complex with a truncated, homogenously glycosilated, gp120 $_{JR-FL}$ (residues 88-492, ΔV1, ΔV2) and a CD4-mimic miniprotein. T_{1-rho}-filtered NOE experiments revealed evidence for a helical conformation in the center of the peptide, induced upon gp120 binding. Saturation Transfer Difference (STD) experiments allowed identification of the Nt-CCR5 residues that participate in gp120 binding and highlight the importance of the sulfated tyrosine residues.

P220

GABARAP directly interacts with Bcl-2: Structural basis of an interaction at the interface between autophagy and apoptosis

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The cross talk between apoptosis and autophagy plays an essential role in the regulation of tumorigenesis and development. One of the most prominent regulators of apoptosis is the anti-apoptotic protein Bcl-2 (B-cell lymphoma 2), which is also able to inhibit autophagy, depending on its localization. Thus, Bcl-2 is one of the nodes connecting apoptosis and autophagy. The present work identifies GABARAP as a direct ligand of Bcl-2. Using NMR spectroscopy, information about the binding surfaces of both proteins were obtained. Based on these data, an atomic model of the complex was generated using HADDOCK. This model reveals that the N-terminal region of the BH4 domain of Bcl-2 is anchored to a prominent hydrophobic pocket of GABARAP.[1,2] In addition, the interaction was verified by co-immunoprecipitation and pulldown assays. Functional studies showed, that over-expression of Bcl-2 inhibited the lipidation of GABARAP, a key step in autophagosome formation. These results support and further define the regulatory role of Bcl-2 in autophagy.

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P221

Dynamics of multi-domains proteins seen by NMR: the di-ubiquitin model

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While recent progresses[1] have been made in understanding intra-domain conformational fluctuations, the evaluation of the relative motions of individual domains of multidomains proteins is still a challenge. We use monomers of ubiquitin, a 76-residues protein, linked by isopeptide bonds between Lys48 and the C-terminal residue Gly76 as a model to assess new methodologies. It has been proposed that this dimer is in equilibrium between a 'closed' state and an 'open' state, where the hydrophobic surfaces of the two monomers are, respectively, in direct interaction or not.[2] The thermodynamic and kinetic parameters of this equilibrium are not yet fully understood. We would like to address here this issue on the basis of our studies by NMR spectroscopy.

We conjugated ^{15}N-labelled ubiquitin monomers, by enzymatic reaction, to obtain two types of dimers. One is a 'linear' dimer, which contains one isopeptide bond, and the other is a 'cyclic' dimer, with two identical isopeptide bonds between the monomers. ^{1}H-^{15}N HSQC spectra suggest that the mono-ubiquitin and the 'cyclic' di-ubiquitin might be, respectively, good models for the "open" and the 'closed' states. We characterized the 'cyclic' dimer structure to confirm its potential as a model for the 'closed' state. The ^{1}H-^{15}N HSQC spectra revealed the symmetric structure of the 'cyclic' dimer. Consequently, by using RDC data, we could obtain an ensemble of possible structures for comparison with structures of the 'closed' conformation. We also used relaxation data to evaluate the dynamics of the various conformations.

Thus, the usage of cyclic dimer has been a tremendous advantage. It allowed us to make the first steps toward an accurate evaluation of the thermodynamic and kinetic parameters of the 'open / close' equilibrium.

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P222

Lipid-Protein Nanodiscs in High-Resolution NMR Studies of Integral Membrane Proteins and Membrane Active Peptides

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Lipid-protein nanodiscs (LPNs) represent a fragment of lipid bilayer (~160 lipid molecules) stabilized by a dimer of human apolipoprotein A1. These particles have discoid shape with characteristic dimensions 10x4 nm. The applicability of LPN based membrane mimetics in high-resolution NMR investigations of membrane proteins and membrane-active peptides was studied using bacterial K+ channel KcsA, isolated voltage-sensing domain of the K+ channel KvAP (VSD) and antibiotic antiamoebin I (Aam-I). The complexes of KcsA with LPN/DMPC, of VSD with LPNs containing DMPC, DMPG, and POPC/DOPG, and of Aam-I with LPNs based on DOPG, DLPC, DMPC, POPC and DLPC/DLPG lipids were formed. The hydrodynamic properties and stoichiometry of obtained complexes were studied using gel-filtration, ^{31}P-NMR spectroscopy, ^{15}N-CSA/dipolar cross correlation and translational diffusion measurements. The complexes demonstrate isotropic motion on the NMR time scale with effective rotational correlation times of ~ 50 ns at 45°C. The 2D ^{1}H-^{15}N-spectra of these proteins recorded in the LPNs environment demonstrated similar signal dispersion but significantly increased line-width as compared to spectra in detergent micelles and bicelles commonly used in NMR spectroscopy. The observed ^{1}HN line-width was about 50-80 Hz for membrane-associated fragments of the molecules and about 20-30 Hz for mobile solvent-exposed domains. The spectra of VSD and Aam-I embedded into LPNs containing pure PC lipids showed an additional selective line broadening, thus suggesting μs-ms exchange processes within incorporated proteins. On the example of VSD it was shown that LPNs can be used as a reference medium in NMR screening of conventional detergent media for studies of integral membrane proteins. The high-quality TROSY spectra obtained for VSD and Aam-I in the complexes with LPNs containing PG lipids suggest the feasibility of direct structural studies of integral membrane proteins and membrane active peptides incorporated into the bilayer of LPN particles.

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P223

Structure and Function of the *E. coli* Rhomboid Protease N-terminal Cytoplasmic Domain

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Rhomboids are unique membrane proteins that use a serine protease mechanism to cleave their transmembrane substrate within the lipid bilayer. The proteolytic activity responsible for these functions is achieved by a core domain comprised of 6 transmembrane (TM) segments. In addition to the core domain, many rhomboids also possess aqueous domains of varying sizes at the N- and/or C-termini, the sequences of which tend to be rhomboid-type specific. The functional role of these domains is generally not well understood; however, we previously uncovered an interaction between the cytoplasmic domain and the catalytic core domain when the bacterial rhomboid protease activity was the highest.[1] To investigate this interaction in greater detail, the *E. coli* rhomboid (GlpG) was chosen based on the availability of X-ray crystal stuctures[2-4] for its core domain. Solution NMR was used to solve the structure of the isolated N-terminal cytoplasmic domain, which is now being used to identify residues that may be involved in domain interactions.

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P224

NMR Characterization of the ^{13}C Enriched Cholesterol Biosynthesized by Yeast

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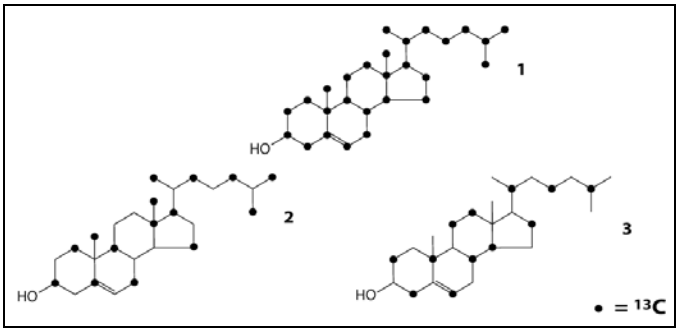

Cholesterol is a very important lipid in all eukaryotes. It has attracted a lot of attention because of its involvement in cardiovascular diseases and because it has been suggested to play an important role in formation of membrane microdomains. Until now, only very limited and expansive sources of labelled cholesterol were available. Previously, we engineered a *Saccharomyces cerevisiae* strain that was capable of synthesizing cholesterol.[1] We present the first efficient biosynthesis and purification of ^{13}C-enriched cholesterol using an improved, stable strain of yeast that produces almost exclusively cholesterol. We produced uniformly labelled cholesterol 1 with up to about 95% enrichment. The source of carbon being glucose we could take advantage of the availability of (1-^{13}C, 99%) and (2-^{13}C, 99%) to selectively favor the enrichment of the metabolitic intermediate AcCoA at the CH_3 or CO positions, which should result to the isotopomers 2 and 3 respectively. These enriched compounds should find many applications in biology.

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P225

Phosphorylation in the Proline-rich region of the neuronal Tau protein induces a change of conformation

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The Tau protein has generated a lot of interest due to its potential causative involvement in the Alzheimer's Disease (AD) pathogenesis and other neurodegenerative diseases. Tau is indeed a neuronal Microtubule Associated Protein (MAP), which has a central role in both physiological (neurone cytoskeletal stabilisation) and pathological (aggregation in Alzheimer disease) processes. Knowing that the neuronal phosphoprotein Tau protein has 40 potential sites of phosphorylation and that it regulates its interaction with microtubules and its aggregation, it is therefore, fundamental to pursue the challenge to understand functional and structural molecular aspects of phospho-Tau's.

Although Tau is described as a globally disordered protein when isolated in solution, previous work using high resolution NMR has shown that local transient structures and global dynamic folding can be characterized, that could be important for its function. These transient conformations could reflect a possible ordered structure once Tau is bound to the microtubules or aggregated. However, little is known about the impact of phosphorylation on these characteristics. We here decided to characterize the conformational changes that are induced by phosphorylations in the regulatory Pro-rich region of Tau. To do so, we have chosen a Tau Fragment to avoid too much overlap in the spectrum but that still remains functional, thanks to the repeats R1 to R3, and contains the Pro-rich domain. This Tau Fragment was fully phosphorylated on the T231 and Ser235 residues by a CDK/cyclin kinase, reconstituting the AT180 epitope found in AD brain or on the S214 site by cAMP-dependent Protein Kinase A kinase. We present here our data on the first structural characterization of a phospho-Tau protein based on the chemical shift values, RDC measurements and Paramagnetic Relaxation Experiments NMR parameters.

P226

Rapid structural characterization of antibody-antigen complexes through experimentally validated computational docking

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If we understand the structural rules governing antibody/antigen interactions in a given virus, then we have the molecular basis to attempt to design and synthesize new epitope to be used as vaccines or optimize the antibodies themselves for passive immunization. Comparing the binding of several different antibodies to related antigens should also further our understanding of general principles of recognition.

To obtain and compare the three dimensional structure of a large number of different complexes, however, we need a faster method than traditional experimental techniques. While biocomputational docking is fast, its results might not be accurate. Combining experimental validation with computational prediction may be a solution.

As a proof of concept, here we isolated a monoclonal antibody from the blood of a human donor recovered from Dengue Virus infection, characterized its immunological properties and identified its epitope on domainIII of Dengue Virus E protein through simple and rapid NMR chemical shift mapping experiments. We then obtained the three-dimensional structure of the antibody-antigen complex by computational docking, using the NMR data to drive and validate the results. In an attempt to represent the multiple conformations available to flexible antibody loops, we docked several different starting models and present the result as an ensemble of models equally agreeing with the experimental data. The antibody was shown to bind a region accessible only in part on the viral surface, explaining why it cannot effectively neutralize the virus.

P227

Investigations into a 69 kDa dimeric Glycosyltransferase: Isotope Labeling and NMR studies

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Glycosyltransferases (GTs) belong to an important class of enzymes that are responsible for the biosynthesis of oligosaccharides, polysaccharides and glycoconjugates. These biomolecules have essential functions which range from structural roles and energy storage to signal transduction, and are ubiquitous in all organisms. GTs catalyze the transfer of a sugar moiety from an activated donor sugar to an acceptor which can be another carbohydrate, lipid, protein, DNA, or natural product. The reaction proceeds either with retention or inversion of the configuration of the anomeric center of the donor sugar. While the catalytic mechanism of inverting GTs is largely established, the mechanism of retaining GTs is still a matter of debate.

As the expression of recombinant and soluble human blood group B galactosyltransferase (GTB) is possible,[1] we have chosen GTB as a model for retaining GTs. Optimized expression conditions based on the labeling technique of Marley et al.[2] were used to obtain high yields of isotopically labeled recombinant GTB, that allowed recording TROSY HSQC spectra with a high resolution. On this basis further NMR experiments were performed including studies of protein-ligand interactions.

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P228

Postprandial effects of sea buckthorn and lingonberry: A metabolomic study of human blood plasma and urine by NMR and statistical methods

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The aim of the study was to find out the effect of berries on postprandial metabolism and absorption after a high fat meal. The clinical study was randomized, single-blind, cross over study, where the subjects were their own controls. The blood has been collected from the study subjects before the test meal and postprandially for 6 hours. Urine was collected before the test meal and after eating pooled until 180 min and from 180 min to 360 min. The meal consisted of yoghurt, rapeseed oil and sea buckthorn or lingonberry (crushed dried whole berries).

The NMR spectroscopic analysis of plasma samples included mainly three experiments: water suppressed ^{1}H spectrum, cpmg spectrum to suppress the signals from large biomolecules, and 1-dimensional diffusion measurement to suppress all small molecule signals. For urine samples only water suppressed ^{1}H spectra were recorded. In addition several 2D techniques were utilized for selected samples to allow the identification of most important signals. NMR spectra were bucketed by Amix-program and further analysed statistically by PCA and PLS-DA methods.

The results obtained thus far show that berries have a significant effect on the postprandial metabolic profile. The absorption of lipids achieves its maximum clearly later after berry meals compared with control meals. In addition some berry specific compounds were identified from NMR spectra. The main compounds were ethyl-β-D-glucose for sea buckthorn and hippuric acid for lingonberry.

Overall this study demonstrates the usability of NMR with statistical methods to provide an effective tool for the analysis of postprandial effects from human blood plasma and/or urine samples.

P229

Cation Movements in G-Quadruplexes

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DNA molecules can adopt besides the well-known B-type double helix several higher-order structures, including G-quadruplex structures. G-quadruplexes are stable structures adopted by DNA guanine-rich sequences that can be found in the promoter regions of a number of genes, including oncogenes, ribosomal DNAs as well as in telomeric DNA regions. In the past years G-quadruplex DNA structures are a subject of great interest since their formation has been suggested to play a role in variety of important biological processes as well as due to their potential therapeutic applications. The main building blocks of G-quadruplex structures are stacks of square-planar arrays of G-quartets, consisting of four guanines that are linked together by eight hydrogen bonds. The presence of cations seems to be prerequisite for G-quartet formation due to their role in reducing repulsions amongst guanine carbonyl oxygen atoms and additionally enhancing base-base stacking interactions. A fairly wide variety of cations is capable of inducing formation of G-quadruplex structures. In general, cations have been localized along the central cavity of G-quadruplex between two G-quartets or in the plane of a G-quartet. Cations inside G-quadruplex structures are not static, but are moving between binding sites and bulk solution. Bigger cations such as ammonium or potassium require partial opening of G-quartets to move through. The use of heteronuclear NMR in combination with ^{15}N-labeled ammonium ion as a non-metallic substitute enabled us to localize $^{15}NH_4^+$ ion binding sites between pairs of adjacent G-quartets and in addition study kinetics of their movement inside a number of G-quadruplex systems. We were able to demonstrate that $^{15}NH_4^+$ ion movement within G-quadruplex core and into bulk solution is influenced by G-quadruplex molecularity as well as by steric restraints imposed by loop residues.[1-3]

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P230

Heterogeneous Hydrogen Bonding and Dielectric Environment of a Transmembrane α-Helix by pH-sensitive Spin-labeling and High Field EPR

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Membrane proteins are exposed to exceptionally large transmembrane gradients of hydrophobic and hydrogen bonding interactions that are known to be essential for these proteins' membrane insertion and folding, thermodynamic stability, and ultimately function. Currently, a rather limited set of experimental data could be found on the effective dielectric gradient and the hydrogen bond network experienced by the protein side chains immersed into the lipid bilayer in a transmembrane orientation. Here we employ an arsenal of advanced spin-labelling EPR methods to profile heterogeneous dielectric and hydrogen bonding environment along the α-helical chain of an alanine-rich WALP peptide that is anchored in a lipid bilayer in a transmembrane orientation. A series of WALP cysteine multants was labeled with a pH-sensitive nitroxide IMSTL (S-(1-oxyl-2,2,3,5,5-pentamethylimidazolidin-4-ylmethyl) ester) that is similar in molecular volume to phenylalanine.[1] The protonation state of this nitroxide could be directly observed by EPR allowing us to follow proton gradient across the membrane in the vicinity of the WALP α-helix, and, thus, to reconstruct the gradient in the effective dielectric constant. These experiments were complemented by measurements of local polarity from characteristic changes in EPR spectra that were enhanced by use of perdeuterated and ^{15}N-substituted nitroxides and high field EPR at 130 GHz (D-band). Formation of hydrogen bonds between the nitroxides and membrane-penetrating water molecules was observed directly in HYSCORE X-band experiments. Taking together these data provide experimental profiles of heterogeneous dielectric and hydrogen bonding environment along a transmembrane α-helix.

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P231

Molecular Mechanism of Phospholipid Transfer by Lipid Transfer Protein Sec14p: Multifrequency High EPR and ENDOR Study

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Sec14p is a major yeast phosphatidylinositol (PI)/phosphatidylcholine (PC) transfer protein that promotes the energy-independent transfer of either PI or PC between lipid bilayers in *vitro*. Although the crystal structure of Sec14p had recently become available, the detailed mechanism of the lipid binding is still unsettled. Here we report on multifrequency electron paramagnetic resonance experiments to analyze dynamics as well as the electrostatic and hydrogen bonding microenvironment for series of doxyl-labeled PC molecules bound by Sec14p in a soluble protein: PC complex. Partially resolved 130 GHz EPR spectra from *n*-doxyl-PC molecule bound to Sec14p were assigned to a hydrogen-bonded and a non-hydrogen bonded nitroxide species. Analyses allowed us to calculate the fraction of hydrogen-bonded nitroxide species and to characterize polarity and proticity profile along the phospholipid-binding cavity of Sec14p. The data suggest that water molecules are drugged into the protein cavity upon the lipid binding. Proposed lipid exchange mechanism indicates that the polarity gradient inside Sec14p cavity contributes to the driving thermodynamic force for extracting a single phospholipid molecule from the bilayer. Proposed mechanism is being confirmed by x-ray crystal structure. Calibration of electrostatic and hydrogen bonding effects on magnetic parameters of doxyl-labeled PC were carried out using structurally similar compound 5-doxyl stearic acid and a series of simple solvents of various polarity. 130 GHz pulsed ENDOR study of the H-bonds formed by 5-doxyl-SA in set of alcohols was conducted to investigate the geometry and strength of the hydrogen bond between the nitroxide radical and solvents and to correlate these parameters with polarity of the solvent.

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P232

^{1}H MRS of Human Bile in the Detection of Cholestatic Liver Diseases: Conjugation Pattern of Bile Acids could be a Diagnostic Indicator

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Bile acids in human bile are conjugated with the amino acids glycine and taurine which helps in the normal bile flow (from liver to the intestine) and protects the liver and bile ducts from the harmful effects of the unconjugated bile acids. In health, the ratio of glycine-conjugated bile acids (GCBAs) to taurine-conjugated bile acids (TCBAs) is generally 3:1.[1] Cholestasis is characterised by an impairment in the normal bile flow which results in various chronic liver diseases including malignancies such as cholangiocarcinoma and gallbladder cancer. It has been reported that during cholestasis, TCBAs are elevated in bile.[2] In this study, we collected bile samples from patients with and without cholestatic diseases and performed ^{1}H MRS on a 360 MHz NMR spectrometer. In additon to the elevation in the levles of TCBAs, we have also observed a decrease in the levels of GCBAs in some patients (Figure 1). The mean ± SD of the ratio of GCBAs to TCBAs in control and cholestatic patients were found to be 2.88 ± 1.47 and 1.47 ± 0.83 respectively. This ratio is considerably reduced in cholestatic patients compared to controls, and hence could be a valuable marker in the early detection of chronic cholestatic diseases augmenting routine liver function tests.

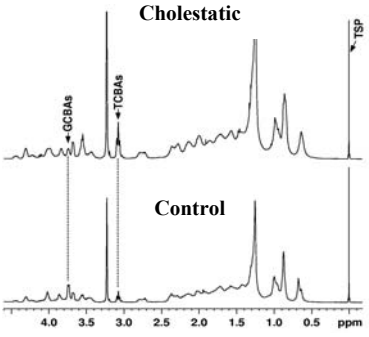

Figure 1: ^{1}H MRS of human bile from control and cholestatic patients showing elevated levels of TCBAs and reduced levels of GCBAs.

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P233

Characterization of the membrane proximal domain from HIV gp41 coat protein in detergent micelles with multi-dimensional NMR

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The membrane proximal ectodomain region (MPER) of the HIV-1 trimeric gp-41 coat protein is an important target for vaccine development. This region contains the epitopes for several broadly neutralizing antibodies against HIV-1, including 2F5 and 4E10. Kinetic studies indicate that the binding of 2F5 and 4E10 to these epitopes is likely assisted by the phospholipid membrane of the virus. We have designed and expressed peptides containing the MPER of HIV-1 with and without trimerization domains and membrane anchoring segments. Biophysical properties of these constructs in detergent micelles are being studied by multi-dimensional NMR and equilibrium analytical ultracentrifugation, along with surface plasmon resonance to probe the binding to the 2F5 and 4E10 antibodies. We have studied a monomer construct with a transmembrane anchor in several detergent micelles and have found that the detergent LMPG yielded good spectra. We have also initiated NMR studies of a trimer complex that does not include a full transmembrane domain in dodecylphosphocholine detergent micelles. Chemical shift data from backbone assignments using a variety of 3D data sets indicate the structure of the MPER domain is disrupted at the membrane interface which may contribute to the antibody recognition process.

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Biophysical characterization and solution structure of the Core Protease Domain of Anthrax Lethal Factor

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The most prominent virulence factor of the disease anthrax is the bacterium's lethal toxin (LeTx) and in particular a 90 kDa Zn-dependent highly specific metalloprotease called Anthrax Lethal Factor (LF).[1] LF exhibits high proteolytic specificity towards vital cellular signal transducers, the family of mitogen-activated protein kinase kinases (MAPKKs) cleaving them close to their N-termini, thus altering of signalling pathways vital for cell cycle.[2] Moreover, the high cleavage specificity of LF against these kinases, often found overexpressed in tumour cells,[3] might provide new insight for possible implication of engineered LF polypeptides in MAPKK-dependent cancer cells cycle regulation.[4] For this reason the LF-MAPKK substrates interaction is important for the understanding of enzyme specificity.[5]

Here we report the recombinant expression and purification of a C-terminal part of LF ($LF_{672-776}$) that harbors the enzyme's core protease domain. The biophysical characterization and backbone assignments (^{1}H, ^{13}C, ^{15}N) of the polypeptide revealed a stable, well folded structure even in the absence of Zn(II), suitable for high resolution structural analysis by NMR.[6] The NMR structure of the metal free catalytic core polypeptide has been also determined exhibiting great similarities with the crystal structures of the corresponding polypeptide both in Zn-free and Zn-loaded forms.

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P235

Ligand induced conformational capture of a synthetic tetracycline riboswitch revealed by pulse EPR

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RNA aptamers are *in vitro* selected binding domains which recognize their respective ligand with high affinity and specificity.[1] They are characterized by complex three-dimensional conformations providing preformed binding pockets which undergo conformational changes upon ligand binding. Small molecule binding aptamers have been exploited as synthetic riboswitches for conditional gene expression in various organisms. In the present study, double electron electron resonance (DEER) spectroscopy combined with site directed spin labeling[2,3] was used to elucidate the conformational transition of a tetracycline binding aptamer. Different sites were selected for post-synthetic introduction of either the (1-oxyl-2,2,5,5-tetramethylpyrroline-3-methyl) methanethiosulfonate by reaction with a 4-thiouridine modified RNA or of 4-isocyanato-2,6-tetramethylpiperidyl-N-oxid spin label by reaction with 2'-aminouridine modified RNA. The results of the DEER experiments reveal the presence of a thermodynamic equilibrium between two aptamer conformations in the free state and capture of one conformation upon tetracycline binding. In the outlook we will report on DEER experiments on DNA containing deoxyadenine residues which were functionalized with the spin label 4-azido-2,2,6,6-tetramethyl-piperidine-1-oxyl *via* azide-alkyne 'click' chemistry.

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P236

Structural characterisation of the C39 peptidase-like domain of the ABC-transporter HlyB

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Haemolysin B (HlyB) from *E. coli* belongs to the family of bacteriocin-associated ATP-binding cassette (ABC)-transporter. In complex with the outer membrane protein TolC and the membrane fusion protein HlyD, the ABC-transporter HlyB translocates the toxin HlyA over inner and outer membranes into the medium without detectable periplasmic intermediates. In general, ABC-transporters consist of two domains, the transmembrane domain and the ATP- or nucleotide-binding domain. HlyB however, like all members of the bacteriocin ABC-transporter family, features an additional N-terminal C39 peptidase-like domain. C39 peptidases are members of the thiol protease family. As a domain of bacteriocin ABC-transporters, those peptidases are assumed to cleave the protein or peptide substrate after a consensus GG motif. Interestingly, HlyB contains a proteolytically inactive C39 domain, as the functional important cystein is mutated to a tyrosine residue. Nevertheless the domain has to play an important role, because deletion of the C39 domain in HlyB abolishes the translocation activity completely.

We investigated the C39 domain structurally and solved its solution structure by NMR. Besides the fact that the C39 domain of HlyB contains a tyrosine residue at the important cystein position, the structure revealed a further distortion of the catalytic triade, stabilized by a tryptophan sandwich. Thus, the role of the C39 in the haemolysin transport system may differ from the canonical role known for homologous domains of other bacteriocin transport systems. We will present the solution structure of the isolated C39 domain of HlyB from *E. coli* and reveal insights in the interaction with the substrate of the transporter, the bacteriocin HlyA.

P237

Characterization of the Binding Mode of SARS Corona Virus spike protein by Saturation Transfer Difference (STD) NMR Spectroscopy

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A defined receptor binding domain (RBD) on the viral spike protein (S) mediates the attachment of SARS Corona Virus to its cellular receptor, angiotensin converting enzyme 2 (ACE2).[1,2] Binding of the SARS spike protein with ACE2 was analyzed by SPR and STD NMR.[3] From a peptide library a hexapeptide from Tyr438 to Leu443, Tyr-Lys-Tyr-Arg-Tyr-Leu (YKYRYL), of S protein was identified to have binding affinity to ACE2 (K_D= 46 µM). This peptide has also strong antiviral activity and can suppress viral proliferation completely at a concentration of 10mM.

STD NMR spectroscopy was used to detect the interaction of YKYRYL and related peptides with the receptor protein ACE2. Furthermore the self aggregation of the viral spike protein was checked. Therefore the binding affinity of YKYRYL to the S protein was investigated by STD NMR.

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P238

Structural stability is critical for inhibitory effect of a cyclic peptide

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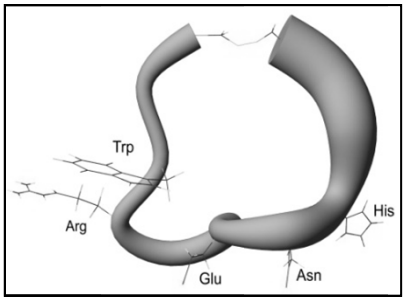
Urokinase-type plasminogen activator, uPA, is a possible target for *in vivo* inhibition in many cancer forms. A small cyclic peptide, upain-1, was found to be a strong, specific inhibitor for uPA.[1]

Our liquid state NMR investigations reveal major differences in the amount of structure of upain variants. A non-inhibiting variant, where the tryptophan was removed, shows no medium or long range interactions. Extension of the peptide at both termini leads to a considerably increased number of interactions, as well as an increase in inhibitory function, even though x-ray studies show no difference in the bound structure.[2]

A low temperature solution structure model was calculated for the extended variant. The cyclic part of the peptide is well ordered, while the extensions are highly mobile. Two sharp turns position the sidechains for interaction with the inhibitory site of uPA. The tryptophan indole ring is positioned perpendicular to the backbone, but may flip out upon binding, stabilising the interaction with uPA.

Our results show a strong structure dependency of the inhibitory effect of the upain peptide. These findings suggest that structure stabilisation of the upain peptide may lead to an even stronger uPA inhibitor.



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P239

Dynamics of a skeletal troponin C – troponin I chimera probed by comparison of experimental and simulated NMR relaxation parameters

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The activation of skeletal and cardiac muscle is triggered by the release of calcium from the sarcoplasmic reticulum. The calcium sensor is the troponin complex that is formed by three subunits: the calcium-binding protein troponin C (TnC), the inhibitory protein troponin I (TnI) and the tropomyosin-associated protein troponin T (TnT). When calcium binds to TnC, the resulting conformational change allows TnC to bind TnI, leading to the removal of the C-terminal region of TnI from actin. Consequential movement of the tropomyosin allows the binding of the myosin head to actin resulting in a power stroke. Regions of these proteins are highly flexible and the importance of these intrinsically disordered sections has been recognized and rationalized.[1]

Structural studies of the muscle system have been very successful in determining the structural organization of most of the molecular components involved in force generation at the atomic level. Although mainly α-helical, the structure and dynamics of TnI remains controversial, particularly in its C-terminal region. Different structures have been presented for this region: a single α-helix observed by x-ray crystallography, a "mobile domain" containing a small β-sheet derived from NMR restraints, and a mainly unstructured region according to NMR relaxation data. To investigate this, we have constructed a skeletal TnC-TnI chimera that contains the N-domain of TnC (1-91), a short linker (GGAGG), and the C-terminal region of TnI (98-182). Our objective is to determine which of the three proposed structures best fit the experimental ^{15}N relaxation data for this chimera. The comparison between experimental and NMR relaxation parameters calculated from molecular dynamic simulations will be presented to assess the validity of the models.

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P240

Characterization of a Novel Coordination Complex between Heme and All-parallel G-quadruplex DNAs

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A G-quadruplex DNA is composed of stacked G-quartets, each of which involves the planar association of four guanine bases circularly connected through Hoogsteen type base-pairings. The size and planarity of a G-quartet are well-suited for interaction with a porphyrin ring through π-π stacking. The complexation of G-quadruplex DNAs withporphyrin or metal-porphyrin derivatives has been studied extensively to characterize their molecular recognition of each other as well as to create catalytic DNAs that exhibit various functions. In the presence of an appropriate [K^+], a single repeat sequence of the human telomere, d(TTAGGG), forms all-parallel G-quadruplex DNA, which further assembles to form a "dimer" through end-to-end stacking of the 3'-terminal G-quartets (Fig. 1).[1] We have demonstrated that heme, the iron(III)-protoporphyrin IX complex, binds to G-quadruplex DNA to form a stable "heme-DNA complex", which exhibits spectroscopic and functional properties remarkably similar to those of hemoproteins. For example, the ferric heme Fe in the heme-DNA complex exhibits a characteristic pH-dependent spin equilibrium between the high spin state, *i.e.*, $S = 5/2$, and the low spin one, *i.e.*, $S = 1/2$, with a midpoint at pH = 8.6 ± 0.3.[2] The structural characterization of the low spin heme-DNA complex revealed that the heme is sandwiched between the 3'-terminal G-quartets of the G-quadruplex DNA, possibly with the formation of novel coordination of the G6-quartet oxygen atoms to the heme Fe in the complex (Fig. 1). This finding provides new insights into the design of the molecular architecture and functional properties of various heme-DNA complexes.

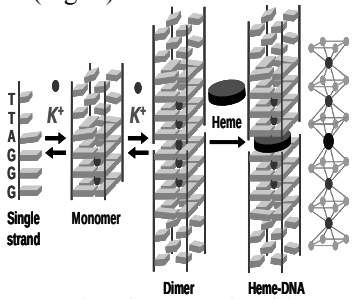

Fig. 1. Schematic representation of the assembly between heme and G-quadruplex DNA. Interaction of oxygen atoms of G-quartets with heme Fe^{3+} and K^+ are schematically illustrated in the right.

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P241
Leaf water content measurements by portable hand-held NMR

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Non-spatially resolved portable NMR is becoming available to study leaf water content (LWC) and distribution of water in different (sub-cellular) compartments, e.g. chloroplasts. These parameters directly relate to plant transpiration, CO_2 uptake, and photosynthesis. Application of portable NMR is not straightforward due to magnetic field inhomogeneities, complex leaf structure and shrinking-elongation movements during changes in LWC. Here we investigate the quantitative relation between LWC and NMR signal intensity as observed by a surface coil. The dehydration of leaves of different plants was studied continuously from the moment they were removed from the plant until their mass became constant. Two approaches were used to follow the wilting process in leaves. One was to use portable NMR while the second was a simple weighing method until the sample has been dried out completely. For the first time we monitored changes in water status of a whole leaf, directly correlated to weight measurements. The NMR signal was obtained from the Free Induction Decay (FID) for measurement of proton signal intensity and by Carr–Purcell–Meiboom–Gill (CPMG) for determination of the spin-spin relaxation time. The NMR results were compared with changes in LWC (Figure 1). The results demonstrate that the NMR signal is uniquely and quantitatively related to LWC, in contrast to observations reported elsewhere.[1]

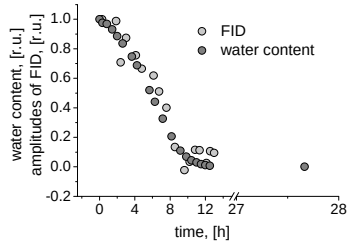

Figure 1. *Wilting of Quercus robur leaf. It is shown that amplitudes of NMR siganl change in a same way as a leaf water content calculated according to the weigh measurements.*

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P242
NMR chemical shifts to characterize protein order and disorder

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Proteins are dynamic entities, displaying conformational flexibility on a wide range of time and length scales. The fact that intrinsically disordered proteins (IDPs) are widespread in Nature,[1] and sustain various functions, puts forward the notion that protein dynamics has evolved for the adaptive benefit of higher organisms, and advances a 'systems view' on protein interaction networks, expanding beyond the traditional 'structure-function' paradigm.[2,3]

Among the great palette of experimental techniques, only NMR spectroscopy offers the unique possibility to relate the structural propensities of disordered proteins and loop segments to biological function and aggregation behavior. Backbone chemical shifts are ideally suited for this task, given that appropriate reference data are available. For this purpose we describe here the first 'random coil' chemical shift database derived from intrinsically disordered proteins, and provide an algorithm to reliably detect functional protein changes.[4]

Our newly developed tool: *ncSPC (neighbor corrected Structural Propensity Calculator)* uses NMR chemical shift data as sole input to determine the molecular conformation of proteins. *ncSPC* can detect and classify areas of disorder more reliably than currently available methods because it can better predict 'random coil' chemical shifts of disordered proteins, and, as a consequence, can more accurately discern local tendencies to adopt canonical secondary structure.[3] The neighbor-corrected structural propensity calculator program (*ncSPC)* will be made available at http://www.protein-nmr.org, and updates to the *IDP 'random coil' chemical shift database* will be posted at the same site.

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P243

Insights into the structure of beta-2 microglobulin fibrils and the role of serum amyloid-P component in their stabilisation

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Dialysis related amyloidosis (DRA) is a serious complication for patients undergoing long term renal dialysis and results in the deposition of amyloid in the joints causing pain and restricted mobility. The major protein component of these amyloid deposits is fibrillar β_2-microglobulin (β_2m). Serum amyloid-P component (SAP) is a protein ubiquitously present in fibrillar deposits and is thought to play a key role in stabilising the fibrillar structures and preventing their clearance by the host's defences.

We are currently investigating the structural transitions that result in the conversion of monomeric β2m into its fibrillar form and identifying sites involved in the interaction with SAP. Magic-angle spinning (MAS) 2D homo- and hetero-nuclear correlation spectroscopy have permitted the assignment of many of the resonances to particular amino acids within the fibrils. Two dimensional homonuclear correlation experiments in the presence of SAP reveal significant changes in the region corresponding to the glutamate sidechains highlighting the importance of these residues in SAP binding.

During the expression of β2m inclusion bodies are formed. Two-dimensional MAS-NMR studies of these inclusion bodies resulted in high quality spectra; however the distribution of chemical shifts appears different to the fibrillar material and exhibits lower resolution. Evidence suggests that this arises from a change in dynamics of the protein rather than the protein being amorphous leading to the possibility that proteins within the inclusion bodies have a defined structure.

P244

Structure and dynamics in the molten globule state of the nuclear coactivator binding domain of CBP

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Native molten globules are the most folded members of the intrinsically disordered proteins. We have characterized the ligand-free state of the nuclear coactivator binding domain (NCBD) in order to understand the mechanism of folding upon ligand binding. Biophysical studies show that despite of the molten globule nature of domain, it has a small cooperatively folded core. Using NMR spectroscopy, we show that despite of the dynamic nature of the molten globule ensemble, NCBD has a well-ordered conformer with specific side chain packing. We show that this conformer resembles the structure of NCBD in complex with one of its ligands, ACTR, and not the structure in the complex with another ligand, IRF-3. This suggests that ACTR binds NCBD by selecting a prefolded NCBD molecule from the ensemble of interconverting structures.

P245

Interaction between the PDZ domain of the microtubule associated serine / threonine kinase 205 and the cytoplasmic domain of the glycoprotein of rabies Virus

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Cell signaling pathways are strategic targets of many viruses during infection. Interactions between a viral protein and the PDZ domains of cellular proteins play a fundamental role in the pathogenicity of the virus as it was observed in other families of viruses (influenza virus,[2] HTLV-1,[3] adenovirus type 9[4]).We address the following question: what are the cellular and viral proteins involved in the perturbation of homeostasis after Rabies virus infection?

The propensity of Rabies virus to induce survival of neurons (virulent strain) or death (attenuated strain) depends on the nature and number of cellular partners of the viral glycoprotein binding-site (PDZ-BS)[5]. The PDZ-BS of the glycoprotein of Rabies virus (Cyto-$G_{virulent}$ or Cyto-$G_{attenuated}$) was identified as a key element in controlling the pathways of survival and apoptosis of infected neurons.[1] The PDZ-BS of these two viruses differs only in one amino acid. This mutation (E -> Q) is enough to shift the cell towards death or survival.

In this study, we focused on the survival protagonists that could compete with the viral glycoprotein. The only partners of the virulent Rabies virus PDZ-BS are two kinases belonging to the MAST family (microtubule associated serine / threonine). To understand the fine structural basis for the specificity of the PDZ-Cyto-G complexes, we determined the 3D structure and the dynamics of the MAST2-PDZ/Cyto-G (13aa) complexes by NMR. The structure of the complex reveals an original binding mode with a very large surface of interaction. One of the perspectives is now to use our knowledges to optimize the sequence of the peptide to enhance their proliferative properties for therapeutic issues.

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P246

Localizing of a substrate analog in a nickel superoxide dismutase biomimetic by REDOR solid-state NMR

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SODs are metalloenzyms which catalyze the disproportionation of the superoxide anion ($O_2^{\cdot-}$) to peroxide and molecular oxygen.[1] Since the NiSOD is structurally not related to other SODs and the coordination sphere is provided by the so called Ni-hook the catalytic mechanism of $O_2^{\cdot-}$ degradation by NiSOD is probably different to other SODs. Metallopeptide based NiSOD biomimetics perfectly match the spectroscopic and functional properties of the native enzyme.[2,3] For our investigations we used metallopeptides which are based on the first 7 residues from the N-terminus of the active form of *S. coelicolor* NiSOD (Fig. 1). Concerning the discussions about an inner- vs. outersphere mechanism REDOR NMR is used to localize the position of cyanide, which we used as a substrate analogue in the NiSOD metallopeptide.[4] For this we synthesized the cyanide adduct of the metallopeptide using different ^{15}N and ^{13}C labelled positions in the peptide backbone and ^{13}C or ^{15}N labelled CN.[4]

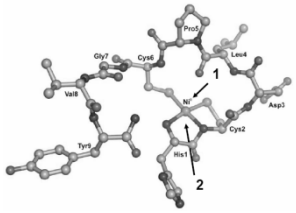

Figure 1 Metallopeptide structure (based on liquid NMR data and computational structure optimising[(1)]) showing two possible cyanide positions (1 and/ or 2)

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P247

Conformational changes induced by phosphorylation out of Effector Domain favors MARCKS antibody interaction

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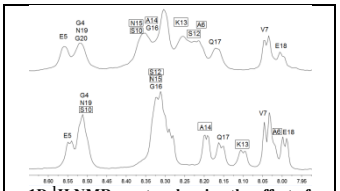
The Myristoylated Alanine-Rich C Kinase Substrate (MARCKS) is a protein present in different tissues, being abundant in nervous system. Although MARKCS is a ubiquitous protein, the development of neuroblast and some types of neurons depend of the cells which have MARCKS isoform phosphorylated at a serine residue located at its N-terminal moiety.[1] In this region was identified a epitope that interacts with the monoclonal antibody mAb3C3. Binding assays of this epitope with mAb3C3 show that only the phosphorylated form (S25p - EKPGEAVAApSPSKANGQENG) maintained the connection properties observed for MARCKS.[2] In this work we used Circular Dichroism (CD) and Nuclear Magnetic Resonance (NMR) spectroscopy to understand how the phosphorylation favors the interaction of the MARCKS peptide with mAb3C3 antibody. NMR and CD spectroscopy data suggest that S25 (S10 in the peptide) phosphorylation does not causes significant structural modification in the peptide structure. The greater ^{1}H chemical shift dispersion observed for the phosphorylated peptide suggests a more ordered structure. This punctual structural modification could be related with the ability of the S25p peptide interacts with the mAb3C3 antibody.



1D ^{1}H NMR spectra showing the effect of phosphorylation on the MARCKS peptides.

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P248

Structural basis for the interactions of the MyD88 TIR domain in TLR4 signaling

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Myeloid differentiating factor 88 (MyD88) and MyD88 adaptor-like (Mal) are adaptor molecules involved in the Toll-like receptor (TLR) 4 signaling pathway in innate immune responses. MyD88 mediates signal transduction from activated TLR4 to downstream components, while Mal has been proposed to act as a bridging adapter between the TLRs and MyD88. The Toll/Interleukin-1 receptor (TIR) domains are known to mediate the interaction between MyD88 and Mal. Here we determine the solution structure of the MyD88 TIR domain. By combining *in vitro* mutational binding experiments with an NF-κB reporter system in mammalian cells, two surface sites of the MyD88 TIR domain are identified as binding interfaces for the TIR domain of Mal. These two sites are distantly located each other, suggesting that the TIR domain of MyD88 simultaneously interacts with two Mal-TIR molecules, which may provide a highly efficient scaffold for signal transduction. The interaction between MyD88 and TLR4 is also examined and it turns out that MyD88-TIR does not directly bind to the cytosolic TIR domain of TLR4, while Mal-TIR does.

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P249

Solution and Solid-State NMR Investigation of Maximin 4, an Antimicrobial Frog-Peptide in Membrane-Mimicking Environments

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Maximin 4 is a 27-residue antimicrobial peptide (AMP) from the Chinese red belly toad *Bombina maxima* with a broad spectrum of antibacterial activity and high selectivity for prokaryotic cells. Similar to other AMPs, its mode of action is thought to be ion channel or pore formation in the bacterial cell membrane. To obtain a high-resolution picture of its interaction with membrane systems, we have conducted a joint solution and solid-state NMR investigation of maximin 4 in membrane-mimicking environments.

Under our experimental conditions, in sodium dodecyl sulfate (SDS) micelles the peptide adopts a helix-break-helix conformation with an unstructured N-terminal segment. The kink between the helices is positioned in the middle of the sequence and is stabilized by van der Waals interactions. The two helices form an approximate L shape with an interhelical angle of ~95º. The solution NMR results in detergent micelles are complemented with the characterization of peptide backbone conformation in phospholipid bilayers using solid-state NMR methodologies. Rotational echo double resonance (REDOR) experiments on specifically ^{13}C and ^{15}N-labeled maximin 4 embedded in multilamellar vesicles of various phospholipid compositions have confirmed helix formation and suggested a deep insertion of the peptide into the lipid bilayer.

Comparison of the structural features of maximin 4 with other well-studied alpha-helical AMPs is presented.

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P250

The Carboxy Terminal of Tubulin: a Versatile Cationic-Partner Binding Domain Regulated by Calcium

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The C-terminal region of tubulin is involved in multiple aspects of the regulation of microtubules assembly. To enlighten the molecular mechanisms of this regulation, we have studied by NMR and other techniques the interaction of the three representative partners of the tubulin C-terminal region, i.e. Tau, spermine and calcium with the peptide fragment comprizing the last 42 residues of α tubulin, (αT-CTD(410-451)).

The NMR study using ^{15}N-^{13}C-double labelled αT-CTD shows that binding of the three tubulin partners involves overlapping amino acid stretches on the C-terminal tubulin region: specifically, residues 421-441 for Tau; residues 430-432 and 444-451 for spermine; residues 421-443 for calcium.

NMR, ITC and cosedimentation experiments show that Tau and spermine have similar micromolar binding affinities while the binding stoichiometry differs (respectively, αT-CTD:spermine = 1:2 and αT-CTD:Tau = 8:1).

Interestingly, calcium, known as a negative regulator of microtubule assembly, can compete with the binding of Tau and spermine to the C-terminal domain of tubulin and twarth the positive effect of these two partners with microtubule assembly in vitro. This observation opens the possibility that calcium may participate to the regulation of microtubule assembly in vivo through a direct (still unknown) or indirect mechanism (displacement of microtubule partners).

The functional importance of this part of tubulin was also underlined by the observation that the α-tubulin mutant deleted from the last 23 amino acid residues does not incorporate properly into microtubules in living HeLa cells. All together, the results provide structural basis for a better understanding of the complex interactions and putative competition of tubulin cationic partners with the C-terminal region of tubulin.

P251

NMR studies of a heterotypic complex of a chicken liver bile acid binding protein showing site selectivity for two different bile acids

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Bile acid-binding proteins (BABPs) are cytosolic lipid chaperones that play central roles in driving bile flow, as well as in the adaptation to various pathological conditions, contributing to the maintenance of bile acid homeostasis and functional distribution within the cell. Understanding the mode of binding of bile acids with their cytoplasmic transporters is a key issue in providing a model for the mechanism of their transfer from the cytoplasm to the nucleus, for delivery to nuclear receptors.

We studied by NMR the behaviour of a chicken liver BABP characterized by the presence of a naturally occurring disulphide bridge (cl-BABP(SS)) whose presence affects ligand-binding properties and backbone dynamics.[1] The cl-BABP(SS) retains the 1:2, protein:ligand, stoichiometry tipical of the cl-BABP but shows a site selectivity for glycocholic (GCA) and glycochenodeoxycholic acid (GCDA), the most abundant bile acids in chiken liver. In order to understand the structural basis of the site selectivity we are currently studying the structure of cl-BABP(SS) complexed with both GCA and GCDA. By comparing HSQC spectra of the homotypic complexes (cl-BABP(SS) with GCA and GCDA alone) and of heterotypic complex (cl-BABP(SS) with GCA and GCDA in the same molecule) we outlined the aminoacids anchoring GCA in the more buried site of cl-BABP(SS) and GCDA in the more superficial one and localized the bile acid binding sites in a preliminary model of the holo cl-BABP(SS) obtained by CS-Rosetta. Data from chemical shift perturbation analysis, backbone dynamics studies and 3D NOESYs will be used for the calculation of the heterotypic complex structure by HADDOCK software, starting from the structure of the holo protein scaffold obtained by CYANA.

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P252

Rational approach for designing unimolecular G-quadruplexes

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G-quadruplexes are four stranded structures consisting of stacks of at least two basic structural elements called G-quartets in which four guanines are H-bonded. G-quadruplexes can form in different genome regions such as telomeric and promoter regions, which makes them potential targets for therapeutics. Apart from that, their characteristics such as structural variability and high temperature stability make DNA G-quadruplexes interesting potential building blocks for nanotechnology. The rules that govern assembly of G-quadruplexes are not yet understood, which makes the exploitation of apparent potential of G-quadruplex as therapeutic targets and building blocks limited.

A deductive system for prediction and control of unimolecular G-quadruplex self-assembly was proposed recently.[1] Two ranges of torsion angle around glycosidic bond define two conformations of guanines - *anti* and *syn*. In G-quadruplexes disposition of the two conformations is interrelated with the structure.

Applicability of prediction and control of G-quadruplex formation with the use of the proposed concept was tested on oligonucleotide sequence d[$G_3TG_3T_4G_3T_3G_3$], which was predicted to fold into a single topology. The self-assembly of the oligonucleotide is controlled through optimal choice of the loop lengths, which directed disposition of guanine conformations in G-quadruplex stem. Solution-state NMR was used to verify and confirm the predicted topology of the formed G-quadruplex and thus evaluate general validity of the rational approach for designing G-quadruplex structures.[2]

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P253

Membrane-based induction of α-helical structure within a subset of peptides derived from HAMP domains

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Since the environment is constantly changing, environmental adaption is crucial in gene regulation for even the most basic organisms to survive. Prokaryotes need to adapt their gene expression profiles and their regulation of gene product activity to match current circumstances. The main system used by bacteria for this task is the two-component system. A linker domain, known as the HAMP domain, is found in many prokaryotic transmembrane signal transduction proteins, connecting the signal input domain with the catalytic core. The HAMP domain is composed of two amphipathic sequences (AS1 and AS2) joined by a connector. This domain is usually found adjacent to the inner leaflet of the cytoplasmic membrane. Because of this close proximity, we examined the influence of several membrane mimetics on peptides containing either AS1 or AS2 from four different HAMP domains: the *Archaeoglobus fulgidus* protein (Af1503), the *E. coli* osmosensor ($EnvZ_{Ec}$), the *E. coli* nitrate/nitrite sensor ($NarX_{Ec}$) and the aspartate chemoreceptor of *E. coli* (Tar_{Ec}). Based on our study these HAMP domains can be divided into two groups considering their membrane interaction features. The group consisting of $NarX_{Ec}$ and Tar_{Ec} is distinguished by showing helical induction in AS1 upon addition of negatively charged large unilamellar vesicles (LUVs) in circular dichroism studies. The secondary structure of AS1 from $NarX_{Ec}$ and Tar_{Ec}, respectively, was further investigated by 2D ^{1}H-^{1}H NMR and two solution structures were calculated. Further, the AS1 peptides of $NarX_{Ec}$ and Tar_{Ec} interact strongly with bicelles as shown by diffusion NMR. The group consisting of Af1503 and $EnvZ_{Ec}$, on the contrary, does not show any helical induction in AS1 upon addition of LUVs, and their AS1 segments do not interact as strongly with bicelles. Interestingly, $NarX_{Ec}$ and Tar_{Ec} are suggested to share a common mechanism of transmembrane signaling involving a periplasmic four-helix bundle.

P254

Why can one metalloprotease overcome elastin and collagen triple helices? Sources of specificity and biophysical properties via NMR and "BINDSIght"

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How does MMP-12 achieve its high specific activity upon fibrils from lungs and arteries in disease? To elucidate the specificity, we developed a general strategy called BINDSIght, for BIoinformatics and NMR Discovery of Specificity of Interactions. Protection from a paramagnetic probe suggests that elastin might enfold MMP-12. Mutagenesis and enzyme kinetics corroborate this. BINDSIght guided choice of mutations at each of ten peripheral locations encircling the catalytic cleft, forming an exosite, and impairing specific activity towards elastin. Eight of the lesions also impair hydrolysis of a triple helical peptide from collagen V. The lesions primarily impair K_m for elastin, and yet the advantage of MMP-12 over its closest paralog (MMP-3) is in k_{cat}. We looked for properties that may be associated with the higher activity and k_{cat} of MMP-12 relative to MMP-3. We monitored backbone relaxation and residue-specific stabilities. Regions surrounding the active sites of both proteases sample conformational substates within msec. The more extensive line broadening in MMP-3 suggests greater sampling of conformational substates throughout the active site, and at more remote sites. This could suggest more excursions to functionally incompetent substates. Hydrogen exchange protection suggests that MMP-3 possesses 2.8 kcal/mol higher folding stability than MMP-12(E219A). The higher stability of MMP-3 coincides with its much lower proteolytic activity. This is consistent with the hypothesis that enzymes often trade stability for higher activity.

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P255

Mapping the structure of the N47A Spc-SH3 amyloid fibrils by H/D exchange

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Amide H/D exchange combined with NMR spectroscopy is a powerful technique to probe the regions of proteins involved in stable structure at single-residue resolution. In this work we have compared the H/D amide exchange rates between the amyloid fibril state[1] and the native state of the N47A mutant of the Spc-SH3 domain under the same experimental conditions.[2]

The results reveal that the protection against H/D exchange is dramatically enhanced in the fibrillar state compared to that in the native state and the patterns of H/D exchange protection are also highly different indicating that the regions of the N47A Spc-SH3 chain participating in the structure of the fibrillar state are markedly different to those in the native state. We have also compared the H/D exchange in two types of fibrils formed during different incubation times and we have observed different degree of protection possibly due to their morphological differences.

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P256

High-resolution structure of pentameric phospholamban and its interaction with the Ca-ATPase using a hybrid solution/solid-state NMR approach

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Phospholamban (PLN) is a single-pass membrane protein that binds to and regulates the Sarcoplasmic Ca-ATPase in muscle cells. In lipid membranes, PLN readily oligomerizes into stable non-covalent pentamers. Dysregulation of the SERCA/PLN complex has been linked to the development of heart failure. Understanding the molecular basis of such interaction is a fundamental step towards the development of new therapeutic approaches.

In order to solve the structure of the SERCA/PLN complex, we are using a hybrid approach[1] that combines solution and solid-state NMR derived structural information. Solution NMR and magic angle spinning NMR are used to measure distances and angular restraints, whereas oriented solid-state NMR gives information about the topology of membrane proteins in lipid bilayers. Here we present the hybrid high-resolution structure of pentameric PLN in lipid and detergent environments. This structure differs markedly from the previous model proposed by Chou and Oxenoid[2] mainly in the orientation of the cytoplasmic regulatory domain as well as in the size and arrangement of the transmembrane pore. Our results exclude the presence of a conducting ion pore and point to the role of pentameric PLN as a storage form of active monomers.

We also present structural data on the interaction between monomeric PLN and SERCA reconstituted in oriented lipid bilayers and lipid vesicles measured by oriented solid state NMR and magic angle magic angle spinning NMR.

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P257

The Influence of Temperature and Binding on the Dynamics of *Ct*CBM11

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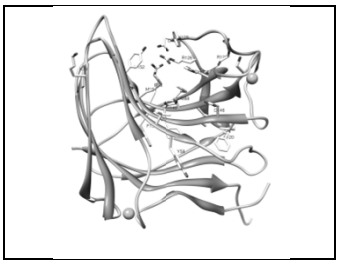
Degradation of cellulose is one of the most important steps in the global turnover process of atmospheric CO_2. Cellulolytic anaerobic bacteria, like *Clostridium thermocellum*, produce large extracellular multi-enzymatic complexes (i. e.) cellulosomes comprising a consortium of enzymes, which contain non-catalytic carbohydrate-binding modules (CBM) that increase the activity of the catalytic module towards the efficient degradation of crystalline cellulose.



In previous work[1] we used STD-NMR to identify the molecular determinants of ligand specificity of family 11 CBMs from *C. thermocellum* (*Ct*CBM11). We showed that the protein interacts more strongly with the central glucose units of cellohexaose (cellohexaose was used to mimic cellulose). In the present work we focus on the residues responsible for ligand recognition by determining the solution structure and describing the backbone dynamics of *Ct*CBM11 at 25 and 50ºC and in the presence and absence of cellohexaose. No modifications were detected on the behavior of amino acids involved in ligand recognition upon temperature change. The analysis of the internal dynamics reveals a generally rigid structure, with some exposed segments which undergo fast (ps to ns) internal motions.

In combination with our previous studies, our results represent a significant improvement in understanding the factors that determine the specificity and the mode of action of *Ct*CBM11 at the molecular level.

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P258

Structural studies on Tau-K19 fibrils using solid-state NMR

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Tau protein is prominent in neurons and plays an important role in neurite outgrowth and axonal transport by stabilizing microtubules which provide the tracks for motor proteins. Interactions of tau with microtubules are regulated by the length of the microtubule binding domain and by phosphorylation. Hyperphosphorylated Tau does not bind microtubules. Instead, it accumulates into fibrils called paired helical filaments (PHFs). PHFs are the primary component of neuro-fibrillary tangles (NFTs) found in the brain of Alzheimer patients.[1]

The molecular fine structure of PHFs is unknown in detail, but represent one of the major goals in the field as it may aid in the development of methods and drugs to prevent aggregation.[2] We employed solid-state NMR to investigate the structure and dynamics of a shorter construct of Tau protein (K19) containing three repeat domains, which is known to form PHFs similar to those formed by the wild type protein.

Our studies indicate that K19 has a well-defined hydrophobic core involving the hexa-peptide[3] region. Secondary chemical shift analysis as well as a model of the K19 core structure based on distance measurements is presented. Mutation studies confirming our model were also performed.

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P259

Probing biologically relevant motions on large multidomain proteins by liquid state NMR

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Developments in biochemistry and liquid state NMR spectroscopy now allow the study of high molecular weight proteins. It thus opens the field for NMR spectroscopists to get information on large proteins with the advantage compared to other techniques to be in conditions close to those encountered in the native environment, i.e in a liquid state and without any additional probe. The work presented here aims at obtaining the dynamical characterization of a multi domain protein of more than 65 kDa for which RX structures are already available. Inter domain motion in this protein is thought to limit the catalytic rate. Several strategies can be used to overcome the fast transverse relaxation rates. A deuteriation protocol followed by the use of the TROSY effect on high magnetic field spectrometers (950 and 800 MHz) led to spectra with sufficient sensitivity for an extensive set of 3D triple resonance and NOESY edited experiments including the usually poorly sensitive HNCACO and HNCANH. The sequential assignment obtained so far is 30% and is still ongoing. The protein sample allows us to record ^{15}N relaxation experiments and to calculate R_1 and R_2 parameters. We can thus estimate the correlation time of the protein and probe possible differences in mobility between domains. In addition ^{15}N relaxation dispersion experiments, which are sensitive to motions occurring on the 100µs-10ms timescale, could be collected. These CPMG based experiments detect the chemical exchange of spins oscillating between two states, even if one of them is low populated. Some amide nitrogens indeed experience chemical exchange and these phenomena are currently under analysis. The results obtained so far show the potential for NMR to contribute to the characterization of the dynamical behavior of high molecular weight multi domain proteins on timescales of biological relevance to their activities.

P260

Structural Basis of the Integrin β_3 Cytoplasmic Tail Phosphorylation and its Crosstalk with VEGF Receptor

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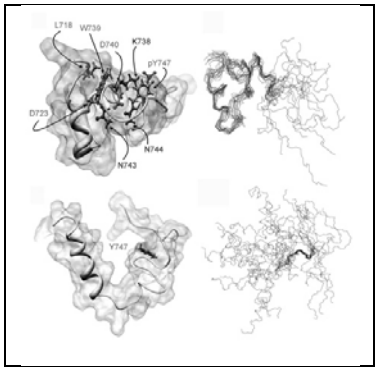

The attachment of adhesion receptors, integrins, to the extracellular matrix is a tightly controlled event. While tyrosine phosphorylation of integrin β_3 cytoplasmic tail (CT) has been shown to play an important role in this process, the molecular basis remains unclear. Here we present the first 3D structure of the full length integrin β_3CT mono-phosphorylated at tyrosine-747. We show that this phosphorylation causes significant conformational rearrangement in β_3CT, surprisingly preventing the α_{IIb}/β_3 membrane-proximal clasp formation which is a key to maintain the receptor in the inactive state. This finding explains how tyrosine phosphorylation may regulate integrin activation by sustaining the receptor in the active state. We also show that the tyrosine phosphorylation enhances the β_3CT binding to Vascular Endothelial Growth Factor Receptor 2 (VEGFR2) – an interaction that has been shown to promote integrin activation and control VEGF-induced angiogenesis. These data provide novel molecular insights into how tyrosine phosphorylation of integrin β_3CT plays multiple roles in regulating integrin activation.

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P261

Std NMR of ternary enzyme-coenzyme-substrate complexes – binding studies of NADH and NADPH to *Candida tenuis* xylose reductase

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Candida tenuis Xylose Reductase (*Ct*XR) reduces its natural substrate xylose to xylitol. This enzyme shows dual co-enzyme specificity with preference of NADPH over NADH to be the hydride donor in this catalysis. Also the accepted spectrum of substrates is inherently broad.[1] To improve the enzyme's activity towards non-natural substrates, a number of *Ct*XR mutants have been produced. However, the co-factor binding was affected. Hence, we focus our investigations onto binding studies of the ternary non-covalent enzyme-coenzyme-substrate interactions by the STD NMR technique.[2]

In these studies we found that intensities of STD NMR signals differ entirely within the non-natural substrates which are accepted by *Ct*XR with different catalytic activity.[3] Therefore, we focused the investigations onto binding of the co-enzymes and substrates during a productive binding mode, leading to the desired biotransformations. For that purpose we studied the STD effects of both co-enzymes. Furthermore we analyzed non-productive substrate binding, not causing any transformation to products. Apart from studies of the natural wild type enzyme we furthermore investigated ternary complexes of some *Ct*XR mutants, also showing variations in STD effects. With these STD NMR data we make some suggestions for ternary binding complexes in the active side of the *Ct*XR wild type and mutant enzymes.

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P262

Mapping the Encounter State of a Transient Protein Complex by PRE NMR Spectroscopy

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Many biomolecular interactions proceed via a short-lived encounter state, consisting of multiple, lowly-populated species invisible to most experimental techniques.[1] Recent development of paramagnetic relaxation enhancement (PRE) nuclear magnetic resonance (NMR) spectroscopy has allowed to directly visualize such transient intermediates in a number of protein-protein and protein-DNA complexes.[2-4] Here we present an analysis of the recently published PRE NMR data[5] for a protein complex of yeast cytochrome *c* (Cc) and cytochrome *c* peroxidase (CcP). First, we describe a simple, general method to map out the spatial and temporal distributions of binding geometries constituting the Cc-CcP encounter state. We show that the spatiotemporal mapping provides a reliable estimate of the experimental coverage and, at higher coverage levels, allows to delineate the conformational space sampled by the minor species. To further refine the encounter state, we performed PRE-based ensemble simulations. The generated solutions reproduce well the experimental data and lie within the allowed regions of the encounter maps, confirming the validity of the mapping approach. The refined encounter ensembles are distributed predominantly in a region encompassing the dominant form of the complex, providing experimental proof for the results of classical theoretical simulations.

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P263

Protein dynamics contribute to the mechanism of Aβ aggregation

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Aβ is widely recognized as a key molecule in Alzheimer's disease, causing neurotoxicity through Aβ aggregates such as Aβ oligomers and fibrils. Aβ40 and Aβ42, composed of 40 and 42 residues, respectively, are the major Aβ species in human brain. Aβ42 aggregates much faster than Aβ40 but the mechanism of such difference in aggregation propensity is poorly understood. Using NMR spin relaxation, we have shown that Aβ40 and Aβ42 have different dynamics in both backbone and sidechain on the ps-ns time scale. Aβ42 is more rigid in C-terminus in both backbone and sidechain while Aβ40 has more rigid methyl groups in the central hydrophobic cluster (CHC: Aβ17-21). These observations are consistent with differences in the major conformations of Aβ40 and Aβ42 monomers derived from replica exchange MD (REMD). To further demonstrate the relevance of dynamics in aggregation mechanism, a perturbation was introduced to Aβ42 in the form of M35 oxidation. After M35 side chain oxidation to sulfoxide, Aβ42 experiences Aβ40-like changes in dynamics. At the same time, M35 oxidation causes dramatic reduction in Aβ42 aggregation rate. Our data have thus established an important role for protein dynamics in the mechanism of Aβ aggregation.

P264

The structure of FgHET-s(218-289) amyloid fibrils by solid-state NMR

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We present the solid-state NMR structure of a distant homolog of the fungal HET-s prion, which is found in the fungus Fusarium graminearum. The domain FgHET-s(218-289), which corresponds to the prion domain in HET-s from Podospora anserina, forms amyloid fibrils in vitro and is able to efficiently cross-seed HET-s(218-289) prion formation. FgHET-s(218-289) and HET-s(218-289) have 38% sequence identity.

Solid-state NMR and hydrogen/deuterium exchange detected by NMR enabled us to calculate the structure of FgHET-s(218-289) using experimental distance restraints with an approach similar to the one used for HET-s(218-289)[1]. We found a high degree of structural similarity between the two fibrils that readily explains why cross seeding occurs here in spite of the sequence divergence. However, there are also several structural differences. These include the prolongation of 2 beta-sheets and the associated shortage of the flexible loop, and a profound structural change of one of the 3 beta-arches.

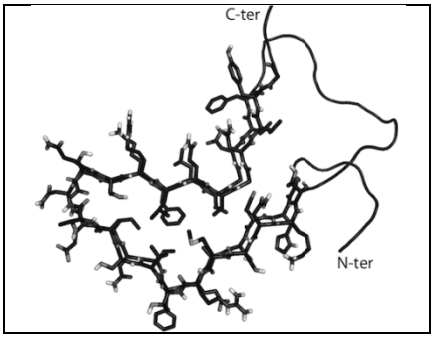

Figure: Stick-representation of the lowest-energy structure of one FgHET-s(218-289) molecule within the amyloid fibril.

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P265
DNA bound ruthenium complex structure determination by NMR

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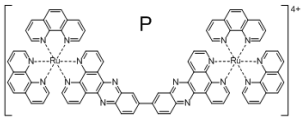
The design and study of DNA-binding molecules have been of great interest for many years due to their possible applications as diagnostic agents, genetic probes, or chemotherapeutics. Optical spectroscopy methods have shown that the binuclear ruthenium complex P (Figure) intercalates into DNA by threading a coordinated ruthenium ion through the DNA base stack, ending up with one subunit in each groove of DNA and the bridging *bidppz* ligand sandwiched between the DNA base pairs.[1-3] We here investigate by NMR spectroscopy the detailed structure of P threaded into a short DNA sequence (CGCGAATTCGCG) at 25 ^{0}C. The NMR spectra of unbound P exhibit symmetry both about the central bond of the molecule as well as within each half-unit. Aggregation of free P is observed in aqueous solution. To obtain further insights into this unusual binding mode, the structural characterization is supplemented by kinetic and thermodynamic studies. Ruthenium complexes strongly binding to DNA and slowly dissociating from the binding sites have been considered for anticancer agent.[4] Structure analysis plays an important role for understanding how ruthenium complex intercalate into DNA and for the design of new medicines.



Ruthenium complexes studied:
[μ-bidppz(phen)4Ru2]4+

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P266
RNA binding and dynamics of the rRNA methyltransferse Nep1

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Nep1 (Emg1) is a highly conserved nucleolar protein with an essential function in ribosome biogenesis. A mutation of a conserved aspartate in the human Nep1 homolog causes Bowen–Conradi syndrome—a severe developmental disorder. Structures of Nep1 revealed a dimer with a fold similar to the SPOUT-class of RNA-methyltransferases. Recently, we were able to show that Nep1 is responsible for the methylation of a conserved N1-methyl-N3-(3-amino-3-carboxypropyl)-pseudouridine in the 18S rRNA.[1]

We initiated NMR studies on *M. jannaschii* Nep1 (*Mj*Nep1), a 48 kDa homodimer, to characterize the RNA binding site and to investigate the effect of the mutation (D56G) responsible for Bowen-Conradi syndrome. As a prerequisite the backbone resonances of *Mj*Nep1 were assigned, which revealed that *Mj*Nep1 exists as an asymmetric dimer in solution. Short RNA oligomers corresponding to the 16S rRNA of *M jannaschii*, which are bound with high affinity and are efficiently methylated by *Mj*Nep1, were used for chemical shift perturbation measurements to map the RNA binding site. To determine the orientation of the bound RNA paramagnetic relaxation enhancement experiments with spin labeled oligomers were performed. Furthermore we compared the dynamcis of MjNep1-wt and the D56G mutant by ^{15}N relaxtion studies.

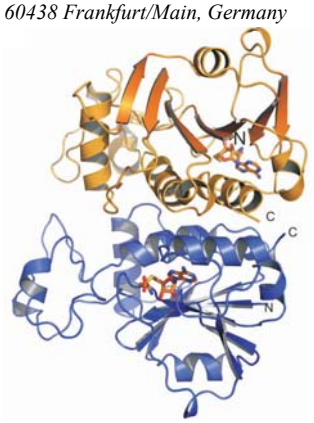

Figure 1: Crystal structure of the Nep1 dimer from *M. jannaschii* [2]

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P267

Structural basis of the interaction between ganglioside clusters and amyloid-β peptide

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Accumulating evidence has indicated that gangliosides interact with amyloid β (Aβ) peptide on neuronal cell surfaces, and thereby promote its assembly, which is considered as a crucial step in Alzheimer's disease. To provide a structural basis for this pathogenic event at atomic resolution, we have designed and conducted NMR studies of the interactions between gangliosides and Aβ using small GM1 micelles as a model system. We characterized the interaction between micelles and Aβ using spin-labeled analogs of this peptide. The paramagnetic relaxation enhancement and chemical shift perturbation data stress the importance of the sugar-lipid interface of the ganglioside clusters for accommodating Aβ.[1] Furthermore, we have performed saturation transfer analyses using deuterated Aβ bound to GM1 micelles. The NMR data revealed that Aβ lies on hydrophobic/hydrophilic interface of ganglioside cluster exhibiting an up-and-down topological mode in which the two α-helices and the C-terminal segment are in contact with the hydrophobic interior, whereas the remaining regions are exposed to the aqueous environment.[2] These findings suggest that the ganglioside clusters serve as a unique platform for binding coupled with conformational transition of Aβ molecules, rendering their spatial rearrangements restricted to promote specific intermolecular interactions.

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P268

Paramagnetic NMR approach to conformational analysis of *N*-glycans

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N-linked oligosaccharides, a major class of glycoprotein glycans, play crucial roles in a variety of biological events including cell-cell interactions and protein quality control. Despite their biological importance, few reports describe 3D structures of the oligosaccharides in solution. The major limitation of the traditional NMR methods is insufficient numbers of conformational restraints of the oligosaccharides provided by the NOE data. In addition, quantitative interpretation of the NMR data associated with dynamic properties of oligosaccharides remains as tasks with more challenge, although it is essential for understanding molecular basis of the various glycan functions. Hence, development of novel NMR methods is highly desirable for detailed characterization of conformations and dynamics of the *N*-linked oligosaccharides.

We herein illustrate an application of paramagnetic effects to NMR characterization of the carbohydrate conformations in solution. An EDTA derivative as a lantanide chelating-tag was covalently attached to the reducing end of *N*, *N*'-diacetylchitobiose, which constitutes the common core structure shared among all the *N*-linked oligosaccharides. Upon complexation with a lanthanide ion such as Tm^{3+} and Ho^{3+}, the tagged disaccharide exhibited spectral changes induced by pseudocontact shift (PCS), offering an opportunity to determine the spatial positions of the individual ^{1}H and ^{13}C nuclei with respect to the paramagnetic center. The experimentally obtained PCS values were in good agreement with those back-calculated from a 3D structure model of this disaccharide, indicating that the common core part of the *N*-glycans is little affected by the tagging. On the basis of these data, we conclude that this lanthanide-tagging method can provide valuable conformatinal informations of a variety of *N*-linked oligosaccharides.

P269

Structural and functional analyses of a male mice-specific pheromone ESP1

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Pheromones are species-specific chemical signals that regulate a wide range of social and sexual behaviours in many animals. The vomeronasal organ (VNO) mediates the pheromonal information via the vomeronasal sensory neurons (VSNs) in mice. We previously identified a male-specific peptide ESP1 (exocrine-gland-secreting peptide 1) secreted into tear fluids that stimulates females' VSNs.[1] Recently, we also elucidated that ESP1 elicits lordosis, a sexual behaviour of females to accept males via its own receptor V2Rp5 (vomeronasal type 2 receptor p5), which is one of the G protein-coupled receptors (GPCRs) expressed in females' VNO.[2,3] ESP1 is the first peptidic pheromone that both of the receptor and the inducing behaviour are revealed. ESP1 also turned out to be a member of a new multigene family which is supposed to convey information on sex, strain and species in rodents.[4]

The aim of this study is to elucidate mechanisms underlying the pheromone-reception system on the ESP family. We report here the three-dimensional structure and the V2Rp5-binding sites of ESP1, based on solution NMR analyses and mutational effects on the VSNs-stimulating activity. A structural model of the ESP1-V2Rp5 complex was constructed by focusing on the identified binding sites and the electric charge distribution on the molecular surface.

The informations on the structure and the receptor-binding sites of ESP1 revealed by our structural and mutational analyses will give a way to elucidate specific ligand-receptor recognition mechanism on the whole ESP family.

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P270

HR-MAS NMR study of *Salmonella enterica* serovar Typhimurium living cells

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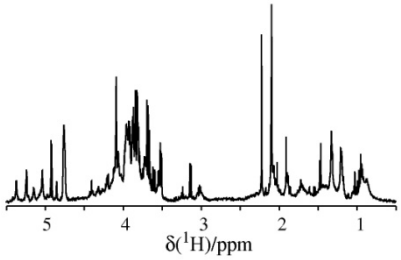
NMR spectroscopy can detect biomolecules directly in the cell, thus avoiding time-consuming processes like isolation and purification, and providing a more realistic description of a cell's status than the one derived from *in-vitro* studies. The resulting spectra can be very complex, and the use of MAS is often required in order to observe narrow lines, mostly because MAS averages the magnetic-susceptibility variations present in such heterogeneous samples.

Here we present a study of the O-antigen of the pathogenic bacterium *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium), where we performed high-resolution MAS (HR-MAS) NMR experiments directly on the living cells. In Gram-negative bacteria lipopolysaccharides (LPS) is a major component of the bacterial outer membrane. LPS is an important virulence factor in pathogenic species. In *S.* Typhimurium the outermost part of the LPS, the O-antigen region, consists of 70-100 repeating [→2)α-D-Manp-(1→4)-α-L-Rha*p*(1→3)-α-D-Gal*p*(1→] units where Rha is rhamnose, Gal galactose and Man mannose. The Man residue is substituted at carbon 3 (C3) with α-linked abequose (Abe) O-acetylated at C2. We found that O-acetylation of Abe dramatically decreases in stationary phase cultures and with increasing pH of the medium. On the other side, high degrees of O-acetylation were observed in late stationary phases if extra glucose (Glc) or Gal was added to the growth media. Our NMR studies also revealed that the acetylation of the Abe altered the structural or dynamic characteristics of the whole O-antigen, providing the first experimental evidences of the effect of O-acetylation on the global physicochemical characteristics of the O-antigen. This correlates with previous findings that O-acetylation alters the immunological properties of *S.* Typhimurium and other Gram-negative bacteria.



¹H NMR spectrum of S. Typhimurium cells in the late exponential phase.

P271

Structural studies on proteins and peptides by relaxation enhancements in a paramagnetic environment

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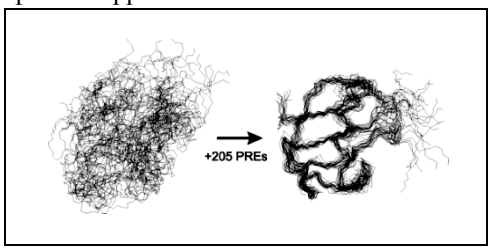
A paramagnetic center yields distance-dependent enhancements of relaxation rates of nearby nuclei. While the paramagnetic probe is often covalently attached to the system under study, it is also possible to add an inert and soluble paramagnetic agent to the solvent and thus make the "environment" paramagnetic. This type of compound allows for a tunable relaxation enhancement simply by variation of the concentration. For our studies we used the water-soluble complex Gd(DTPA-BMA),[1] which is inert towards proteins and cannot penetrate their interior. The overall paramagnetic relaxation enhancement (PRE) of a specific nucleus depends on the combined effect of the entire paramagnetic environment. It yields an immersion depth-dependent parameter. We developed an approach that allows the structure determination of proteins using these PREs together with limited NOE data sets.[2] We obtained structures of two model systems (ubiquitin and maltodextrin-binding protein) employing PREs and NOEs of exchangeable protons only. This approach should also be suitable for systems of high molecular mass where PRE restraints and NOEs between exchangeable protons can be obtained even on perdeuterated samples. Besides soluble proteins, relaxation enhancements in a paramagnetic environment can also be used in a quantitative way to obtain the orientation and location of micelle-bound peptides.[3]



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P272

Chicken ileal bile-acid-binding protein: a promising target of investigation to understand binding co-operativity across the protein family

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BABPs (Bile Acid Binding Proteins) are intracellular transporters able to bind ligands with different stoichiometry, selectivity and co-operativity.[1-3] The present work addresses the study and proposes a mechanism for the multi-site interaction of bile acids with chicken I-BABP (ileal BABP) with the aim of elucidating the determinants of ligand binding in comparison with homologous proteins from different species and tissues. A thermodynamic binding model describing two independent consecutive binding sites is derived from isothermal titration calorimetry experiments and validated on the basis of both protein-observed and ligand-observed NMR titration data. It emerges that a singly bound protein is relatively abundant at low ligand/protein molar ratios assessing the absence of strong co-operativity. Both the measured energetics of binding and the distributed protein chemical-shift perturbations are in agreement with a first binding event triggering a global structural rearrangement. The results described in the present study point to the presence of a protein scaffold which is able to establish long-range communication networks, but does not manifest positive-binding co-operativity, as observed for the human protein. We consider chicken I-BABP a suitable model to address the molecular basis for a gain-of-function on going from non-mammalian to mammalian species.

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P273

Solution NMR Structure and RNA interaction studies of the Human SBDS Protein

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We determined the solution structure and backbone dynamics of the human SBDS protein and describe its RNA binding site using NMR spectroscopy.[1] SDS (Shwachman-Bodian-Diamond syndrome) is an autosomal recessive genetic syndrome with pleiotropic phenotypes including pancreatic deficiencies, bone marrow dysfunctions with increased risk of myelodysplasia or leukemia and skeletal abnormalities. This syndrome has been associated to mutations in the SBDS gene, which encodes a conserved protein showing orthologs in Archaea and eukaryotes.[2] Significant conformational exchange was observed in the NMR dynamics experiments for the flexible linker between the N-terminal and the central domains, and these experiments also reflect the relative motions of the domains. RNA titrations monitored by heteronuclear correlation NMR experiments and chemical shift mapping analysis identified a classic RNA binding site at the N-terminal domain that concentrates most of the mutations described for the human SBDS.

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P274

3D structure of EMAP II protein in solution reveals high flexibility and exposure of N-terminal cytokine motif which is partially buried in crystal structure

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Endothelial and monocyte activating polypeptide II (EMAP II) is the proinflammatory cytokine which induces endothelial cells apoptosis and reveals strong antitumor activity *in vivo*.[1] Analyzing crystal structure of EMAP II by MD simulations we postulated the rearrangement of structural loop Lys116 – Lys123 in solution which resulted in stabilization of the adjacent α-helix and extension helix by four residues (Pro120 – Lys123). In present work, we describe the high-resolution 3D structure of EMAP II obtained by multidimensional NMR spectroscopy in solution. In order to make all sequence-specific assignments, the standard 3D NMR experiments were supported by 4D NMR HNCOCA / HNCACO datasets[2] acquired using arbitrary sampling in evolution time space.[3] According to our NMR data, the Val6 – Thr18 cytokine motif in EMAP II protein reveals much higher accessibility in comparison with known EMAP II crystal structures where it is partially buried. Solved 3D structure also supports our observations about α-helix extension by four residues in Pro120 – Pro130 region which does not observed in some EMAP II crystal structures. Our data shown that the flexible region of EMAP II structure (Lys116 – Pro130) may be involved in the formation of *t*RNA binding site of this polypeptide.

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P275
Using ^{19}F HRMAS techniques to study Krebs cycle intermediates

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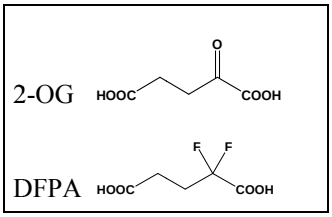
The Krebs cycle, a well-conserved metabolic pathway, provides precursors for the synthesis of a variety of metabolites and macromolecules. Recent studies indicate that some intermediates of the Krebs cycle, such as 2-oxoglutarate (2-OG), act as signaling molecules involved in the regulation of different cellular activities in bacteria, plants and human. Most of these studies have been carried out *in vitro*, and the signaling function of such metabolites is difficult to establish *in vivo* because they are rapidly metabolised. To circumvent this difficulty, we have recently synthesized a nonmetabolizable analogue of 2-OG : the 2,2-difluoropentanedioic acid (DFPA).[1,2] This analogue can be easily traced *in vivo* by ^{19}F High-Resolution Magic Angle Spinning (HRMAS) techniques. In particular, we show here preliminary results obtained by Pulsed Gradient Spin Echo (PGSE)[3] and ^{1}H-^{19}F HOESY experiments to elucidate the interaction between DFPA and its putative receptor NtcA.



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P276
Sp140 Plant HomeoDomain (PHD) finger: a structural and functional study

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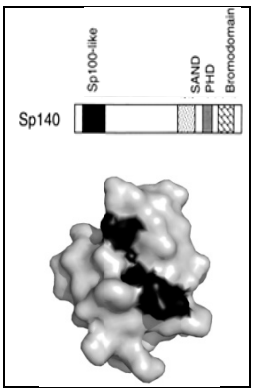
The plant homeodomain (PHD) finger is a conserved zinc-binding domain found in many chromatin-remodelling proteins. Mutations targeting PHD fingers have been associated with cancers, immunological and neurological diseases.[1] PHD fingers can work as SUMO E3 ligase or as readers of epigenetic marks, mainly on histone H3. They are functionally diversified and work in a combinatorial fashion with other nuclear interacting domains, such as bromodomains. The leukocyte-specific Sp140 protein is a component of the PML-NBs in mature B cells, plasma cells and some T cells[2] and contains a tandem PHD-bromodomain. It is implicated in chronic lymphocytic leukemia, but its real function is still unknown. We expressed Sp140 PHD finger in *E.coli* and we are solving its structure in solution. Its ^{1}H-^{15}N-HSQC spectrum showed the presence of cis/trans isomerization of one of its peptidyl-prolyl bonds. The isomerization was further confirmed by means of Pro-Ala mutation.



In vitro SUMOylation tests performed on the tandem PHD-bromodomain expressed in *E.coli* revealed that one SUMO moiety can covalently bind to a lysine of the tandem. By NMR titration we also demonstrated that SUMO E2 enzyme Ubc9 binds to one face of the PHD finger (mapped in black on the PHD structural model). We are currently verifying the hypothesis that Sp140 PHD finger works as SUMO E3 ligase for the adjacent bromodomain. We are also investigating its role as epigenetic reader, by means of NMR and fluorescence titrations which test the domain affinity towards modified and unmodified H4 and H3 tails.

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7.2 *In vivo*/Imaging

Posters

P277

Longitudinal analysis of macrophage activity in inflammatory bowel disease using perfluorocarbon nanoemulsion and ^{19}F MRI

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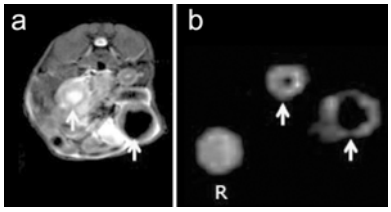
Methods to non-invasively diagnose and quantify inflammatory bowel disease (IBD) can potentially aid in our understanding and treatment of this serious disease. In a mouse model, we demonstrate that quantitative and longitudinal monitoring of IBD is possible *in vivo* using ^{19}F MRI following perfluorocarbon (PFC) nanoemulsion delivery intravenously (i.v.). The nanoemulsion droplets are taken up by circulating monocytes and macrophage that participate in inflammatory events *in vivo*, resulting in an accumulating of ^{19}F at inflammatory loci. IBD was induced in IL10$^{-/-}$ mice (n=6) by feeding piroxicam-doped chow for 14 days. The PFC nanoemulsion (VS1000, Celsense, Inc., Pittsburgh, PA) was then injected i.v., and longitudinal ^{1}H and ^{19}F images were acquired in anesthetized mice on days 2, 9, 16, 23 and 30 post-injection at 11.7 T. Additionally, excised colon tissues were imaged *ex vivo* using MR microcopy. Colon tissues were subjected to H&E histology and immunohistochemistry to look at macrophages (F4/80), neutrophils, monocytes (Ly6C) and endothelial cells (CD31), and RNA was extracted to measure macrophage load using qRT PCR. A thickening of the colon wall was observed in ^{1}H images (panel a), and patchy ^{19}F signals were observed (panel b, R=external reference). Longitudinal quantification of ^{19}F in the colon showed increasing ^{19}F signal from days 2 to 16 and then decreased thereafter. H&E staining displayed pancolitis with heavy mononuclear cell infiltration. Immunofluorescence of colon tissues showed that PFC was localized within macrophage exclusively. The qRT PCR revealed a linear correlation between macrophage RNA and ^{19}F signal in the same tissue samples. Overall, *'in situ'* macrophage labeling using PFC nanoemulsion can enable visualization and quantification of a wide range of acute inflammatory lesions with high specificity.



Acknowledgments: NIH R01-CA134633, R01-EB003453, P01-HD047675, and P41-EB001977.

P278

Hyperpolarized ^{129}Xe NMR for Analysis of Blood-compatible Xenonizer Membranes

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NMR of hyperpolarized ^{129}Xe has found a great variety of applications in many fields of research,[1] mainly due to the broad chemical shift range and the lipophilic character of the large xenon atom allowing, in principle, for dissolution of the gas in liquids. However, especially for medical use such as in blood studies, the dissolution process must not lead to bubbles and should exert as little mechanical stress on the fluid as possible. The so-called "xenonizer" setups[2] have turned out to meet these requirements to a wide extent. Basically, they consist of hollow fiber membranes in oxygenator modules and have been proven to be feasible for more complex experiments on dissolved hyperpolarized ^{129}Xe.[3]

In order to further enhance the efficiency and biocompatibility of the xenonizers, a home-built system featuring different membrane materials has systematically been analyzed for various biologically relevant solvents ranging from water and isotonic saline solution to porcine plasma, whole blood, and highly concentrated erythrocytes. The suitability of the studied membrane types has been characterized with regard to the significance of the parameters relevant for different applications, e.g. highest signal-to-noise ratio, fastest exchange of depolarized and freshly hyperpolarized ^{129}Xe in the solvent, lowest hemolytic effect, etc.

Based on the results presented, experiments employing xenonizers can further be improved. Moreover, they lay the foundation for future studies of the complex processes within the xenonizer setup itself as well as for the understanding of xenon-blood interactions and the resulting anesthetic effect.

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P279 (*)

Toward molecular imaging using ^{129}Xe NMR-based biosensors

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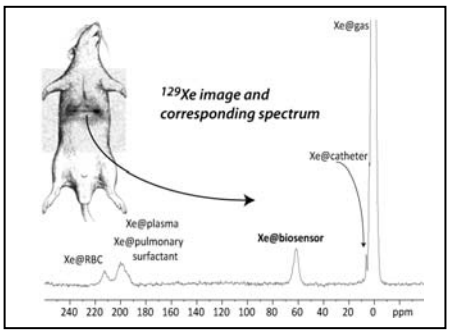

A promising molecular imaging modality proposes to detect hyperpolarized xenon transported to biological targets *via* host molecules functionalized with adequate ligands.[1] Research for developing potent biosensors[2] and fast imaging techniques taking full advantage of the hyperpolarization[3] aim at alleviating the sensitivity problem. Whereas the proof-of-concept of this approach was already established by the specific detection of proteins or nucleic acids at low concentration in solution, none of these ^{129}Xe NMR studies were developed directly with biological cells or *in vivo*. First, in order to assess its potential for detection of specific cellular surface receptors, we have built ^{129}Xe NMR-based sensors of the transferrin receptors and studied their interaction with eukaryotic cells through NMR combined with fluorescence.[4] Then, in order to check the validity of the 'post-labeling' concept where xenon delivered in a second step enables localization of the biosensor previously injected, we have instilled cryptophanes (ideal xenon host molecules) in rat lungs. The spatially-resolved spectrum of laser-polarized xenon inhaled by the animal some minutes after reveals the signal of the noble gas in the biosensor (see figure).

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4. Manuscript in preparation

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In situ MR imaging of food during continuous heating

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MR imaging can overcome the limitations of destructive methods for the in situ monitoring of structural changes and mass transfer in foods during heat treatment. For this application, a nonmagnetic adiabatic heating system is necessary. Moreover, a continuous control over the measuring conditions is needed to prevent the signal-to-noise ratio (SNR) worsening with rising temperature. A nonmagnetic experimental device was designed based on circulating water heated linearly from 20 to 75 C. The tank containing the meat sample and the circulating water was heat-insulated from the transmitter-receiver antenna by a PTFE® tube. During the experiment the SNR loss of ~40% due to concomitant variations in the intrinsic NMR parameters of the muscle outweighed the ~15% loss caused by the extrinsic experimental variations. To obtain sufficient contrast in the muscle and monitor its deformation at 4.7T, the imaging sequence made use of the difference in magnetic susceptibility between the network of conjunctive tissue and the muscle fibres. This sequence featured bipolar gradients to cancel the signal from the mobile protons in the circulating hot water. Velocity mapping by MRI showed that the distribution of mean velocities in the heating water was uneven in the tank, which explains the persistence of minor artefacts in the low velocity areas. In addition, numerical temperature simulations showed that the heat exchange varied little at the surfaces of the sample over the range of velocities measured. These conditions therefore ensured homogeneous and reproducible heating of samples. An acceleration strategy based on the BRISK method[1] was developed to achieve a time resolution of 1'45'', a mean SNR of ~40 and a voxel size of 0.25×0.25×2 mm^3. In these conditions, structural changes and juice transfer in slowly cooking meat samples can be monitored in an original and robust way.

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Acknowledgments: This work was funded by the EU project ProSafeBeef (www.prosafebeef.eu/asp/).

P281 (*)

Region Specific Frequency Differences Between Water And Metabolite Resonances Within The Human Brain

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Recent studies showed that frequency shifts responsible for phase contrast in gradient echo MRI are dependent from the brain tissue architecture.[1] The aim of this work was to examine possible regional differences in water to metabolite frequency distances with proton Chemical Shift Imaging (CSI) without water suppression.

Unsuppressed in-vivo CSI data were collected at 3T MR scanner (Siemens, Erlangen) from 14 healthy volunteers at the level of lateral ventricles. Parameters of CSI acquisition were as follows: TE/TR = 144/1350 ms, voxelsize of 5x5x10 mm. Water and metabolite peaks were fitted with Gauss-Lorenz function and resonant frequencies were evaluated.

Fig. 1 shows the example of water to N-Acetyl Aspartate (NAA) frequency distance map. One can notice that the frequency distance for white matter is lower than for grey matter. This can be seen in case of all volunteers and for all metabolites. However, this tendency is strongest in case of water to NAA frequency distance.

Figure 1

We showed that there are regional differences in water to metabolites frequency distances between gray and white matter. This observation is confirmed by phase images and proves that CSI without water suppression may provide important additional information.

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Acknowledgments: Study supported by the German Research Foundation (DFG KL 1073/7-1).

P282

Automated Parahydrogen-Induced Polarizer (PHIP) Employing Low Field NMR Spectrometer, Tunable RF Circuit and *in situ* Detection

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Parahydrogen-Induced Polarization (PHIP) of ^{13}C and ^{15}N enriched contrast agents allow for real time metabolic imaging. However, PHIP technical implementation and routine use is limited to very few research groups in the world capable of in-house design and development of PHIP equipment. Previously published PHIP polarizer designs capable of up to 20% nuclear spin polarization utilize un-tuned *RF* coils and have lacked onboard receivers. As a result, these first generation polarizers require tedious calibrations of B_1 and B_0 fields, which are often accomplished on an external high field NMR instrument. Moreover, any adjustments of polarizing conditions (reaction time, B_1, etc.) as well as the quality control of the hyperpolarized compounds also require this access to high field MR systems that are not always available or of a significant cost.

Here, we present a PHIP polarizer design based on the commercially available Kea2 low-field NMR system (Magritek, New Zealand) operating at 49 mT. A double-tuned *RF* circuit operating at 2.02 MHz (^1H) and 0.51 MHz (^{13}C) allows for *RF* transmission and direct NMR detection of PHIP, Fig. 1. B_1 and B_0 calibrations and *in situ* quality assurance of PHIP contrast agents (Fig. 1) are carried out within minutes without access to high field NMR equipment. This design on the Kea2 platform (<$100k total cost) can be readily replicated by other research groups.

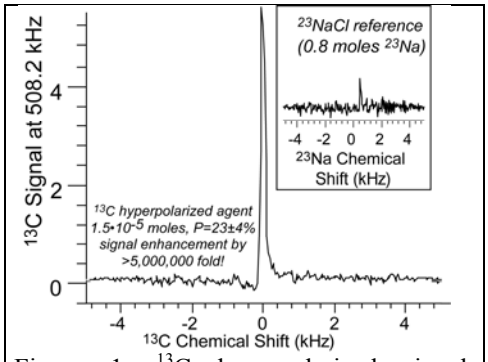

Figure 1. ^{13}C hyperpolarized signal, $P_{produced}$~40%, detected after 32 s *in situ* and referenced to ^{23}Na signal obtained from NaCl sample at 49 mT spectrometer.

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P283
Metabolomics and Its Applications

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In the era of genomics and proteomics, metabolomics offers a unique way to probe the underlying biochemistry of physiological and pathological conditions. Cancer metabolomics studies the global variations of metabolites involved in the development and progression of malignancy. Cancer metabolomics uses techniques such as magnetic resonance (MR) methodologies to discover disease biomarkers that may have the potential to assist disease detection and diagnosis, as well as to predict the courses of progression. In the past ten years, utilizing the intact tissue MR spectroscopy method that we developed, my laboratory has engaged in the studies of human malignancies of prostate, breast, brain, lung, etc. Examples from these studies will be presented to illustrate the power of cancer metabolomics in detecting human prostate cancer through metabolomic imaging[1] and in predicting prostate cancer recurrence using metabolomic profiles.[2] Further demonstration of the ability of human serum metabolomic profiles in characterization of lung cancer will also be discussed.[3]

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Acknowledgments: PHS/NIH Grants: R01CA115746, R01CA115746-S1, R21CA141139, and MGH A. A. Martinos Center for Biomedical Imaging.

P284
Depth Profile Imaging using Single-Sided RF Coils – A Novel Application

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Complementary to conventional magnetic resonance techniques, muon spectroscopy[1,2] uses an implanted 100% spin polarised spin-½ positive muon either as a probe of the local magnetic environment or to act as a proton analogue in diffusion studies and chemistry. RF techniques can be applied;[3] however, excitation is indiscriminate over the volume of implanted muons (typically of ~20mm diameter and 0.5mm depth for a material of mass density 1g.cm^{-3}), making it difficult to study highly inhomogeneous systems.

The purpose of this present work was to examine whether the experimental setup described by Casanova and Blumlich,[4] and used with great success for the NMR-MOUSE®, could be applied to the RF μSR technique to introduce depth discrimination and localise the μSR signal to comparatively thin volume sections. With the permanent magnet orientated such that the field lines are parallel to the incident muon beam, an RF surface coil located on a pole face is ideally positioned to accept the muon beam. Results will be presented that demonstrate this novel application of imaging methodology.

A valuable aspect of this work is the evaluation of surface coils for RF μSR measurements. Typically, cavities are constructed as a flattened solenoid (~24x24x2mm) both to match the profile of the incoming muon beam and contain the sample. While simple to make, they have the disadvantage that a fraction the incident beam is stopped in the coil winding to give a background signal. Surface coils remove this problem, and results will be presented to evaluate performance.

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Acknowledgments: The Neutron Scattering and Muon Spectroscopy Integrated Infrastructure Initiative (under EU Framework Programmes 6 and 7) is thanked for supporting this project. Iain McKenzie, Zaher Salman and Nigel Clayden are thanked for useful discussions.

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Magnetically oriented nanovesicles as MRI CEST agents

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The growing interest aroused by CEST agents resides in the possibility to develop innovative diagnostic protocols currently precluded with the use of conventional MRI contrast agents (e.g. multiple probe detection). It has been shown that nanosized scaffolds are excellent candidates for the class of CEST agents. In particular, nanovesicles have been used either as carrier for the probe itself or as a source of mobile protons, provided by the water molecules contained in their inner cavity, properly shifted by the addition of a paramagnetic shift reagent (the so-called LipoCEST agents).[1] The latest approach has proved extremely effective for increasing the sensitivity of the CEST agent. For *in vivo* applications, it is particularly important to deal with agents displaying a very large separation between the resonance of the intraliposomal water and the bulk signal. This task has been tackled by generating non-spherical vesicles in which the intraliposomal water resonance receives a substantial contribution from bulk magnetic susceptibility effects.[2] Despite this progress, the preliminary *in vivo* experiments using LipoCEST agents have highlighted some limitations in their applicability. In fact, the rapid uptake of liposomes by macrophages and their subsequent degradation occurring in cellular organelles, determines the disappearance of the CEST contrast. However, this drawback may be turn into an opportunity to shed light into biological processes. In fact, the exploitation of the multicontrast (T_1, T_2 and CEST) ability of paramagnetic liposomes has allowed the evaluation of the intracellular fate of the vesicles (and their content) in the tumor microenvironment.[3]

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P286

Magnetic Resonance Spectroscopy Method for the Detection of Metabolites and Macro-molecules in Human Brain at 7 Tesla

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The increased frequency separation at high magnetic field strength, 7 Tesla, can provide signals of high signal-to-noise ratio which allows the detection of various brain metabolites and macro-molecules.[1-3] Model metabolite solutions,[4] prepared from twenty metabolite models of different concentrations, was used in a combination with automatic shimming, water suppression adjustments and STEAM-VERSE sequence (TR=3000 ms, TE=20 ms, TM=15 ms and VOI= 1 mL) to make the Basis –Set for use with Linear Combination Model. The *in vivo* spectra acquired from human brain at 7 T can then be quantified using LC-Model.[5,6] The results provide a powerful tool for routine proton magnetic resonance spectroscopy of the human brain at high magnetic fields.

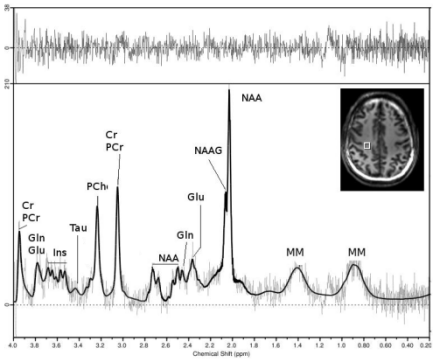

Fig. in vivo human brain metabolites quantification using LC-model at 7 Tesla.

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P287

In vitro and in vivo characterization of polymer-water interaction studied by an off-resonance MRI - synthetic copolymer gels and breast carcinoma

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Introduction: In order to clarify the characteristics of tissue water protons in MRI, i.e., biopolymer-water interactions in living systems, we studied the correlations between cross-relaxation rate (CR) and physical state of water in synthetic copolymer gels for in vitro study, and between CR and histological parameters of carcinoma in human breast for in vivo study.

Materials and Methods: Fourteen synthetic copolymer gels (rod-shaped, 15 x 100 mm) were composed of any two or three monomers among HEMA, GMA, N-VP and MMA. Water contents were adjusted to 18.4 - 83.0% changing monomer composition. Twenty-seven patients with histologically confirmed invasive ductal carcinoma of the breast were participated. These carcinoma were classified based on their histological features, i.e., extent of fibrosis in the intercellular matrix, dysplastic changes of nuclei and mitotic index. Breast MRI was conducted using a 1.5 Tesla MRI clinical scanner. To evaluate CR values, an off-resonance MR technique for preferential saturation of the immobile protons was used (off-resonance irradiation at 7, 19 or 75 ppm apart from the frequency of water resonance).

Results and Discussion: In the model system such as copolymer gels, there was a good correlation between CR values and the hydrophilicity of various copolymer gels, especially the CR-7 (CR at 7 ppm) values were found to be more separable parameter depending on the different hydrophilicity of the samples. In the breast carcinoma, CR-7 values were correlated well with the intracellular characteristics, whereas CR-19 values were correlated with the histological intercellular structure. Intracellular macromolecules such as DNA, RNA and proteins contain a large number of OH groups. The CR-7 values are increased with increasing dysplastic changes of nuclei and mitotic index, suggesting that CR-7 values might reflect the amount of bound water or the degree of hydration with the intracellular macromolecules.

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Cellulose Liquid Crystalline Defects Probed by MRI

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Cellulosic liquid crystal solutions were found to exhibit spontaneous twist due to intrinsic curvature.This twist enables fibres electrospun from the liquid crystal phase to mimic the shapes of the tendrils from climbing plants.[1, 2] In order to investigate the origin of the intrinsic curvature of the fibers different confined cellulosic solutions were studied by means of nuclear magnetic resonance imaging (NMRI).

Solutions of cellulose in cholesteric liquid-crystal phase, which generate curved fibers, showed a heterogenous structure in cross-section with hard "islands" predominantly located closest to the tube walls and never in the middle of it. Confined solutions of isotropic cellulosic solutions showed a homogeneous cross-section.

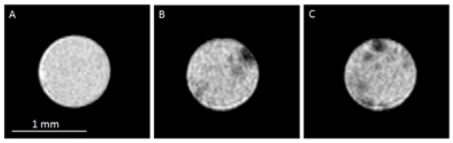

Fig. 1 Cellulosic solutions in a glass capillary. **(A)** through **(C)** are MR images of the cross-section of a capillary filled with: **(A)** an isotropic cellulosic solution (20%w/w, APC/DMac), **(B-C)** a liquid crystalline solution (60%w/w, APC/DMac) in subsequent sections. Solutions were sheared for 10 min ($\dot{\gamma} = 20s^{-1}$) and frozen before MR measurements.

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NMRD profiles of silica based contrast agents at high magnetic fields

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Nanosized periodic mesoporous silica (PMS) materials loaded with gadolinium have shown promising properties as potential contrast agents for magnetic resonance imaging (MRI).[1-3] Knowledge of the field dependence of T_1, known as Nuclear Magnetic Relaxation Dispersion (NMRD), is needed to model the detailed mechanisms of paramagnetic relaxation in these systems. However, as MRI is moving towards higher magnetic fields it is important to also map the corresponding NMRD profiles at these fields. The objective of the work presented is to measure the NMRD profiles of PMS materials in a wide range of magnetic fields, with a particular emphasis to measurements at high fields (> 1.0 T). This has been achieved using two different NMR techniques and instrumentation; a dedicated Field Cycling NMR relaxometer, which allows measurements of relaxation data from 0.01 to 40 MHz (200 uT to 1.0 T), and a new cryogen-free, variable field, superconductive magnet for measurements in the field range from 40 to 80 MHz (1.0 to 2.0 T). Common for all of the obtained NMRD profiles is that the maximum in relaxivity occurs at higher magnetic field strengths (50-60 MHz or 1.2-1.5 T) than normally observed in gadolinium-based contrast agents. Also, the relaxivity of the materials stays above 20 mM^{-1} s^{-1} even at 2.0 T (80 MHz). The very characteristic relaxation behavior of the PMS materials at high fields would not have been possible to observe without the use of the new cryogen-free, variable field, superconductive magnet.

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P290

Determination of rCMR(O_2) and rCBF in the Human

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Determination of Regional Cerebral Metabolic Rate of Oxygen (rCMR(O_2)) and Regional Cerebral Blood Flow (rCBF) is important for the understanding of physiological and pathophysiological processes in man as aging, sleep and drug effects on the brain. Quantitative determination of rCMR(O_2) and rCBF consist of direct means for determining the extent of infarct core and penumrba in the diagnosis of stroke and could be useful in the evaluation of vascular dementia and neoplastic processes.

By means of ^{17}O MRI we determined rCMR(O_2) and rCBF in the human voxel by voxel using a clinical 1.5T GE scanner.[1-3]

^{17}O imaging was carried out using Fast Multi Planar Gradient Recalled (FMPGR) and 3 Dimensional Projection Reconstruction (3DPR)[4,5] pulse sequences at 1.5 and 3 Tesla using GE clinical scanners.

Data analysis was carried out by a least square fit of a whole body reflow simulation (WBRS)[6] to the experimental data. The method could be extended to other organs.

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Relaxivity Studies of Potential Nanoparticulate MRI Contrast Agents: Lanthanide Based Metal Organic Frameworks and Polyoxometalates-Silica Nanocomposites

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The potential use of aqueous suspensions of two new kinds on Ln(III)-containing nanoparticles made up of hybrid materials as MRI contrast agents was explored by studying the relaxometric properties of their aqueous suspensions. Paramagnetic porous metal organic $[Ln(H_2cmp)(H_2O)]$ frameworks (MOF) (H_2cmp=(carboxymethyl) iminodi(methylphosphonic acid)) particles, with sizes defined by SEM, TEM and DLS, were studied.[1] The water ^1H r_1 values are very small in all cases, while r_2 are larger, proportional to μ_{eff}^2 of the Ln(III) and dependent on τ_{CP} (the time interval between two consecutive refocusing pulses in the train of 180° pulses applied in a CPMG pulse sequence), saturating at values 3 to 5 times lower than r_{2p}*. This was explained by the static dephasing regime (SDR) theory. r_2 relaxivity also increases when particle size decreases. Nanoparticles of Ln(III)-containing polyoxometalate (POM) encapsulated in a silica shell have very low r_1 values, while r_2 depends on μ_{eff}^2 and is independent of τ_{CP}. This is in agreement with the outer-sphere relaxation mechanism, since the particles are small enough to satisfy the condition for motional narrowing. Both kinds of particles studied, despite the presence of one water molecule in the inner-sphere of each framework Ln(III) ion, show very low r_1 values. This inefficiency can be attributed to inadequate exchange with bulk water due to hindered diffusion through the frame. The large r_2 values at high magnetic fields make these particles very efficient as potential MRI contrast agents for T_2-weighted imaging.

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The Advantages and Drawbacks of Contrast enhancement in MRI by magnetic nanoparticles: The Inflammatory Response to Iron Nanoparticles in MRI study of murine model

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Magnetic iron oxide nanoparticles (NPs) are widely used in MRI for diagnostic of various diseases including inflammatory lung complications. The inflammatory response of contrast agent used in the nanoparticulate form of superparamagnetic iron nanoparticles through intratracheal route of administration to mice was studied. The images of lung-bronchial tract of mouse were detected with Avance II spectrometer (Bruker) at 11.7 T. The measurements were carried out using gradient-echo sequence (GEFI ORTO), multiscan-multiecho (MSME). The T_1, T_2 –weighted images were obtained with help of RARE-T_1 and Turbo-RARE-T_2 scanning regimes. Iron NPs were synthesized by gas decomposition of iron pentacarbonyl. NPs were about 30 nm in diameter with outer Fe_3O_4 shell (2-3 nm) and inner Fe core. The metabolomic effect of the iron nanoparticles was studied upon orotracheal administration of NPs (1mg/mouse) to nonbread mice. The low intensity signals in response to NPs were observed as grey level images. The inflammatory response to NPs and its suppression by anti-inflammatory drug was also confirmed by the total cell number in bronchoalveolar lavage (BAL), the relative quantity of macrophages and neutrophils and metabolic characteristics of BAL fluid (pH, redox potential, lactate, total protein, proteomic profile). The protein composition of BAL fluid of BALF and plasma probes were carried by PAGE, HPLC and NMR spectroscopy methods. The inflammatory response to instillation of iron NPs is characterized by the increase of total protein, neutrophils relative count, ethanol and lactate in BALF. Pharmacological substance IL-1ra may be one of contra-inflammatory means to reduce lack of iron NPs-lung inflammation in MRI.

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Water flow from soil to roots investigated by MRI

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Water flow in soils is one of the most important functions that control the water supply for root and plant growth. Since flow velocities in soils are mostly too slow to be monitored directly by MRI flow velocity imaging, we used Gd-DTPA as tracer for the first time to visualize flow processes in soils[1]. Apart from its chemical stability it turns out that the main advantage is the anionic net charge in neutral aqueous solution which hinders the adsorption at soil mineral surfaces and therefore avoids retardation. To obtain optimal measurement parameters the relation between signal intensities, tracer concentrations, repetition time t_R und echo time t_E were investigated in a preliminary study. The images were measured with a spin echo multi-slice sequence with strong T_1-weighting (e.g.: t_R = 0.2s, t_E = 2.7 ms, 0.16 × 0.16 × 0.9 mm^3 voxels).

Water flow has been investigated during i) infiltration and ii) injection experiments of unsaturated model soils with maize and lupin plants. During the infiltration experiment we observed initial rapid homogeneous wetting of the bulk soil from the bottom, whereas the immediate vicinity of the root is not reached by the tracer. After this initial period a continuous enrichment of the tracer is observed in this region within the next hour, but no uptake by the plant. Furthermore, injection experiments were performed to decouple the wetting from the transport process. Under bright illumination different steps could be observed: Dispersive spreading of the plume in the soil, followed by directed flow to the root system, enrichment in the cortex of the roots, and final transport upwards in the xylem. Under dark conditions spreading of the plume is still present, but Gd-DTPA is now taken up so slowly that it is not enriched in the cortex but actively transported with the water to the xylem. With these results active and passive uptake of Gd-DTPA can be distinguished and conclusions on the water flux processes can be drawn. The soil structure determining the flow process was additionally investigated by relaxometric imaging (t_E = 4 ms, n_E = 64, 0.32 × 0.32 × 0.6 mm^3 voxels). These first results show that the root-soil interface possesses a small zone of higher water content und homogenous relaxation times around lateral roots corresponding to the extension of the root hair zone and may serve as water buffer for roots.

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P294

Magnetic Resonance Imaging of Oscillating Electrical Currents

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Direct imaging of neuronal activity by MRI would resolve many issues inherent to fMRI, yet common techniques rely on phase measurements which cancel in the presence of oscillatory magnetic fields. We demonstrate experiments to directly image oscillating currents by MRI. The approach[1] rests on a resonant interaction between an applied RF field and an oscillating magnetic field in the sample and, as such, permits quantitative, frequency-selective measurements of current density without spatial or temporal cancellation. We apply this method in a current loop phantom, mapping its magnetic field and achieving a detection sensitivity that is at the threshold of that required for detection of neuronal currents. The spectroscopic control afforded by the experiment allows us to independently image current-induced magnetic fields of different frequencies, and we further demonstrate how ramped and phase modulated spin lock radiation can enhance the sensitivity and robustness of the experiment. We introduce a Fourier imaging experiment in which the spatial variation of the magnetic field can be readily measured.

Finally, we perform a remotely-detected analogue of the experiment, in which we measure the effects of currents in small volumes of flowing water. By separating the encoding and detection steps of the MRI experiment, remote detection allows the separate optimization of each. We are thus able to overcome filling factor, magnetic susceptibility, and other limitations to achieve high spatial and temporal resolution without sacrificing sensitivity. In this way, we can easily acquire high-resolution images which display features of the phase distribution that may otherwise be compromised by spatial averaging when viewed at lower resolutions.

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Quantification of the water exchange between vessels and parenchyma cells in xylem of diffuse-porous viburnum tree

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Water exchange between fluid in xylem vessels and surrounding them parenchyma cells in diffuse-porous viburnum tree was non-invasively investigated by Magnetic Resonance Imaging (MRI). The signal from vessels and parenchyma cells was discriminated on the basis of different in T_2 and diffusion coefficients. From the diffusion-T_2 correlated measurements follows that two peaks detected at observation time 20 ms and corresponding to water in different types of cells (component with faster diffusion coefficient and longer T_2 is water in vessels and component with slower diffusion coefficient and shorter T_2 is water in parenchyma cells) gradually associate in one peak with increase of observation time (Δ). Such behaviour is typical for exchange between components. It is known that exchange between the compartments results in a decrease of the relaxation times of both components, an increase of the amplitude of the flowing water (water in xylem vessels) and a corresponding decrease of the amplitude of stagnant water (in parenchyma cells). Since the signal from flowing fluid in porous media is sensitive to susceptibility effects and the amplitude of this component uncorrected decreases with increasing Δ, this component can not be used for determination of exchange. The amplitude of stagnant water in parenchyma cells after correction on apparent T_1 (T_{1app}) strongly increased with increasing of Δ. Since exchange between component with different T_1 is accompanied by a decrease of the T_{1app}, the correction of the amplitude of stagnant water on this value results in overcorrection of this amplitude. The application of the T_1 value without exchange (T_{1real}) results in that the corrected amplitude of stagnant component becomes independent on Δ. From the difference between T_{1app} and T_{1real} the mean residence time of water in parenchyma cells can be calculated.

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Apparent diffusion anisotropy in rat cerebellum is altered at short effective diffusion-times using oscillating-gradient diffusion-tensor MRI

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It has long been hypothesised that restricted (or hindered) motion of water molecules is responsible for the anisotropic image contrast in diffusion-tensor MRI, in particular in white matter. It has also been recognised that in that case, by varying the length or separation of the motion-probing gradients (MPGs), it should be possible to alter the contrast of *in vivo* images and thus common measures of diffusion anisotropy to probe tissue microstructure. Most previous efforts were unable to find *in vivo* evidence for the restricted-diffusion hypothesis (eg Clark[1]), but recent work using a technique that utilises rapidly oscillating MPGs added to a standard spin-echo sequence, has demonstrated some of the characteristics of restricted diffusion for in vitro samples, and normal and diseased rat brain.[2-4] However, the technique has never been applied to examine alterations to apparent diffusion anisotropy as the MPG frequency is increased. In this study, an oscillating MPG sequence was applied to investigate changes to the apparent diffusion tensor, fractional anisotropy and mean diffusivity in rat cerebellum. The gradient frequencies were in the range 30-200 Hz and corresponded to effective diffusion times of 1-8 ms.[2] The results clearly showed that the mean diffusivity increased with MPG frequency, which is a characteristic expected of the restricted/hindered diffusion model. Other indices of diffusion anisotropy were also visibly altered by changes to the MPG frequency. Given sufficient gradient-set performance, it is anticipated that normal and pathological *in vivo* tissue structure can be probed with this technique.

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Intracellular water lifetime measured by diffusion weighted and dynamic contrast enhanced MRI

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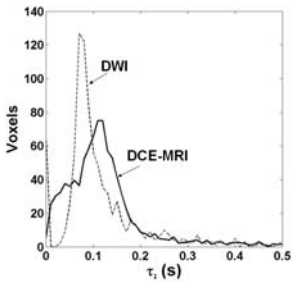
Exchange of water between intracellular and interstitial compartments is an important factor to be considered in estimating physiologically relevant parameters from dynamic contrast enhanced (DCE)-MRI data using a pharmacokinetic model.[1] While it has been demonstrated that intracellular water lifetime (τ_i) can be estimated from DCE-MRI data, it has not been shown whether the estimated τ_i can be compared with any other *in vivo* measurement method. In this study, we measured τ_i using both DCE-MRI and diffusion weighted imaging (DWI) in BALB/c mice (n=3) with 4T1 mammary carcinoma. DCE-MRI data were acquired using a 3D FLASH sequence with 3.8s/frame for 12 min and were analyzed using the adiabatic approximation of tissue homogeneity model[3] with full water exchange model.[4] DWI experiment was performed with a constant gradient strength of 150 mT/m and seven diffusion times from 15 ms to 200 ms. From DWI data, τ_i was estimated as negative inverse slop of the regression line of the natural logarithm of data between 75 ms and 200 ms.[5] The plot on the right shows comparison of τ_i histograms from tumour lesion measured by both methods. The medians (25th - 75th percentile) of τ_i from DCE-MRI and DWI were 112 (72 – 150) and 90 (71 – 146) ms, respectively. To our best knowledge, this is the first *in vivo* imaging study to measure τ_i using both DWI and DCE-MRI and to report a good agreement between their estimates.



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Liquid State Dynamic Nuclear Polarization for MRI applications: An In-bore Approach

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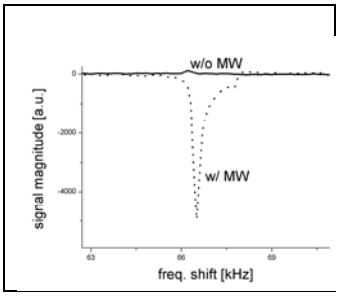
Contrast and scan time being key issues in Magnetic Resonance Imaging (MRI), several approaches can be taken to improve the Contrast-to-Noise Ratio (CNR) of MRI images. Among those, hyperpolarization techniques are very promising, one of which is Dynamic Nuclear Polarization (DNP).

In DNP, hyperpolarization of nuclei is achieved by microwave irradiation of electron spins ("radicals") transferring their larger Boltzmann polarization to the nuclei – in this study via the Overhauser Effect.



We present a liquid state DNP polarizer operating in flow through mode at a magnetic field strength of 1.5 T (42 GHz microwave frequency), compatible with a standard 1.5 T medical imaging magnet. Compared to other approaches, where the polarization of the sample takes place in a separate magnet placed well outside the imager bore, e.g. the Oxford Instruments HyperSense, there are major technical differences. Out-of-bore solid state polarization buildup time is in the range of an hour, making it a one-shot procedure. However, liquid state polarization buildup time is in the range of seconds, allowing for a flow through design providing a constant flow of polarized sample. Additionally, any system polarizing outside the imager bore has to shuttle the sample into the imager. This is a process roughly on a timescale of the nuclear T_1, making polarization loss during that period significantly large. On the other hand, polarizing in the liquid state at room temperature and 1.5 T will yield less polarization enhancement than an external system operating at a magnetic field optimized for the polarization process in the solid state at low temperature. The work presented outlines the design of the DNP system, comprising of the flow-through resonator and an appropriate microwave source, and demonstrates its performance in enhancing water proton NMR signals.

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Implementation of MRI to pharmacopoeial method of evaluation of controlled release dosage forms – experimental results, *a priori* and *a posteriori* modeling

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Combined MRI and flow-through cell dissolution testing (US Pharmacopoeia 4) is a very promising tool for investigating controlled release (CR) oral dosage forms.[1,2] Two studies were performed on the MR system equipped with dedicated flow-through dissolution cell combined with MR probe with respect to *a priori* and *a posteriori* modeling.

In the first study, equetiapine fumarate, HPMC based, compressed tablets were used as a subject. The results were compared with theoretical *a priori* model proposed by Ju et al.[3] It concerns swelling of uncross-linked polymers (e.g. HPMC) and is based on polymer disentanglement concentration and diffusion layer in dynamic medium conditions. Model proposed by Ju et al. allowed proper identification of the regions of the swelling matrix in MR images. Qualitative agreement between temporal changes of dry glassy polymer, swollen glassy polymer and gel regions (evolution profiles of polymeric matrix) as obtained experimentally by MRI and theoretically calculated by Ju was confirmed.

In the second study, subjects were different L-dopa, non-compressed HPMC based formulations. They were prepared to give identical results (dissolution profiles) as studied by means of dissolution test only[2]– test commonly used in pharmaceutical industry. MRI in the flow-through cell allowed differentiating between formulations. Moreover, matrix evolution profiles were applied to develop single (surrogate) *a posteriori* model for investigated formulations.

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P300

Iron(III) EHPG complexes as potential T_1 contrast agents for MRI

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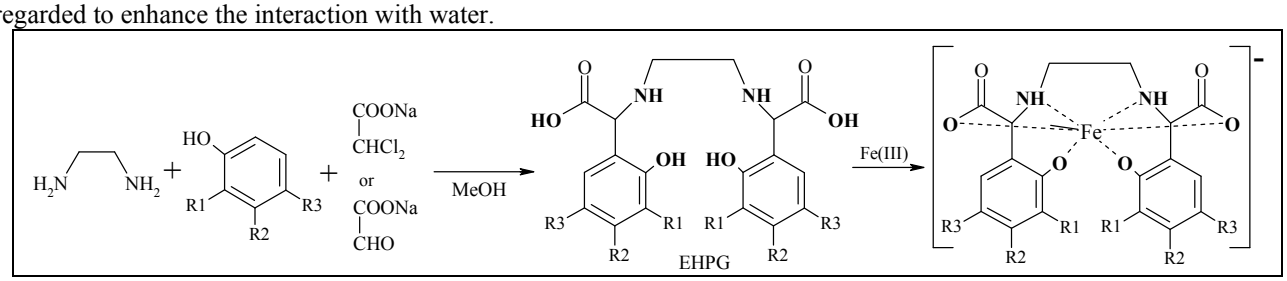
Non-gadolinium contrast agents are under intensive research.[1] Iron is very attractive due to its role in organism, but its applications are still limited. So, a series of hexadentate aminoacidic ligands – *N,N'*-ethylenebis[2-(*o*-hydroxyphenyl)glycine] EHPG[2] derivatives have been synthesised. Most of the ligands carried polar groups, which were regarded to enhance the interaction with water.



Synthesis of EHPG derivatives; R1, R2, R3: -OMe, -NHAc, -Me, -Ph, ...

The ligands were complexed with Fe(III) and the resulting complexes have been separated. Isomeric structures were collected. T_1 measurements have been recorded in aqueous media. T_1 relaxivity at 300 MHz were in the range of 0.5-1.8 mM^{-1} s^{-1}. The models are promising and are under further investigation.

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Study of the effect of paramagnetic ions on the T_2 distribution curves of oils

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The models frequently used to correlate the viscosity of oils to the ^{1}H transverse relaxation times (T_2) in low-field NMR experiments do not take into account the possible effects associated with the presence of paramagnetic ions.[1,2] The aim of the present work was thus to analyze the influence of Fe^{3+} ions on the T_2 distributions recorded for Fe-doped oil samples. The low-field ^{1}H NMR experiments were conducted in a MARAN Ultra spectrometer, from Oxford Instruments, operating at 2.2 MHz for ^{1}H. The T_2 measurements were performed using the CPMG pulse sequence and the T_2 distributions were computed by the inverse Laplace transform method. The T_2 distributions corresponding to the crude and the doped oil samples are shown in Figure 1. In the case of the crude oil, two peaks were observed, associated with the water and oil fractions. The presence of Fe^{3+} ions caused the lowering of the T_2 values associated with both phases – an effect especially strong for the water contribution –, leading to the overlapping of the two peaks. This result clearly illustrates the influences of paramagnetic ions on the T_2 distribution profiles for oil samples, which can lead to erroneous models for the correlation between T_2 values and oil viscosity.

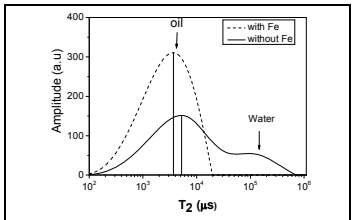

Figure 1- T_2 distribution curves.

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P302

Methods for groupwise analysis of functional MEMRI data

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This study addressed the functional mapping of deep brain regions activated by odours in rats. For this purpose, manganese-enhanced MRI (MEMRI) was chosen. This method uses manganese (Mn) as an exogenous contrast agent.[1] Manganese, a calcium analogue, is recruited by activated neurons and then slowly eliminated. This allows stimulations to be performed on conscious animals and images to be acquired under anaesthesia. Image contrast depends on intra-neuronal Mn concentration, and so reflects neuronal activity throughout the stimulation period. However, Mn remanence prevents control and activation state images being obtained in a single-subject experiment. Images have to be acquired through multi-subject studies and spatial variation of Mn concentration assessed by groupwise image comparison. Two image processing steps are crucial for such an analysis: (i) brain segmentation and (ii) inter-subject normalization. We have developed an original image processing sequence comprising (i) a semi-supervised brain segmentation method based on fast adjustment of an average three-dimensional brain model obtained from 20 manual segmentations and (ii) an iterative algorithm normalizing images in both spatial and intensity dimensions, independently of an *a priori* image target.

This latter algorithm integrates the AIR package intra-modal inter-subject registration method,[2] which was optimized by exhaustively searching for the best cost function/deformation model pair. Preliminary results indicate that our image processing sequence, associated with a voxelwise statistical test for comparing means, highlights deep brain regions involved in odour processing.

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P303
NMR Imaging Study of the Supported Catalyst Preparation

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NMR imaging was applied to study the transport of the precursors of an active component $(Ni(H_2O)_6^{2+}$ and $Ni(edtaH_x)^{(2-x)-}$ complexes, x = 0, 1, 2, edta = ethylenediaminetetraacetic acid) into a γ-Al_2O_3 support pellet during the preparation of the supported Ni/γ-Al_2O_3 catalyst via a dry impregnation technique. The possibility to study the transport of these precursors is based on their paramagnetic influence on the relaxation times of a solvent (water) in the pores of the support. Therefore, the transport of these complexes can be studied detecting the T_1- or T_2-weighted ^1H NMR images. The transport of $Ni(H_2O)_6^{2+}$ into a γ-Al_2O_3 pellet during its impregnation with an aqueous $Ni(NO_3)_2$ solution was characterized not only qualitatively but also quantitatively. For these purposes, a calibration curve showing the dependence of the intensity of the ^1H NMR signal in the T_2-weighted images of γ-Al_2O_3 pellets after their impregnation with the aqueous solutions of different Ni^{2+} concentrations on the concentration of Ni^{2+} in the impregnation solution was constructed. The bulky edta ligand shielded the paramagnetic effect of Ni^{2+} on the ^1H NMR signal, and a T_1-weighted image detection protocol was required to follow the transport of $Ni(edtaH_x)^{(2-x)-}$. The combination of NMR imaging with UV-VIS microspectroscopy allowed the visualization of both $Ni(H_2O)_6^{2+}$ and $Ni(edtaH_x)^{(2-x)-}$ with the complementary information on the dynamics and adsorption/desorption phenomena within γ-Al_2O_3 support bodies. In particular, the data on the interaction of different Ni complexes with the γ-Al_2O_3 surface and with each other were obtained. When we used a stoichiometric ratio of Ni^{2+}: edta, in 2-3 h of impregnation a uniform distribution of $Ni(edtaH_x)^{(2-x)-}$ in the support pellet was observed. When an excess of a Ni salt was used, an egg-shell Ni distribution with a uniform $Ni(H_2O)_6^{2+}$ distribution was established in 2 h.

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P304 (*)
A Xenon-based Molecular Sensor Assembled on an MS2 Viral Capsid Scaffold

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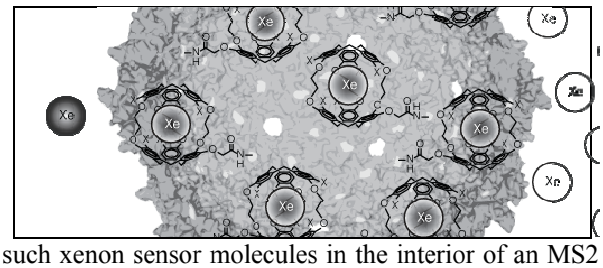

In MRI, anatomical structures are most often differentiated by variations in the bulk magnetic properties of water. Alternatively, exogenous contrast agents can be attached to chemical moieties that confer affinity to molecular targets; the distribution of such contrast agents can be imaged by magnetic resonance. Xenon-based molecular sensors are molecular imaging agents that rely on the reversible exchange of hyperpolarized xenon between the bulk and a specifically targeted host-guest sensor molecule. We have incorporated ~125 such xenon sensor molecules in the interior of an MS2 viral capsid, conferring multivalency, improved aqueous solubility, probable biocompatibility, and other properties of the viral capsid to the sensor[1]. The resulting signal amplification, coupled with the Hyper-CEST detection scheme[2] and highly-efficient, frequency-selective saturation pulses, facilitates the detection of sensor at 0.7 pM, the lowest to date for any molecular imaging agent used in magnetic resonance. This amplification promises the detection of chemical targets at much lower concentration than would be possible without the capsid scaffold.

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P305

Xenon Concentration Dependent Signal Enhancement in Hyper-CEST

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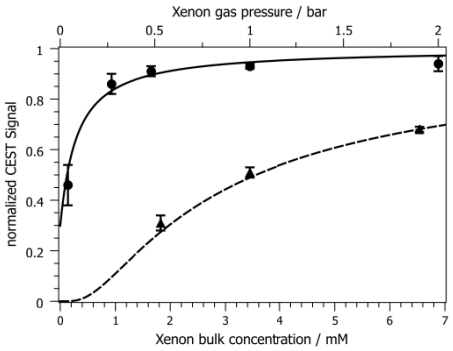
The hyper-CEST method promises tremendous potential on molecule-specific MR using hyperpolarized ^{129}Xe and functionalized cryptophane cages as a biosensor.[1] The approach relies on the steady exchange of Xenon atoms between cage and bulk solution: upon saturation of caged ^{129}Xe a continual reduction of the bulk magnetization occurs, amplifying the sensitivity of biosensor detection in comparison to a direct measurement. Optimal performance of hyper-CEST is thus key to biomedical applications at minuscule molecular concentrations. Here the enhancement of the saturation efficiency with decreasing bulk xenon concentration is reported. In the figure the experimental results for biosensor concentrations of 5 µM (triangles) and 0.5 µM (dots), respectively, are presented. The sensor[2] was dissolved in 2 mL PBS in a valved NMR tube, allowing ^{129}Xe bulk concentration to be varied by setting its gas pressure in the range of 0.04 to 2 bar. Instead of being destroyed by cw-radiation, the magnetization of caged ^{129}Xe was inverted by a train of 1000 selective RF pulses separated by a period of the order required for a complete turnover of the cage population. Data are normalized by bulk signal amplitudes obtained by applying the inversion pulses far off-resonance. The lines are fitted curves based on a model for the exchange process.



The enhanced sensitivity of biosensor detection at reduced bulk ^{129}Xe concentration may improve hyper-CEST in various applications, particularly in MRI, for contrast enhancement or shortened measurement times.

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P306 (*)

Towards Understanding Transverse Relaxation Mechanisms of Tissue Water in Human Brain

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Transverse relaxation time T_2 of the water molecule in human body is one of the sources of tissue contrasts in MRI, and utilized for disease diagnosis as well as functional mapping of the brain. While the magnetic environments and mobility of the water molecule which are thought to regulate the T_2 process are complicated *in vivo*, we recently found that the apparent transverse relaxation rate $R_2^\dagger$ (= $1/T_2^\dagger$) of tissue water in human brain is well described as a linear combination of the regional non-hemin iron concentration [Fe] and the macromolecular mass fraction f_M (= 1 – water fraction), $R_2^\dagger = \alpha[Fe] + \beta f_M + \gamma$, where α, β, and γ are coefficients[1]. Further, experimentally determined coefficients of α, β, and γ at 1.9, 3, 4.7 and 7T depended on the static field B_0 in unique ways (figure). Coefficient α showed a linear dependence on B_0, which is exactly the same as observed in solutions of the ferritin molecule. β appeared to depend on B_0 in a quadratic manner, suggesting mechanisms of chemical exchange and/or diffusion. γ was almost independent of B_0, suggesting the classical dipole-dipole mechanism.

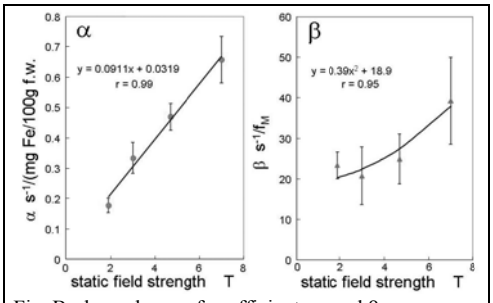

Fig. B_0 dependence of coefficients α and β.

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P307

Quantitative T_2 MRI study of HPMC based drug delivery systems

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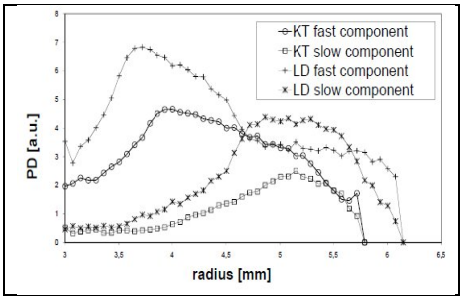
The application of MR Microscopy in the study of pharmaceutical systems has steadily evolved.[1,2] In the presented study, MRI time-resolved approach to measure spatial distribution of T_2 and Proton Density (PD) during hydration was used. Quantitative data analysis in terms of biexponential T_2 fitting was applied in order to distinguish regions with different hydration levels and water mobility. Pure HPMC tablets and HPMC tablets with addition of active substances, L-dopa and Ketoprofen (freely *vs*. poorly soluble), were tested. In all cases, two components (short and long) of T_2 decay were fitted. Significant differences between formulation with KT and LD were found. In the case of LD, hydrated area consists of several separated sub-areas (layers) with well-defined properties. In the case of KT, continuous spatial change of MR parameters was observed instead. Application of biexponential approach leads to much better understanding of the evolution of the swelling/hydrating tablet structure than previous monoexponential data fit.[3] Subpixel level heterogeneity is clearly visible allowing more accurate estimation of the hydration process dynamics. In combination with other pharmaceutical testing methods, obtained results may provide useful information in the preparation of a dosage form with certain properties.



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P308

Dendrimer-based SPIO nanoprobes for Magnetic Resonance Imaging

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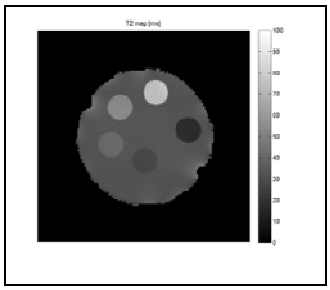
Dendrimers are a class of monodisperse hyperbranched and ordered polymers suitable for several biomedical and pharmaceuticals applications. They have been used as drug carries, vaccine adjuvants, gene delivery systems and imaging agents. Multiplicity of functional groups (amine or carboxyl) on their molecular surface allows to functionalize dendrimers efficiently with peptides, antibodies or chemical spacers. Great efforts have been made in order to develop new probes designed for Magnetic Resonance Imaging and for cellular magnetic labeling. Dendrimers seem to be good candidates for this latter purpose due to their good biocompatibility and low cytotoxicity. The aim of this study was to synthesize and characterize Dendrimers – based Superparamagnetic Iron Oxide nanoparticles (SPIO) probes for MRI. Magnetite (Fe_3O_4) nanoparticles core were prepared following Massart co-precipitation reaction, and polyamidoamine dendrimers (PAMAM) were grown on the core surface via divergent synthesis. Reaction cycles were repeated until generation fourth (G4) was reached. Dendrimers - based SPIO nanoparticles characterization was made by means of FTIR, potentiometric titration and MRI phantoms analysis. MRI data shown a decrease in relaxivity proportional to PAMAM generation, suggesting that the formation and growth of dendrimers branches influence SPIO relaxivity. Further studies are under way to substantiate preliminary data.



P309

Diffusion of Exchangeable Water in Novel Hardened Polymer/Cement Dispersions

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Reactive cement-in-polymer dispersions (p/c) consist of a water or alkali soluble polymer and non-hydrated cement. These newly developed materials are used as coatings for various systems of rovings, leading to the formation of highly resistant composites designed for demanding applications in the building industry. The formation of the composite matrix is triggered by hydrating the p/c dispersion; the contact with an aqueous environment leads to the solvation of the polymer and, subsequently, to the exposure of the cement particles. No information is yet available on the properties of the composite, such as porosity, transport times or the behavior of fluxes of matter, information known to be essential for predicting the materials behavior and resistance.

In the present study, the diffusion of water in several poly(vinylacetate)/cement dispersions has been monitored in real time using ^{1}H NMR spectroscopy and single point imaging. The diffusion phenomenon has been observed indirectly, by immersing the H_2O saturated samples into D_2O. The relaxation parameters of the water protons have allowed the acquisition of SPI profiles of the water in the composites with sufficient temporal resolution to monitor the H_2O/D_2O exchange process. The apparent diffusion coefficient has been determined for samples with different compositions at several temperatures using a 1D theoretical diffusion model.[1] The data were used to obtain information about the microstructure and to estimate the activation energy for the diffusion process.

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P310

Ultrafast velocity mapping in planar micro-structures

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NMR has proven to be a powerful tool for the characterization of flow in micro-fluidic setups like lab-on-a-chip mixers or reactors.[1-4] Such devices contain planar arrays of microscopic channels and chambers that need optimization to maximize throughput. So far, the full potential of NMR to monitor the flow pattern inside these structures has not been reached. Various obstacles, e.g. B_0-inhomogeneities generated by the micro-structures, B_1-inhomogeneities imposed by the use of designated surface rf coils and the high flow velocities in micro-devices, have prevented the implementation of ultra-fast velocity mapping techniques based on multi-echo generation.

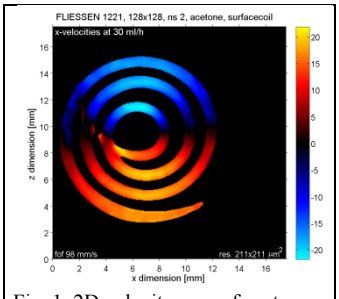

Fig. 1. 2D velocity map of acetone flow inside a spiral phantom.

In this work, we exploit the advantages of the FLIESSEN pulse sequence[5] (FLow Imaging Employing a Single Shot ENcoding). It is a combination of an ultrafast RARE-based acquisition with frequent updates of velocity-encoding and exhibits a high resilience to the above-mentioned inhomogeneity and high-velocity effects, while simultaneously allowing high spatial and temporal resolution. The performance of this technique is demonstrated on a phantom that bears all characteristics of a micro-device. Using a planar surface rf coil in combination with the FLIESSEN pulse sequence, high-fidelity 2D velocity maps were obtained within seconds (cf. Fig. 1).

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P311
Shear-thinning fluids in porous media with transverse permeability discontinuity

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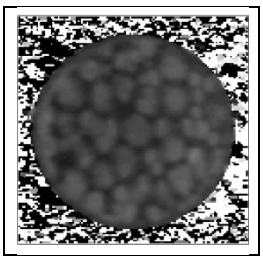
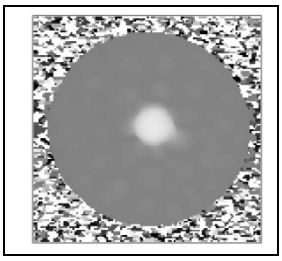
Flow of shear–thinning fluids was studied in a model porous medium with transverse permeability discontinuity by MRI velocimetry methods.[1] Fluids under study (0.25% and 0.5% Xanthan gum aqueous solutions) were also characterized





rheologically. A model porous medium was reticulated polyurethane foam with 5 pores per inch (PPI) structure. The transverse permeability discontinuity was produced by cutting a cylindrical channel (diameter of the channel was the length of the average pore) in a bulk porous medium that is coaxial with the main axis of the bulk sample. MRI velocimetry experiments were performed at different bed thickness of the porous medium, with different fluid composition and flow rate. We found out that the obtained axial velocity profiles differ drastically from the profiles expected for Newtonian fluids. The power law behaviour was apparent in the open channel while the porous medium was characterized by the preferential flow through tortuous channels formed by the structure of the porous medium. A velocity profile originated from an individual flow channel differed form the major channel flow. Interfacial velocities were increased with the increase of the bulk flow rate for both fluids. Data were processed with Prospa 2.1 (Magritek, New Zealand). Obtained velocity fields were of very satisfactory quality thus providing a good experimental avenue for refining present theoretical models for power-law fluid flow behaviour in porous media where a discontinuity in permeability is present.

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Acknowledgments: Alan Raudseep, VUW, NZ.

P312
Slow flow in natural porous media monitored by MRI

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Besides the gas phase, water is the universal transport medium for nutrients and contaminants in soils. The corresponding flow processes are characterised by slow flow velocities and sensitivity for external disturbances. Therefore MRI in combination with tracer is very convenient for non-invasive monitoring. Necessary is a tracer which behaves conservatively, e.g. it should not interact with the solid matrix and a good contrast should be achievable. For these reasons we have chosen the chemically stable complex Gd-DTPA containing the strong paramagnetic Gd^{3+} ion. In a preliminary study the relation between signal intensity, tracer concentration, repetition time t_R und echo time t_E was investigated. For the following experiments we applied a spin echo multi slice sequence with strong T_1-weighting (e.g.: t_R = 0.2s, t_E = 4.8 ms, 1.3 × 1.3 × 2 mm^3 voxels).

Measurements on the flow of Gd-DTPA in natural porous media were performed under two different boundary conditions: Gravitationally and evaporation driven flow. In the first case a model column consisting of an inner highly conducting core of medium sand surrounding by a less conductive out core of silt was irrigated from top under steady state conditions. In doing so the tracer plume moved homogenously only through the inner core. This behaviour was validated by soil physical simulations based on the basic parameters: structure, density, water characteristic, and hydraulic conductivity. The second set-up for gravitational flow was a natural soil column of a sandy loam also irrigated under steady state conditions. The short relaxation in this system required the further reduction of t_R = 0.05 s and t_E = 1.9 ms. In contrast to the model column the flow behaviour is more complicated. First of all the plume moved along defined pathways. Local flow velocities are higher than the average flow velocity which is characteristic for preferential flow phenomena. The third set-up is a continuation of the first one, i. e. the infiltration of the tracer plume is stopped after a certain distance and the following upward flow, which is now driven by evaporation from the surface, is monitored. The previous assumptions about internal redistribution of tracer near the surface and deeper in the porous system, as implemented in previous soil physical simulation, are validated by MRI, which allows a non-invasive look inside the "black box".

P313

In vivo and *in vitro* NMR-based metabolomics for studying clioquinol treatment effects on brain metabolism in Alzheimer's disease mouse models

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Alzheimer's disease (AD) is one of the most important causes of progressive dementia in the elderly, characterized by a slow and progressive impairment of cognitive functions. Nowadays, there is neither curative medicine, nor effective and valid animal model for the evaluation of new medicines against AD. It could thus be important to discover efficient diagnostic tools allowing revealing the disease before the first signs of cognitive decline. Metabolomic offers a global approach for describing metabolic changes. Metabolomic analysis consists in identification and as much as possible quantitation of the largest set of metabolites.

The goal of this study was to detect global metabolic perturbations related to the development of AD with two NMR approaches: (i) *in vivo* localized Magnetic Resonance Imaging (MRI) and ^{1}H Magnetic Resonance Spectroscopy (MRS) and (ii) *in vitro* ^{1}H Nuclear Magnetic Resonance (NMR). The metabolomic profiles of hippocampus in 3 months-old AD (AppSwe Tg2576) and healthy mice were compared after two months of treatment with a solution of clioquinol or with the vehicle alone. The hippocampus was first analyzed with MRS and then, after mouse sacrifice, it was extracted and analyzed by ^{1}H NMR. Both *in vivo* and *in vitro* data were treated with univariate and multivariate statistical approaches using surpervised or unsupervised methods.

This study enabled the comparison of cerebral metabolites detected by *in vivo* and *in vitro* NMR for both treated and untreated mice. The metabolic changes observed were similar with both techniques. Moreover, clioquinol induced metabolic changes in transgenic mice only whereas vehicle alone had no effect.

P314 (*)

A Robust Protocol for Diffusion-Weighted Functional MRI on Rodents at 7 Tesla

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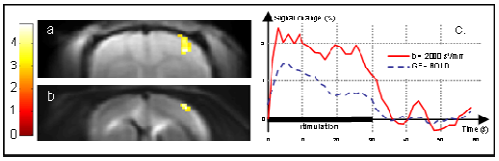
A small decrease in the water diffusion coefficient during cortical activation has been previously reported in humans using diffusion functional MRI (DfMRI)[1]. It has been suggested that DfMRI is a *direct* marker of neuronal activation through changes in tissue structure (i.e. cell swelling). Hence, this method could improve the temporal and spatial resolution of fMRI as compared to the *indirect* conventional blood oxygen level-dependent (BOLD) method. Using DfMRI in small animals is challenging; the only small animal DfMRI studies published to date have been done on cats[2]. In this work, we establish a robust protocol for observing the DfMRI activation in rats at 7T (Pharmascan, Bruker). In this protocol, we use medetomidine anesthesia through intravenous injection. Being an alpha-2-adrenoreceptor agonist with dose-dependant effect, medetomidine interacts less with neurotransmission and causes less hemodynamic instability. We first inject a bolus of 110 µg/kg, wait for 20 minutes, then proceed to administer a continuous infusion of medetomidine. Within a 2-hour period, the injection rate is gradually increased from 100 µg/kg/h to 300 µg/kg/h. We determined that these values ensure the optimal activation, both in strength and duration. In our experiment, we alternate between a GE EPI sequence for BOLD response and a DW-SE EPI sequence for DfMRI response. The parameters used are [TE=10 ms, TR=1500 ms] for BOLD and [TE=24ms, TR=1500 ms, ∂=2.5ms, Δ=12.5 ms, b=2000 s/mm^2] for the DfMRI, respectively. Forepaw stimulation is performed in a paradigm consisting of 5 blocks (30s rest, 30s activation), with 10 minutes rest between sessions to prevent habituation. Typical activation maps for BOLD and DfMRI are shown in Figures a and b, respectively. The corresponding time courses are displayed in Figure **c**. Following stimulation, we observe a significant change in the DfMRI signal (2%, p=0.005), with good spatial localization of the activation.



In conclusion, we present a robust DfMRI protocol for rodents at 7 T.

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P315

MRI Thermometry Based on Encapsulated Hyperpolarized Xenon

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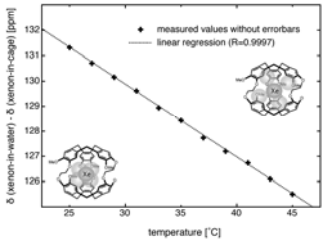
Noninvasive, accurate and spatially resolved temperature measurement in the human body is a desirable technology for many biomedical applications, including the monitoring of hyperthermic treatment of cancer and the detection of vulnerable atherosclerotic plaques. We demonstrate a new approach to MRI thermometry using encapsulated hyperpolarized xenon. The method is based on the temperature dependent chemical shift of hyperpolarized xenon in a cryptophane-A cage. The shift is linear with a slope of 0.29 ppm/°C (see figure) which is perceptibly higher than the shift of the proton resonance frequency of water (ca. 0.01 ppm/°C) that is currently used for MRI thermometry. Using spectroscopic imaging techniques, we collected temperature maps of a phantom sample that could discriminate by direct NMR detection between temperature differences of 0.1 °C at a sensor concentration of 150 µM. Alternatively, the xenon-in-cage chemical shift was determined by indirect detection using saturation techniques[1] (Hyper-CEST) that allow detection of nanomolar agent concentrations.



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P316

13-interval stimulated echo multi slice imaging for flow investigations on quartz sand

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In the context of climate change and linked phenomena like stronger varying weather conditions (draught, strong rain) the understanding of root water uptake in soils is very important for securing nutrition. One step to learn how root water uptake occurs is to study the water flow in soil towards plant roots.

Magnetic Resonance Imaging (MRI) is potentially the most powerful analytical tool for non-invasive three dimensional visualization of flow and transport in porous media.[1] Local velocity in porous media has been measured so far by combining phase encoding of the velocity with fast imaging methods,[2-4] where flow velocities in the vascular bundles of plant stems were investigated. Since their cells impose almost no limitation to flow, their MR signal is hardly inferred by internal field gradients. The situation in the surrounding soil, a natural porous medium, is different, since there internal magnetic field gradients are not negligible.

In this work we account for the existence of these gradients by employing bipolar pulsed field magnetic gradients[5] for velocity encoding. This method opens the possibility to study flow through sand (as a model system for soil) at flow rates relevant for the water uptake of plant roots.

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P317

Determination of hydraulic properties of model soil column using combined magnetic resonance imaging and multi-step-outflow experiments

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Knowledge of the hydraulic properties is essential for all simulation studies and the prediction of water and solute flow in the vadose zone. In general, there is a wide range of measurement techniques available for the estimation of soil hydraulic parameters. Unfortunately, all known setups do not count for the heterogeneity which might be included in the observation sample. Soils contain large heterogeneities in terms of refilled earth worm borrows, root channels, and/or inclusions of different material.

In this study, we aim to accurately and reliably determine soil hydraulic properties of a strongly heterogeneous soil sample by combining a classical multi-step-outflow (MSO) experiment with magnetic resonance imaging (MRI). A laboratory MSO experiments was performed on a model coaxial sample filled with sand and sand-clay mixture. MRI images at 4.7T (200 MHz) were recorded during each pressure step, to provide information about the soil water distribution at specific locations within the soil sample, using a pure phase-encoding MRI sequence which ensured the desired linearity between signal amplitude and water content at different pressures, e.g. various water saturations. The recorded cumulative outflow and water content data were used as input parameters in the inversion. For the inversion the hydrological model HYDRUS-2D was coupled with a global-optimization algorithm, namely the shuffled complex evolution (SCE-UA) algorithm.

The results show conclusively that the combination of the two MRI and MSO methods leads to a unique estimation of the retention and hydraulic conductivity functions of two materials simultaneously. These results could have applications in understanding the hydraulic behavior of heterogeneous agricultural soils, clay or lignite imbedded soils, and forest-reclaimed mine soils.

P318

Progress in CW & Time Domain Functional EPR Imaging: Recent applications In Tumor Oximetry and correlation with MRI

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We have been developing CW and pulsed EPR imaging methods at 300 MHz for application in small animal functional imaging research.[1] Our main thrust is the development of rapid FT and CW EPR imaging in mice models of tumor to non-invasively and quantitatively evaluate the perfusion of spin probes and spatially resolved tissue oxygenation based on the trityl radical analogs. In the time-domain we use the Single Point Imaging (SPI) strategy that involves global phase encoding (in all dimensions) and Fourier reconstruction leading excellent resolution and efficient band-width coverage compared to frequency encoding. Using interleaved multi-gradient SPI we are able to generate excellent spin distribution images, and oxygen maps derived from spatially resolved oxygen-dependent relaxation with a resolution of ± 2 mm of Hg. Mice tumor images take about 5 minutes using the trityl, Oxo63. We have also devised means of co-registering functional MRI and EPR oximetry at 300 MHz without the need to move the subject from the resonator aiding us to look at spin perfusion, oxygen distribution from EPRI and correlation of the same with respect to MRI T_2-weigheted, blood-volume, and diffusion images.[2] We are also examining the effect of anti-angiogenic tumor drugs as well as the effect of radiation via the tumor oximetry and MR profiles. For addressing in vivo redox-sensitive spin probes with large line widths we have also developed fast CW imaging using Rapid Scan combined with rotating gradients for spectroscopic imaging. Recent developments in combining the SPI and Spin Echo modalities promise excellent resolution in spin and oximetric images.

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P319
Cellular PTMs states of p53

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Post-translational protein modifications (PTMs) strongly modulate the activity of the human oncoprotein p53 [1]. More than 30 PTMs have been reported for p53, among which 10 Serine/Threonine phosphorylation sites have been identified in the N-terminus, intrinsically disordered transactivation domain (TAD). In addition, multiple Lysine acetylation reactions have been mapped to the C-terminal, disordered region of the protein. Here, we employ high-resolution NMR spectroscopy to study those PTM events *in vitro* and *in vivo*.

Our results indicate that N-terminal protein phosphorylation occurs in a region of prominent Proline *cis/trans* isomerization. Cellular PTM states of p53 were markedly different depending on whether isolated protein fragments, or full-length (FL) p53 constructs were studied. Thus indicating that cross talk between the N- and C-termini may 'steer' the establishment of these PTMs. The fact that different modification patterns in different cancer cell extracts were observed could path the way for future in-cell NMR applications to identify cancer types, or to annotate stages of cancer progression.

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P320
Nanostructured Nanoparticulate Inorganic Contrast Agents for Medical Imaging

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Medical diagnostics, such as magnetic resonance imaging (MRI), are routinely conducted with the administration of image enhancing contrast agents. The two known types of contrast agents are, T1 and T2 contrast agents which are classified based on their mechanism of providing improved relaxation to proton spin states. T1 agents have traditionally consisted of a polydentate ligand, chelated to a paramagnetic centre. In recent years the research focus has shifted towards the use of nanoparticulate (NP) contrast agents. NP agents offer improvements such as higher loading, improved water exchange, slower molecular rotation and surface functionalisation.[1, 2] All of which contribute to an improved proton relaxivity.

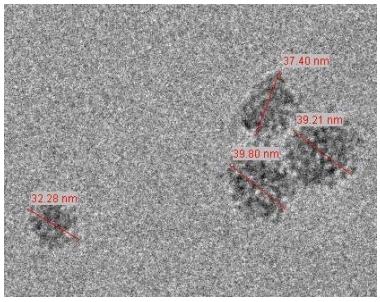

Figure 1: Cyro-TEM image of porous silica particles

In this work, porous colloidal silica with varying amounts of gadolinium doping, pore size, shape and pore hierarchy were prepared via various acid-catalysed hydrolysis-condensation approaches, with the inclusion of templating agents to generate the required porosity. In our findings, the porous gadolinium silicates produced have a high surface area, of up to 900 m^2g^{-1} and pores sizes between 2–5 nm in diameter (Figure 1). The gadolinium loading varied from 1 wt% to 10 wt% with respect to silica. Structural features of the samples are correlated to relaxivity measurements to determine which physical properties of NPs are most influential on the relaxivity of inorganic contrast agents.

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P321

MRI of water transport in (woody) plants: the short and the long

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Hydraulic conductivity of axial and radial water transport on different length scales provide key information to validate eco-biophysical plant models for evaporation. Such models are of importance from ecophysiological and environmental point of view. Flow in (woody) plants can be described by Darcy's law. However, there are many violations to Darcy's law when applied to living (woody) plants and key aspects of the relationships between pressure, flow / flow conducting area and (exchange with) water storage pools are not well understood.

New intact plant MRI methods are now available to study water dynamics on different length and time scales, covering sub-cellular, cell-to-cell and long distance transport.[1-3] In this way flow characteristics like velocity, volume flow and flow conducting area can be measured per pixel, even in pixels that contain flowing and non-flowing fluid. 2D correlated propagator-T_2 MRI[4] results in information about the relation between flow and pore or vessel size. The flow driving force, the plant water potential is obtained from quantitative water content images.[2] Propagator measurements can also be applied to quantify exchange between the stagnant (cell water) and flowing water pools, even in the presence of susceptibility differences and differences in relaxation times between exchanging flowing and stagnant water pools. Diffusion measurements on the long time limit gives access to cell-to-cell transport or tissue permeability.[3]

An overview will be presented of such methods as applied to study xylem and phloem hydraulics and dynamics, xylem air embolisms as a function of water potential and the interplay with storage pools during e.g. drought stress.

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P322

Towards ^{1}H and Hyperpolarized ^{13}C Metabolic Imaging at 14T: Building blocks of a capable High Field NMR spectrometer

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Metabolic imaging of biomarkers in preclinical animal models and human tissues at high field has benefited greatly from recent advancements in multiple components of the NMR spectrometer. Significant improvements and development of state of the art hardware such as ^{1}H /^{13}C quadrature probes and detectors, vertical bore animal position systems, plus the implementation of high performance gradients (fast switching, high strength) and strong homogeneity corrections (2nd and 3rd order shimming) have allowed for dramatically increased spatial resolution (anatomic imaging), high-resolution Dynamic Contrast Enhanced imaging (DCE) and multi-direction Diffusion Weighted Imaging (DWI), as well as kinetic analysis using DNP sensitivity enhanced ^{13}C metabolic imaging.[1] Additionally, software developments in the areas of ultra fast echo-planar sequences (3D EPI), including efficient large bandwidth pulses, selective data collection, and reconstruction algorithms have further advanced these system hardware improvements.

Here we demonstrate a fusion of the necessary hardware and software to overcome the limitations of high-field spectroscopic imaging and accomplish dynamic hyperpolarized metabolic imaging.

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P323 (*)

Microfluidic Gas-Flow Profiling Using Combined Parahydrogen-Induced Polarization and Remote Detection MRI Techniques

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NMR has several advantages as compared with conventionally employed optical methods in microfluidic flow profiling. It does not require the use of markers and allows versatile experiments providing dynamic and spectroscopic information. However, NMR measurements using a large coil around the microfluidic device are very challenging because of low sensitivity resulting from the low filling factor of the coil and low sensitivity of large coils. The issue is even worse when gases are investigated, and thermally polarized compound are used. Here we demonstrate that the substantial sensitivity enhancement (10^4 – 10^5) provided by combining parahydrogen-induced polarization (PHIP) and remote detection (RD) NMR technique enables gas-flow visualization in microfluidic devices. Hyperpolarized propane obtained in heterogeneous hydrogenation reaction[1] was utilized in the experiments. The PHIP RD MRI experiments turned out to be one to two orders of magnitude more sensitive than the corresponding RD MRI experiments performed using hyperpolarized xenon, leading to the dramatically shortened experimental times. In addition, the developed technique allows one to perform scientifically and technologically more fascinating studies, because parahydrogen can naturally take part in many important chemical reactions, including those performed with the use of microfluidic devices.

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7.3 Solid State and Materials/Quadrupolar Nuclei

Posters

P324

Chemical shift prediction: Helping solid-state NMR, from protein expression to data analysis

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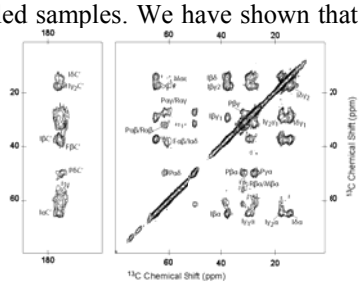
Among the major problems in determining the 3D structure of membrane proteins by solid-state NMR is their overexpression and the analysis of the carbon-carbon correlations from uniformly labelled samples. We have shown that selective labelling of the membrane protein MscL, using cell-free expression, represents a crucial help in improving data sets obtained by NMR in the solid-state.[1] Since the membrane protein MscL is highly hydrophobic, many of its amino acid chemical shifts are overlapped. We therefore need a strategy to choose the specific labelling patterns that will produce better-resolved spectra.[2] Based on the specific algorithm SPARTA,[3] we have grouped the predicted chemical shifts according to the nature of the amino acid. We have combined this approach with comparative modelling,[4-6] to evaluate the chemical shift distribution of amino acids along the protein sequence. We show that this strategy helps us optimize the labelling scheme of our protein, but also the identification and assignment of amino acids.



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P325

Characterization of Cellulose-Silica Hybrid Materials by Advanced Solid-State NMR Techniques

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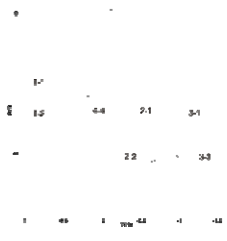
Organic-inorganic hybrids are known for their interesting properties, such as mechanical stability, optical and thermal behaviour.[1] It is possible to use natural cellulose as organic component. For silicon-based inorganic components, the sol-gel process is often chosen as synthetic method,[2] using different mixtures of silanes. A major issue during the study of such hybrid systems concerns the understanding of the interaction between cellulose and the silica network. In the present work, cellulose-silica hybrids were prepared using tetraethoxysilane $(EtO)_4Si$ mixed up with different types of monosubstituted triethoxysilanes. Apart from ^{13}C and ^{29}Si CP/MAS NMR experiments, heteronuclear correlation studies, e.g. ^{1}H-^{13}C Lee-Goldberg heteronuclear correlation (LG-HETCOR) experiments, and ^{13}C-^{29}Si double resonance NMR experiments on ^{13}C labelled samples were performed. In combination with double quantum filtered back-to-back (BABA) correlation spectra (figure) and ^{17}O double-quantum MAS spectra (MQMAS), the results provide new insights into the molecular composition and interaction at the inorganic-organic interface.



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P326
^{195}Pt Solid State NMR of Platinum(II) Dialkyldithiophosphates

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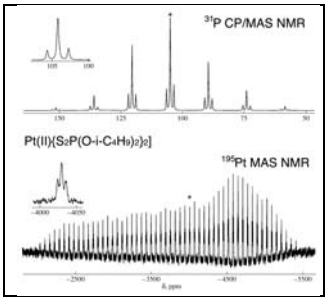
In our previous studies on natural crystalline mineral cooperite (PtS) we employed ^{195}Pt solid-state (both MAS and static) NMR to characterize the structural state of platinum(II) in square-planar chromophores [PtS_4].[1] As attractive models for this mineral and for a variety of structural states of Pt(II) observed in different materials, such as Pt-Pd catalysts based on nanoparticles with surface defects, we further studied the structures of selected platinum(II) *O,O'*-dialkyldithiophosphate complexes.[2,3] In the presented work we summarize a large set of data (^{31}P and ^{195}Pt CSA, $^{2}J(^{195}Pt\text{-}^{31}P)$) obtained from a variety of ss-NMR experiments (^{31}P MAS, high resolution ^{195}Pt MAS and static NMR), SIMPSON simulations and CASTEP calculations for Pt(II)-DTP complexes with different alkyl groups: ethyl, *iso*-propyl, *iso*-butyl, *sec*-butyl and *cyclo*-hexyl.



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Solid state NMR of Silks

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Common silk is obtained from the domestic silkworm *Bombyx mori*, but many other silkworms and spiders produce silk protein fibers with wide varieties of primary structure and chemical/physical properties. The dragline silk from the Japanese spider *Nephila clavata* has a tensile strength approximately 5 times greater than that from the wild silkworm *Samia cynthia ricini,* although both silks are block copolymers of polyalanine and glycine-rich regions.[1] Here we show, using solid-state NMR and X-ray diffraction, that the two polyalanine regions have different packing arrangements, arising from the different lengths of the polyalanine regions: $Ala_{5\text{-}6}$ for *N. clavata* and $Ala_{12\text{-}13}$ for *S. c. ricini* although both polyalanine regions are anti-parallel β-sheet structure. This result opens the possibility for rationally designing silk fibroins of different tensile strength.[2,3]

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P328

"Solid State NMR/GIPAW/VASP" Method: The Study Case of Pure and Carbonated Hydroxyapatite Structures

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Apatitic structures based on the $Ca_{10}(PO_4)_6(OH)_2$ formula are of prime importance in biology, as apatite is the main mineral phase of mammal bones and teeth. In general, the detailed structure of apatite is highly complex as apatitic structures can adapt very easily a large variety of anionic (CO_3^{2-}) and/or cationic substitutions (Na^+, Sr^{2+}…).

In this communication, we show that ultra fast MAS ^{1}H experiments (up to 67 kHz) at high fields leads to ultimate resolution in the apatitic structures, though the OH line can be considered as inhomogeneously broadened by the homonuclear dipolar coupling (in the Maricq-Waugh sense).

VASP models of hexagonal HAp structures, including various geometries for the hydroxyl groups were used as starting points for GIPAW *ab initio* calculations of CSA and quadrupolar parameters for ^{1}H, ^{31}P, ^{17}O and ^{43}Ca nuclei. We show that the GIPAW calculated values are in excellent agreement with experimental and that the ^{1}H calculated chemical shifts are sensitive to local geometrical characteristics. These results are highly promising as powder XRD is usually insensitive to protons location. H-relaxed XRD structures have been shown also to be suitable for GIPAW calculations, whereas non-relaxed structures led to unrealistic ^{1}H isotropic chemical shifts. We demonstrate also that in the case of "isolated" OH groups (as in HAp), the established correlation between $\delta_{iso.}(^1H)$ and hydrogen bonding should be closely reinvestigated.

Moreover, the observed distributions have been clearly identified at high field (700 MHz) using 2D double and triple resonance CP MAS experiments and fully enriched $^{13}CO_3$ substituted apatites (^{1}H-^{31}P-^{13}C and ^{1}H-^{13}C-^{31}P).
We strongly believe that the "NMR/GIPAW/VASP" method can be easily extended to a large variety of anionic and cationic substitutions in apatitic structures, leading to original insight into their intrinsic complexity.

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Investigations of the liposome/silica interface through advanced CP MAS experiments combined to DFT modeling and first principles calculations

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In this contribution, we present the solid state NMR study of an original nanocomposite combining liposome and silica where the lipid bilayer is covered by a silica shell that finds its application in the administration of highly hydrophobic drug.[1] The characterization of such hybrid materials is fundamental but also very challenging in reason of the complex chemical nature of the sample. Solid state NMR spectroscopy appeared as a well suited technique as it can probe independently the mineral and the organic part, as well as the interaction between these two. In particular, the silica/liposome interface is studied by ^{1}H-{X-^{1}H} Double CP experiments (where X = ^{29}Si or ^{31}P) that consists in two consecutive CP transfers, from ^{1}H to X and then back to ^{1}H. The second transfer, from X to ^{1}H, allows the investigation of X-^{1}H proximities by varying the corresponding contact time $t_{CP}2$ and allows saving considerable experimental time when compared to conventional 2D HETCOR. The results show unambiguously a close proximity between the silica and the phosphatidyl choline (PC) headgroup of the lipid and evidence the presence of silanols (Si-OH) and water that ensure the stability of the material through hydrogen bonding network. Furthermore, DFT modeling allows us to describe the adsorption of a PC headgroup on an amorphous silica surface confirming the presence of interfacial water. This result combined to GIPAW NMR parameters calculations gives a precise assignment of the ^{1}H experimental resonances and allows us to understand the local dynamic of the adsorbed lipids through the confrontation of experimental and calculated ^{31}P CSA parameters. This multiple technique approach provides fundamental information on the interaction of the liposome with the silica shell evidencing the crucial role of interfaced water through the H-bond network.

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P330

Structural quality of 3D protein structures determined by MAS solid-state NMR spectroscopy

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During the last decade, solid-state NMR (ssNMR) spectroscopy has grown into a prime instrument in structural biology. Solid-state NMR can now rely on a complete toolbox that allows approaching not only high-resolution structural information, but also internal dynamics of immobilized, insoluble proteins, such as fibrils and membrane proteins. Since 2002, when the first structure of a protein was determined by Magic-Angle Spinning (MAS) ssNMR[1], more than twenty other structures have been deposited in the Protein Data Bank (PDB). Still, interpretation of solid-state NMR data is often complicated by a lower spectral resolution (compared to solution NMR) and by the difficulty to measure precise inter-atomic distances.

To investigate the *bona fide* aptitude of solid-state NMR for high-resolution structure determination, we performed a survey of the actual quality of 3D protein structures solved by MAS ssNMR. Using well-established measures of structural quality, various quality scores were evaluated and compared, not only to common standards (often related to X-ray crystallography data), but also to the range of value observed in solution NMR structures. As judged by the constant improvement in quality over the different generations of structures, our results clearly established that MAS solid-state NMR now produces true high-quality structures and competes well with solution NMR in terms of overall structural quality. Moreover, through two examples, we will show how a better application of structure calculation methods, initially developed for solution NMR, could allow to drastically enhance the quality of protein structures determined from MAS solid-state NMR restraints.

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P331

Experimental and Simulated ^{11}B MAS Spin-Echo Dephasing for Lithium Diborate

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The feasibility of *J* homonuclear correlation experiments for the specific case of the half-integer quadrupolar nucleus, ^{11}B (I = 3/2), in the model crystalline compound, lithium diborate, $Li_2O.2B_2O_3$ is considered. At natural abundance, 80% of boron nuclei are ^{11}B, with the remainder (20%) being ^{10}B. In this study, the effect of ^{11}B-^{11}B dipolar couplings on ^{11}B spin-echo dephasing was investigated using samples with three different degrees of ^{11}B depletion/enrichment: 5%, 25% and 100%. The effect of quadrupolar coupling strength was also investigated as lithium diborate contains one high-C_Q site (2.5 MHz) and one low-C_Q site (0.5 MHz). Spin-echo dephasing curves were recorded using MAS rates between 5 kHz and 20 kHz. The experimental results were complemented by density-matrix simulations and first-principles calculations of the $^2J_{BB}$ couplings.

The ^{11}B spin-echo dephasing was seen to be faster for the low-C_Q site, a quadrupolar-based effect reproduced by simulation. Increasing the spinning speed prolonged the dephasing time for the high-C_Q site more than for the low-C_Q site. This effect was not reproduced in the two-spin simulations as multiple noncommuting homonuclear dipolar couplings were not accurately represented. The removal of such couplings by isotopically depleting ^{11}B caused much longer dephasing times for both sites. Despite the longer dephasing duration, a *J*-coupling modulation of the spin-echo was not detected, setting an upper bound for the $^2J_{BB}$ couplings in lithium diborate. This result is confirmed by first-principle calculations using the CASTEP code.

P332
NMR of nanoclusters in relaxors

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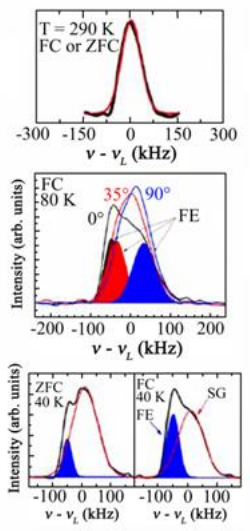
Relaxors represent one of the great unsolved problems of solid state physics. They are characterised by the appearance of polar nanoclusters embedded in a neutral matrix. The system is characterized by random bonds and random fields and a broad frequency distribution. The nanoclusters are at high temperatures randomly oriented. Their symmetry is lower than the macroscopic symmetry of the crystal. Here we show that the nanoclusters are dynamic entities which gradually freeze-out as the temperature decreases. The Pb^{207} NMR spectrum above 290 K is isotropic and of a Gaussian lineshape.[1] Two- dimensional (2D) separation of interactions experiments show that the spectra are frequency distributions and are composed of a large number of individual Pb^{207} lines with different chemical shifts. Below 209 K an anisotropic Pb^{207} line suddenly appears in addition to the isotropic one in the field cooled (FC) as well as zero field cooled (ZFC) spectra. Its angular dependence in the external magnetic field follows the $(3\cos^2\vartheta - 1)$ law. Here ϑ determines the orientation of the eigenframe of the chemical shift tensor $\underline{\sigma}$ with respect to the crystal fixed frame. The anisotropic line requires a shift of the Pb ions in a given preferred directions, i.e., [111]. The slow anisotropic component sees the instantaneous polar cluster distribution, whereas the fast isotropic component sees the time averaged distribution and represents the neutral matrix. The dynamic nature of both the isotropic and the anisotropic polar nanoclusters is also seen by the spin-spin relaxation (T_2) measurements.



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P333
Dynamics of gel and coagel phases of ascorbic acid derivatives by means of ^{2}H and ^{13}C NMR

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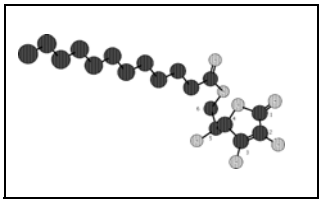
Ascorbic acid amphiphilic alkanoates (ASCn) combine the extraordinary radical-scavenging properties of ascorbic acid (Vitamin C) with the possibility of forming a rich variety of supramolecular assemblies in water, which can represent an ideal environment for the solubilization of hydrophobic and sensitive drugs.[1] Depending on alkanoate chain length (n), amount of water and temperature, ASCn can form liquid micellar solutions, gel and coagel, whose properties have been investigated by means of several techniques but are still not fully understood. Here we present an NMR study of the gel and coagel phases of ASC12 in water, mainly aimed at investigating the structural and dynamic properties of water and alkylic chains in the two phases, whose understanding could also help in clarifying the mechanims of the phase transitions. The NMR study was based on the analysis of ^{2}H and ^{13}C spectra, acquired in the gel and coagel phases of ASC12 in D_2O. Two different epimers, D-ASC12 and L-ASC12, were studied, in order to also clarify previously observed different behaviours related to the different ascorbic acid headgroup chirality.[2] ^{2}H-NMR spectra unambiguously showed the completely different dynamic properties of water in the two phases, while from ^{13}C spectra it was evident that the phase transition is also associated with a strong modification of the dynamic properties of the alkylic chains of ASC12. Some differences were observed between D- and L-ASC12, which were related to possible different conformational features and hydrogen-bond interactions.



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P334

Preliminary studies of the dynamics of an elastin mimetic peptide, (VPGVG)3 by deuterium NMR spectroscopy

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Deuteron NMR spectroscopy is a well known experimental method for characterizing dynamics in a broad range of solid and semi-solid systems. The T_1, T_2 and T_{1Q} relaxation times yield information about the correlation times and can be used to identify separate components and processes. The line shapes of one-dimensional quadrupolar echo and alignment echo together provide a sensitive probe for measuring molecular motions with correlation times in range of 10^{-8}s to 100s.[1] The polypentapeptide $(VPGVG)_n$ serves as a useful model for characterizing structure and dynamics of elastin, a major protein constitute of connective tissues.[2,3] In this poster, we report on a preliminary experimental study of a deuterated $(VPGVG)_3$ peptide by deuteron NMR spectroscopy. For hydrated and dry samples, we have successfully measured the correlation times for the H^{α} on the Glycines as a function of temperature. Using available simulation tools, we obtained the quadrupolar coupling coefficient $\delta \approx 120$kHz as well as other parameters in the two-site rotational model[4]. Experiments of this and other deuterated peptides are ongoing and will be used for providing a direct measurement of the dynamics occurring in this system.

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P335

Recognition of multiple CH···π interactions in high melting macromolecular adducts by Solid State NMR

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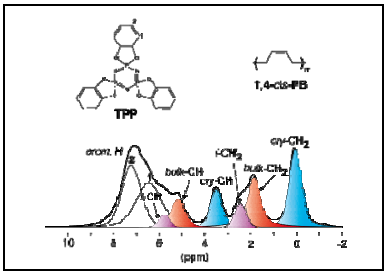
High melting macromolecular adducts self-assembled by solvent-free mechanochemical or thermal treatments of a crystalline host and few polymers are stabilized by cooperative CH···π interactions, as demonstrated by ^{1}H MAS and 2D solid state NMR. Quantitative ^{1}H MAS NMR spectra recorded at 600 MHz with 35 kHz spinning speed revealed important features of the nanostructured materials. For example, the remarkable resolution allowed us to achieve the simultaneous identification of the aromatic host (TPP) and the crystalline, intercrystalline and amorphous phases to which the polymer (1,4-*cis*-PB) belongs. The hydrogen nuclei of the polymer chains confined to the crystalline nanochannels resonate notably upfield by 1.8 ppm compared to the bulk polymer hydrogens. This large shift derives from the diamagnetic susceptibility generated by the aromatic rings of the host facing the polymer chains in the nanostructured crystalline adduct. 2D PMLG HETCOR NMR experiments designed for high resolution both in hydrogen and heteronuclear domains (^{13}C, ^{31}P) were applied successfully for the description of host-guest systems. This multinuclear approach allows a detailed description of the role of weak interactions cooperating to fabricate polymer nanostrucured materials that exhibit exceptional thermal stability.



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P336

Transport properties of New Membrane Materials for PEM Fuel Cells Investigated by PFG and Electrophoretic NMR

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In polymer electrolyte fuel cells (PEM-FC), mainly perfluorosulfonic polymers such as Nafion are used as membrane materials. Current attempts to increase the operation temperature of PEM fuel cells are also driving the development of membranes with high proton conductivity and stability at high temperature and low humidification.[1] Sulfonated poly(phenylene) ionomers have been showing to be an important advance in this direction thanks to their high proton conductivity and stability under low relative humidities and high temperature conditions.[2, 3]

The high degree of sulfonation of these polymers leads to the development of a microstructure characterized by very narrow hydrated, hydrophilic domains which are well connected on longer scales resulting in low water transport coefficients, water diffusion and also electroosmotic drag. Under PEM-FC conditions, the protonic current through the membrane produces an electroosmotic water current in the same direction that leads to a depletion of water at the anode, resulting in an increased membrane resistance and consequently reduced fuel cell performance.[4,5] It is therefore important to know the magnitude of the electroosmotic drag in order to optimize the water management in the membrane. Electrophoretic-NMR has been applied to measure electroosmotic drag (Kdrag), in polymer electrolyte membranes. The method and the measurements of Kdrag as a function of water content are going to be presented for Nafion 117 (state-of-the-art polymer) and for the sulfonated poly-sulfones.

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P337

Revealing and Quantifying Defects in the Cationic Ordering of Mg/Al Layered Double Hydroxides

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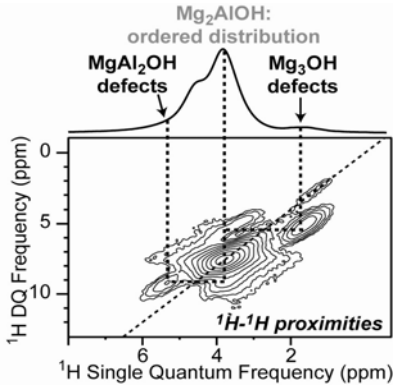
Cationic ordering is believed to have crucial effects on many of the physico-chemical properties that make layered double hydroxides (LDHs) materials of considerable interests as host structures for drug delivery systems, nanocomposite materials, or for catalysis. Here we first unambiguously confirm that solid-state ^{1}H NMR at ultra-fast (60-65 kHz) magic-angle-spinning (MAS) frequencies can be used to distinguish and quantify ordered environments and Mg-clustering in the layers, as very recently proposed.[1] We demonstrate by combining series of solid-state ^{1}H and ^{27}Al one- and two-dimensional NMR measurements with DFT calculations that although globally ordered, the cationic distributions in Mg/Al LDHs nevertheless contain small amounts of Al clustering. The corresponding small amounts of Al atoms misplaced with respect to the perfect ordered cationic distributions are quantified and showed to counterbalance well the number of misplaced Mg atoms for Mg to Al ratios of 2, and to rapidly disappear with decreasing Al contents. This establishes that, although not favoured, Al-Al close contacts are not excluded in LDHs materials, a finding that will strongly impact our vision of the local acidity of these materials and their widely exploited anion exchange and reconstruction properties.



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P338

Protection of cellulose-based material via the sol-gel route: Characterization by solid-state NMR spectroscopy

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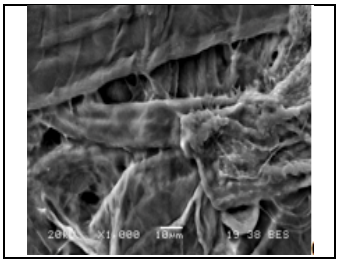
Organic materials based on cellulose, such as paper and wood, undergo deterioration due to several factors.[1] It is thus important to develop coating materials that increase the durability of the substrate without affecting its physical features. The deposition of alkoxysilane-derived sols, obtained through the sol-gel route seems promising to fulfil all the requirements.[2] We use a hydrophobic inorganic layer, both to reduce the humidity that attracts fungi and to limit fire flame propagation, obtained via dip-coating of the substrate in methyl-functionalized silane sols. A detailed study on the molecular level of the produced material will be presented. The structure of the coating material is examined by solid-state ^{29}Si NMR experiments, comparing spectra from bulk gels and the related thin film deposited on the cellulose sheets. Solid-state ^{13}C NMR spectroscopy is used to prove the non-destructivity of the process and to determine the amount of deposited material. In combination with other available data - from FTIR, contact angle and mechanical measurements - structure-property relationships are derived in order to find the optimum coating material.



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P339

Effects of magic angle spinning on dynamic nuclear polarization in solids

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Dynamic Nuclear Polarization (DNP) combined with solid-state magic angle spinning (MAS) NMR is becoming more routine thanks to recent developments in the group of Griffin et al.,[1,2] and the commercialization of a Solid-State DNP-MAS NMR spectrometer by Bruker BioSpin.[3] A current trend in solid-state MAS NMR is to spin at ever-higher MAS frequencies (currently ~70 kHz) in order to improve resolution in solids. Since DNP in the solid state will almost certainly follow this trend, it is important to examine the effects of increasing MAS frequencies on DNP efficiency. As recently noted by Rosay et al.,[3] the DNP enhancements increase from ν_{rot} = 0 to ~3 kHz and then decrease to about 50% of the maximum value from ν_{rot} = 3 to 15 kHz. We investigated the effects of increasing spinning frequencies up to 16 kHz on the DNP enhancements ε, proton T_1's, and the build-up time constant τ_{DNP}, for samples with various concentrations of radicals and partly protonated/deuterated solvents. Greater understanding of these effects should lead to more effective DNP enhancements at higher spinning frequencies.

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P340

Multifrequency Ferromagnetic Resonance (FMR) of Co/Al2O3 Catalysts

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Supported cobalt nanoparticles are employed as catalyst for the well-known Fischer-Tropsch Reaction (FT), in which hydrogen and CO are converted to liquid hydrocarbons. It is of interest for the petroleum industry to understand the influence of particle nanostructure and composition on the yield, the final products and the catalyst lifetime. Particle size, composition of the supporting material and the presence of oxides are considered to play a major role in the performance and deactivation of the catalyst. Magnetic techniques have been applied to either confirm results from XRD, electron microscopy and chemical analysis, or investigate some peculiar aspects of the catalyst structure, such as the distribution of cobalt particles, the role of the supporting material (alumina) and the deactivation mechanism. We present preliminary results of a study of Co/Al2O3 nanocomposites used in FT catalysis, obtained by Ferromagnetic Resonance (FMR) measurements performed at different resonant frequency (X, Q and W bands). Stabilized alumina carriers, metallic (ferromagnetic) cobalt and its (antiferromagnetic) oxides were tested, together with some reduced catalyst samples. Different temperatures (from lowest, 5K, to room conditions) were considered, and the FMR results compared to those obtained by magnetometry techniques. The FMR spectral response of the catalysts is attributed to the metallic cobalt nanoparticles (NPs). The results confirm the presence of strong magnetic anisotropies of the nanocomposites. At low field (X-band), forward and backward FMR scans evidenced irreversibility effects, attributed to the magnetic anisotropy field of the NPs which is large compared to the external magnetic field. At high field (W-band) the situation is reversed, the lineshapes become clearly defined and the irreversibile effects are strongly reduced. The FMR lines broaden and shift to lower field values upon decreasing the temperature. The high magnetic moment of the sample at resonance can heavily perturb the working conditions of the spectrometer and cause lineshape distortion. Particular care should be used in selecting the amount of sample and the microwave power employed.

P341

Structural characterization of acid and sodium Naproxen in the solid state by means of 2D MAS NMR ^{1}H-^{13}C correlation experiments

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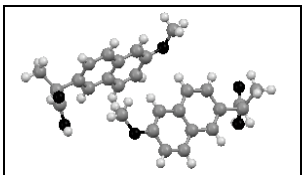
The sensitivity of nuclear properties to the local environment makes Solid State NMR a powerful technique for the investigation of local structural features of single molecules, as well as of crystal packing, for a wide variety of substances and materials. The elucidation of the structure in the solid phase has a particular importance in the pharmaceutical field, since most drug formulations are solid. The anhydrous crystalline forms of Naproxen [(*S*)-(+)-2-(6-Methoxy-2-naphthyl)propionic acid] and its sodium salt, widely used non-steroidal anti-inflammatory drugs, have been here investigated through ^{1}H and ^{13}C MAS 1D spectra and 2D ^{1}H-^{13}C MAS-J-HMQC and ^{1}H-^{13}C FSLG-HETCOR experiments. ^{13}C spectra could be assigned and interesting ^{1}H-^{13}C correlations through scalar and dipolar couplings were found. A strong difference in the chemical shift of the aromatic protons between acid and salt was clearly observed, which could be ascribed to different intermolecular effects of the aromatic ring currents, in turns ascribable to the different crystal packing of the two forms.[1] Dramatic intermolecular ring current effects on ^{1}H chemical shifts are indeed expected in crystalline polycyclic aromatic systems, but the experimental evidences reported so far in the literature are very few.[2]



Crystallographic cell of Naproxen

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P342

Solid-State ^{1}H and ^{13}C MAS NMR Investigations of European Coals

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In this study, the results of solid-state NMR (SSNMR) investigations of two sets of European coals (from Germany and the Czech Republic) are summarized. We have performed solid-state ^{1}H and ^{13}C magic angle spinning (MAS) NMR as well as ^{1}H-^{13}C heteronuclear correlation (HETCOR) experiments on these coal samples to determine their structures. Rank of these coals was obtained by straightforward ^{13}C one dimensional cross polarization (CP) with MAS NMR experiments and compared with results from the petrographical analysis; the results from both experiments are in well agreement. The carbon aromaticity (protonated, alkylated, phenolic or condensed) and the ratio between ternary and quaternary aromatic carbons were classified, for example by ^{1}H-^{13}C HETCOR experiments. In addition, solid-state ^{1}H MAS NMR results obtained with very high spinning rates are presented. This leads to the conclusion that SSNMR is a powerful method to determine structural features of coal.

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P343

Identification of Spin-filtering Defects in Ga(In)NAs by Optically Detected Magnetic Resonance

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Generating electron spin polarization/coherence at room temperature is one of the most important as well as the most challenging issues for future spintronics and spin-based quantum information technology. Spin filtering has been demonstrated by employing ferromagnetic metals, diluted magnetic semiconductors, quantum point contacts, quantum dots, carbon-nanotubes and multiferroics, etc., though so far unfortunately mostly with a limited efficiency and primarily at a low temperature or under applied magnetic fields.

Here we demonstrate a new approach for an efficient spin filter by defect-engineering in a non-magnetic semiconductor, which is capable of generating >40% electron spin polarization at room temperature without requiring external magnetic fields. We provide experimental proof for the physical mechanism leading to the observed spin filtering effect, i.e. an electron spin-polarized defect, such as a Ga self-interstitial in dilute nitride Ga(In)NAs, can effectively deplete conduction electrons with an opposite spin orientation and can thus turn the non-magnetic semiconductor into an efficient spin filter. The identification of the spin-filtering defects is unambiguously established from optically detected magnetic resonance studies, by their unique spin-resonance signatures derived from the hyperfine interaction between the localized unpaired electron spin and nuclear spins (I=3/2) of the Ga atom with two naturally abundant isotopes ^{69}Ga and ^{71}Ga. We demonstrate how the spin-filtering effects can be engineered by varying the concentration of the responsible defects, which can be achieved during the growth or by post-growth treatments. We show that the spin filtering effect remains effective for quantum wells as narrow as 3 nm. The present work has thus demonstrated the potential of such a defect-engineered, switchable spin filter as an attractive alternative to generate, amplify and detect electron spin polarization at room temperature under the conditions desirable for practical device applications.[1]

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P344

Correlation between ^{1}H MAS data and melting point alternation in acid-base co-crystals

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The phenomenon of melting point alternation in *n*-alkanes and in most end-substituted *n*-alkanes has been known for decades.[1] Physical properties such as solubility and sublimation enthalpy that are related to the solid state also exhibit an alternating pattern, whereas those related to the liquid state show monotonic behaviours.

Here we present that the melting point alternation of α,ω-alkane dicarboxylic acids (HOOC-$(CH_2)_{n-2}$-COOH, n= 4-10) is maintained in co-crystals of the same acids with 1,2-bis(4-pyridyl)ethane (BPA), which contains an even number of carbon atoms in the chain, while it is reverted in co-crystals with 1,2-bis(4-pyridyl)propane (BPP), which contains an odd number of carbon atoms in the aliphatic chain.

^{1}H DQ MAS Solid-state NMR spectroscopy has been able to detect the similarity of all structures in terms of the main packing feature,[2] while the co-crystal nature of all the adducts has been ascertained by means of ^{13}C and ^{15}N CPMAS NMR spectra.[3] The hydrogen bond strength seems to be the parameter influencing the melting point alternation since, at least from the ^{1}H MAS NMR data,[4] the highest melting points are associated to strong hydrogen bond interactions and *vice versa*.

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P345

Solid-State NMR Studies of Biomimetics to Understand Templating and Binding of the Mineral Phase in Bone and Calcified Plaques

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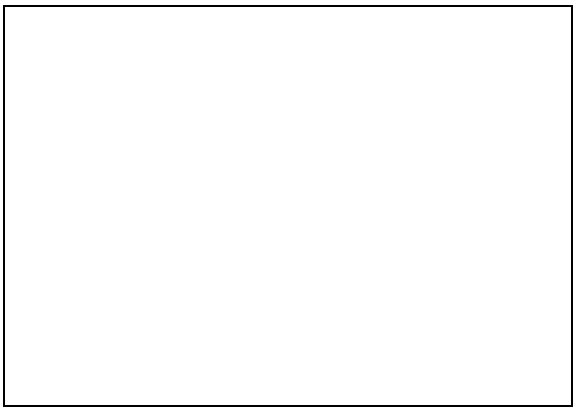
Bone is a lightweight biomaterial composed primarily of a protein (collagen) matrix bound with nanocrystals of a calcium phosphate phase which is commonly described as a substituted hydroxyapatite. How the mineral crystals are formed and the nature of the subsequent binding between them and the collagen matrix remain a matter of considerable debate.

This work describes the development of synthetic mimics of bone and calcified vascular plaques (which are bone-like in their molecular structure) and the detailed characterisation of these by various solid-state NMR methods, including ^{1}H-^{31}P and ^{13}C–^{31}P HETCOR and ^{13}C{^{31}P} REDOR. In particular, we examine the ability of different biomolecules to template hydroxyapatite and the nature of the interactions which allow this process to occur. We also examine the binding of molecules to nanocrystalline hydroxyapatite to develop a description of surface reactivity.



^{13}C{^{31}P} REDOR of alendronate bound to bone mineral

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P346

Browning reaction of sugars revealed by solid-state NMR

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The browning reaction of sugars in the presence of amino acids, or amines with organic acids, has long been of interest in food chemistry, biochemistry and agricultural chemistry. In addition, such reactions have been recognized as potentially important in prebiotic chemistry. However, the structure of the "melanoidin" polymer formed in the browning reaction is not completely understood. Recently, we have observed browning at mild temperatures (65°C) without amines. This presents a new opportunity to characterize the polymer structure because the solid state NMR (ssNMR) spectrum is simplified by the absence of pyrrole signals. Using melanoidins formed from selectively ^{13}C-labeled sugars we find that the furans produced by the browning reactions are embedded in complex hetero-polymers. Polymers for ssNMR analysis were prepared under argon, using initially dry reactants to obtain high yields. Sugars included D-ribose, 2-deoxy-D-ribose, D-fructose, D-glucose, D-galactose, and D-mannose. On their own, none of the sugars forms a dark product within 90 days. Pulverized with dry oxalic acid, pentoses generally form dark product faster than hexoses, and among the hexoses, the ketose fructose forms dark product faster than the aldoses. Due to the slow reaction rates and the low yields from the aldohexoses, we focus our spectroscopy on the polymers produced from D-ribose, 2-deoxy-D-ribose, and D-fructose. By comparing 1D ^{1}H-^{13}C cross polarization, ^{1}H-^{13}C dipolar dephasing, and double quantum filter experiments, we conclude that the furan units act to crosslink sugar molecules rather than form homopolymers. The details of the formation of furan units and the cross-linking of sugar molecules vary between ribose, deoxy-ribose and fructose, but the results are similar.

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P347

Intermolecular host-guest interactions in macromolecular systems determined by two-dimensional solid state NMR spectroscopy

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Additives can modify the physical-chemical and/or mechanical properties of macromolecular systems without altering their fundamental chemical character. To characterize the host-guest interactions in macromolecular systems different solid-state NMR spectroscopy techniques are used depending on the properties of the investigated materials. Their physical properties and the molecular motions can vary on an extremely wide range. This work reports results obtained by two-dimensional solid-state NMR techniques on a soft hydrogel and on a rigid biopolymer system. Proximity was determined in a thermoresponsive host–guest gel system and the polymer–phenol distance was calculated using solid-state ^{1}H–^{1}H CRAMPS NMR spectroscopy and rate matrix analysis.[1] Differences were found in the plasticizing effects of two commonly used softening materials (glycerol and PEG 400) on amorphous chitosan films by two-dimensional ^{1}H-^{13}C frequency-switched Lee-Goldburg (FSLG) HETCOR experiments and density functional theory calculations.[2,3]

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P348

Solid State NMR Methods for the Study of ^{7}Li -^{1}H and ^{7}Li-^{13}C Distances

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In polymers, binding of lithium was shown to affect differently carbonyl and ether carbons.[1] In the context of bound lithium in solid state a special interest to us is the binding of lithium to proteins in human bipolar disease. Thus retrieving structural information about the binding sites is a most desirable goal. For lithium complex in solution ^{7}Li -^{1}H distances were estimated using triple quantum (TQ) filtered NMR.[2] In solids bound ^{7}Li has quadrupolar coupling that can be as large as 100 kHz. Such values present a situation where the excitation is neither selective nor non-selective under common conditions in solid state NMR. This leaves open the question of method of choice in structural studies of bound lithium. For distance determination the internuclear dipolar interaction has to be measured. Thus, we examined experimentally and theoretically several MAS NMR methods that are expected to be affected by the dipolar interaction: (I) REDOR involving ^{13}C and ^{7}Li; (II) TQ dephasing of ^{7}Li; (III) four quantum filtration (4QF) consisting of TQ for ^{7}Li and single quantum (SQ) for protons. As the structure of the complex of lithium with Kryptofix 211 is well known and the static spectrum of ^{7}Li in it could be measured (we obtained for the quadrupolar coupling 80kHz and for the anisotropy factor 0.46) it was used for the above tests. The major conclusions are: (1) Simulations show that all the above methods are capable of determining distances with accuracy of 0.2Å (2) Experimentally all the methods were found to be sensitive to the interaction of ^{7}Li with either ^{13}C (REDOR) or with ^{1}H (TQ dephasing and 4QF) (3) Theoretically and experimentally excitation of the ^{7}Li TQ coherence is more effective using the following five pulses sequence: 90-τ–(SQ)-90-delay(TQ)-90-τ(SQ)-90-delay(M_z)-90-acq(TQF-IP, for static case[3]) with $\tau=0.5/\nu_r$ than the commonly used two selective pulses (2SP). The TQF-IP sequence has shown an improvement of 100% over the 2SP scheme with efficiency of 27%.

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P349

^{13}C and ^{29}Si-Solid State NMR Study of silicon Nano-Scale Ionic Materials

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A new class of nanomaterials termed Nano-Scale Ionic Materials (NIMs) is attracting widespread research interest, as it displays interesting and unique properties include negligible vapour pressures, fluidity in the absence of solvent, tunable physical states with changes in chemical composition and tunable electrical, thermal, rheological & optical properties.[1-4] ^{13}C Cross-polarization under magic angle spinning (CP MAS) and ^{29}Si magic angle spinning (MAS) NMR spectroscopy has been applied to the study the structure and stability of three Si Nano-Scale Ionic Materials. To investigate NIMs stabilities, ^{29}Si NMR spectra were recorded at 300, 343, 383 and 400 K. The results show that the studied NIMs materials are stable over the temperature range of 300 to 400 K.

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P350

µg-Analysis of silicates using magic angle micro coil spinning technique

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In the past decades silicate sol-gel chemistry has grown into a major research area among other things due to the importance of coatings. These substances are essential for a multitude of production areas, such as thermal barrier coatings and in electrical devices as organic light emitting diode (OLED).[1] It is a matter of common knowledge that solid-state NMR is a powerful tool to gain information about the chemical structure. Applying solid-state NMR for characterization of small amounts materials often suffers from the small quantities. In the range of µg with simultaneous consideration of relatively big rotors - even 1.3 mm - the bad filling factor leads to a poor signal to noise spectrum in addition to a long measuring time. A recent approach to achieve qualitatively good NMR spectra from µg amounts in a reasonable time scale is the method of "magic angle coil spinning" (MACS) introduced by Sakellariou et al.[2]

For structural clarification in the sol-gel chemistry, solid-state NMR has been used to obtain information on the microscopic level.[3] Thus, a work combining the new technique of MACS with the analysis of small amounts such as thin films can help to reveal new information about the nature of oxygen bridges. It is obvious that the use of micro coils can withdraw open questions about the sol-gel thin film formation. ^{17}O labeled samples were characterized using ^{1}H and ^{17}O solid-state NMR in 2.5 and 1.3 mm standard rotors as well as the MACS design for comparison.[4]

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P351

Spin dependent transport in thin film solar cells: an electrically detected ESEEM study

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Silicon-based thin-film solar cells offer the potential for a significant cost reduction in photovoltaic power generation due to a low temperature deposition process, low material consumption and the use of low-cost substrates. Despite these benefits, efficiencies of thin-film silicon devices are up to now significantly below those of their silicon wafer-based analogues. Major limiting factors in thin film devices are defects which are acting as excess charge carrier traps or recombination centers. Hence, the identification of their structure and influence on charge carrier transport is one of the most pressing problems of solar energy research. Defect states in solids are often paramagnetic or can be made paramagnetic which opens the possibility to investigate them by means of Electron Paramagnetic Resonance (EPR). However, standard EPR methods fail to detect defect states in fully processed thin film solar cells, containing less than 10^6 paramagnetic sites. We recently managed to circumvent this limit by employing pulsed electrically detected magnetic resonance (pEDMR) and thereby lifting the detection limit to ~ 10^4 spins. In order to further exploit the potential of pEDMR, we implemented for the first time an electrically detected electron-spin echo envelope modulation (ED-ESEEM) scheme. With this novel method at hand we succeeded to identify the dominant spin-dependent charge carrier transport pathways and the participating paramagnetic defects in amorphous/microcrystalline silicon solar cells at cryogenic temperatures and characterize them via their hyperfine interactions.

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On the use of nuclear quadrupole resonance for quantum computing

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The NMR experiments usually performed for quantum computing and quantum information processing employ liquid substances or oriented liquid crystals.[1] A promising alternative to this is the use of nuclear quadrupole resonance (NQR) at zero or small external magnetic fields, with the obvious benefit of lower cost in comparison to high-field NMR. The different spin dynamics involved in NQR experiments demands the development of specific experimental procedures for implementing the basic tasks in quantum computing. Thus, this work aims at exploring the possibilities of the use of NQR for such purpose. The spin dynamics was numerically simulated for a spin 3/2 system, using the quadrupole coupling parameters of ^{35}Cl nuclei in a single crystal of $KClO_3$. First, the NQR spectra corresponding to different external magnetic fields were simulated and compared to the experimental spectra. Next, the selective excitations of the transitions between the energy levels of the spin 3/2 system (illustrated in Fig. 1) were considered. Although the levels are degenerate in the absence of an external magnetic field, selective excitation is possible with a combination of circularly polarized single (P_S^+ and P_S^-) and double (P_D^+ and P_D^-) quantum RF pulses. The effects of these pulses were numerically simulated by using average Hamiltonian theory in the interaction frame, allowing the generation of pseudo-pure states as well as some simple quantum gates.

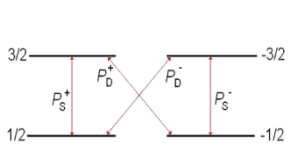

Fig. 1: Energy levels for a spin 3/2 quadrupole system at zero external magnetic field.

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P353

A complete investigation of dynamic properties of organic molecules in the solid state from a global analysis of spectral features and relaxation times

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The molecular dynamics of a solid drug strongly affects its pharmaceutical properties and other important characteristics as solid state degradations/reactions or drug-excipient interactions.[1] Solid State Nuclear Magnetic Resonance (SSNMR) is an important technique for the physical characterization at molecular level of small organic molecules such as many active pharmaceutical ingredients, which can be also directly investigated in their final formulation, and in particular it offers several approaches to study molecular dynamics in a wide range of frequencies.[2] An extensive characterization of molecular dynamic properties of two forms of Ibuprofen, acid (IBU-A) and sodium salt (IBU-S),[3] obtained through SSNMR techniques is here presented. A deep characterization of motions with characteristic frequencies from Hz to MHz was achieved through ^{13}C isotropic chemical shift and chemical shift anisotropy line shape analysis, ^{13}C and ^{1}H longitudinal relaxation time in rotating frame ($T_{1\rho}$) measurements, ^{13}C and ^{1}H longitudinal relaxation time (T_1) measurements. Combined analysis of all the data provided either qualitative or quantitative information about the motions of the various molecular fragments (phenyl ring, methyl groups, aliphatic chains). Beside the detailed results obtained for Ibuprofen, this study represents, to the best of our knowledge, the first case in which the molecular dynamics of organic solids in the wide range of motional frequencies accessible by SSNMR has been fully characterized. The flexibility and generality of the approach here proposed makes it a potential powerful tool for the characterization of the internal motions occurring in organic molecules in the solid state.

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P354

Quantification of the metallic-to-semiconducting ratio in single-walled carbon nanotube samples using cobalt porphyrine EPR spectroscopy

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A simple and quantitative spectroscopic technique for the determination of the ratio of metallic to semiconducting single-walled carbon nanotubes (SWCNTs) in a bulk sample is based on the measurement of the electron paramagnetic resonance (EPR) spectrum of cobalt(II)octaethylporphyrin (CoOEP) probe molecules adsorbed on the SWNTs.[1] Signals from both CoOEP molecules on metallic and on semiconducting tubes are easily distinguished and accurately characterized in this work. By applying this technique to a variety of SWCNT samples produced by different synthesis methods, it is shown that these EPR signals are independent of other factors such as tube length, defect density and diameter, allowing the intensities of both signals for arbitrary samples to be retrieved by a straightforward least-squares regression. The technique is self-calibrating in that the EPR intensity can be directly related to the relative number of spins, and as the two components were also found to occur in a constant relative intensity, independent of the procedure used in rinsing off the excess (unbound) molecules (i.e. not preferential removal from one or the other type of CNTs), the intensities of both signals are proportional to the surface area, and hence the mass fraction of M and SC tubes respectively. Using this method, for some samples metallic-to-semiconducting ratios differing strongly from the usual 1:2 ratio were found.

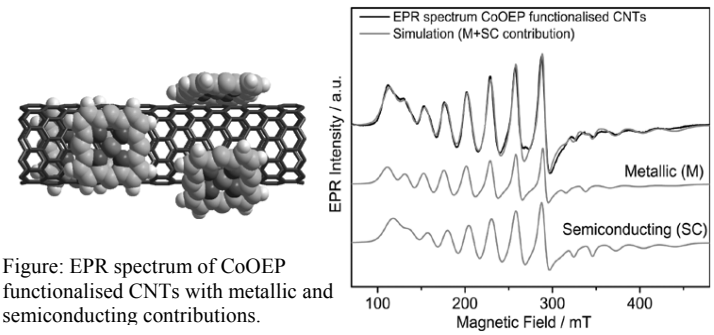

Figure: EPR spectrum of CoOEP functionalised CNTs with metallic and semiconducting contributions.

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P355

Investigation of the Timescale and Geometry of Motion of a Microcrystalline Protein by Dipolar Codex Nmr

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Knowledge about protein dynamics is essential for the understanding of their biological function. Solid-state NMR techniques, in comparison to liquid-state NMR, proved to be very powerful to investigate a wide range of correlation times of protein dynamics[1] due to the fact that fast motions are not overlapped by the isotropic Brownian tumbling. Molecular motions cover a wide time scale. To study motions in the slow limit of ms to s the CODEX (Centerband-Only Detection of Exchange) sequence[2] is one of the most often applied pulse sequences in solid-state NMR.

Here we used the basic principles of CODEX but employed the heteronuclear coupling instead of the CSA as a probe for molecular mobility. With the so called Dipolar CODEX[3] experiment we investigated a microcrystalline protein, namely, the SH3 domain of α-spectrin. The experiments presented here show side resolved information about the time scale of motion as well as the geometry of motion. For quantitative information about the amplitude of motion, knowledge about the residual dipolar coupling is necessary. To obtain side resolved information we applied a new REDOR based HSQC sequence. Our results obtained by means of this experiment fit well to data published in literature.[4]

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P356

The Impact of Side Chains on Charge-Carrier Mobilities in Perylene Derivatives Studied by High-Resolution Solid-State NMR Methods and Models

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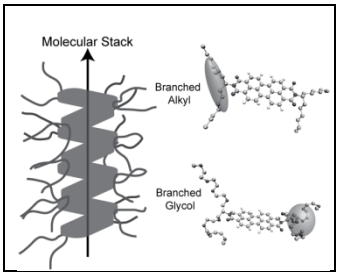
In the emerging area of nano-scale electronics, the application of stacks of molecules as molecular conducting wires seems highly attractive. Such wires are typically based on polycyclic aromatic hydrocarbons[1] and, as we have recently shown, the conduction performance along these stacks critically depends on the size, shape, and periphery of the individual molecules and pitch angles between successive molecules.[2] In this contribution we investigate the impact of attaching different side chains (symmetrically or non-symmetrically) to a number of perylene derivatives by combining the information available from solid-state NMR, WAXS, and MD simulations. We show that the choice of side chains strongly influences the phase behaviour of the resulting materials and that this choice on the molecular level can lead to different pitch angles between successive molecules.[3-5] This can be understood in terms of the gyration tensor (see picture).[6] With these new insights it should be possible to rationally design perylene-based molecular stacks with optimized charge carrier mobilities.



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P357

Multinuclear Solid State NMR Characterisation of Zinc and Bismuth Incorporation in Borosilicate Glass Systems

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The structures of borosilicate glasses, used as components of automobile windscreens, are being studied using multinuclear solid state NMR. Some of these materials are already commercially used, although their properties need to be improved. The aim is to produce a new glass with much higher acid resistance which can pass a new industry test, while keeping the firing temperature the same as that for conventional windscreen glasses.

Two series of model samples have been made in order to understand the roles of Bi and Zn in the glass networks of systems close to the commercial compositions. The samples are sodium borosilicates; in the compositions Bi or Zn are substituted for boron and NMR has been used to determine whether they also substitute the role boron in the glass network, forming bonds, or whether they form less bonds and make the network less connected. Bloch decay ^{11}B, ^{23}Na and ^{29}Si experiments and two dimensional ^{11}B MQMAS studies have been undertaken to understand the evolving structural changes with increasing metal content. It has been found that as the metal content increases the glass networks become less connected and so Bi and Zn are not taking the place of boron in the glass network.

Acknowledgments: JRH, MES and JVH acknowledge Johnson Matthey. The NMR spectrometers and probes used in this research were obtained through Birmingham Science City: Innovative Uses for Advanced Materials in the Modern World (West Midlands Centre for Advanced Materials Project 2), with support from Advantage West Midlands (AWM) and part funded by the European Regional Development Fund (ERDF).

P358

Solid-state NMR as a tool to aid and correct diffraction-based structure solution

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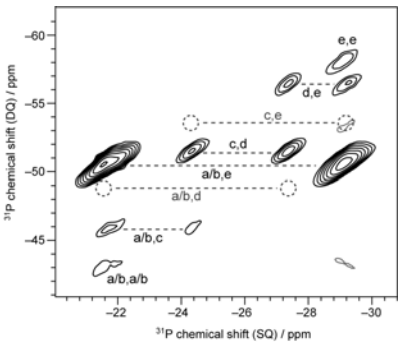
Although historically crystallography and diffraction-based studies have been virtually synonymous, the complementary role of solid-state NMR in characterising crystalline solids is increasingly recognised. We present a ^{31}P NMR and powder X-ray diffraction study[1] of $(MoO_2)_2P_2O_7$. Like many framework pyrophosphates, this material exhibits subtle phase transformations, from the high-symmetry structure at high temperature, to a complex superstructure at low temperatures. The structural distortions are subtle and very challenging to determine from Bragg diffraction only.



The exquisite sensitivity of NMR parameters to local environment is an ideal complement to Bragg diffraction. Even simple 1D ^{31}P NMR spectra are informative, revealing an incommensurate phase between high and low temperature structures. 2D NMR and measurements of $^2J_{PP}$ couplings via spin-echo experiments give detailed insight into the structural distortions involved in the phase changes, and allows potential structure solutions to be validated. First principles (Gaussian/CASTEP) calculations are used to connect NMR observables with structural features. Finally we present a case where ^{1}H and ^{13}C solid-state NMR combined with CASTEP calculations are used to evaluate XRD structure determinations of terbulatine sulfate, clearly demonstrating the superiority of one solution protocol over the other.[2]

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P359

Proton-Detected Non-Uniformly-Sampled 4D DREAM for Solid-State NMR Protein Structure Determination

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Solid-State NMR has recently been established as a structure determination method for non-soluble and non-crystalline proteins. In fully protonated samples, the strong dipolar couplings lead to broad ^{1}H lines, but with perdeuteration, highly resolved ^{1}H spectra of proteins can be obtained.[1] Compared to widely used ^{13}C-detection, ^{1}H-detection benefits from higher sensitivity due to the higher gyromagnetic ratio.

Here, we demonstrate, using a crystalline sample of ubiquitin, that ^{1}H-detected ^{1}H-^{1}H DREAM[2] spectra can successfully be used for structure calculation. Unambiguous long-range restraints were obtained from a 4D non-uniformly sampled (NUS) ^{1}H-^{1}H DREAM spectrum of deuterated ubiquitin with valine and leucine methyls labelled as $^{13}CD_2H$.[3] This spectrum was recorded in 3 days on 2 mg of protein.

Because of the unambiguity of assignments in four dimensions and the high sensitivity due to direct proton detection, we expect this approach to be applicable to proteins significantly larger than ubiquitin.

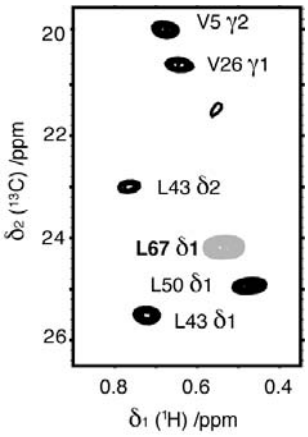

Figure 1 Plane from NUS 4D DREAM spectrum of [U-^{2}H^{15}N; VL-$^{13}CD_2H$]-Ubiquitin. Negative peaks are shown in black, the positive diagonal peak in grey.

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P360

Multinuclear NMR Study of the SEI on the Li-$FeSn_2$ Negative Electrodes for Li-ion batteries

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The aim of this work is to investigate the composition, formation and evolution processes of the solid electrolyte interphase (SEI) on the Li-$FeSn_2$ negative electrodes for Li ion batteries. In this system, lithiation gives rise to a conversion of the material to Li-Sn alloys and Fe nanoparticles. The presence of such super paramagnetic Fe nano particles causes a great challenge in the NMR characterization. The visibility of the ^{7}Li NMR signal associated with Li-Sn alloy has been studied as a function of the magnetism by varying both the species of the transition metal (Fe, Co, Ni and Mn) and the experimental temperatures. A series of samples at different stages of lithiation or aging have been investigated. ^{7}Li in combination with ^{1}H, ^{19}F and ^{31}P MAS NMR provided important information about the composition of the SEI layer and the intermediate species during lithiation process.

P361

^{13}C CPMAS NMR: CP kinetics of model compounds from *Actea racemosa*

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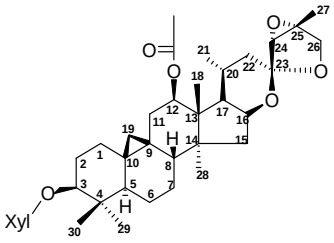
Acteae racemosae extract is effective botanical preparation for relief of vasomotor symptoms of menopause, such as hot flashes and night sweats. The main group of compounds are triterpene glycosides,[1] which are thought to be responsible for the pharmacological effect of this plant, although further studies are needed. Since there are no data for the solid phase of triterpene glycosides, the aim of this research was thus to register and interpret the ^{13}C CPMAS spectra of four model triterpene glycosides: deoxyactein (1) shown in the Figure, cimigenol xyloside (2), cimicifugoside H-1 (3) and 24-acethylhydroshengmanol xyloside (4).



In addition we analyzed the cross-polarization kinetics profiles for four compounds, which can be acknowledged as model substances, since they represent four types of triterpene skeletons found in *A. racemosa*. This enabled the differentiation of the CH, CH_2, CH_3 and quaternary groups, as well as gaining interesting structural information.[2]

The unambiguous assignment of ^{13}C CPMAS NMR signals of the studied compounds allows fast identification of triterpene glycosides isolated from *A. racemosa*, without any preparation procedures. The samples removed from rotor can be used for further studies of biological and pharmaceutical activity.

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P362

Magnetic properties and spin dynamics of heterometallic entangled Cr_7Ni molecular nanomagnet

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Molecular nanomagnets represent a class of magnetic systems that received a lot of attention from physicists and chemists in the last 20 years, both for their fundamental properties and possible applications.[1] Among them, the high-spin clusters received most of the attention but recently another class of compounds, the high-symmetry molecular rings, attracted theorists and experimentalists for its connection to the problem of quantum computation.[2] Particularly, antiferromagnetic Cr_7Ni-based rings show good characteristics for quantum computing, i.e. long decoherence times, low anisotropy and effective low spin at low temperature. The heterometallic Cr_7Ni molecular spin cluster contains seven Cr^{3+} and one Ni^{2+} centres, arranged in an octagonal ring and held together by fluoride and carboxylate ligands,[2] interacting via a n.n. antiferromagnetic (AF) coupling (J~17K) to give an effective spin ground state S = ½ spin at sufficiently low temperature. By synthesizing a system where a Cu^{2+} dimer links pairs of Cr_7Ni clusters, one is in presence of "entanglement" between pairs, thus giving a key requirement for implementing quantum information processing (QIP) applications.[2] We investigated the magnetic properties and spin dynamics of the entangled heterometallic Cr_7Ni-Cu_2-Cr_7Ni cluster by studying the magnetic susceptibility, and the ^{1}H- nuclear magnetic resonance (NMR) spectra and relaxation rates (spin-lattice, T_1 and spin-spin, T_2) in the temperature range $1.5 < T < 300$ K at different applied magnetic fields, and as a function of frequency at room temperature. The NMR results obtained in the entangled Cr_7Ni cluster have been compared with those of pure Cr_7Ni molecular spin cluster.

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P363

Applications of ^{1}H and ^{31}P Ultra Fast MAS NMR Spectroscopy in Structural Study of Phosphoroorganic and Organometallic Compounds

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During last ten years a remarkable development in MAS (Magic Angle Spinning) probe-heads technology has been achieved. In modern probe-heads, ''ultra-fast" (UF) regime of more than 60 kHz sample spinning is reached using commercially available 1.3 mm rotors. Under UF conditions, the MAS frequency exceeds the width of the ^{1}H-^{1}H dipolar interaction narrowing the ^{1}H linewidths. This feature, make possible to utilize ^{1}H Solid State MAS technique in structure elucidation of solids employing pulse sequences which found number of spectacular applications in the liquid phase.

In this communication we report application of ^{1}H MAS NOESY and ^{1}H RFDR NOESY for investigation of two model samples, O-phospho-L-threonine and Grubbs-Hoveyda metathesis catalysts ortho-isopropoxybenzylidene[1,3-bis(2,4,6-trimethylphenyl)-2- imidazolidinylidene]dichlororuthenium. Both spectra were recorded with spinning rate 60 kHz and RF power during the mixing time equal to 60 kHz and 120 kHz for the latter sequence. From comparative analysis of both sequences the usefulness of ^{1}H RFDR NOESY experiment in structural study of heterorganic samples is apparent.

In the second part we discuss the problem of ^{1}H→^{31}P cross-polarisation under UF MAS employing hard and soft pulses. From our study it is concluded that low power RF pulses allow very effective cross-polarization according to double quantum (DQCP) and zero quantum (ZQCP) mechanism when the $\omega_{1H} + \omega_{1P} = n\omega_r$ and/or $\omega_{1H} - \omega_{1P} = n\omega_r$ conditions are fulfilled. Study of standard decoupling sequences TPPM, SPINAL, XiX and CW confirmed the applicability of low power decoupling (ca 25 kHz) for study of phosphoroorganic compounds. The influence of rotary resonance effect on quality of decoupling is discussed. Finally, the ^{1}H -^{31}P SPECIFIC-CP band selective experiment carried out for phosphoroorganic sample with distinct phosphorus sites (P=S and P=O) is discussed.

P364

A Study of the Lithium Environment in Optically novel $LiTaO_3$: A Solid State 7Li NMR Study

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Lithium niobate (LN) is one of the most important crystals, being the equivalent in the field of optics, nonlinear optics and optoelectronics to silicon in electronics. Lithium tantalate (LT) has almost the same crystal structure but different physical properties and is sometimes used as a replacement for LN, particularly for shorter-wavelength applications, although it is true to say that LN is by far the more widely used of the two materials at present.

Both LN and LT can be synthesized with a large range of stoichiometry, ranging from strongly lithium deficient to lithium abundant. The structural mechanisms behind the influence of this stoichiometry on the observed optical properties are investigated using ^{7}Li solid state NMR, using conventional MAS, static (broadline) spin-echo and saturation-recovery T_1 relaxation measurements.

These static, relaxation, and MAS experiments show that there are stoichiometrically driven structural and dynamic changes in LT, coinciding with the compositions that exhibit novel optical properties.

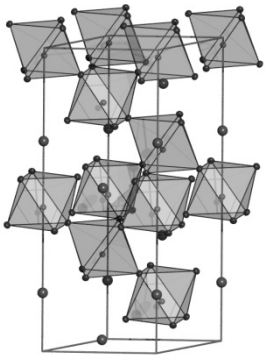

Figure 1: The Ilmenite structure of $LiNbO_3$ and $LiTaO_3$, forming with NbO_6 octahedra (highlighted) and lithium in the interstices

P365

Electronic and magnetic properties of V_2O_5 and $Li_2V_3O_7$ nanotubes

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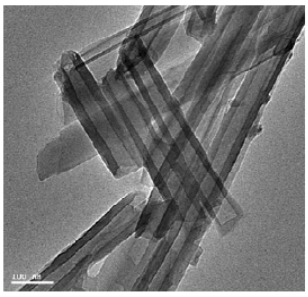
We report the electronic and magnetic properties of V_2O_5 and $Li_2V_3O_7$ nanotubes. 51V MAS NMR spectra have been obtained by using a ultra fast MAS probe with a cylindrical 1.2 mm rotor at B0= 11. 7 T. The spinning frequency was 40 kHz. The lithium vanadium oxides have so called a ladder-type crystal structure. The vanadium ions, each roughly situated in the center of a pyramid of 5 oxygens, form layers in which 1D vanadium chains are assembled in a network of two-leg ladders. From the 51V MAS NMR, we could determine two different sites of vanadium in $Li_2V_3O_7$ nanotube. It is in good agreement with the $Li_2V_3O_7$ crystal structure. Vanadium oxides are frequently of mixed valence. The electronic and magnetic structure of $Li_2V_3O_7$ consists of a double-chain charge ordered configuration, magnetic V4+ chain and nonmagnetic V5+ double chain. The magnetic property of V_2O_5 nanotube shows a paramagnetic, whereas, $Li_2V_3O_7$ nanotube clearly shows a ferromagnetism at room temperature. This result implies that the ferromagnetic properties are due to the charge compensation from Li which might be increased the electron doping at vanadium atoms in pyramidal sites. The lithium atoms act as electron donors.



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P366

Structural analysis of Alumino-silicates inorganic polymer systems by solid state NMR

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The stability of amorphous aluminosilicate inorganic polymer (AIP) systems with regard to the structural role of water molecules incorporated in inorganic matrix. It is shown that even small changes in the manufacture dramatically affect mechanical properties and the overall structural stability of AIP systems. If the required quantity of water is admixed to the reaction mixture during the initial period of AIPs synthesis the resulting amorphous aluminosilicate matrix undergoes extensive crystallization (zeolitization) during the artificial aging. On the other hand, if the required amount of water was added to the reaction mixture during the final periods of the system preparation the inorganic matrix was resistant to the structural changes and remained amorphous even after a long-term hydrothermal treatment.

An understanding and disclosure of the fine relations between structure, processing and post-processing of various types of geopolymer requires application many techniques of solid-state NMR spectroscopy. In our work we focussed our attention to the precise localization of water molecules in inorganic framework. That is why we used not only simple one-dimensional experiments on various nuclei like ^{1}H, ^{23}Na, ^{27}Al, ^{29}Si but we rather bet on two-dimensional multiple-quantum experiments that were modified (REDOR) to indirectly detect water molecules that are closed in selected structural units. By this way we were able to identify structurally important units that are responsible for their stability/instability. We tested large scale of NMR techniques involving also ^{1}H-^{1}H correlation experiments, cross-polarization transfer as well as relaxation experiments to locate and describe properties of clusters of water molecules.

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P367

Broadband NMR above 1 GHz at ultra high magnetic fields up to 34 Tesla

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We present recent research projects of broadband NMR above 1 GHz and at high magnetic fields up to 34 T at the *Laboratoire Nationale des Champs Magnetiques Intenses* in Grenoble. With the commissioning of NMR instrumentation covering the frequency range up to 1.5 GHz and working at temperatures as low as 0.4 K, we are able to offer general purpose NMR options up to 34 T, the highest currently available field at Grenoble.

We used our experimental setup first for condensed matter ^{1}H NMR experiments on azurite ($Cu_3(CO_3)_2(OH)_2$) at low temperatures (0.4 K).[1] This compound has been recently recognized as a model system for the frustrated antiferromagnetic Heisenberg spin-1/2 chain of "distorted diamond" geometry. Its most prominent feature is an extended plateau in the magnetization curve at 1/3 of the saturation magnetization, which is a purely quantum state without classical analogue. Our experiment revealed detailed microscopic information on the transition from the 1/3 plateau into the fully polarized state at 34 T.

With the same experimental setup we have also studied for the first time the paramagnetic relaxation enhancement of ^{1}H in diamagnetic species interacting with paramagnetic metal complexes in solution above 1 GHz. This provides a deeper insight into the intermolecular recognition movements, in particular those governing the efficiency of MRI contrast agents.

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P368

Polarization transfer and relaxation in solid state systems-Imidazolium based molecular crystals as an example

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Performing proton field cycling experiments for solid state systems containing nuclei possessing quadrupolar moments one can observe two essentially different phenomena: relaxation and polarization transfer.

When one experimentally observes polarization transfer effects ('magnetization dips'), one can immediately conclude that a part of the considered molecule is rigid (exhibits slow dynamics), or that there is a residual dipole-dipole coupling between the participating spins. A detailed quantitative analysis of the polarization transfer pattern gives the quadrupolar parameters and details of the molecular structure provided that an appropriate theoretical model is available.[1-3] A complete theory of field dependent relaxation processes in such systems has also recently been proposed.[3,4]

We shall outline the basic ideas of the mentioned theories of polarization transfer and relaxation. To demonstrate how one can profit from polarization transfer and relaxation experiments, we shall apply the theoretical approaches to imidazolium (Im) based molecular crystals: $Im_3Bi_2Br_9$, $Im_3Sb_2Br_9$ and $Im_5Bi_2Cl_3$.

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P369

NMR quadrupolar splitting from stretched gels: quantum mechanics of $^{23}Na^{+}$ *z*-spectra

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The nature and extent of interactions of cations with macromolecules in and around cells is poorly understood. However, quadrupolar nuclei such as ^{23}Na have important characteristics that manifest themselves as split peaks in NMR spectra from anisotropic media such as various stretched gels; these are good models of ordered biological systems.[1, 2] The splitting is due to the electric quadrupole moment of the nucleus interacting with the average electric field gradient tensor of the partially aligned macromolecules in the stretched gel. Radio-frequency (RF) radiation, applied at offsets across the whole NMR spectrum generates a '*z*-spectrum' or partial-saturation envelope. With a stretched gel, containing $^{2}H_2O$, the *z*-spectrum shows the most marked dip, not at the position of one or other of the two obvious peaks, but exactly in the centre between them.[3, 1] With $^{23}Na^{+}$, the *z*-spectrum shows the most marked suppression of the 3:4:3 triplet when the irradiation is applied on: (1) the satellite transitions (type S dip); (2) exactly in the middle between the central peak and either of the two satellites (type D dip); and (3) on the central transition peak (combined type S and T dips). *z*-Spectra fitted using an adaptation of the theory in refs. 1 and 4 enabled consideration of the combined effects of RF irradiation at various offsets from the centre peak, and residual quadrupolar interactions.[1,2] Thus we give a quantum mechanical explanation for the form of $^{23}Na^{+}$ NMR *z*-spectra and ascribe the type S, D, and T dips to single, double, and triple quantum transitions, respectively. We discuss possible *in vivo* applications.

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P370

Solid state NMR studies of paramagnetic cyclam complexes

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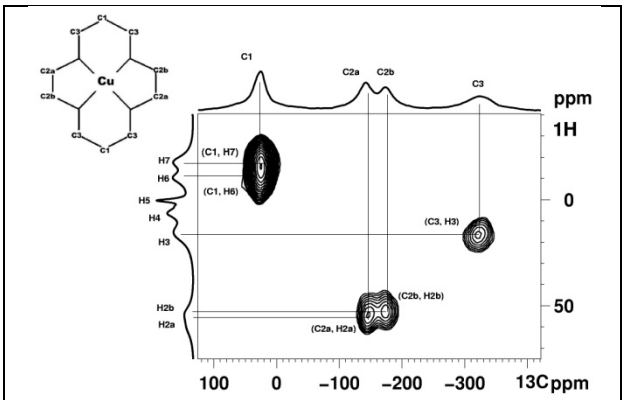
Cyclam (1,4,8,11-Tetraazacyclotetradecane), a macrocyclic organic molecule, forms stable coordination complexes with *d*-block transition metals like Cu^{2+} and Ni^{2+} in various structural conformations. We compare these paramagnetic complexes by ^{1}H and ^{13}C NMR under MAS frequencies that are high enough to decouple the proton network. Under these conditions the hyperfine shifts overcompensate line broadening by paramagnetic relaxation, so that the spectral dispersion is sufficient to resolve most signals in ^{13}C and even ^{1}H spectra. ^{1}H-^{1}H together with ^{1}H-^{13}C correlation experiments allow signal assignment. The paramagnetic relaxation rates of C- and H-nuclei depend on their distances to the metal center and should therefore in principle contain structural information. To assess their suitability as structural constraints, they are compared with the corresponding theoretical values. There are two parameters necessary for their calculation, the correlation time of the electron-nuclear dipolar interactions and the metal-nucleus distances. The former were determined by means of EPR, the latter by *ab-initio* and DFT calculations and compared with crystallographic data.



Dipolar INEPT ^{1}H-^{13}C correlation spectrum of the Cu-cyclam complex.

P371

Pulsed NMR Approach to Bulk Polymerizations of Methacrylic acid, Methyl methacrylate and 2-hydroxyethyl methacrylate

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Pulsed NMR is an efficient technique to follow transition of states, such as a bulk polymerization, a curing reaction of epoxy resin continuously and non-destructively in terms of spin mobility. We carried out bulk polymerizations of methacrylic acid (MA), methyl methacrylate (MMA) and 2-hydroxyethyl methacrylate (HEMA) to follow spin-lattice relaxation time (T_1) and spin-spin relaxation time (T_2) at different temperatures(40-60°C). For those monomers, three kinds of T_2; T_{2S}(short), T_{2L}(long) and T_{2M}(intermediate) were obtained and these relaxation times decreased as the polymerization proceeded. T_{2S} of which final value is *ca.* 20μs corresponds to the polymer produced. Fractions corresponding to above three relaxation times, F_S, F_L and F_M, respectively changed also. Fs gradually increased and then showed a sigmoidal increase while F_L decreased reciprocally to the time course of F_S. In the early stage of reaction, the concentration of entanglement of polymer chain (network) is not sufficiently high enough to decrease the mobility of proton spin. The reaction proceeds further, the entanglement becomes rich enough to restrict the mobility of spin. The polymer yields are found to be comparable to F_S for MA, MMA, and HEMA throughout the reactions at different temperatures. The time courses of F_S for these three monomers are similar to each other. T_1 is also a parameter sensitive to this kind of transition caused by entanglement of polymer chain. T_1 of MA, MMA and HEMA showed a linear decrease at the beginning of the reaction and showed a constant value corresponding to the "gel effect" occurred in bulk polymerization. The time course of T_1, T_2 in these polymerizations was well explained by BPP theory.[1] The progress of bulk polymerization, therefore, was found to be sharply reflected on the time course of T_1, T_2 and its fractions and can be evaluated in terms of spin mobility. The effect of difference in side chain structure for three monomers on the formation of network structure was also discussed in conjunction with the results of DD/MAS NMR.

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P372

^{95}Mo solid state NMR study of molybdenum cluster compounds, a combined experimental and computational approach

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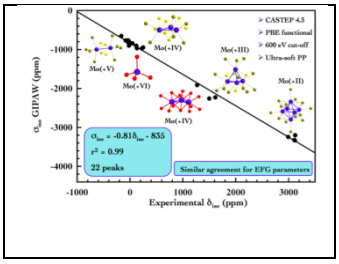
Transition metal clusters are chemical units containing three or more metal atoms held together by metal-metal bonds (Cotton, 1964). From structural and electronic structure point of view, these compounds stand on the threshold between molecular and bulk chemistry. Some of these compounds exhibit interesting properties – e.g. superconductivity, thermoelectricity, catalytic or intercalation properties.[1] During the last few years, new routes for the synthesis of nanostructured cluster compounds have been developed. In order to get a better structural understanding of these materials, we developed a characterisation approach based on a combination of experimental ^{95}Mo NMR experiments and DFT calculations of ^{95}Mo NMR parameters[2] (high field experiments using sensitivity enhancement techniques 18.6T, HS-QCPMG, ^{95}Mo CSA and EFG calculated using the GIPAW method). As a first step, we validated this approach on a series of well-crystallized cluster compounds of various nuclearities. We, then, applied this method to get insights on the controversial structure of $Mo_6S_{8-x}I_x$ nanowires.[3]



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P373

Solid-state NMR Studies of Vinyl Polymer/Silica Colloidal Nanocomposite Particles

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Solid-state NMR spectroscopy is well suited for investigating the nature of the molecular interactions in heterogeneous systems, such as organic-inorganic nanocomposites. For example, a recent study by Agarwal et al.[1] found evidence for hydrogen bonding at the interface in nanocomposites manufactured by polymerizing 4-vinylpyridine in the presence of an ultra-fine silica sol. In this presentation improvements in heteronuclear solid-state NMR correlation experiments which allow the interrogation of next-generation nanocomposite particles are described. A combination of state-of-the-art CRAMPS (in this case the windowless DUMBO-1 homonuclear decoupling sequence[2]) and a selective cross polarization scheme (in this case Lee-Goldberg CP[3]) is used to obtain high resolution proton-carbon-13 and proton-silicon-29 correlation spectra. The former can be used to assign the proton resonances in the polymer phase, while the latter allows the identification of the interactions responsible for adhesion to the silica surface. Colloidal "core-shell" nanocomposite particles were synthesized by in situ aqueous (co-)polymerization of styrene and/or butyl acrylate in the presence of a glycerol-functionalized silica sol. Polymer protons are found in close proximity (< 5 Å) to Q^3 silanol sites at the surface of the silica particles, indicating that either styrene or butyl side groups are entangled with the surface-functionalizing molecules. For the poly(styrene-co-*n*-butyl acrylate)/silica nanocomposite butyl acrylate co-monomers are preferentially located at the surface of the silica particle to the exclusion of styrene, suggesting a specific interaction between the butyl acrylate co-monomer and the functionalized silica surface. The most likely possibility is a hydrogen bond between the ester carbonyl and the glycerol functionalizing groups, although this cannot be observed directly.

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P374 (*)

Surface Enhanced NMR Spectroscopy by Dynamic Nuclear Polarisation

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Solid-state NMR is a powerful technique for the structural characterization of inorganic and hybrid materials, offering the possibility to directly investigate both the bulk, and the surface functionalities (adsorbates, grafted compounds or incorporated organic fragments for example). However the concentration of the NMR active nuclei often remains relatively low. This strongly limits the characterization power of solid-state NMR in surface chemistry. Here we show how high-field Dynamic Nuclear Polarization (DNP)[1] can be implemented to yield a significant increase (up to a factor fifty) in the NMR sensitivity of molecular organic functionalities of hybrid nanoporous materials. For ^{13}C CPMAS spectra, DNP enhancements between 10 and 50 could be observed for the NMR signals of the organic fragments, depending on the concentration and nature of the radical (TEMPO or TOTAPOL) dissolved in a 90:10 D_2O/H_2O solution.

Only minor line broadening was observed at the optimum carbon-13 enhancements. In order to extend the applicability of the method to water sensitive materials, the use of an aprotic solvent was investigated. Enhancement up to a factor 10 was observed using toluene. 2D $^{1}H–^{13}C$ correlation spectra acquired on surface organic fragments at natural abundance will be presented. They provide essential information for the detailed characterization of these mesostructured materials.

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P375

^{6}Li MAS NMR spectroscopy and first-principles calculations as a combined tool for the investigation of Li_2MnSiO_4 and Li_2FeSiO_4 polymorphs

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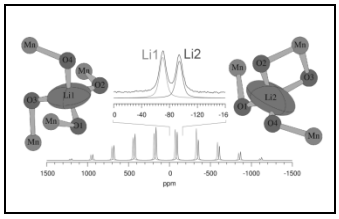
In the past two decades many new materials for positive electrodes in lithium ion batteries have been thoroughly investigated. In our laboratory two materials from a new family of transition metal silicates, Li_2FeSiO_4 and Li_2MnSiO_4, have been successfully prepared and preliminary tested. Although the primary motivation for preparation of these materials was their low price and safety, it was hoped that at least the Li_2MnSiO_4 analogue could open exciting new prospects in the search for high-capacity cathode materials.

Because Li_2MnSiO_4 and Li_2FeSiO_4 are prepared as powders, their structural analysis is difficult. Moreover, the as-prepared samples are most often mixtures of diffferent polymorphs, since the differences in formation energies of the latter are very small. In both cases, in case of Li_2MnSiO_4 and in case of Li_2FeSiO_4, at least three different polymorphs were isolated so far.

In this work we investigate polymorphism of Li_2MnSiO_4 and Li_2FeSiO_4 by solid-state NMR. We show that ^{6}Li MAS NMR spectroscopy is perfectly suited to investigate purity of Li_2MnSiO_4 and Li_2FeSiO_4 samples and to distinguish among different polymorphs. With the aid of first-principles calculations we relate isotropic shifts and spinning-sideband patterns observed by NMR spectroscopy to structural motifs in the polymorphs. In this way we verify the proposed structural models and demonstrate that first-principles calculations can reproduce NMR observables for periodic systems containing abundant paramagnetic centers.



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P376

Validation of the Villain's conjecture in Gd(hfac)$_3$NITEt fully frustrated XY helimagnet: a NMR study

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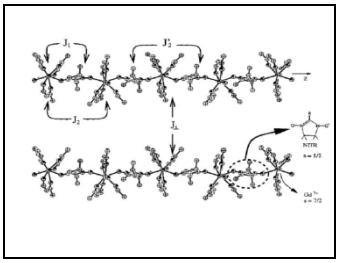
In this work we studied the quasi-1D molecular XY helimagnetic chain Gd(hfac)$_3$NITEt (in short Gd-Et) consisting of a regular pattern of Gd(hfac)$_3$ moieties (S_{Gd}=7/2) alternated to nitronyl-nitroxide organic radicals NITEt(s_{rad}=1/2)[1,2] and characterized by a strong magnetic frustration, because of the competition between n.n. and n.n.n. exchange interactions.[3] Gd-Et fulfils the Villain's conjecture,[4,5] as showed by MUSR, magnetic susceptibility and Specific Heat investigation.[6] To implement the study of spin dynamics, we present here a low temperature NMR investigation of Gd-Et performed through ^{1}H nuclear spin-lattice relaxation rate (NSLR) $1/T_1$ and ^{1}H absorption spectra measurements at low applied magnetic field H=0.1Tesla. The data respectively display a sharp peak and a sudden broadening of the linewidth at T~1.9K confirming the occurrence of the 3D helical spin phase transition at the expected temperature.



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P377

Efficient MAS ^{23}Na-^{29}Si HMQC with dipolar recoupling scheme and ^{29}Si-^{29}Si 2Q-1Q experiments for the spectral editing of multiphased systems

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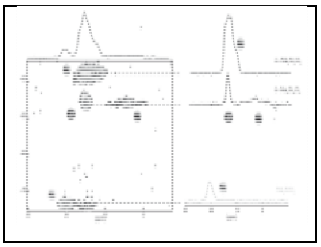
Several polymorphs have been observed during preparation of sodium silicates crystals,[1] thus the ^{23}Na MAS NMR spectra are very complicated due to the strong overlapping of the different signals. We propose a new technique to separate their respective contributions using a HMQC with $SR4_1^2$ recoupling scheme.[2,3] Therefore, three separate phases can easily be assigned[1,4,5] to the two polymorphs α and δ of sodium disilicate ($Na_2Si_2O_5$) and to the sodium metasilicate (Na_2SiO_3).



We also performed experiments on a glass of composition $16Na_2O$-$84SiO_2$ using homonuclear 2Q-1Q correlation with J-coupling and the dipolar recoupling scheme $SR26_4$.[6] It has been shown that this kind of glass undergoes phase separation[7] after heat treatment. Thanks to these correlation experiments on a ^{29}Si 100% enriched sample (Cortec) we see that phase separation has already started.

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P378

^{2}H MAS NMR study of metal-insulator transition in a conductive organic complex (EDO-TTF)$_2$PF$_6$

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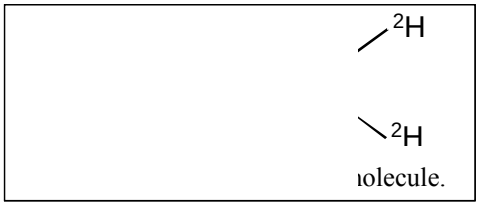
In order to study the mechanism of metal-insulator transition in (EDO-TTF)$_2$PF$_6$ at 280 K, where EDO-TTF means ethylenedioxytetrathiafulvalene, ^{2}H MAS NMR measurement were carried out in deuterated (EDO-TTF-d$_2$)$_2$PF$_6$ (Fig.1) for various temperatures from 250 K to 308 K. ^{2}H peaks corresponding to the conducting (-2 ppm) and insulating (7 ppm) phases were separately observed, and their area intensities varied with temperature, as shown in Fig.2. A model based on the percolation theory is proposed to explain the macroscopic conductivity from the temperature dependence of the domain fraction of the conducting phase.



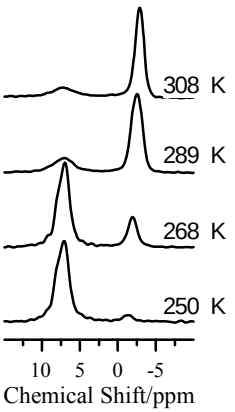


Fig.2. Temperature dependence of ^{2}H MAS NMR of (EDO-TTF-d$_2$)$_2$PF$_6$.

P379

Silicon pin solar cells investigated by multi-frequency EDMR

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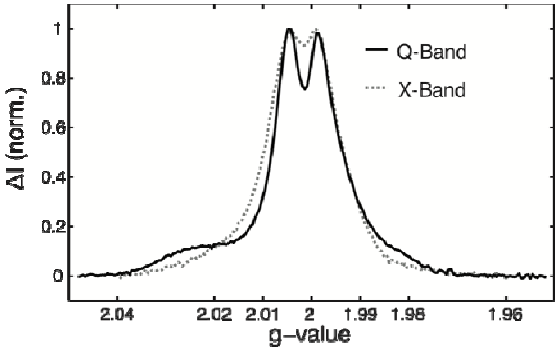
Electrically detected magnetic resonance (EDMR) can be used to investigate paramagnetic centres influencing charge transport in semiconductors even at concentrations well below the sensitivity threshold of conventional electron paramagnetic resonance (EPR). This technique measures conductivity changes in the sample that occur when spin transitions cause an enhancement or a quenching of currents. EDMR was e.g. successfully employed to microcrystalline Si pin solar cells in X-band (9.7 GHz) .[1]

We present the application of EDMR to Si pin solar cells at Q-band frequency (34 GHz). We could demonstrate a gain of spectral resolution (see figure). With multi-frequency EDMR we distinguished between field-dependent and field-independent interactions. Further, we realized EDMR in a non-resonant setup at 94 GHz (W-band) and will show first results.



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Acknowledgments: This work was supported by the German Federal Ministry of Education and Research (BMBF network project EPR-Solar 03SF0328C).

P380

Analysis of the ^{7}Li NMR signals in monoclinic $Li_3Fe_2(PO_4)_3$ and $Li_3V_2(PO_4)_3$

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The monoclinic $Li_3Fe_2(PO_4)_3$ and $Li_3V_2(PO_4)_3$ phosphates are materials for positive electrodes in Li-ion batteries. They also have interesting structures to test and improve the understanding of Li NMR signals in paramagnetic compounds. The position of such signals is governed by the transfer of electron spin density from the transition ion to the Li nucleus (Fermi contact shift). These mechanisms are based on delocalization and polarization effects which induce positive and negative shifts respectively.

We have characterized $Li_3Fe_2(PO_4)_3$ by Li NMR. To understand the signals observed, we have analysed the electron spin density transfer mechanisms: (i) by considering the geometry of the Fe 3d orbitals (distorted octahedral site and Li ions in three different sites), (ii) by using DFT calculations. We compare our analysis to the one very recently reported by Davis et al.[1] These analyses have been extended to $Li_3V_2(PO_4)_3$ studied by NMR by Cahill et al.[2,3]

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Acknowledgments: This work benefits from a grant by Agence Nationale de la Recherche (France) (ANR-09-BLAN-0186-01). Florent Boucher and Laurent Le Pollès are gratefully acknowledged for fruitful discussions, as well as Marie-Flora Coustou for technical assistance. Region Aquitaine is acknowledged for financial help for NMR equipment and M3PEC for computing facilities.

P381

A Multifrequency (170, 222.4, 331.2 GHz) EPR study of Fe^{2+} and Mn^{2+} in a $ZnSiF_6{\cdot}6H_2O$ single crystal at liquid-helium temperatures

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A liquid-helium temperature study of Fe^{2+} and Mn^{2+} ions has been carried out on a single crystal of Fe^{2+}-doped $ZnSiF_6.6H_2O$ at 35, 20, 17, 10 and 5K at 170, 222.4 and 333.2 GHz. The spectra are found to be an overlap of two magnetically inequivalent Fe^{2+} ions, as well as that of an Mn^{2+} ion. From the simulation of the EPR line positions for the Fe^{2+} (d^6, S=2) ion the spin-Hamiltonian parameters were estimated for the two inequivalent Fe^{2+} ions at various temperatures. From the relative intensities of lines the absolute sign of the fine structure parameters has been estimated. As well, the fine-structure and hyperfine-structure spin-Hamiltonian parameters for the Mn^{2+} ion, present as impurity at interstitial sites, were estimated from its hyperfine allowed and forbidden line positions.

P382

X_0 shim coil for presice adjustment of magic-angle

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For a precise and reproducible magic-angle adjustment system in solid-state NMR, we developed a shim coil which generates a homogeneous field along the x-axis against the external field of z-axis (Figure 1).[1] For better homogenity, we adopt a saddle-shape Helmholtz coil and wound it around the probe cap of a MAS probe. To chase the angle between the sample's spinning axis and the magnetic field, we simply alter the current applied to that coil. This realizes the angle adjustment without any backlash, which is associated with the conventional adjustment system using mechanical gears. The angle range confirmed by NMR observation of ^{13}C chemical shift anisotropy was within ±0.06 with applying ±7.2 A for our 7.05 Tesla magnet and the precision of the angle setting is ca. 0.00002. We call this coil as "X_0 shim coil" corresponding just as Z_0 shim coil. It'd like to be useful for any experiments which demand accurate magic-angle such as STMAS.[2]

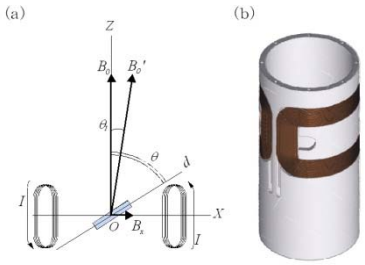

Figure1(a) Illustration of precise adjustment of magic-angle. B_0: external field, B_x: field produced by X_0 shim coil, Total field B_0' is adjusted with θ–θ_1.(b) Sketches of X_0 shim coil prototype made by a rectangular wire.

We examined the angle adjustment by obtaining CSA pattern of ^{13}C NMR. Even if the magnitude of the magnetic field along z-axis produced by the X_0 shim coil may be quite small rather than the external field of 7.05 Tesla, it would deteriorate the spectral resolution even at the order of ppm. We will show the calculational results of the dimensional parameters' dependence of the magnetic field along z-axis.

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2. Gan Z., *J. Am. Chem. Soc.*, 122, 3242 – 3243 (2000)

Acknowledgments: This work is financially supported by CREST in Japan Science and Technology Agency.

P383

Study of natural melanins by solid-state NMR

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Melanins are a class of pigments obiquitously found in the animal and plant kingdoms. They are associated with a variety of biological functions, such as pigmentation of skin and hair, photosensitization, photoprotection, metal ion chelation, camouflage, thermoregulation and free radical quenching. In addition, melanins have been observed to display interesting non-biological functions as insulators, semiconductors, ion-exchange resins, redox polymers, etc.[1] Despite their importance, natural melanins resist structural analysis by conventional spectroscopic techniques because of their substantial insolubility and opacity. Moreover, the extraction of melanins from the proteic matrix present in melanocytes is a very difficult task, typically ending up with leaving variable amounts of proteic fragments still linked to the melanoprotein, which indeed further limits its observability. Notwithstanding the almost unique possibility offered by solid-state NMR of investigating materials lacking long-range order in a non-invasive way, its use in the study of melanin structure is still quite sparse in the literature.[2] Here, we present preliminary results of the characterization of samples of natural melanins extracted from human hair by means of 1D and 2D solid-state NMR techniques. Insights into the morphology of the proteic and melanin domains are obtained with the help of high-resolution ^{13}C techniques, ^{1}H spin-lattice relaxation times measurements, ^{13}C dipolar filter and ^{1}H-^{13}C 2D-WISE experiments.

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Acknowledgments: G. M. gratefully thanks the European Commission for funding (Marie Curie IEF-FP7-PEOPLE-2008-237339).

P384

Structural and Orientational Determination of the Antimicrobial Peptide Phenylseptin-1 in Membrane-Mimicking Environment, Using Conjoint Solution and Solid-State NMR Techniques

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Phenylseptin-1 (Phes-1) is an antimicrobial peptide isolated from *H. punctatus*, an anuran species found in South America. Phes-1 has activity against both *Gram*-positive and negative bacteria,[1] but its mechanism of action is not fully understood. Hence the need to determine both its most probable structures and the way it interacts with membranes, in order to understand some of its biological functions.

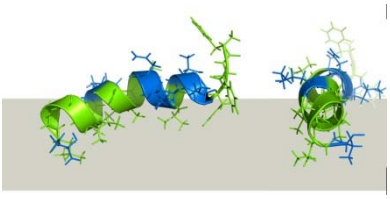

Figure 1- orientation of Phes-1 on the bilayer surface

For this work, NMR experiments were performed in both solution and solid in mechanically aligned POPC bilayers. The study of Phes-1 in solution was performed in the presence of DPC and the structures, calculated with geometric restraints derived from NOEs and chemical shifts. Through solid-state NMR, one could get the ^{15}N chemical shift and deuterium quadrupolar splitting to calculate the tilt and rotational pitch angle constraints, which determine the overall backbone orientation.[2] Fig. 1 shows the best fit for these calculated constraints.

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Acknowledgments: CNPq, FAPEMIG, CNRS, CNRMN.

P385

^{11}B NMR Studies on Phase-separated Sodium Borosilicate Glass

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[b]*National Institute of Advanced Industrial Science and Technology, Ikeda, Osaka 563-8577, Japan*
[c]*University of Hyogo, Himeji, Hyogo 671-2280, Japan *Present affiliation: Office of Society-Academia Collaboration for Innovation, Kyoto University, Yoshida-Honmachi, Sakyo-ku, Kyoto 606-8501, Japan*

To study micro-structure of phase-separated sodium borosilicate glass, which is a starting material for preparing porous glass, ^{11}B NMR under magic-angle spinning (MAS) is applied. So far, it has been postulated that a ^{11}B MAS spectrum of sodium borosilicate glass consists generally of four or five peaks; two peaks with appreciable second-order quadrupolar broadening are attributed to trigonal boron species and the other two or three without significant quadrupolar broadening are ascribed to tetragonal borons.[1] Further, one of the two trigonal boron sites has been attributed to boron in boroxol rings and the other one to boron in nonring sites.[1] In this study, we applied one dimensional (1D) ^{11}B MAS NMR experiments and two-dimensional (2D) ^{11}B-^{11}B homonuclear correlation experiment under MAS at 21.8 T. Fig.1 shows the observed ^{11}B-^{11}B 2D exchange spectrum of a phase-separated sodium borosilicate glass sample. The spectrum exhibits strong cross peaks between a trigonal boron at ca.14 ppm and a tetragonal boron at ca. 0 ppm , indicating that these borons are in close proximity. We assign these borons to those of borate structures, and further attribute these to the main components in the boron-rich phase, that is to be removed by acid leaching used in preparing porous glass.[2]

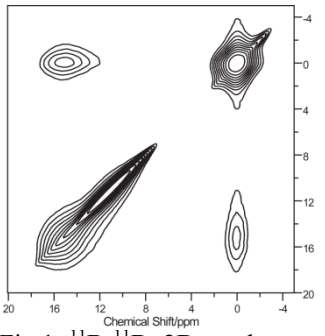

Fig.1 ^{11}B-^{11}B 2D exchange spectrum of phase-separated sodium borosilicate glass.

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P386

Planar Microresonators for Ferromagnetic Resonance Measurements of Nanoobjects

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Magnetic resonance experiments are based on the interaction between magnetic moments (spins) in the sample and applied microwave magnetic fields. This interaction is relatively weak. Even if in the ferromagnetic samples almost all spins are polarized, thus increasing the total magnetization of the sample, about 10^{12} spins are necessary for detection. These detection limits are too high for measuring single nanostructures. We increase the sensitivity of the inductive detection by using small resonators for excitation and detection, which concentrate the microwave field in the sample region. They provide efficient conversion of microwave power into magnetic field and, conversely, an efficient conversion of magnetic flux from the precessing magnetization into a traveling electromagnetic field to be detected by the spectrometer.

The resonators with loop diameter of 20µm are produced by electron beam lithography, followed by the evaporation of the metallization layer. The same procedure is used for placing the single 5x0.5x0.05µm^3 permalloy ($Fe_{0.2}Ni_{0.8}$) stripe into the resonator. The achieved limit of detection is about $3 \cdot 10^7$ spins, or, in terms of minimal detectable magnetic moment, $2 \cdot 10^{-16}$Am^2. The measured spectra are in good agreement with theoretical simulations. The planar technology used for the production of the microresonator and the sample placing, allows for further miniaturization of the resonators loop. From our simulations, the detection limit should decrease linearly with the loop diameter. Reducing the loop size down to 2µm and keeping the noise at the thermal noise level would allow to detect FMR signal of ~10 nanocubes with ~10nm edges.

Acknowledgments: Financial support by DFG (SU-192/18-2) is acknowledged.

P387

Magnetization Transfer among the Backbone ^{15}N Spins in Macroscopically Aligned Proteins for Spectroscopic Assignment and Distance Information

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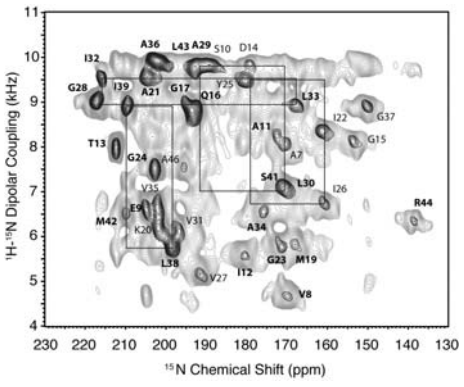
Magnetization transfer among the dilute spins can be achieved using a highly coupled proton network. This effect can be observed in both rotating[1] and static[2] samples. The recently developed methods for macroscopically aligned systems based on mismatched Hartmann-Hahn (MMHH) magnetization transfer[2,3] have been applied to the uniformly ^{15}N labelled Pf1 phage protein. Using the MMHH method, cross peaks have been built up for nearly all adjacent ^{15}N spins of the backbone. Notably, a 20% optimal mismatch was found to induce the crosspeaks for the adjacent ^{15}N spins; whereas for the more distant ^{15}N spins in a NAL crystal, (separated by as much as 6.7 Å as opposed to about 2.8 Å for the ^{15}N spins in a protein backbone) a 10% optimal mismatch amplitude was found[2,3]. This may allow one to deduce distance information from the magnitude of the optimal mismatch. Detailed many-body simulations (involving as many as 12 spins) have been performed using the coordinates of the previously published structure[4] (pdb ID 1ZN5) for short- and long-range transfers throughout the Pf1 backbone, which support the experimental observations. Moreover, the MMHH method can serve as a purely spectroscopic means of assignment for solid-state NMR spectra of macroscopically aligned proteins.



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Acknowledgments: We wish to thank Prof. S. J. Opella for providing the Pf1 sample. Supported by grants from NC Biotechnology Center and NSF.

P388

Solid-State NMR investigations of Paramagnetic Jarosites ($KB_3(SO_4)_2(OH)_6$), B = V(III), Cr(III), Fe(III))

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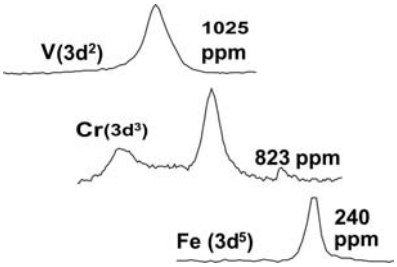

Solid-state NMR (SSNMR) studies of paramagnetic samples give detailed insight into the local environment around the NMR nuclei investigated. This can be used to probe how structural defects affect the magnetic properties and the nature of binding between e.g., a molecule and a paramagnetic surface or a biomolecule. However, interpretation of the paramagnetic shift is not straightforward and the modelling of these open shell systems is a challenge.

The isostructural series of jarosites with V(III), Cr(III), and Fe(III), have a very similar local environment around the B ion. Thus, they represent an excellent model system for studies of how the electronic structure affects the SSNMR spectrum. The main difference between the three jaorosites is their d-electron configuration, which is $3d^1$, $3d^2$, and $3d^5$ for V(III), Cr(III), and Fe(III), respectively resulting in quite different magnetic properties and ^{2}H paramagnetic shift (see figure). This work focuses on interpretation of the ^{2}H MAS NMR of these jarosites and relating them to the electron configuration of the transition metal as well as local structural and magnetic properties. Variable temperature ^{2}H MAS NMR studies will be presented and linked with results from susceptibility measurements.

P389 (*)

^{1}H-^{14}N 2D solid-state NMR under very fast MAS: A few minutes observation for a sample less than 1 mg

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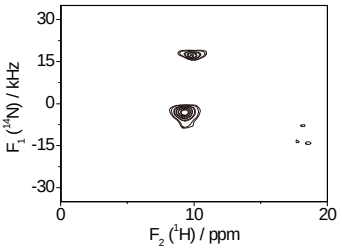
Sensitivity and resolution enhancement of ^{1}H detected ^{14}N HMQC NMR in solid state is presented. Several works have been published on ^{1}H-^{14}N HMQC under moderate MAS.[1] Recently we have developed very fast MAS modules up to 80 kHz.[2] This very fast MAS has following advantages: 1) Elongated ^{1}H T_2 relaxation time, leading to sensitivity and resolution enhancement in both direct (^{1}H) and indirect (^{14}N) dimensions. 2) Wide spectral width of the indirect dimension collected rotor-synchronous manner. 3) High sensitivity per unit volume. 4) Strong ^{14}N rf field, leading to efficient excitation. 5) Efficient ^{1}H-^{14}N heteronuclear decoupling which is not perferctly accomplished by a simple π pulse at the middle of the indirect dimension.



^{1}H-^{14}N HMQC spectrum of a small peptide, glycyl-L-alanine under 70 kHz MAS is shown. The sample volume is 0.8 μL. Sixteen rotor-synchronized t_1 points of 2 scans each were collected. Heteronuclear dipolar recoupling of $SR4^2_1$ is applied[3] to the excitation/reconversion intervals. We will also present various applications of this new technique including several silks.

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Acknowledgments: This work has been supported by SENTAN JST.

P390

^{129}Xe NMR Study of the Framework Flexibility of the Porous Hybrid MIL-53(Al)

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Among metal-organic framework compounds the MIL-53 family (MIL standing for Materials of Institut Lavoisier) have been reported as very promising candidates for CO_2 capture and storage. Their porous structure, consisting of one-dimensional channels, changes from an open form (large pores, lp, 8.6×8.6 Å^2 cross section) to a closed form (narrow pores, np, 2.6×13.6 Å^2 cross section) and back upon adsorption of some gases (H_2O, CO_2, certain hydrocarbons), but not others (H_2, CH_4). This phase transitions can be easily detected in the sorption isotherms by the presence of two steps with hysteresis.

We have shown, that xenon is also able to induce a structural transformation of MIL-53 materials between a (lp) to a (np) form. The Xe adsorption isotherms show two steps corresponding to lp/np and np/lp double structural transition depending on temperature and pressure.

^{129}Xe NMR has been used to study this transition induced by the adsorption of xenon. Two lines corresponding to xenon in lp and np pores are observed at xenon pressures above 400 Torr. Line np features the anisotropy of the chemical shift due to single file diffusion regime of xenon atoms in the narrow pores. As xenon adsorption increases, the intensity of line np increases at the expense of that of line lp but the latter does not disappear proving that the structural transformation from lp to np form is not complete. Further increase in pressure leads to reemergence of the line a due to np/lp transition.

Estimation of the extent of structural transformation as a function of pressure shows that at 298 K the lp-np transformation rate reaches a limit value above ca. 600 Torr when about 30% of the channels remain open.

P391

Surfactant assembly characterization via ^{13}C natural abundance solid-state NMR

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Amphiphilic molecules and water form well-characterized, water-rich phases such as micellar solutions and liquid crystals, but also water-poor solid phases like crystals, hydrates, and glasses. The latter are technically, pharmaceutically, and biologically important, for example in washing powders, tablet formulations, and the lipids in the outer layer of the human skin. In all of the mentioned cases, the water content is determined by equilibration with a more or less humid atmosphere, which often leads to a coexistence of liquid and solid phases - a regime so far notoriously difficult to fully characterize. Liquid-state NMR is a powerful tool for the investigation of the water-rich regime, because obtaining the spectra with good resolution is relatively simple. However, the solid or anisotropic phases result in broadened signals and only the advancement of the technical possibilities of fast MAS has made high resolution, solid-state NMR achievable. Furthermore, the study of natural abundance ^{13}C samples has been made easier with the invention and perfection of polarization transfer techniques and heteronuclear decoupling schemes. Consequently, the study of low-water regime on molecular level has become possible. In this work a combination of three NMR experiments has been designed and tested as a potential phase identification tool.[1] The Direct Polarization (DP) experiment provides the information about all the ^{13}C nuclei in the sample, while the CP and refocused INEPT provide selective information, on molecular level, about the solid and fluid parts of the sample. Combining the spectra from the three experiments is enough to differentiate between solid crystalline (with differences in the case of different hydrates), solid amorphous, anisotropic liquid crystalline and isotropic phases. Phase coexistence and glass transition can also be identified in the samples. The method has been thoroughly tested on a series of CTA complex salts and further applied to various systems. In all the systems phase recognition was possible and in some phase coexistence was observed. Differences between different hydrates (in the same system) were also clearly seen and so was the glass transition.

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P392

Low temperature ^{1}H NMR measurements of the molecular magnet F2PNNNO

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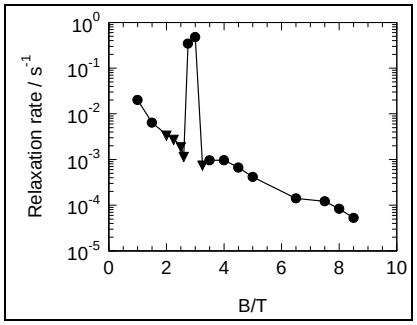

The spin-lattice relaxation rate of protons in the molecular magnet F2PNNO has been measured at very low temperature. This work was motivated by the question of whether significant nuclear spin polarization could develop in samples held at very low temperatures in a high magnetic field, in the timescale of a typical neutron scattering experiment (a few days).[1] One feature of some molecular magnets is the appearance of level crossings at particular values of applied magnetic field strength, where the various magnetic energy levels cross.[2] At level crossings, spin lattice relaxation is enhanced which therefore leads to rapid spin polarization. We studied a single crystal of F2PNNNO in magnetic fields of 1.5 to 8.5 T, using a broad-band NMR spectrometer at temperatures between 1.8 K and 400 mK. Although we could achieve much higher B/T ratios, the relaxation rate was practically too slow to be measured. As a function of field at fixed temperature (1550 mK), we observe a rapid increase in relaxation rate by a factor of over 100 at around 2.9 T (see figure). We ascribe this feature to the S=0/S=1 level crossing. However, even with this increase in relaxation rate, the time to polarize at 2.9 T and lower temperature becomes very long, and further, the situation is exacerbated at higher field as the dependence is at least B^2. We were able to perform a rudimentary search for other level crossings up to 13 T by field cycling, but no evidence for others was found.

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Acknowledgments: We would like to acknowledge A. Zheludev for providing the sample studied.

P393

Phase Solitons in the Spin Ground State of Overdoped Manganites. Direct NMR Evidence

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The role of stripes in the electronic and magnetotransport properties of hole-doped transition metal oxide (TMO) compounds, such as high T_c cuprates, nickelates and manganites[1,2] is a central issue in the physics of strongly correlated electron systems.

An important controversy in the physics of these systems is whether the charge ordered ground state in overdoped manganites, an important sub-class of TMO materials, is a regular arrangement of charge stripes, or (according to latest experiments) a uniform incommensurate charge and spin density wave. A clarification of this fundamental question and the examination of its relevance with the stripe phase in high T_c cuprates and nickelates might have important consequences on our basic understanding of the stripe phase in hole-doped TMO materials. At the same time, recent theoretical works predicted that the ground state in overdoped manganites is modulated by an incommensurate (IC) orbital and charge soliton lattice. However, no experimental evidence for this important prediction has been reported so far. In this work, we present latest Nuclear Magnetic Resonance (NMR) results,[3] which provide direct evidence that the spin ground state in La based overdoped manganites is IC modulated with phase solitons. At higher temperatures the solitonic superstructure is replaced by a uniform spin density wave, subjected to coherent slow fuctuations, which show a striking similarity with slow fluctuations in the striped phase of high T_c cuprates and nickelates.

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P394

^{13}C and ^{15}N CPMAS NMR Studies of Biologically Active Alkaloids from *Uncaria tomentosa*

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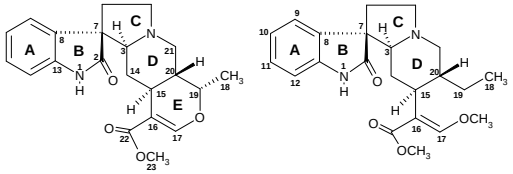
Oxindole alkaloids, isolated from the bark of *Uncaria tomentosa* [Willd. ex Schult.] Rubiaceae, are considered to be responsible for the biological activity of this herb. Five pentacyclic and two tetracyclic alkaloids were studied by solid-state NMR and theoretical GIAO DFT methods. The ^{13}C and ^{15}N CPMAS NMR spectra were recorded for mitraphylline, isomitraphylline, pteropodine (uncarine C), isopteropodine (uncarine E), speciophylline (uncarine D), rhynchophylline and isorhynchophylline. Theoretical GIAO DFT calculations of shielding constants provide arguments for identification of asymmetric centers and proper assignment of NMR spectra. These alkaloids are 7*R*/7*S* and 20*R*/20*S* stereoisomeric pairs. Based on the ^{13}C CPMAS chemical shifts the 7*S* alkaloids (δ C3 70-71 ppm) can be easily and conveniently distinguished from 7*R* (δC3 74.5-74.9 ppm), also 20*R* (δC20 41.3-41.7 ppm) from the 20*S* (δC20 36.3-38.3 ppm). The *epiallo*-type isomer (3*R*, 20*S*) of speciophylline is characterized by a larger ^{15}N MAS chemical shift of N4 (64.6 ppm) than the *allo*-type (3*S*, 20*S*) of isopteropodine (δN4 53.3 ppm). ^{15}N MAS chemical shifts of N1-H in pentacyclic alkaloids are within 131.9-140.4 ppm.

Fig. 1



The calculations were performed in the Interdisciplinary Center for Mathematical and Computational Modeling (ICM) at Warsaw University under the computational grant G14-6.

P395

Solid-state NMR Investigation of Solvent/Block Copolymer/Silica Surface Interactions on the Formation of Mesoporous Silica

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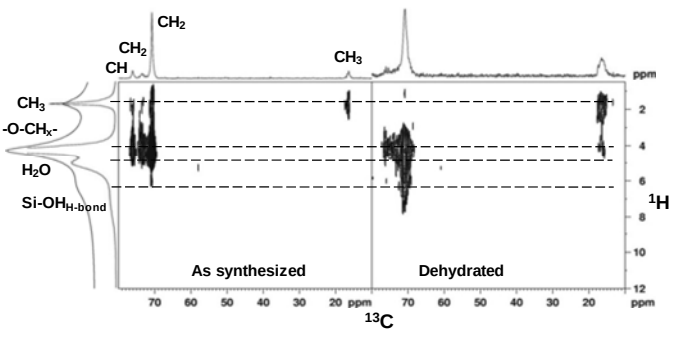
The structure and dynamics of block copolymer Pluronic F127 and solvent, water, in a mesoporous silica (SBA16) are characterized by multinuclear multidimensional solid-state NMR spectroscopy. The combination of chain motion and fast magic angle spinning averages the proton-proton dipolar interactions of the block copolymer chains in the as synthesised SBA16. However, selective dehydration of the material resulted in a confined segmental motion of the copolymer chains within the mesoporous channels. ^{1}H and ^{13}C spectra of the dehydrated sample showed considerable peak broadening, indicative of decreased mobility, while the 2D HETCOR NMR suggested non-covalent interactions between the copolymer and silica surface. Insights from the solid-state NMR investigations on the formation, structure and dynamics of this complex system lead to the engineering of mesoporous materials for wider applications.



P396

Dynamic correlations between susceptibility gradients and T_2-relaxation as a probe for wettability properties of liquid saturated rock cores

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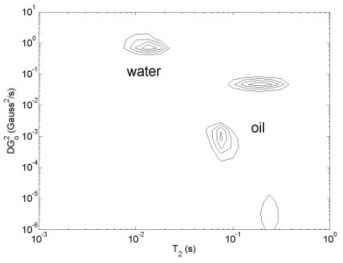
The oil/water wettability is an important property of rock core samples from oil reservoirs. The dynamic behaviour (relaxation and diffusion) of the NMR signals from liquids confined in such rock core samples is sensitive to surface-interactions and can potentially be used for characterization of wettability properties.[1] We explore the use of correlations between susceptibility gradients and T_2-relaxation (G_0-T_2) as a method for characterization of wettability.[2] We compare the G_0-T_2 correlations to regular T_2 measurements, and to measurements of diffusion-T_2 (D-T_2) correlations. The measurements were performed on a Maran DRX 12 MHz spectrometer. The sample studied was a water-wet sandstone rock core. T_2, G_0-T_2, and D-T_2 measurements were compared in water-saturated and partly-oil-saturated states. Compared to the other measurements, G_0-T_2 correlations are more sensitive to differences in surface interactions between oil and water (Figure: oil-saturated state). The G_0-T_2 measurement is therefore very promising as a probe for wettability properties of liquid saturated rock cores.



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Acknowledgments: We are thankful to Dr. Ketil Djurhuus at CIPR, University of Bergen, for assistance in the preparation of the rock core.

P397

Dynamic Nuclear Polarization at 263 GHz: Experimental Methods and Applications

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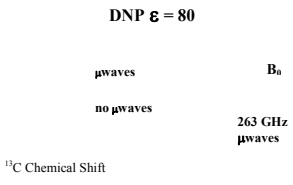
Dynamic Nuclear Polarization (DNP) can be used to substantially increase the sensitivity of NMR experiments by transferring the higher Boltzmann polarization of unpaired electron spins to nuclear spins. The polarization transfer is accomplished by irradiation of unpaired electrons with microwave at or near the electron Larmor frequency. We have developed a spectrometer for DNP experiments at 263 GHz microwave frequency, 400 MHz ^{1}H frequency, of solids and have measured signal enhancements up to a factor of 80 at 100 K using the biradical TOTAPOL.[1] Microwaves are generated with a high power gyrotron, transmitted to the NMR probe via corrugated waveguide, and irradiated onto the sample in a 3.2 mm rotor for magic angle spinning DNP-NMR experiments. This contribution focuses on DNP transfer efficiency and applications to biological solids. DNP signal enhancements have been measured as a function of sample temperature, microwave power, and sample preparation parameters. The nuclear and electron relaxation times have also been investigated for insight into the dependence of DNP efficiency as a function of temperature. Additionally, a range of samples have been successfully polarized including small peptides, soluble proteins, membrane proteins, and large biological complexes.



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P398

A solid-state NMR study of molecular properties of specific pharmaceutical materials based on solid solutions and dispersions of active pharmaceutical ingredients in polymer matrix

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In this contribution a solid-state NMR study of structure and segmental dynamics of solid dispersions and solid solutions of active pharmaceutical ingeredients (API) in polymer matrix is presented. In many clinical studies it has already been demonstrated that higher efficiency of APIs significantly reduces menace of many diseases. Unfortunately a lot of pharmaceutical substances exhibit low solubility in water. That is why current pharmaceutical research focuses on increasing solubility and thus also bioavailability of these substances.

Among many procedures how to improve dissolution rates of poorly water-soluble drugs, the transformation from their crystalline state to more soluble amorphous or nanocrystalline solid dispersion and/or solid solution represents one of the most promising ways.

In our work we focussed our attention on the study of structural properties of APIs in the prepared solid polymer dispersions exhibiting increased solubility. As typical models of APIs with relatively high solubility we used L-ascorbic acid, while ±α tocopherol nicotinate and acetylsalicylic acid represented model compounds with low solubility. Several procedures were used to combine these model compounds with polymeric nontoxic water soluble matrix (e.g. PEG, PVP etc). In some cases the observed drug-polymer interaction significantly enhanced dissolution rates of the APIs. Structural reasons of the increased solubility in amorphous solid dispersions were subsequently probed by wide range of ^{13}C CP/MAS NMR, ^{13}C-^{1}H HETCOR and relaxation experiments. The obtained results were correlated with morphology of the systems monitored by Raman microscopy.

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P399

New Experimental Facility for Broadband Solid State NMR and NQR in Zagreb, Croatia

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Broadband NMR spectroscopy of correlated electronic systems is nowadays a key experimental technique for determining fundamental properties of new materials applicable in the growing variety of new technologies.

We present here a newly established laboratory for broadband NMR/NQR spectroscopy at the University of Zagreb (http://nmr.phy.hr) (Zagreb, Croatia). The laboratory is established and equipped within the European FP7 project SOLeNeMaR[1] and with the support of Croatian Ministry of Science, Education and Sport.[2]

At present, the laboratory is equipped with two Tecmag Apollo spectrometers and an Oxford Instruments 12 tesla wide-bore sweepable magnet of medium homogeneity (10 ppm/cm^3). The spectrometers cover frequency range 0.5 – 500 MHz and they are equipped with pulsed 1 kW amplifiers covering the whole frequency range. Cryostats cover temperature range 1.5 K – 400 K with sample space 6.25 cm in diameter, both for zero field measurements (NQR) and for measurements in magnetic field (NMR).

The laboratory is open to proposals for scientific cooperation.

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P400

Measurement of Heteronuclear Dipolar Couplings - A Study of Cross Polarization Dynamics for Separated Local Field Experiments

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Separated local field (SLF) NMR experiments are used extensively for the study of structure and dynamics of oriented molecules. One of the popular SLF techniques is the use of spin evolution under dipolar coupling during cross polarization transfer which gives rise to the dipolar cross peaks in a 2D experiment. This for a two spin system, can be understood to arise from evolution under mutually commuting zero and double- quantum subspaces. Under the conditions of exact Hartman-Hahn match, the evolution in the zero-quantum sub space contributes to the dipolar cross peak while the evolution in the double-quantum sub-space contributes only to the axial-peak. Increasing the cross peak intensity enhances the sensitivity of the experiment, while suppression of the axial-peak enables resolution of cross-peaks arising from small dipolar couplings. This requires that the initial density matrix is essentially of zero-quantum in nature which can, for example, be achieved by Polarization Inversion (PI). Subsequently, the dipolar oscillations are monitored with the removal of the homonuclear dipolar couplings by a suitable decoupling scheme. In this presentation different schemes for initial polarization are considered. The experiments have been carried out on simple oriented systems and the relative cross-peak to axial-peak intensities in all the cases have been obtained. Along with the simple CP pulse sequence other pulse sequences namely CP with polarization inversion (PI-CP), equilibrium X-nuclear- polarization enhanced cross-polarization (EXE-CP) and PISEMA have been considered. Experiments on oriented liquid crystalline samples have been carried out and the performance of the different schemes has been compared. Other possible approaches such as the use of adiabatic cross-polarization are also indicated. The information obtained from the present study is expected to be useful for optimizing and improving methods used for the measurement of heteronuclear dipolar couplings in static oriented samples.

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Structural Investigation of Metal-Organic Frameworks with Solid-State NMR Spectroscopy

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Metal-organic frameworks (MOFs) are a new class of porous solids[1,2] that consist of metal ions linked together by organic binding ligands. In the last decade they have been growing increasingly popular because of their vast potential in a variety of applications[3] such as heterogeneous catalysis, gas separations and storage, heat storage, drug delivery, etc.

We used ^{1}H, ^{13}C, ^{27}Al and ^{25}Mg solid-state NMR spectroscopy as a supplement to X-ray diffraction for determining the structure of new type of MOF materials when the samples were poorly crystalline or totally amorphous. Moreover, ^{1}H NMR spectroscopy enabled us to specify the amount of water molecules adsorbed in pores of the material, since they are invisible to XRD.

Further, we used NMR spectroscopy to observe the framework formation and crystal growth of some well known Al and Mg MOF materials. We were able to observe the assembly of individual building blocks into coordination polymers during the synthesis procedure. NMR investigation of MOF structures was also combined with other spectroscopic techniques such as X-ray absorption spectroscopy to get a further insight in the immediate surroundings of different metal ions.

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P402

Solid State Nuclear Magnetic Resonance Study of Apatite Oxide Ion Conductors

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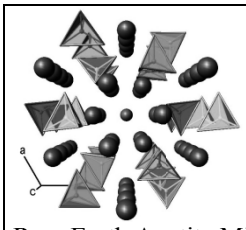
Materials displaying high oxide-ion conductivity have attracted considerable interest due to technological applications in solid oxide fuel cells (SOFC), oxygen sensors and separation membranes.[1] ^{17}O solid state NMR data have been recorded for the apatite series $La_{8+x}M_{2-x}(GeO_4)_6O_{2+x/2}$ ($0 < x < 1.0$). For x = 0 a single NMR resonance is observed at a chemical shift of ~δ 175 ppm; as the La:M ratio is raised the interstitial oxygen content also increases and a second chemical shift at ~δ 300 ppm is observed. This has been attributed to the formation of a GeO_5 unit via the presence of O interstitial species.


Rare Earth Apatite Minerals

An increase in intensity of the low field resonance is observed with increasing x, which is thus consistent with an increase in oxide ion content. These data have been used to predict the number of GeO_5 units and Frenkel-type disorders.[2] The increased intensity in this low field peak is shown to correlate with enhanced conductivity. ^{17}O labelling shows bias towards the GeO_4 and interstitial oxygen speciation, and not the two channel oxygen's thus suggesting that the route of conductivity is due to the mobility of the oxygen's around the germanium centres. Hence, ^{17}O solid state NMR has given an insight into the conduction pathway and environment of the varying oxide-ion conductors.

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Structure analysis of heterogeneous catalysts by multinuclear solid state NMR

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Synthesis of crystalline porous aluminosilicates and mesoporous silicas remain the most promising area in heterogeneous catalysis related research for the past several decades.[1] A number of innovative recipes with novel approaches have been reported under the theme of hydrothermal synthesis. In this work we will discuss the textural features of three different synthesized catalysts based on aluminum-silicates (MCM-22) and mesoporous silicas (HMS and SBA). The two silicas were prepared with distinct organic precursors (DDA and P-123). The samples were characterized concerning their textural and structural properties using physisorption measurements and x-ray diffraction besides the MAS NMR. All solid state MAS NMR spectra were recorded on a Varian Infinity-Plus 400 spectrometer and the ^{13}C, ^{27}Al and ^{29}Si MAS experiments were carried out under appropriated quantitative conditions.[2]

The ^{29}Si spectra showed five characteristic resonance peaks at -92, -97, -100, -110 and -120ppm, designated Q^0, Q^1, Q^2, Q^3 and Q^4 according to the number of OSi groups bounded. It was shown that Q^3 species seems to play the major contribution to the MCM-22 performance. In the other hand, Q^0, Q^1, Q^2 are the important defects for the SBA while Q^0, Q^3 for the HMS activities. The ^{27}Al spectra displayed two tetrahedral sites at 56ppm for all MCM-22 samples. It was observed that organic templates remaining after thermal treatment as detected by ^{13}C MAS spectra. This fact has added an important understanding of the systems route synthesis and performance.

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P404

Homonuclear decoupling for high-resolution proton solid-state NMR with very fast MAS

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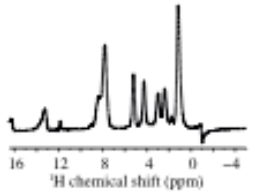
Protons are the nucleus of choice for Nuclear Magnetic Resonance studies due to their high natural abundance and high magnetogyric ratio. Yet, the dense dipolar-coupled network of protons results in spectral line broadening in most solids, obliterating the possibility of obtaining high-resolution chemical shift spectra. Radio-frequency field irradiation sequences have been specifically designed to remove the proton homonuclear dipolar couplings. Combined with Magic Angle Spinning (CRAMPS), these decoupling sequences are efficient for rigid crystalline samples.

However, there is still a need for better proton homonuclear dipolar decoupling sequences to make use of the ^{1}H NMR information for larger molecules. We show that the high spinning rates available today (70 kHz) can be successfully combined with decoupling sequences designed in a static framework (DUMBO) or at moderate MAS rates (eDUMBO-1_{22}). We also present a new decoupling scheme, which was developed by experimental screening of random starting points, followed by experimental optimization of the best candidates, directly using windowed acquisition of ^{1}H NMR spectra with a very fast MAS rate of 65 kHz. Line widths of 150 Hz (0.3ppm at 500 MHz) can be obtained for ultra-fast spinning probes on a range of fields (500, 800 and 1000 MHz). Experiment and calculations support the hypothesis of a joint radio-frequency and MAS averaging regime, where the large scaling factor contributes significantly to the overall performance of the decoupling.



^{1}H spectrum acquired at 800 MHz using the eDUMBO-PLUS sequence.

P405

Distance estimations in switched-angle spinning solid-state NMR

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We present a spin-echo based experiment which combines the selection of desired spherical tensors[1] with frequency-selective pulses and switched-angle spinning to enable the robust estimation of internuclear distances.[2,3] Experiments are conducted using a DOTY switched-angle spinning probe which permits a precise mechanical switching of the rotation angle up to 20° off the magic angle, along with independent measurement of the spinning angle via a Hall-effect device.[4]

Spherical tensor selection is introduced into the pulse sequence by rotations around an appropriate set of Euler angles. This is accomplished by converting the Euler angles into a set of phases that can be applied to the pulse sequence. The effect of the inserted rotation is a modulation of the general spherical signal component according to the corresponding Wigner matrix element. The desired internuclear distance can then be chosen by carefully calibrating the frequency-selective pulses. Subsequently, the experiment is carried out at several spin-echo evolution times, which yields characteristic build-up curves for each selected component of the NMR signal. This build-up is sensitive to the dipole-dipole coupling strength; a phenomenon that we demonstrate may be exploited for reliable internuclear distance estimation.

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P406

Polymers under mechanical stress- an NMR investigation

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Local order and dynamics in polymers under mechanical stress is studied by low-field NMR. Permanent magnets in a Halbach arrangement permit NMR investigation without the limits present in high-field NMR. In particular the confined stray field permit the application of NMR in a stretching apparatus and a rheometer. The major drawback of low-field NMR, the lack of chemical shift resolution, is not a problem, because in the study of known materials properties other than their chemical composition are of interest.

The crystalline and amorphous fractions of semicrystalline polymers are distinguished by their transverse relaxation times. Under mechanical load there is a significant shortening of the transverse relaxation time, which partially relaxes with time, when the load is kept constant.

Mechanical load on elastomers results in partial chain ordering and consequently reduced chain mobility. The resulting stronger residual dipolar couplings show up the stronger buildup of double quantum coherences and in a shortening of the slower component of the transverse relaxation time.

The interaction with paramagnetic moieties in the fillers in polymer nanocomposites has a strong impact on the longitudinal relaxation time. Delaminating filler particles under mechanical stress results in a shorter T_1 of the protons in the polymer, because the contact area between the filler and the polymer increases.

P407

Solvent-swelling in polymer dispersions – novel insights from spectrally resolved studies

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In a recent publication,[1] acetone-induced swelling of polyurethane (PU) dispersions was studied by means of low-field TD-NMR. The information from the relaxation times measured in dispersions spiked with different quantities of acetone suggested various stages of the swelling process which were attributed to complete incorporation of acetone into the particles and to distribution of acetone between the particles and the continuous phase.

The same type of polymer dispersions was now studied by means of low-field medium resolution (MR)-NMR spectroscopy and by high-field spectrally resolved pulsed field gradient (PFG) NMR diffusometry. In all cases, the acetone added to the dispersions could be spectrally well resolved and the integration of the acetone line in the MR-NMR spectra showed a good linear relationship between the peak area and the amount of added acetone. As different acetone populations were expected on the basis of the relaxation time findings, this result was quite surprising. This lead to further studies involving PFG NMR on both acetone-spiked dispersion samples and serum samples produced by centrifugation. In these experiments, indications for a partial dissolution of the particles due to acetone addition were found. Furthermore, surprisingly high acetone-diffusion coefficients were found even for those samples in which the relaxation results suggested complete incorporation of acetone into the particles. Taking all the results together, a more detailed model of the acetone-containing dispersions is suggested in which dissolution effects and the formation of transient co-continuous networks of acetone-rich polymer particles are used to explain the findings.

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P408

Resolution improvement in protein MAS solid state NMR via rotor-synchronized spin-echo pulse sequence

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Application of the solid-state NMR spectroscopy to characterize structure and dynamics of immobilized biological macromolecules becomes more attractive. Improving resolution is a necessity for a progress in the field. Recently we used a strong level of deuteration of the alpha-SH3 domain to obtain high resolution proton detected spectra without high power decoupling. This approach resulted in spectra possessing line widths of ca. 10 Hz and 20 Hz in the ^{15}N and ^{1}H dimension, respectively. Analysis of the dynamics of this protein[1] shows that local correlation times and motional amplitudes are highly similar in both solid and solution states. One would expect that the line width in the solid state should be smaller compared to the line width obtained in the liquid phase. However this expectation is in a strong contradiction with experimental facts. Our aim is to identify the origin of this broadening and to find an experimental approach to eliminate these factors and improve resolution. It has been shown before that application of a Carr-Parcell-Meiboom-Gill (CPMG) pulse train during free induction decay in solids can efficiently refocus inhomogeneous contributions and dramatically narrow the resonance line width.[2] We followed this approach to remove possible residual dipolar couplings, CSA and the anisotropy of the magnetic susceptibility. We applied a CPMG pulse train on the ^{15}N channel in 2D proton detected ^{15}N,^{1}H correlation experiments. The approach was applied to highly deuterated microcrystalline sample of the alpha-SH3 domain on a spectrometer operating at ^{1}H Larmor frequency of 400 MHz and a MAS frequency of 20 kHz. On average, the ^{15}N line width was decreased by a factor 1.5. Currently we continue to collect experimental data to establish the efficiency of this approach at different experimental conditions.

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P409

Structural evolution in glassy arsenoselenides

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Solid state ^{77}Se (I = 1/2) NMR spectroscopy was applied to verify chemical ordering evolution during physical ageing in vitreous v-As_xSe_{100-x} (x=10, 18, 23, 30, 40) prepared by conventional melt-quenching. All measurements were carried out at room temperature on ASX 300 Bruker spectrometer operating at 57.3 MHz with a 2.5 mm Magic Angle Spinning probe rotating at 22 kHz. Due to breadth in NMR lines, a Hahn spin echo sequence was applied to refocus whole magnetization. The recycle time was 30 s in view of slow longitudinal relaxation. The obtained experimental spectra were simulated using the Dm2000nt version of the Winfit software.

Only three NMR lines with character chemical shifts at about 860, 580 and 380 ppm were recorded in the studied glasses, they being attributed to different Se sites (–Se–Se–Se–, –Se–Se–As= and –Se–As–Se–, respectively). The relative intensities of these lines extracted from Gaussians fitting are in good accordance with XPS data, testifying in a favour of "chain crossing" structural motives, which dominate in a whole range of Se-rich glass compositions from stoichiometric As_2Se_3 to pure Se. No any evidences for double-bond anomalies such as optimally-constrained quasi-tetrahedral $Se(AsSe)_{3/2}$ structural units were found. This implies incorrect assignment of $29<x<37$ compositional range showing margin non-reversing heat flow in temperature-modulated DSC[1] to possible reversibility window in this system, but is in full agreement with our previous conclusion on optimally-constrained isostatically-rigid glassy network near x=40.[2]

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P410
^{13}C CP MAS NMR characterization of thiophene-based copolymers

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Two π-conjugated copolymers have been analyzed by means of solid state ^{13}C CP MAS NMR :
a) Copolymer –(Tf-Cbz-Tf)n- Tf: Tiophene CBz: Carbazole
b) Copolymer –(Tf-Flu-Tf)n Tf: Tiophene Flu: Fluorene
Both of them have been obtained with two different synthethic routes:

1) Pd-catalyzed Suzuki coupling reaction of the $B(OH)_2$ and Br terminated monomers; this synthetic route guarantees polymers in a neutral state.

2) The second route, reaction with FeCl3 in chloroform has, as result, polymers with a ferric level of the order of percent; in this case the ferric residues, assuming to be Fe^{3+} ions, are probably coordinated to the polymer and the polymer is in a sort of p-doped state.

The ^{13}C CP-MAS spectra have been completely assigned on the basis of the assignments obtained on the correspondent neutral polymers in solution. What is evident on the basis of the comparison between the spectra obtained with the two synthetic routes is the missing of certain signals in the MAS spectra of the copolymers obtained through $FeCl_3$ synthetic route. These signals can be shifted away or extremely broadened for effect of the interaction with Fe^{3+} ions. In the two copolymers the coordination sites are different. In copolymer a) the coordination is preferentially on the nitrogen of the carbazole moiety as the missing signal is the first $–CH_2$- directly bonded to the nitrogen. In copolymer b) the preferred coordination site is the position in the middle of the two thiophene rings probably stabilized by the two S atoms. In this case the missing signals are the internal quaternary carbons of the thiophene moiety. The coordination is strictly confined in the depicted positions as the other resonances of the spectra are not absolutely influenced by the Fe^{3+} coordination.

Further insight about the effect of iron interaction has been obtained through vibrational and electronic spectroscopy.

P411
Mg based metal hydrides for hydrogen storage. A NMR perspective

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Metal hydrides are studied for their reversible hydrogen storage properties. MgH_2 has a high storage capacity of 7.6 wt-% hydrogen but suffers from slow sorption kinetics. To overcome this barrier, Notten[1,2] and his group showed that the kinetics can be improved by alloying magnesium with a minimum of 20 at-% of a Transition Metal (TM) element. NMR has the advantage over conventional diffraction based techniques that long-range ordering is not a constraint. Advanced ^{1}H and ^{2}H–{^{45}Sc} TRAPDOR NMR experiments[3] on $Mg_{0.65}Sc_{0.35}D_2$, reveal the existence of small Mg- and Sc-rich clusters at the nanometer length scale within the overall coherent crystal structure. However, scandium is an expensive element. A less costly alternative would be to use titanium. 2D Exchange Spectroscopy (Exsy)[4] of ball-milled $Mg_{0.65}Ti_{0.35}D_{0.6}$ powders reveals the presence of small TiD_x domains which are in close contact with MgD_2, in addition to macro-phase separated TiD_2. The 2D Exsy of RF magnetron co-sputtered $Mg_{0.65}Ti_{0.35}D_{1.2}$ films shows exchange of a major fraction of deuterium atoms from Ti-rich sites to Mg-rich sites. In another approach to improve the sorption kinetics of MgH_2, nanometer sized MgH_2 is encapsulated in a carbon matrix. Problems arising from susceptibility and skin depth are reduced by working at a lower magnetic field. The hydrogen from nano- and bulk-MgH_2 phases is distinguished by their spin-lattice relaxation (T_1). In combination with other techniques, NMR gives a complete picture of nano-structured materials for hydrogen storage.

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P412

Sensitivity-enhanced high-resolution NMR of half-integer quadrupolar nuclei at 30 Tesla

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More than 75% of all NMR active nuclei exhibit a nuclear quadrupole moment ($I > 1/2$). For low-sensitivity, half-integer quadrupolar nuclei in strong electric field gradients, high magnetic fields can overcome their two major problems, their low sensitivity and the second order quadrupolar line broadening of their central transition in the NMR spectra.

In our contribution we present new options of sensitivity-enhanced solid state NMR of these nuclei at 30 T in resistive magnets at the *Laboratoire National des Champs Magnétiques Intenses* in Grenoble. We summarize our efforts to adapt our facility to the standards of high-resolution NMR, since resistive high field magnets provide lower spatial field homogeneity and temporal field stability as compared to superconducting magnets. In addition, resistive magnets suffer from high operating costs due to their electric power consumption of more than 20 MW. In view of these constraints, we developed tailored and sensitivity-enhanced NMR probes providing strong excitation fields, implemented the CPMG sequence, improved spatial field homogeneity by a passive ferroshim and eliminated the long term field drifts using an NMR lock. At present we offer to external users NMR options up to 30 T covering all relevant quadrupolar nuclei with a resolution of the order of 10 ppm for a standard 5 mm sample size. As a first benchmark experiment we present recent ^{91}Zr NMR studies on a series of inorganic Zr compounds at 30 T. The obtained results contribute to a systematic and quantitative determination of the relation between structural parameters (bond lengths, bond angles, coordination geometry) and NMR parameters (chemical shift and quadrupole tensors) of Zr compounds.[1]

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P413

Novel silica-reinforced rubbers obtained by sol-gel processes: A Solid State NMR study

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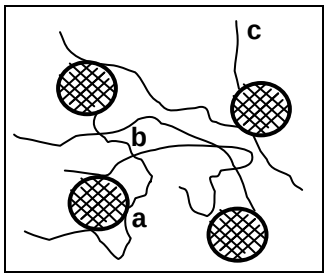
The study of silica-reinforced rubbers is increasing attention due to the specific properties exhibited by these composites, especially in the field of tyres. In this work, new composites obtained by in situ generating silica through sol-gel process in synthetic polyisoprene matrices were investigated by means of Solid State NMR, in order to obtain a characterization of their structural and dynamic properties at a "molecular" level, and compare them with their "macroscopic" features (morphologies, mechanical properties, etc.).[1] Low-resolution proton (^{1}H) NMR has been used successfully in the study of rubber–filler interactions.[2] Here ^{1}H T_2 relaxation was investigated in low resolution, in order to characterize the dynamic properties of the polymer and the effect of the presence of sol-gel silica. The pure cis-1,4-polyisoprene rubber, composites containing variable amount of silica and the composites obtained at different times of sol–gel reactions were studied. Three regions with different chain mobility have been detected and related with sample composition and silica-polymer interactions. Additional dynamic and structural information were also obtained from ^{1}H T_1 low-resolution measurements and high-resolution ^{29}Si and ^{13}C spectra.



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P414

Homonuclear SQ – DQ correlations in solids. Applications to detection of ^{31}P –^{31}P spatial relations in palladium-phosphane complexes

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Recent advances in solid state NMR hardware and theory, in particular the development of effective homonuclear decoupling schemes[1] make possible the study of intramolecular and intermolecular ^{31}P–^{31}P spatial relations. We have synthetized two-, three-, and four-spin systems, resolved the single crystal X-ray structures (if not known) to gain access to the experimental P-P distances and tested the limits of BaBa based SQ-DQ sequencies[1] in revealing internuclear correlations. The ^{31}P-^{31}P dipolar coupling values varied between 200 and 800 Hz. Pairs with large isotropic chemical shift differences (up to 100 ppm) and nuclei with significant chemical shift anisotropy values (up to 32 -34 kHz) were also tested at 9.4 T and rotation frequencies up to 25 kHz.

We concluded that while the double-quantum excitation efficiency is sufficient for two-spin systems with internuclear distances up to 4.8 angströms, for three-spin systems with larger distances this is not always the case, furthermore three-spin relaxation effects can interfere. DQ excitation times larger then 500-600 μs did not bring any improvement.

The long T_1 relaxation of the P atoms can (must) partially be overcome by cross-polarization. Even so, mostly due to the long phase-cycling the time requirements are relatively high (at 9.4 T, 2.5 mm, ~ 6 -12 hours). The method is capable to detect spatial correlations in three- and four–spin systems up to 4.7 angtröms. The achievable DQ excitation band-width limits the applications to systems with about 50 ppm isotropic chemical shift ranges.

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P415

Efficient Decoupling and Recoupling in Solid State NMR at Very High Static Fields and Spinning Speeds

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We present new schemes for solid-state NMR with efficient heteronuclear decoupling and homonuclear recoupling at magnetic fields up to 23.5 T (1000 MHz) and spinning frequencies up to 64 kHz.

A new heteronuclear decoupling method, dubbed PISSARRO (Phase Inverted Supercycled Sequence for Attenuation of Rotary ResOnance),[1] was designed to quench deleterious rotary resonance recoupling effects that occur at high spinning frequencies. It proved to be more effective than XiX, TPPM, SPINAL-64 and CW decoupling methods in quenching rotary resonance effects and offers improved decoupling efficiency over a wide range of rf amplitudes. Moreover, we demonstrate its efficiency for low rf amplitudes regime at spinning speeds up to 64 KHz, which is particularly useful for heat-sensitive samples.

We also show new applications of our recently developed phase-alternated recoupling irradiation scheme (PARIS).[2] PARIS recoupling offers an attractive alternative to the popular DARR scheme because of its inherent immunity to the inhomogeneity of the rf field, its ability to achieve recoupling with rf amplitudes well below the rotary resonance condition, and its capacity to promote efficient magnetization transfer even when the spinning frequency ν_{rot} exceeds the difference of isotropic chemical shifts between spectrally distant carbons, so that the rotational resonance condition cannot be fulfilled. In particular, we show that an extension of the basic PARIS approach to a phase-shifted version dubbed PARIS-xy allows efficient broadband magnetization transfer with moderate rf amplitudes even at spinning frequencies as high as ν_{rot} = 60 kHz and magnetic fields up to 23.5 T (1 GHz for protons).

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P416

Multifrequency EPR studies of dangling-bonds in hydrogenated amorphous silicon

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Hydrogenated amorphous silicon (a-Si:H) is utilized in thin film solar cells replacing the cost-intensive crystalline silicon wafer technology. Light-induced degradation of a-Si:H (Staebler-Wronski effect - SWE), arising from an increase of dangling bonds (db) reducing excess charge-carrier lifetime, presents a serious limitation for this promising material. The most crucial step in further device optimization is the identification of the db generation mechanism. To address this question, we applied high-resolution multifrequency EPR to identify the microscopic structure of dbs. The increased Zeeman-resolution at higher frequencies allows by comparison with the low-frequency spectra to distinguish between field-independent (resolved and unresolved hyperfine interactions) and field-dependent linewidth contributions (g-tensor and g-strain). Simultaneous fit analysis of multifrequency spectra yields striking results, which indicate that the db g-tensor possesses rhombic symmetry, a fact which contradicts earlier db models relying on axially symmetric sp-hybrid orbitals.[1]

The translation of these spectroscopic data into a microscopic picture is not straightforward. DFT calculations are performed to test several structural models, judging their significance by comparison of calculated magnetic parameters with the experimental results gained in this study.

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P417

Characterization of hydrogel network of PEG-(meth)acrylate based functional copolymers by ^{1}H and ^{13}C NMR relaxometry and spectroscopy

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Amphiphilic copolymers containing poly(ethylene glycol) (PEG) segments have attracted much attention due to their unique hydrophilic properties, inhibition of unspecific cell adhesion, and their potential use as drug-delivery systems. The stress-strain behavior of the segments in the hydrogel network was investigated by measurements of transverse ^{1}H relaxation. Various amphiphilic copolymers based on PEG and methoxy methacrylate, individually functionalized with thiols were studied. The relaxation measurements provided residual dipolar couplings as a function of mechanical stress. They are explained by the theory of network elasticity in the non-Gaussian approximation. For comparison, residual van Vleck moments were measured from double-quantum (DQ) NMR. Each of these properties is correlated with the cross-link density of the hydrogel networks extracted from ^{13}C direct polarization MAS spectra. The hydrogel network properties are also discussed in terms of the nature of cross-linker.

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Micro-MAS NMR: Towards high-resolution NMR of nanoliter volume solid samples

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Nuclear Magnetic Resonance (NMR) spectroscopy suffers from its intrinsically low sensitivity compared to other techniques, which precludes study of mass-limited samples. Considering the potential of NMR in providing local structural information and insight into molecular dynamics, many approaches have been used to enhance the sensitivity. Here, we focus on miniaturized NMR detectors to enhance sensitivity and their application to achieve high-resolution solid-state NMR of mass-limited samples.

Magic Angle Spinning (MAS) is widely used in combination with multi pulse decoupling sequences to average out anisotropic interactions and obtain high spectral resolution. MicroMAS (μMAS) combines the versatility of MAS with the superior sensitivity, provided by very small detection coils (solenoidal coils of diameter around 200 – 450 μm), for samples with nanoliter volumes. Furthermore, the very strong radio frequency (rf) fields that can be generated by these microcoils facilitate a much broader excitation bandwidth and decoupling efficiency.

The use of fused-silica capillaries as sample holders for μMAS, results in spectra without any ^{1}H background signal. It proved possible to obtain ^{1}H spectra of 40 - 80 nL sample volumes in an only few scans. We obtained high-resolution ^{1}H spectra employing different homonuclear decoupling sequences on powdered samples (L-alanine & tripeptide AGG) as well as single-crystal (L-tyrosine.HCl). Furthermore, we demonstrated the feasibility of indirectly detecting low-sensitivity X-nuclei such as ^{13}C via protons with natural isotope distribution.

The key factor determining the resolution of solid-state NMR spectra of rare spins such as ^{13}C in protonated materials is usually the quality of proton spin decoupling. A significant averaging of these interactions can be achieved by decoupling sequences such as CW, TPPM, SPINAL decoupling under MAS. Here we discuss the context of these decoupling sequences at high rf field strengths using μMAS and finding the observable ultimate line width in solids.

P419

Structural changes in the antifilarial drug diethylcarbamazine citrate in solid state studied by ^{13}C-CPMAS NMR– preliminary results

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Filariasis is an endemic disease occurring in most of the tropical countries, including northern Brazil. It is mainly caused by a parasitic filarial worm named *Wuchereria bancrofti*, which is transmitted by mosquitoes.[1] The parasite lives in the lymphatic system and it causes the inferior members lymphoedem, known as elephantiasis. Diethylcarbamazine (DEC) citrate is widely used to treat this disease by killing the adult worms. DEC exists as polymorphic forms, and this polymorphism may affect the solubility as well as its bioavailability. Recently, it was described some phase transitions in solid state,[2] and the results can be related to these possible differences. Preliminary results obtained for unformulated drug show a mixture of two different phases. According to the conformation of piperazinic and ethyl groups, in relation to the carbonyl group, the conjugation between both nitrogen atoms and carbonyl group bonded to each other can be unfavoured. In an unconjugated system the nitrogen nuclei remains more shielded and the NMR signal related to the other carbon nuclei are shifted to lower δ(ppm). The presence of citrate should favour the conjugation. Hence, it is possible to use ^{13}C-CPMAS, in a fast way, to monitor phase transitions in this drug, according to temperature variation, and also estimate possible changes in their properties, especially for formulated drugs.

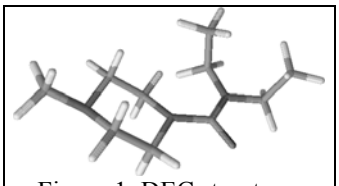

Figure 1. DEC structure.

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Acknowledgments: FAPESP (2009/13860-2), CNPQ (475903/2009-9), FIOCRUZ-Far Manguinhos.

P420 (*)

Solid-State Nitrogen-14 Nuclear Magnetic Resonance Enhanced by Dynamic Nuclear Polarization using a Gyrotron

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By combining indirect detection[1] of ^{14}N with dynamic nuclear polarization (DNP),[2] the signal-to-noise ratio can be dramatically improved and the recovery delay between subsequent experiments can be shortened. MAS spectra of glassy samples of the amino acid proline doped with the stable bi-radical TOTAPOL[3] at 100 K were obtained in a 400 MHz solid-state NMR spectrometer equipped with a gyrotron for microwave irradiation at 263 GHz. DNP enhancement factors on the order of e ~ 40 were achieved. The recovery delays can be reduced from 60 s without radicals at 300 K to 6 s with radicals at 100 K. Thus, DNP allows one to reduce the acquisition times of ^{13}C-detected ^{14}N spectra from several days to a few hours.

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P421

TM-Al-TM groups trapped in cages of decagonal approximants

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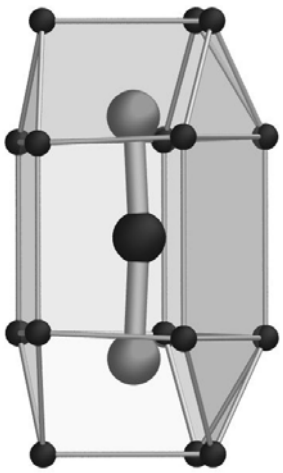

We present the ^{27}Al NMR spectroscopic study of the $Al_{13}TM_4$ family of four-layer decagonal approximants, including the orthorhombic o-$Al_{13}Co_4$, the monoclinic $Al_{13}Fe_4$, its ternary derivate $Al_{13}(Fe,Ni)_4$ and the monoclinic $Al_{13}Ru_4$. Single crystals were grown by Czochralski technique and from each, three bar-shaped specimens were cut with there long edges along crystallographic direction. The so-prepared samples enabled us to measure ^{27}Al (spin I = 5/2) central-line (1/2 → −1/2) NMR rotation patterns that exhibit peaks belonging to magnetically equivalent ^{27}Al crystallographic sites within the unit cell. Most lines in rotation patterns overlap; exception is one line with a stronger shift. Since this line is well resolved from the rest ^{27}Al intensity in the NMR rotation patterns, we are able to determine its EFG- and magnetic shielding tensors for all four samples. The asymmetry parameters of the tensors are small for all compounds, amounting 0,01 − 0,11. This demonstrates that the tensors are almost axially symmetric around particular crystallographic direction, which is compatible with the structural detail of nearly linear TM-Al-TM atomic groups (with TM = Co, Fe, Ni, Ru) in an approximately axially symmetric chemical environment. The asymmetry parameters of EFG tensors were reproduced theoretically by a point-charge calculation and confirm ionic bonding of the TM-Al-TM group to the cage atoms. The above results show that the traditional description of the $Al_{13}TM_4$ decagonal approximant phases in terms of 2D atomic layers stacked along the perpendicular crystallographic direction is a convenient geometrical approach to describe their complex structure, but is not appropriate for description of their physical properties.

Acknowledgments: The authors would like to thank Prof. Dr. Peter Gille for the sample preparation.

P422

Understanding the crystal structure in layered aluminum hydroxides by high-resolution solid-state NMR and first-principles calculations

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Layered double hydroxide (LDH) – a synthetic clay, which finds numerous applications, as for example, flame-retardant filler in polymer nanocomposites. To quantitatively characterize the structural changes in the different stages of LDH modification by a regeneration method a combination of the ^{27}Al MAS and 3QMAS NMR has been applied. The ^{27}Al NMR signals are attributed to either 4-fold (tetrahedral) or 6-fold (octahedral) aluminum sites, where in the latter two different environments are discriminated in both pristine and organically modified LDHs. Evidence for two distinct octahedral sites is found in the central transition (MAS), in the satellite transition (MAS and two-dimensional one pulse TOP) and in 3QMAS. To understand the structure a pure aluminium hydroxide – gibbsite – has been used as a model, exhibiting the presence of two equally populated sites characterized by different quadrupolar coupling constants (4.2 MHz and 2.4 MHz) and similar chemical shifts in both the ^{27}Al MAS NMR spectrum and DFT calculation. This observation is explained by the different hydrogen bonds, in which the hydroxyls forming the corresponding octahedron around each aluminum site are involved. ^{1}H CRAMPS allows distinguishing non-equivalent hydrogen sites reported in the gibbsite crystal structure.

Insight into molecular mobility has been gained from ^{1}H $T_{1\rho}$ experiments detected with chemical shift resolution. Applying a numerical inversion of the Laplace transform allows discriminating two contributions in LDH, overlapped in the ^{1}H chemical shift dimension. Based on the data for gibbsite they are attributed to metal hydroxides and highly mobile interlayer water. Variation in the local proton environment linked the presence of Mg or Al sites has been found to influence the proton mobility, which increases upon incorporation of surfactants. To probe the spin diffusion between protons, spin exchange experiments using radio-frequency driven recoupling (RFDR) have been applied.

P423

Hydrogen diffusion in Zr-based bulk metallic glasses

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At the time when sources of green energy are hardly expected, we have investigated the $Zr_{50}Cu_{40-x}Al_{10}Pd_x$ (x = 0-7 at.%) bulk metallic glasses (BMGs) characterized by a high capacity of the hydrogen storage. Up to H/M ~1.6 (hydrogen-to-metal) ratio guarantees the utility of a BMG as a hydrogen storage medium, whereas the weak interaction of hydrogen with the zirconium atoms as the majority chemical element in the investigated BMGs assures easy mobility of the hydrogen. Due to unique mechanical properties (like combined hardness and elasticity) caused by the presence of the palladium, the hydrogen storage in the Zr-Cu-Al-Pd BMGs provides safe way of hydrogen storage, in contrast to the classical 700-bars tanks proposed as an alternative by the car industry. Our aim was to determine whether the hydrogen in the amorphous structure is chemically bound to the metallic ions and hence statically distributed over the material or the hydrogen atoms can move freely through the amorphous structure and their spatial distribution is dynamic. We have performed measurements of the hydrogen self-diffusion constant D using ^{1}H NMR spectroscopy. Within the temperature interval 380-420 K, the D values are between $7*10^{-10}$ and $8*10^{-9}$ cm^2/s and show a classical (Arrhenius) over-barrier-hopping temperature dependence. The average hopping activation energy of all the samples amounts to E_a = 576 ± 15 meV. The hydrogen diffusion constant of the $Zr_{50}Cu_{40-x}Al_{10}Pd_x$ BMGs is comparable in magnitude with other Zr-based BMG. No correlation between the diffusion constant and the Pd content in the samples could be observed within the experimental precision. We did not notice any variations in the magnetic and physical properties associated with the Pd content as well. However the presence of the Pd in such compounds is the source of: 1) Pauli spin susceptibility of the conduction electrons and 2) non free-electron-like electrical transport visible in the negative temperature coefficient (NTC) of the electrical resistivity.

P424

Effects of Electrical and Ionic Conductivity on MAS-NMR of Quadrupolar Nuclei in γ-Cuprous Iodide

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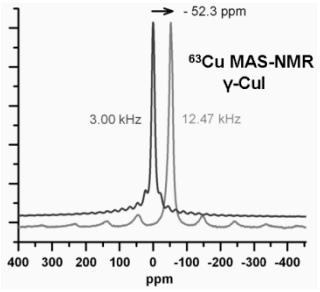

We have used variable-temperature MAS-NMR to investigate γ-CuI, a Cu^+-ion conductor at elevated temperatures as well as a wide bandgap semiconductor. Puzzling anomalies are seen in the ^{63}Cu, ^{65}Cu and ^{127}I MAS-NMR of γ-CuI, whose chemical shifts depend strongly upon the square of the spinning-speed as well as the particular sample studied.[1,2] By using the ^{207}Pb resonance of lead nitrate mixed with the γ-CuI as an internal chemical shift thermometer we show that frictional heating effects of the rotor cannot alone account for the observations. Instead, we find that spinning the electrically-conductive (unintentionally doped) p-type semiconductor in a magnetic field generates electric currents over the entire rotor that can resistively heat the sample by over 200° C. These induced currents and their associated heating effects are disrupted in samples containing inert filler material. A theoretical analysis and simulation accounting for these heating effects will be presented.

In addition to the dramatic consequences of *electrical conductivity* in the sample, *ionic conductivity* also influences the spectra. All three nuclei exhibit quadrupolar satellite transitions extending over several hundred kilohertz that reflect defects perturbing the cubic symmetry of the zincblende lattice. Broadening of these satellite transitions with increasing temperature arises from Cu^+ ion motion modulating the electric field gradients and thus interfering with the formation of rotational echoes. This broadening can be quantitatively analyzed using a simple model to yield an activation barrier for the Cu^+ ion dynamics.

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7.4 Small Molecules/Pharma & Metabolomics

Posters

P425 (*)

Ligand–Receptor Binding Affinities from Saturation Transfer Difference (STD) NMR Spectroscopy: The Binding Isotherm of STD Initial Growth Rates

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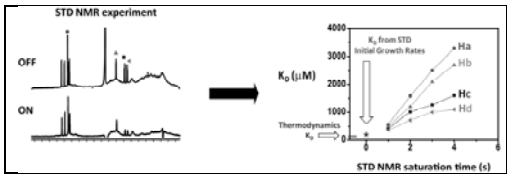
Among ligand-observed NMR methods for protein-ligand interactions, the saturation transfer difference (STD) NMR experiment has demonstrated to provide high sensitivity and robustness.[1] In this method, a selective radiofrequency irradiation saturates only protein NMR signals, and the transfer of this saturation to any binder is revealed by difference spectroscopy. STD NMR titration experiments might be employed to derive binding affinities. Interestingly, STD NMR is well appropriated for weak kinetics exchange, where other physical techniques might meet their detection limits. However, direct approaches have failed so far to get correct values of equilibrium dissociation constants (K_D) from STD NMR titrations, as the magnitudes of the determined constants have shown dependence on the particular STD signals chosen to build the corresponding binding isotherms. Indirect determinations, by competitive titrations, deliver accurate K_D values, as long as a competitive ligand, with previously known affinity, is available for the protein under study.

We have carried out a detailed study of the factors responsible for biases in K_D determinations by direct STD NMR titration experiments and have identified ligand longitudinal relaxation and fast rebinding processes as the main sources of artifacts in K_D measurements. As a result, we have developed a new protocol based on the use of STD initial slopes to get the binding curves, and have demonstrated its ability to cancel out all spurious factors, allowing to get the thermodynamic dissociation constant, independently of the experimental conditions or chosen ligand signal (see figure).[2]



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P426

The Lyotropic Behavior of the Catanionic Sodium Taurodeoxycholate Mixed Micelles Studied by Means of Multinuclear NMR and Rheology

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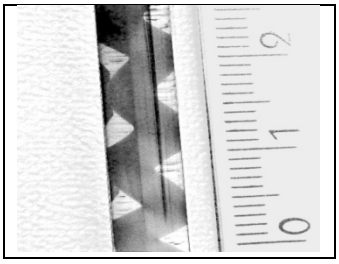
Sodium taurodeoxycholate (STDC) is an endogenous surfactants of the bile acids salts family. Their quite peculiar structure is responsible for the generation of numerous intriguing supramolecular architectures. Here we added to STDC micelles a double tail cationic surfactant, either DDAB or gemini 12-6-12, at a fixed 5:1 ratio of – to + charges, with the overall surfactant concentration in the range 1-30 wt% in D_2O. The effect of the two surfactants on the STDC micelles was explored by means of the relaxation times of ^{23}Na and ^{81}Br of the counterions and by ^{1}H and ^{13}C. ^{14}N of the alkylammonium head-groups resulted a very sensitive probe, able to discriminate between the two surfactants, in fact its line-width increases at concentrations higher than 15%, and much more strongly in the 12-6-12 containing systems. The effect is due to slower tumbling of cylindrical micelles, hindered more efficaciously by the surrounding aggregates in the case of the gemini, as enlightened by rheological measurements.



The ^{2}H and ^{23}Na spectra of the LC phase formed at T< 10 °C are reported and discussed in comparison with those of the STDC-D_2O binary system[1]. Striking is the macroscopic helical pattern observed inside the NMR tube, provided alignment in the magnetic field has been carried out. The whitish (black in the figure, which corresponds to photo negative) matter is birefringent when observed between crossed polarizers. It probably corresponds to a dispersion of LC droplets in the isotropic micellar phase and possesses such visco-elastic properties to undergo a twisting undulation under the effect of a flow induced by the temperature gradient.

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P427

Novel strategies against Anthrax toxin entry and activity

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B. anthracis is a rod shaped bacterium which infects humans through the respiratory system, skin, or digestive tract and that can be highly lethal depending upon its entry route into the human body. The bacteria release a toxin that kills host macrophages, consisting of three virulence factors: protective antigen (PA, 83 kDa), edema factor (EF, 89 kDa) and lethal factor (LF, 90 kDa). Although antibiotics such as ciprofloxacin are effective against *B. anthracis*, high levels of the secreted toxin may remain in circulation for several days which continues to damage the host even after the bacteria may have been killed[1,2]. Hence, our goals are to develop and implement novel strategies to counteract the entry and the activity of the Anthrax toxin. Using an siRNA screen of 7,000 human genes, we have identified a number of host genes that are essential for the entry of the toxin. These include the proprotein convertase Furin, responsible of processing PA, and Caspase-8, whose role in toxin entry is yet not fully understood. Based on these observations, we will report on our preliminary studies aimed at the identification of novel and effective anti-toxin agents.

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P428

Spin probe dynamics in relation to free volume and relaxation dynamics in small molecular H-bonded glass-former: G l y c e r o l

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A combined study of the free volume microstructure and local dynamics in prototypical H-bonded type glass-forming system - *glycerol (GL)* - by means of the spin probe method is presented. The rotation dynamics of the smallest spin probe of nitroxide type *2,2,6,6-tetramethyl-1-piperidinyloxy* (*TEMPO*) was measured by using electron spin resonance (ESR) and the spectral parameter of mobility, $2A_{zz}$ (T), and the rotational correlation time, τ_c (T), were compared with the annihilation behavior of the atomistic ortho-positronium (o-Ps) probe from positron annihilation lifetime spectroscopy (PALS) and the related free volume as well as with the relaxation one from broadband dielectric spectroscopy (BDS).

Three regions of the distinct rotation dynamics of the spin probe *TEMPO* in *GL* were found. Two regions in low - T region within the s l o w motional regime with an acceleration at the characteristic ESR temperature, T_{X1}, being close to the characteristic PALS temperature, T_{b1}, is related to the secondary β process *above* T_g. The subsequent s l o w to f a s t regime transition at the characteristic temperature, $T_c \cong T_{50G}$, both being close to the characteristic PALS temperature, T_{b2}, is connected with the primary α process in the *glycerol*. Further, the spectral temperature parameter of mobilitt, T_{50G}, is connected with the o-Ps lifetime, $\tau_3(T_{50G}) = 2.1$ ns in good agreement with the empirical rule $\tau_3(T_{50G}) = 2.25 \pm 0.15$ ns found for a series of several van der Waals-bonded type small molecular and polymer glass-formers. This suggests that the slow to fast transition of the *TEMPO* is related to free volume fluctuation of about 110 A^3. Finally, in high - T region the fast motional regime of the spin probe *TEMPO* is fully coupled with the structural relaxation of the *glycerol* matrix.

P429

Structure, dynamics and bioavailability of N-methylated cyclopeptides

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N-Methylation of peptide bonds is long known and has often been used to modify biological properties of bioactive peptides. However, it has become evident only recently that multiple N-methylation is a novel technology to improve the pharmacological properties of peptides and in extreme cases even achieve oral bioavailability such as found for Cyclosporin.

Here we present recent structure investigations of a melanocortin receptor subtype 1 selective fourfold N-methylated MT-II derivative,[1] of a cyclic fourfold N-methylated somatostatin analog and of a number of N-methylated cyclohexaalanine cyclo(-a-A-A-A-A-A-) peptides. N-methylation led to a decreased flexibility of some of the cyclopeptides under investigation, which was recognized immediately by an increase in the dispersion of otherwise similar chemical shifts (e. g. of α protons). When the solution conformation stabilized by N-methylation was similar to the receptor bound state, high potency and receptor subtype selectivity were obtained. Interpretation of the cyclohexaalanine peptides bioavailability as monitored by diffusion over Caco-2 monolayers in terms of a structural model is less straight forward. Currently, a model is under development that correlates the bioavailability of the cyclohexaalanine peptides with their structural and dynamical properties.

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P430

Practical Aspects of Timecourse DOSY

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Analysis of chemical reactions is a daunting task which has been tackled by a variety of methods with different degrees of success and complexity. One of the latest methods is Timecourse DOSY,[1,2] which aims to extract NMR spectra, diffusivities and kinetic information through PARAFAC (Parallel Factor analysis).[3] In order to perform this multiway decomposition it is necessary to obtain high quality data. Critical requirements such as temperature stability and sufficient signal-to-noise ratio are discussed, along with illustrative examples at the limits of successful decomposition. A Matlab-based toolbox has been created to facilitate the task of pre-processing data to optimise data quality and to post-process PARAFAC results, providing publication quality plots with minimal intervention.

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P431

Determination of the Conformation of the Key-Intermediate of a Pd-catalyzed allylic substitution from RDCs

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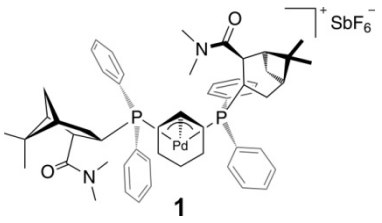
Residual Dipolar Couplings (RDCs) are becoming increasingly important in the determination of configuration and conformation – including conformational flexibility[1,2] – of small organic compounds.[3] Until recently, however, RDCs have not been applied to gain insight into conformational analysis of reaction intermediates.[4]

Here we report on the determination of the conformation of the intermediate 1, which could not be determined based on conventional NMR restraints.



1

We therefore oriented the very sensitive intermediate **1** in high molecular weight PBLG[5] and fitted several $^{1}D_{C\text{-}H}$ to possible diastereomorphous representations of conformers. This resulted in a much better fit for one conformer than for the others. As a cross-validation, we examined each fragment of the complex individually and determined their orientation with respect to each other using local orienting tensors.[3,4] With the RDC data we also obtained the populations of the diastereomorphous conformations of the flexible cyclohexenyl ring of **1**.[4]

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P432

In situ oxidation of sulfides to sulfoxides and sulfones. Determination of sulfoxide configuration using NMR spectra and DFT calculations

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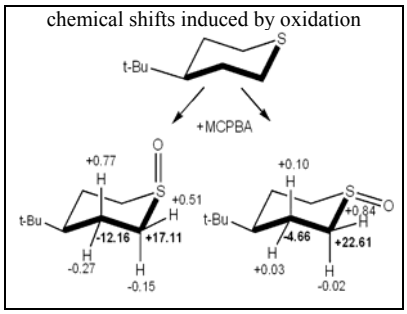
The interest in determining the absolute configuration of chiral compounds stems from the fact that it often determines important chemical and physical properties, biological activities and pharmaceutical use of these compounds.

Direct NMR methods for configuration determination (*J*-values, NOE) cannot be used in the case of sulfoxides. Indirect methods based on the formation of dynamic diastereomeric complex with some chiral reagents showed as not reliable. Therefore we decided to develop a method combining experimental and *ab initio* calculated chemical shifts for distinguishing of the S=O group configuration.



Sulfoxides and sulfones can be efficiently prepared by *in situ* oxidation of corresponding sulfides with *meta*-chloroperbenzoic acid (MCPBA) in NMR tube without separation and isolation. Different methods of calculation were tested on simple model compounds[1]. Here we present our results obtained with sulfoxides of 1,6-anhydro-β-D-thiahexopyranoses, cyclodipeptides with thiapipecolic acid containing a sulfur atom in various position of the ring, 4-*tert*-butyl-thiane and thiaadamantanes. It can be shown that ^{1}H and namely ^{13}C chemical shift differences, induced by oxidation of sulfide to sulfoxide, are characteristic for given configuration of S=O group. Our results show a very good agreement between experimental and DFT calculated chemical shift differences even for computationally "cheap" methods.

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Acknowledgments: Financial support from Czech Science Foundation (project 203/09/1919) is acknowledged.

P433

'Solution State Crystallography' Quantitative 3D Molecular Structure Determination by NMR Spectroscopy

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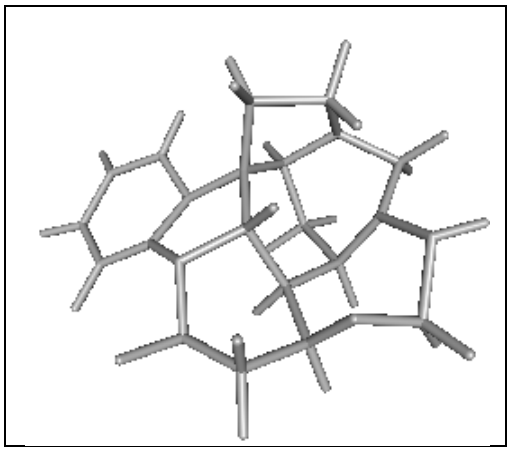
De novo 3D molecular structure elucidation *in solution* is demonstrated using data derived only from NMR spectroscopy *without the need to pre-determine the bonding skeleton*.



A purely distance-geometry approach is used whereby internuclear distances are employed to generate both connectivity and conformational information *simultaneously* on the basis of NMR spectroscopic data. We demonstrate that rigid interproton distances can be determined from NOE data with accuracies of <0.1 Angstroms in most cases, allowing discrimination between myriads of possible skeletal isomers *without the need to pre-determine the bonding skeleton*. The methodology is equally appropriate to flexible systems and over a range of molecular frameworks – limitations and applications will be discussed.

Remarkably this technique works without relying on interpretation of traditional solution-state semi-qualitative information such as chemical shift or coupling constants, although these could aid connectivity determination in more complex cases. This solution-state technique complements the high precision and intermolecular information available from solid-state crystallography, with structure elucidation in a more convenient state of matter.

P434

New findings in the understanding of Red wine astringency using NMR tools

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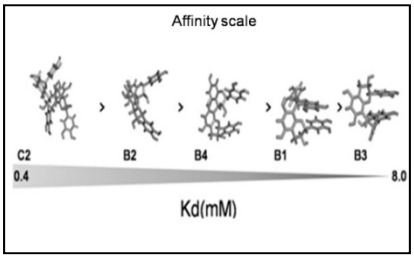
Astringency characterized by a dry, rough or even pucker sensation, is considered as a proof of red wine quality. During half a century, studies proposed that this sensation is the result of an interaction between tannins (mainly procyanidins) and saliva proteins[1] (mainly Proline-Rich Proteins (PRPs)). Procyanidins are polymers of flavan-3-ol that are drastically polydisperse in size and chemical structure. The aim of the present work is to establish an affinity scale between various chemically synthesized procyanidins[2] (four different C4-C8 dimers, and a trimer) and a representative Proline-Rich Protein model peptide[3]: $IB7_{14}$ which is a 14 residues fragment of the whole IB7 PRP containing three such repetitive units. Different NMR approaches were used in a wine mimicking medium in order to characterize this interaction: chemical shift variation and DOSY experiments were performed in parallel with all-atoms molecular dynamic calculations. For the first time, we have been able to build an affinity scale. Results show that the higher the polymerization degree, the more dynamic the 3D structure, the stronger the affinity. Secondly, we show that tannin-protein complexes could be formed in two ways. Below their CMC, tannins bind specifically to salivary proteins. Above the CMC, the specific interactions are still present, but tannins can also form micelles and create hydrophobic interactions. Finally, we evidenced a possible macromolecular networking, leading to precipitation of the complexes in relation with the astringency scale. C2 and B2 tannins precipitate $IB7_{14}$ whereas B3 does not.



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P435

Comparison of Chromatographic NMR and Liquid Chromatography

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The association of the addition of a chromatographic-like solid phase support and of DOSY experiments can provide enhanced discrimination in the analysis of mixtures by NMR.[1] This Chromatographic NMR has been shown to be even able to outperform LC in specific cases.[2] The reason for such possible difference is unveiled in this presentation, being linked to the phase ratio. In the course of this study, the interplay of vapor, liquid and adsorbed phases in determining the mass transport in porous materials were highlighted.[3]

These theoretical findings allow a more accurate comparison between LC and Chromatographic NMR, which thus can be also used as a prediction tool for column behavior and classification. Examples along these lines will be shown.

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Acknowledgments: We are grateful for financial support to the Agence National de la Recherche (Grant ANR-08-BLAN-273-01) and to Region PACA (APO-G 2009).

P436

Prediction of ^{13}C and ^{15}N chemical shifts by DFT-GIAO QM methods: Application to Structure Elucidation of some Heterocyclic Compounds

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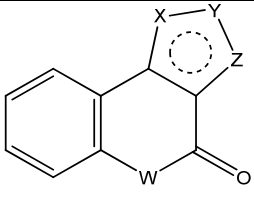
The GIAO (Gauge Including Atomic Orbitals) DFT (Density Functional Theory) method is applied at various levels of theory for the calculation of ^{13}C and ^{15}N chemicals shifts for some heterocyclic systems. Prediction of chemical shifts appears to be accurate and useful; the comparison between experimental and theoretical data is performed using the linear regression method. Thus, we were able to confirm the regiochemistry of the reaction products of some acylderivatives with 1,2-bisnucleophiles[1,2] whose structures have been determined also by 1D- and 2D NMR experiments (HSQC, HMBC, H2BC, 1D-NOESY).[3] For the "right" assignments almost perfect correlations (0.999-1.000) between experimental and theoretical data are obtained and the "correct" structure can be unambiguously chosen between the possible isomers just by comparison of carbon CSs. Analysis of ^{15}N CSs is also particularly useful and there is no need of detailed assignment as for ^{13}C. In this communication we will extend the analysis to the reaction of the same substrates with 1,4-bisnucleophiles like ethane-1,2-diamines and 2-aminoethanol for the obtainment of seven mebered fused heterocyclic systems with potential biological activity.



W = O, NMe
X = N, Y = O, Z = CH
X = O, Y = N, Z = CH
X = O, Y = CH, Z = N
X = N, Y = NR, Z = CH
X = NR, Y = N, Z = CH

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P437

NMR Crystallography of Heteroorganic Host-Guest Complexes

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Host–guest chemistry describes supramolecular systems in which one chemical compound (the "host") forms a cavity in which molecules of a second compound ("guest") are located. In such complexes, two or more molecules (or ions) are held together in unique structural relationships by forces other than covalent bonds. They are often used as models for understanding the three-dimensional structure of large molecules (such as proteins, nucleic acids) which in many biological processes bind specifically but transiently to one another. In this communication we show that approach called "NMR crystallography" is invaluable for analysis of host-guest systems. NMR crystallography incorporates the wide variety of information available in solid-state NMR experiments into the process of crystal structure determination. Employing two host systems; N-(4,19-dioxo-2,8,15,21-tetraoxa-5,18-diazatricyclohexacosa-1(25),9(14),10,12,22(26),23-hexaen-26-yl)-benzamide and bis[6-*O*,6-*O*'-(1,2;3,4-diisopropylidene-α-D-galactopyranosyl) thiophosphoryl] dichalcogenides (with S-S, S-Se and Se-Se in dichalcogen bridge) we show that the number of ^{13}C, ^{15}N and ^{31}P peaks and their relative intensities reflect the number and occupancies of unique crystallographic sites in the structure and may be used to identify possible space groups. ^{13}C NMR provides information about quantity of organic guest molecules incorporated into host lattice for multi-component systems. ^{31}P-^{31}P PDSD and POST-C7 2D correlations provide information about connectivities and distances between atoms, in the form of dipolar couplings between nuclei. Analysis of ^{13}C and ^{15}N principal elements of chemical shift tensors is used to analyze strength of nonbonding interactions (hydrogen bonding). The example showing that vapors of guest molecules are able to change space group of solid host molecules is impressive.

P438

Interaction of Ln^{3+} – Based MRI Contrast Agents with HSA using Saturation Transfer Difference NMR Techniques

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Saturation transfer difference (STD) NMR[1] allows the direct study of the interaction between ligands and proteins. This method was applied to interaction studies of potential angiographic MRI contrast agents with human serum albumin (HSA), providing powerful data to study kinetics and binding sites of ligands to the protein, the validation of binding epitopes, and estimation of affinity constants. The ligands studied in the present work were La(BOPTA), La(DTPA-cholate) and La(NAPHTO-EGTA). Competitive assays with two known inhibitors (warfarin and ibuprofen) were performed to identify the binding sites of the compounds. It was found that the binding of La(BOPTA) and La(DTPA-cholate) occurs mainly through site I of HSA. However, La(NAPHTO-EGTA) was not displaced by either of the two inhibitors, indicating that its binding occurs at a different HSA site, that is neither site I nor II. We also used STD-NMR to show that one can distinguish between two enantiomers of the same lanthanide complex, SSS-(Δ)-$[Y.L^1]^{3+}$ and RRR-(Λ)-$[Y.L^2]^{3+}$, in their binding to HSA.[2] Knowing that recent crystallographic studies of HSA complexes have suggested that "drug site II" is the most stereo-differentiating binding site, we used the inhibitor dansyl sarcosine to perform competitive STD studies, which confirmed this as the binding site of both enantiomers.

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P439

MyMRs: Map your Magnetic Resonance stuff!

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Due to the widespread diffusion of magnetic resonance (MR) equipment, it is sometimes difficult to keep track of all the magnetic resonance instrumentation available in a country. Paradoxically, while a high number of recent and less recent instruments may be present in a country area, they are often unknown to the occasional user (and sometimes even to some specialists), because of lack of communication within the scientific community. The problem may be exacerbated by the well known "bad practics" of letting information spread only within groups of "homogeneous" scientists, so that – so to speak – chemists talk only to chemists, physicists to physicists and so on.

As a step toward the solution of this problem, the GIRM (the Interdivisional Group of Magnetic Resonance of the Italian Chemical Society) has begun a project called MyMRs (http://www.soc.chim.it/gruppi/girm/mymrs), based on the well known Google maps, by which the MR instruments are graphically displayed as placeholders with different colors (yellow = NMR spectrometer, red = relaxometer, green = EPR spectrometer, blue = MRI equipment). All relevant information is obtained from the MR community through a form connected to a spreadsheet *via* the "Google docs" web site, converted to KML format with a simple BASH script and eventually shown in a baloon when a placeholder is clicked. The authors – who are member of the GIRM board – thank all Italian and foreign collegues who will want to provide data about their MR equipment.

P440

^{13}C NMR Spectra of Natural Products Enhanced by SVD

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Advances on natural products chemistry requires that ever small amounts of compounds to be considered for structure elucidation. Considering the central role that NMR plays for structure elucidation in organic chemistry, obtaining spectra with good signal-to-noise ratio is an essential requirement. The trivial solution is to increase the signal-to-noise ratio through signal averaging. In this scenario of always shrinking available samples, however, the time required for signal averaging to obtain a desirable ^{13}C NMR spectra can eventually become too long to be carried out: since the S/N ratio increases as $\sqrt{n}$, where n is the number of scans, an increase of S/N of one order of magnitude requires a 100-fold acquisition time! The quest for higher sensitivity has always been an intrinsic part of the development of NMR, concerning either instruments and processing techniques. Improvements in instrumentation, especially in probe design, has provided a steady increase in sensitivity.

On the other hand, the enhancement of spectra through data processing, such as the use of window functions, is routinely used in ^{13}C NMR. Indeed, the extraction of clean signal from noise-contaminated data is of crucial interest to many fields of science and technology. A method developed by Kunikeev and Taylor called harmonic inversion noise reduction uses a noise reduction pre-processor to "clean" data followed by a harmonic inversion spectral estimator.[1]

We have successfully applied the idea of a noise reduction pre-processor to the analysis of natural products. Data were subjected to a noise reduction process by single value decomposition (SVD) using Mathlab®. The filtered data were later Fourier transformed to yield the enhanced (filtered) spectra, as will be shown.

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Acknowledgments: CNPq, CAPES, FAPESP.

P441

Optimisation of Conditions for Accurate Diffusion NMR Measurements

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Diffusion Ordered NMR spectroscopy (DOSY) is used to observe the self-diffusion of a molecule in solution,[1] which is related to a range of physical properties, including size, shape and aggregation. This technique is being applied to many areas of chemistry including mixture analysis, reaction monitoring, molecular association, and MWt estimates,[2] some of which involve quantitative analysis of the measured diffusion coefficient. However, particularly in low viscosity solvents, these measurements can be inaccurate due to the presence of convention.[3] This poster describes a simple experimental method to detect convection, and recommendations on sample preparation to minimise such effects. We also propose a method to correct data that has over-estimated diffusion coefficients due to convention.

We demonstrate how the acquisition of DOSY measurements can be readily automated, increasing the throughput of such analysis. We also highlight how the use of a Matrix-Assisted DOSY[4] can be used to enhance resolution of species with similar diffusion coefficients.

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P442

Study of a NOP Receptor Antagonist in Interaction with Cellular Membrane Models

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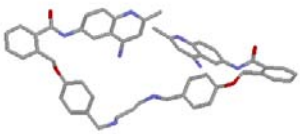
The NOP receptor belongs to the family of the G-coupled opioid receptors which have been demonstrated to form omo end heterodimer on their activation. Bivalent ligands potentially acting on dimer receptors are a unique possibility of eliciting different cellular response in respect to the activation of the monomer.

The bivalent ligand selected for this study consists of a monomeric moiety corresponding to the non competitive NOP antagonist JTC-801 [*N*-(4-amino-2-methylquinolin-6-yl)-2-(4-ethylphenoxymethyl)benzamide] and a diaminopropane spacer (Figure 1). It maintains the monomer affinity in the nanomolar range (IC_{50} 31 nM) and it is also active as a non competitive selective antagonist of the NOP receptor.



Ligand conformation in the presence of liposomes

High resolution 1D and 2D tr-NOESY NMR experiments were performed to calculate the internuclear distances within the ligand structure in the presence of DPC micelles and DMPC/cholesterol liposomes mimicking the cellular membrane.

Distances from NMR data were used as conformational restraints for molecular modelling calculations and the preferred conformations of the ligand in interaction with the membrane models were predicted.

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P443

Investigation of sterically stabilized (Stealth) liposomes by Diffusion Ordered NMR Spectroscopy

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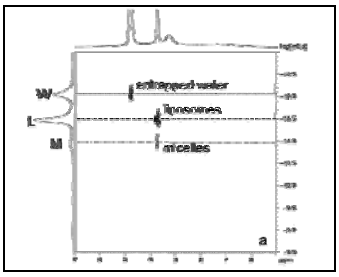
The present investigation explores the scope and applicability of DOSY for the investigation of sterically stabilized liposomes composed of DPPC, cholesterol and diblock PEG based copolymers with short lipid mimetic units. DOSY was applied for discrimination between the liposomes and micelles formed in liposome suspension and for the determination of their size. The relative amount of polymer incorporated in liposomes and in micelles, an important characteristic of these systems, was also determined. Lidocaine HCl encapsulation and release were monitored through time dependence of the diffusion profiles.



Mean hydrodynamic diameters of 120 nm and 38 nm were determined for liposomes and micelles, respectively. The relative fractions of polymer incorporated into liposomes, micelles and other smaller aggregates obey the ratio 88:11:1. Within 24 hours, about 10 % of the quantity of lidocaine encapsulated in liposomes is released in the volume phase.

Thus, DOSY is suitable for determining liposome sizes and discriminating between all structures generated in liposome suspensions. The main advantage of DOSY using a high-intensity gradient probe (max. *ca* 1200G/cm) is that it allows to determine precisely very low diffusion coefficients and to investigate the restricted diffusion of internal water phase, not accessible using standard methods. In addition DOSY turns out to be very convenient for tracing drug release kinetics and could be particularly useful for the investigation of drug release without appropriate chromophore systems.

P444

Degradation of Luminol, Epr and Electronic Structure Calculation in the Characterisation of Carboxyphenylporphyrins

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Metal carboxyphenylporphyrins: TcPPM, M = H, Fe, Co, Ni, Cu, and Zn, free and anchored on TiO_2 were synthesized and characterized by degradation of luminol, EPR spectroscopy and electronic structure calculation. The EPR spectra were measured in X- (9 GHz) and Q-bands (34 GHz) at room temperature and at 77 K. The EPR spectra of TcPPH, TcPPCo, TcPPNi and TcPPZn exhibited only one line attributed to free radicals, while in TcPPM anchored on TiO_2 (TcPPM/TiO_2) the signal showed lower intensity than the free porphyrins (about of 300 times less intense), which is in agreement with the number esteem of molecules in the free and anchored porphyrins. The EPR spectra of TcPPCu and TcPPFe exhibited resolved lines of Cu and Fe species respectively. For TcPPCu, the spin Hamiltonian parameters were accurately determined: $g_{/\!/}$=2.186; $g_{\perp}$=2.055; $A_{/\!/}$=183 Gauss; $A_{\perp}$=8 Gauss and $A'_{Cu\text{-}N}$~16 Gauss. EPR spectra indicated that the bond formed between the carboxyl group of the porphyrins and TiO_2 did not influence the signals of the porphyrin ring. The calculated Hfcc's showed an excellent agreement with experimental results. The degradation of luminol with visible light evidenced the formation of superoxide anion radicals (O_2•-) with M-tetra(4-carboxyphenyl)porphyrins in solution (TcPPM) and anchored on TiO_2 (TcPPM/TiO_2). Supported porphyrins showed higher photoactivity than the porphyrins in solution.

Acknowledgments: COLCIENCIAS and CAPES for the financial support of grants; and the EPR Division of Bruker-BioSpin GmbH (Germany) for recording the EPR spectra in Q-band.

P445

Intermolecular ^{h}J Scalar Couplings Provide an Insight into the Geometric Arrangement in the Hydrogen Bonding Network of Acylguanidines

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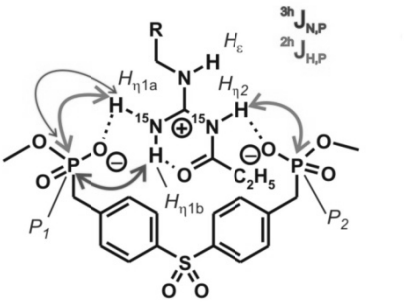

Despite the crucial role of H-bonding networks in enzymatic reactions, ligand recognition and organocatalysis, the detailed understanding of H-bonding in solution is rather limited. The effect of acylation of guanidinium moieties on the binding mode and H-bond strengths has been investigated, because acylguanidines provide significantly improved pharmacokinetics, activities and selectivities in several receptor families.[1]

Using an artificial arginine receptor, initially developed by T. Schrader,[2] as a model system, we were able to detect hydrogen bonds directly to individual guanidine and acylguandine protons with the help of 1D- and 2D-^{1}H,^{31}P-HMBC.[3,4] The quantification of 1D, 2D and 3D correlations caused by $^{2h}J_{HP}$ and, for the first time in non-biomolecules, $^{3h}J_{NP}$-couplings, leads ultimately to an insight into the spatial arrangement of the NH-OP H-bonds and indicates an end-on binding mode.

The effect of carboxylation of the tweezers shall be investigated, thus a more detailed understanding of the corresponding binding motives may be reached, leading to explicit insights into the binding motives of the guanidine function in G-protein coupled receptors.

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P446 (*)

Metabolic signatures of lung cancer in biofluids: an NMR-metabonomics study

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Lung cancer is the leading cause of cancer death, its poor prognosis being related to asymptomatic development and late detection. Hence, there is great need for novel biomarkers that can aid in the early detection of lung cancer. In this study, NMR-metabonomics, which has established value in oncology research,[1] is applied for investigating lung cancer metabolic signatures in blood plasma and urine.

Biofluid samples from lung cancer patients (n=70) and a control healthy group (n=61) were analysed by high resolution ^{1}H NMR (500 MHz), and their spectral profiles subjected to multivariate statistics, namely Principal Component Analysis (PCA), Partial Least Squares Discriminant Analysis (PLS-DA) and Orthogonal Projections to Latent Structures (OPLS)-DA. In the case of blood plasma, OPLS-DA resulted in reasonable discrimination between patients and controls, mainly due to increased levels of lactate, glycoprotein and LDL+VLDL, and lower levels of HDL and histidine in cancer compared to healthy subjects. Interestingly, very good discrimination between cancer and control groups was achieved by multivariate modeling of urinary profiles. The metabolites contributing to class separation were mainly creatinine, phenylacetylglycine, citrate, dimethylamine (elevated in patients), and hippurate, N-methylnicotinate and creatine (reduced in patients relatively to controls). These results show, for the first time to our knowledge, the promising potential of NMR-metabonomics for finding putative biomarkers of lung cancer in biofluids, collected in a minimally invasive way, which may have important diagnostic/prognostic impact. Correlations between the biofluids metabolic profiles and the tumours histological type and stage are also explored.

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Acknowledgments: FCT-Portugal, for funding (FCT/PTDC/QUI/68017/2006, SFRH/BD/63430/2009); INDASA for collaboration in biofluid collection.

P447 (*)

Heat-Bath Cooling of Spins in Amino Acids

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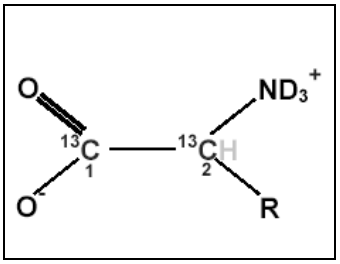
Algorithmic cooling of spins[1] generates, without external cooling, spin ensembles that are highly polarized in magnetic fields. We applied heat-bath cooling,[2] a key building block of algorithmic cooling, to 1,2-$^{13}C_2$-amino acids (glutamic acid and glycine), where the α protons relax much faster than C1. For each amino acid, both ^{13}C signals were enhanced simultaneously. In addition, the total entropy of each spin-system (including all protons) was reduced following a significant delay before measurement, thus bypassing Shannon's entropy bound. The effect of adding Magnevist®, a gadolinium-based contrast agent, was evaluated.



The pulse sequences used for heat-bath cooling of glycine and glutamic acid are expected to be suitable for other amino acids. More extensive heat-bath cooling and algorithmic cooling are potentially feasible in the presence of Magnevist or other paramagnetic MRI contrast agents. Algorithmic cooling holds the potential to improve in vivo ^{13}C spectroscopy of slow metabolic processes not accessible by hyperpolarization.

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Acknowledgments: We thank Dr Dorith Goldsher for a sample of Magnevist. This research was supported by the Israeli Ministry of Defense, the Promotion of Research at the Technion - Israel Institute of Technology, the Wolfson Foundation, and the Institute for Future Defense Research.

P448

In solution NMR studies reveal enhanced affinity of anti HIV-1 antibody 2G12 for multivalent gold-glyconanoparticles compared to monovalent glycan ligands

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The human antibody 2G12 neutralizes a broad range of human immunodeficiency virus type 1 (HIV-1) isolates by binding a dense cluster of carbohydrate moieties (*high-mannose* glycostructures) of the gp120 virus envelope glycoprotein.[1] Solid state studies revealed that 2G12 is highly specific for the terminal moieties of these *high-mannose* (Manα1→2Man).[1,2] Nevertheless, its carbohydrate specificity is less restrictive than originally believed,[1] and binding to different arms (D1 and D3) of branched oligomannosides has been described, which relaxes the constraint of an exact match of the oligosaccharides with respect to the multivalent binding site of the antibody.[2]

Our research is supported by in-solution NMR ligand-based techniques (Saturation Transfer Difference and transferred NOESY)[3] and theoretical calculations. We have characterized the interactions between 2G12 and synthetic oligomannosides, structural motifs of the natural *high-mannose type* glycans presented on the surface of gp120. Based on these results, multivalent oligomannoside functionalized gold nanoclusters (*manno*-glyconanoparticles), prepared to mimic the glycan clustering in the gp120,[4] have been studied pursuing to improve the affinity of the monovalent ligands.

In the present work we study by competition STD NMR experiments in solution the affinities of these glyconanoparticles towards 2G12 in the presence of monovalent ligands. Enhancement of affinity due to the so-called *cluster effect* was observed for some glyconanoparticles, and the effect of ligand surface density at the glyconanoparticle on affinity will be discussed.

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P449

Analysis of Furosemide, Metronidazole, and Chloramphenicol by Using NMR and UV/VIS Techniques

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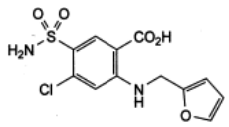
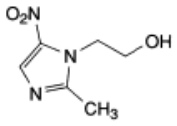
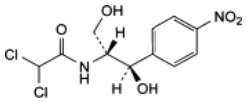
Nuclear Magnetic Resonance (NMR) spectroscopy has become an important tool in analysis of pharmaceutical products. This includes drug identity, structure of raw materials and finished pharmaceutical products, drug discovery[1] etc. As drug molecules absorb radiation either in the ultraviolet or in the visible region, Ultraviolet/ Visible (UV/Vis) spectroscopy is used in determination of active ingredients in drugs,[2] reaction kinetics of drug degradation etc. The major goal of this work was to use NMR and UV/Vis techniques in primary checking of the quality of some of the commonly dispensed drugs (Furosemide "**F**", Metronidazole "**M**", and Cloramphenicol "**C**") which are used by patients.



Furosemide "**F**"



Metronidazole "**M**"



Cloramphenicol "**C**"

Although analysis of **F**, **M**, and **C** was performed without further purification, ^{1}H, ^{13}C, and DEPT-135 NMR spectra shows the suitability of NMR technique for characterizing such drugs. The percent content of the active ingredient of **M** and **C** fell within the limited range of British Pharmacopoeia (B.P.) 2007, whereas, the percent content of **F** exceeded the range of B.P. 2007.

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P450

Pure Shift DOSY

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Diffusion-ordered NMR spectroscopy (DOSY) allows the spectrum of a mixture to be resolved into individual components on the basis of their diffusion coefficients. Good results require well-resolved spectra; peak overlap in the frequency dimension, almost unavoidable in ^{1}H NMR, leads to artefacts such as peaks appearing in compromise positions in the diffusion dimension. Signal overlap and its attendant problems can be greatly reduced by simplifying the proton spectrum to give a homodecoupled or 'pure shift' spectrum.

Pure shift techniques based on homonuclear 2D J spectroscopy[1] have been long available but are all more or less unsatisfactory. The properties of the phase twist lineshape that is inherent to the technique[2] necessitate the use of severe weighting functions and absolute value display, so that 45° projection of absolute value 2D J spectra yields pure shift spectra with broad lines and distorted intensities. The introduction of the Zangger-Sterk pulse sequence element[3] has led to significant improvements. This combination of selective pulse and magnetic field gradient, simultaneously slice- and shift-selective, allows a subset of the spins to be treated as heteronuclei which can then be manipulated independently of the rest of the sample. A number of pure shift experiments[4, 5] have been developed that show resolution of complicated multiplets and pure absorption mode pure shift spectra. The extension of such sequences to produce 2D[4] and 3D pure shift DOSY experiments will be demonstrated, alongside a number of illustrative examples and applications.

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P451

^{57}Fe NMR study of polymer coated γ-Fe_2O_3 nanoparticles

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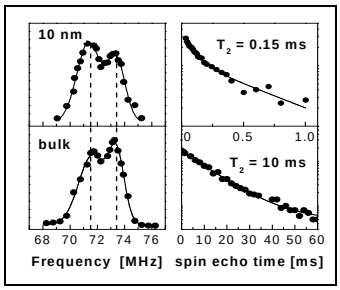

The study of the magnetic properties of nano-sized materials has been an active field of research for over fifty years. Recently, ferrofluids (colloidal dispersions of magnetic nanoparticles) attracted strong interest due to their use in many technological and biomedical applications. In this work, ^{57}Fe nuclear magnetic resonance in single-domain, ferrimagnetic γ-Fe_2O_3 coated nanoparticles of 10 nm diameter is reported. Zero-field spectra and T_2 spin-spin relaxation times have been measured as a function of temperature in the liquid helium range. The spectra could be readily resolved into two hyperfine field components corresponding to tetrahedral and octahedral sites. The similar observed hyperfine fields between the bulk and nanoparticles samples indicate similar magnetic structure, whereas the effective T_2 relaxation time of the nanoparticles is found two orders of magnitude shorter than the bulk material. It is shown that the dramatic reduction of T_2 when the particle diameter decreases is due to slow thermal fluctuations in the longitudinal magnetization of the nanoparticles in the low temperature limit.[1]

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P452 (*)

SERF-filtered experiments: new enantio-selective tools for deciphering complex spectra of racemic mixtures dissolved in chiral oriented media

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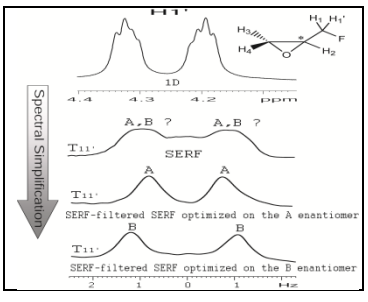

The increased development of enantio-selective synthesis has intensified the need for suitable methods for determining enantiomeric excesses and elucidating the absolute configuration of chiral compounds. Our laboratory has developed since more than fifteen years now,[1] an original and powerful NMR methodology based on chiral ordering solvents to differentiate and to quantify enantiomers, being much more robust than traditional liquid state NMR techniques. However, analysis of 1D ^{1}H spectrum of enantiomers mixtures dissolved in chiral oriented media is usually hard to decipher. Thus, we have implemented NMR experiments like selective echoes[2] (SERF) to simplify ^{1}H spectrum in chiral oriented media. However, these experiments are not convenient to assign dipolar couplings to each enantiomer in case of racemic mixtures, since signals intensity (integral) remains identical. To overcome such limitations, we have developed a new set of experiments baptised SERF-filtered techniques for oversimplifying the spectra. Among them, we will demonstrate that ^{1}H-SERF-filtered SERF experiment allows to assign all ^{1}H-^{1}H dipolar couplings to each enantiomer by enhancing the spectral resolution, especially in case of very close couplings that could not be separated with SERF experiments (see Figure). These new enantio-selective tools permit for the first time to get a coherent ensemble of dipolar couplings for each enantiomer, being necessary to access the molecular orientation.

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P453

Conformational Studies on β-ACC containing Tripeptides in Solution by NMR and MD

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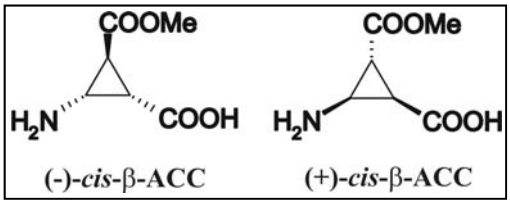
In biological systems and organocatalysis specific conformations of peptides often provide high activities and selectivities. In this context, derivatives of the unnatural cis-β-aminocyclopropanecarboxylic acid (β-ACC) have been shown to stabilize secondary structure elements even in short peptide sequences.[1] Therefore, foldamers containing β-ACCs are used in medicinal chemistry as analogues for the neuropeptide Y with high selectivity for the Y1 receptor.[2] Additionally, tripeptides with β-ACC and proline like residues showed organocatalytic activity in inter- and intramolecular aldol reactions yielding high diastereo- and enantioselectivities.[3]



Here, we present the structure investigations of Pro–Pro–(-)-β-ACC–OBn and Pro–(-)-β-ACC–Pro–OBn and Hyp(OBn)–(+)-β-ACC–Pro–OBn.[4] To stabilize intramolecular interactions in this ultrashort peptides, the NMR studies were performed in $CDCl_3$ at lower temperatures. The NOESY distance and RDC restraints were refined by a spindiffusion relaxation matrix calculated with AUREMOL and molecular dynamics simulations were done with CNS.

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P454

DENA: a New Sequence Element for Unambiguous Chemical Shifts in Aliased HQSC Spectra

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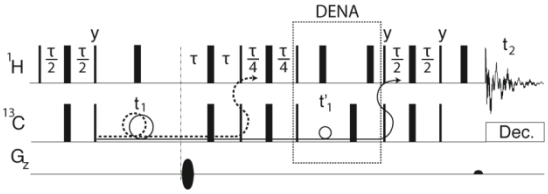
In normal ^{1}H-^{13}C 2D spectra, signals have low resolution in the carbon dimensions, which causes low precision (XXX.X) carbon chemical shifts. In aliased 10 ppm spectra,[1] chemical shifts are quite precise (??X.XXX) but ambiguous because of the violation of the Nyquist theorem. Reducing the spectral window from 10 to 9.9 ppm results in a quantized shifting the signals corresponding to the aliasing order. Overlapping 10 and 9.9 spectra provides easy access to the unknown digits (??). To do this in a single experiment the new DENA (Differential Evolution for Non-ambiguous Aliasing) sequence divides the magnetization of SE-HSQC experiment in two parts and let the carbon chemical shift of one part to evolve slightly more. Combined with multiplicity edition, the DENA-HSQC experiment advantageously replaces 1D DEPT-135 spectra.



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P455 (*)
Multinuclear Nanoliter NMR Spectroscopy in a Microfluidic Chip

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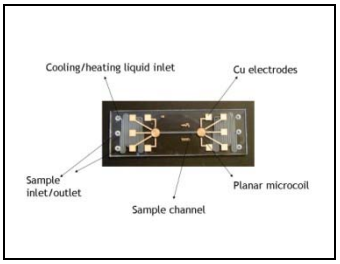
The amplitude of the NMR signal is maximized when the filling factor is optimal; therefore, an intense effort has been devoted over the past decade to the development of microcoil NMR probes for the analysis of mass-limited and volume-limited samples. We previously reported the study of supramolecular interactions using ^{1}H and ^{19}F NMR spectroscopy in a microfluidic chip equipped with a planar transceiver microcoil.[1,2] The on-line monitoring of a microwave-assisted cycloaddition was also described as a proof-of-concept for the hyphenation of small-volume NMR spectroscopy to other techniques.[3] Here we present the design of a second generation of microfluidic chips for nanoliter NMR spectroscopy, as well as the corresponding chip holder. The new setup allows a precise positioning of the microprobe inside the bore of the magnet, and enables the use of plug-and-play electrical connections on one side, and microfluidic connections on the other side. A sample cooling/heating channel was also included for variable temperature experiments. The possibility of detecting different nuclei combined with the advantages of working on-flow opens the path for many lab-on-a chip applications, including real time monitoring of chemical reactions using sub-microliter volumes of reagents.



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P456
^{1}H Photo-CIDNP and DFT Study of Monoreduced Ru(II) Complexes

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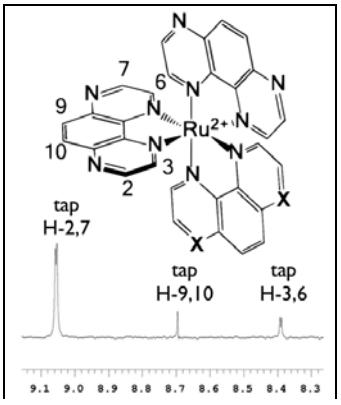
Polyazaaromatic Ru(II) complexes comprising at least two TAP (1,4,5,8-tetraazaphenantrene) ligands are known to photo-oxidise certain amino acids, such as tyrosine and tryptophane, and the guanine nucleobase.[1] Recently, some of us showed that these photo-reactions give rise to ^{1}H CIDNP (Chemically Induced Dynamic Nuclear Polarization) for H-2 and H-7 of the tap ligands.[2]

The photo-reactions of such complexes with hydroquinone have been studied by steady-state ^{1}H photo-CIDNP experiments. In agreement with the previous study, major signal enhancements are observed for tap H-2,7 but some photo-CIDNP is also detected for the tap H-3,6 and H-9,10. These experimental results are compared to the hyperfine coupling constants determined for the corresponding monoreduced Ru(II) complexes by DFT (Density Functional Theory) calculations that were carried out both *in-vacuo* and using a continuum model for water solvation.[3]



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P457

Demixing of Severely Overlapped Spectra through Multiple Quantum NMR

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We present an NMR approach to help in the analysis of complex mixtures by capitalizing on the simplified spectra associated with high-quantum orders, up to the limiting case of the maximum-quantum (MaxQ) observable coherence, which is always a unique singlet constituting the identification of molecular fragments. This approach performs best in the case of signals concentrated in a very narrow frequency range, which is a challenging situation commonly encountered in many relevant analytical problems such as the characterization of extraction fractions (oil, plants, tissues), biological fluids, or environmentally relevant samples. As a demonstration, we apply the MaxQ-NMR analysis to a test mix of 11 environmentally relevant molecules and to a mix of phenolic compounds.

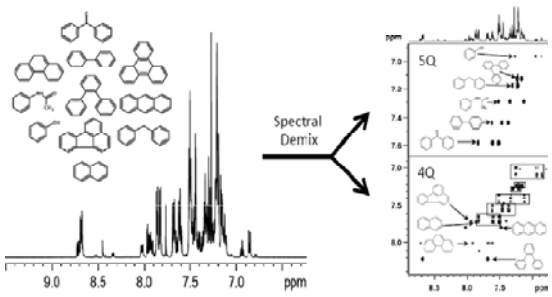

Fig1: Spectral demix of 11 molecule test mixture. 1D spectrum of the mix contains 80% of signals concentrating at 7.1-8.1 ppm (left). 5Q-1Q (top right), 4Q-1Q (bottom right) depict spectroscopic isolation of molecular fragments.

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Acknowledgments: We are grateful to the Agence National de la Recherche (ANR) for the financial support under grant ANR-08-BLAN-273-01 in the region PACA, France and to Spectropole (Federation des Sciences Chimique FR 1739) for privileged spectrometer time. Dr. Corinne Delaurent, C&S, ISM2, Marseille, France, is acknowledged for providing environmentally relevant molecules.

P458

In situ ^{1}H MAS NMR study of the mechanism of H/D exchange reaction between molecular hydrogen and Brønsted acid sites of Zn- and Ga-modified zeolite BEA

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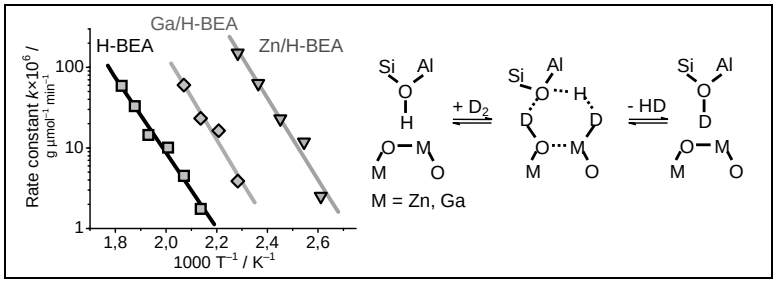
^{1}H MAS NMR has been used to study the mechanism of H/D exchange reaction between D_2 and acid OH-groups of zeolite BEA. The kinetic of the exchange reaction has been monitored *in situ* for both pure acid-form and Zn-, Ga-modified zeolite samples. Remarkable increase of the rate of the H/D exchange has been found for Zn- and Ga-modified zeolites as compared to that for pure acid-form zeolite. The rate of exchange for Ga/H-BEA zeolite is one order of magnitude higher as compared to that for H-BEA zeolite. The exchange reaction on Zn/H-BEA zeolite occurs 3 orders of magnitude faster as compared to H-BEA zeolite. Moreover, the temperature threshold of the H/D exchange reaction on metal-modified zeolite samples is decreased by 100 K. Promoting effect of metal on the rate of H/D exchange was rationalized by preliminary dissociative adsorption of molecular hydrogen on metal oxide species or metal cations. Dissociatively adsorbed hydrogen is further involved in the exchange with the acid OH-groups located in vicinity of metal species.



Acknowledgments: This work was supported in part by Russian Foundation for Basic Research (grant no. 07-03-00136). AAG is grateful to Zamaraev International Charitable Scientific Foundation for financial support.

P459

Monitoring alumina synthesis by operando relaxometry RMN

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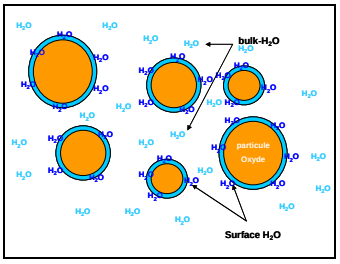
Alumina are widely used as support for catalyst in refining, petrochemicals and fine chemical process. The support selection is made taking into account the constraints of the process, such as pressure drop, diffusional limitations and surface properties.[1]



The aim to develop new refining processes and new catalysts able to transform heavy products into light product which can be directly included into fuel product (gasoline, kerosene and gas oil) is a challenge for IFP. The catalysts which are used are usually bifunctional materials composed by an acid active phase (the support) and an hydrogenating metallic phase. The improvement of existing catalyst and the development of new materials imply to have powerful analytic tools following up their genesis.

In this study, relaxometry NMR technique has been used to follow up boehmite particles synthesis by swing pH synthesis method.[2] This technique appears as a powerful tool to in situ follow up the surface particles amount, the binding layer thickness, the aggregation state and a second phase formation. This technique is an interesting way to drive and chose optima synthesis conditions to obtain the better compromise between the amount and the size of particles and the concentration of reactant. We are able to drive the phenomena of nucleation, growth and sedimentation and at the same time to estimate in-situ a specific area, an S / V ratio[3] or a mean aggregates radius.

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P460

Ufo-qNMR: A fast and efficient approach for quantitative analysis by ultrafast 2D NMR

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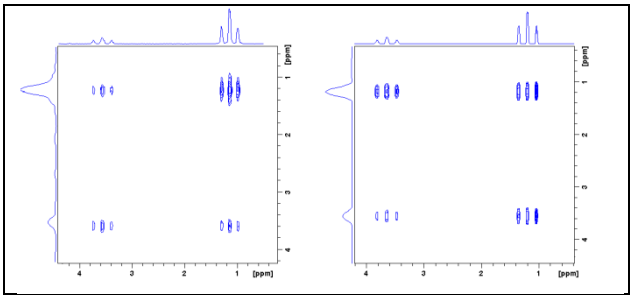
2D NMR is a powerful tool for precise quantitative analysis of complex mixtures presenting large overlap on 1D spectra. However, the long experiment durations characterizing conventional 2D NMR methods make them unsuitable for precise quantitative analysis, as the precision of long experiments is highly affected by spectrometer instabilities. Moreover, these durations are incompatible with quantitative studies of short timescale phenomena.

We have recently demonstrated the reliability of ultrafast 2D NMR[1] as a quantitative tool for fast and precise quantitative analysis of mixtures. Ultrafast Optimized Quantitative NMR (ufo-qNMR)[2] is characterized by an excellent precision and linearity. We will illustrate the principles and the quantitative potentialities of ufo-qNMR for a variety of applications. We have recently developed an ultrafast zTOCSY method with adiabatic spin lock to measure specific ^{13}C enrichments for ^{13}C-labeled metabolites in a single scan. We will also show the application of ufo-qNMR for following a fast chemical process: the mutarotation of D-glucose in aqueous solution by ultrafast HSQC.



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P461

Probing the Reactivity of Benzaldoxime Esters as Photoinitiators by Magnetic Resonance

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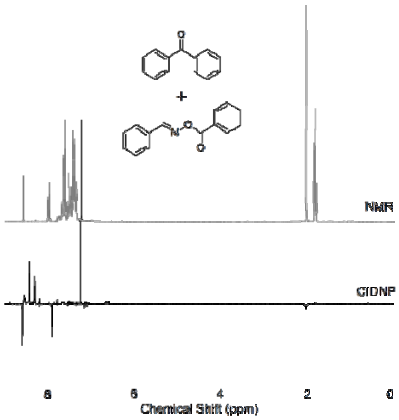
Photoinitiators are key compounds in light curable polymerization systems by generating the reactive (radical) species. A novel initiating system, comprising benzophenone and a benzaldoxime ester displays an excellent efficiency.

The mechanism of the photoinduced reactivity of this initiating system was investigated using time resolved EPR (TR-EPR) and **C**hemically **I**nduced **D**ynamic **N**uclear **P**olarization (photo-CIDNP) spectroscopy.

Benzophenone is acting as triplet sensitizer, greatly enhancing the light harvesting process compared to the pure benzaldoxime ester or other sensitizers.[1,2] The combination of TR-EPR and CIDNP offers conclusive and consistent insights into the reaction sequence of the photoinitiating system and the subsequent reactivity towards acrylate monomers. Thus information on the photophysical basis and the chemical nature of the active species can be elucidated.



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P462

2D NMR exchange experiments of natural porous media with portable Halbach-Magnets

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Mobile NMR has its origin in well-logging. By now there are numerous applications of mobile NMR in materials analysis and chemical engineering where, for example, unique information about the structure, morphology and dynamics of polymers is obtained, and new opportunities are provided for geo-physical investigations.[1] In particular, dynamic information can be retrieved by two-dimensional Laplace exchange NMR, where the initial NMR relaxation environment is correlated with the final relaxation environment of molecules migrating from one environment to the other within a so-called NMR mixing time t_m.[2]

Relaxation-relaxation exchange experiments were performed with saturated and un-saturated soil samples at low and moderately inhomogeneous magnetic field with a simple, portable Halbach-Magnet. By executing such exchange experiments for several mixing times and inverting the results to 2D T_2 distributions (reminiscent of joint probability densities of transverse relaxation times T_2) with the help of the inverse 2D Laplace Transformation (ILT), we observed characteristic exchange processes: Soils consisting mainly of silt and clay components show predominantly exchange between the smaller pores at mixing times of some milliseconds. There exists also weaker exchange with the larger pores observable for longer mixing time. In contrast to that fine sand exhibits 2D T_2 distributions with no exchange processes which can be interpreted that water molecules move within pores of the same size class. These results from fully saturated samples are compared to exchange at partial saturation.

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P463

Residual Chemical Shift Anisotropy (RCSA): A Tool for the Analysis of Configuration of Small Molecules

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Nuclear Magnetic Resonance (NMR) has been enhanced in recent years by weakly orienting molecules in anisotropic solution and thus recovering anisotropic NMR parameters such as residual dipolar couplings (RDCs) and residual chemical shifts anisotropies (RCSAs). In this communication, we describe an approach to measure RCSAs reliably, using the Kuchel scalable alignment device,[1] and to interpret RCSAs structurally using CSA tensors calculated from Density Functional Theory (DFT) combined with the Gauge Independent Atomic Orbital (GIAO) methodology.

We conclude that RCSAs deliver orientation information which can be used to determine conformation and configuration of molecules. For estrone, we could show that RDCs and RCSAs together, but not individually, allowed a clear differentiation of estrone from 13-epi-estrone. We have introduced a robust way to measure RCSAs based on adjustment of two alignments in NMR tube, using the stretching apparatus, referencing to a carbon of the molecule, and using differences of CSA tensors for the back calculation. Further ways of changing alignment in the same sample tube will be beneficial for this approach. We expect that RCSAs will be measured and used in the future whenever RDCs are measured to improve the determination of conformation and configuration of small molecules.

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Acknowledgments: We thank Dr. B. Luy for helpful discussions and for providing the stretching apparatus, the DFG which supported the research under the auspices of the Graduiertenkolleg GK 782 (to C.G.) and the Forschergruppe FOR 934 (to C.G.). F.H. thanks CNPq and Alexander von Humboldt Foundation. A.N.V. thanks Xunta de Galicia for financial support and Ministerio de Ciencia e Innovación for a "Ramón y Cajal" contract.

P464

NMR Studies of the Dynamics of Porous Inorganic Capsules

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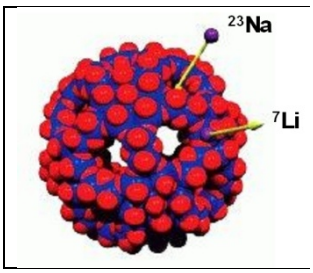
Extended heteronuclear NMR studies have demonstrated that porous inorganic molecular capsules of the type

$$[[(Mo^{VI})Mo^{VI}_5O_{21}(H_2O)_6]_{12}[Mo^{V}_2O_4(SO_4)]_{30}]^{72-}$$

behave like artificial cells concerning cation transport through molecular channels.[1,2] The extension of these studies focusses on other types of exchange reactions which are monitored by various NMR experiments. $^{1}H/^{31}P$-NMR titration spectra show, that internal anionic spacers can be exchanged against other anions with higher affinities. EXSY spectra demonstrate, that there remains an exchange equilibrium between the "free" anions in the solvent and the internal spacer molecules, and that the characteristics of this exchanges are influenced by the pH-value.



Additionally, previous studies of capsules with organic molecules[3] as part of the internal hollow space are extended to a compound with a longer alkyl chain. These compounds can be studied nicely by various experiments with ^{1}H and ^{13}C-NMR spectroscopy. While the DOSY and variable temperature spectra demonstrate the stability of the capsules in solution, an interesting behaviour of a compressed alkyl chain in a restricted compartment can be nicely studied with NOESY/ROESY-spectra.

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P465

Modeling of Peptides and Small Rings using Time-Averaged NMR Restraints

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Over the years, NMR has proven to be an excellent technique for the structure determination of many different types of organic compounds. For flexible molecules however, classical NMR-based modeling techniques fail to calculate accurate structures and new techniques, e.g. time-averaged restrained a molecular dynamics simulations (tar-MD), have to be explored. In this study, we use this latter technique to investigate the conformation of a very potent opioid mimetic (K_i=1nM) using NOE distance restraints. Results show a clear conformational equilibrium between an open and closed form that is corroborated using independent NMR measurements.[1]

Furthermore, we present the first conformational study using tar-MD of five-membered rings using $^3J_{HH}$ scalar coupling data. Because of the presence of five-membered rings in many molecules of biological relevance, their conformational analysis is of considerable interest. Until recently, a well-established mathematical procedure that fits two rigid conformations to the available experimental data was generally followed for this purpose. This so-called pseudorotation analysis approach is not without problems however, as chemically unrealistic conformations are sometimes generated from the data. We present our achievements on the use of tar-MD simulations as a generic tool to determine a realistic distribution of ring conformations that agree well with experimental $^3J_{HH}$ scalar coupling data.[2]

As tar-MD can now routinely be applied using desktop CPU power within an acceptable time, it should be considered a valid alternative for NMR-based structure determinations for a broad variety of flexible systems.

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P466 (*)

Unraveling multi-step reactions by real-time DNP-NMR

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NMR spectroscopy provides an extraordinary level of chemical information through observation of chemical shift and correlations in high-resolution spectra. However, NMR spectroscopy often requires signal averaging, and applications to the study of rapid reactions have been limited in scope. Hyperpolarization techniques, foremost dynamic nuclear polarization (DNP) now enable the measurement of NMR spectra of reactant and product species in a single scan, in samples at typical concentrations encountered in chemical or biochemical reactions, and of non-isotopically enriched compounds. Molecular species can readily be distinguished, enabling the investigation of multi-step reactions in real time. Chemical polymerization reactions, which involve the sequential addition of monomer units, can be studied either as a whole or step by step, without the need for specific isotope labeling techniques (Figure 1). Kinetic parameters and mechanisms in enzyme catalyzed reactions can be determined by comparing peak intensities of substrate and product, as evidenced on examples of trypsin, as well as uronate isomerase catalyzed reactions. Structural and mechanistic information is readily obtained through hyperpolarized NMR correlating chemical shifts in the traditional way through space, or over time between the reactant and the product species. Finally, the use of polarized or non-polarized reporter molecules included in the reaction mixture, as well as the effect of spin relaxation can provide dynamic information on intermediate species, even if these are not directly observable due to time scale or concentration limits.

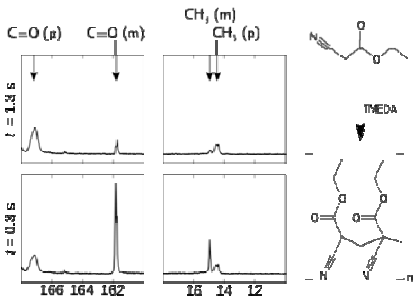

Figure 1: Real-time DNP-NMR of cyanoacrylate polymerization

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P467

^{13}C NMR study on anion-cation interaction of paramagnetic ionic liquid 1-alkyl-3-methylimidazoliun tetrachloroferrate

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The ^{13}C-NMR spectrum of paramagnetic ionic liquid of 1-alkyl-3-methylimidazolium tetrachloroferrate $[C_n mim]FeCl_4$, which is liquid state in room temperature, is observed at about 500 ppm lower field than the spectra of diamagnetic substances. The contact shift is inversely proportional to the concentration of the paramagnetic sample in a solution. Assuming a pseudo-contact shift for the shift, the distance between the anion and each carbon in the $[C_n mim]^+$ cation can be estimated.[1] The distances between the anion and all carbons in the cation decrease monotonously with increasing concentration in the solution (Fig.). The relative distances between the anion and carbons in the cation change dependent on the species of the solvent. As a result, by using a paramagnetic anion as a probe, information of the anion-cation interaction and structural information of the alkyl branch in solution can be obtained from the contact shift of carbons in the$[C_n mim]^+$ cation.

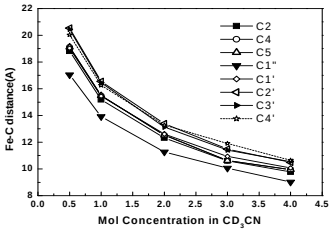

Fig. Distances between Fe and carbons in the $[C_4mim]^+$ cation depend on concentration in CD_3CN

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P468

Using ^{19}F NMR for structure elucidation

Kirsten A. M. Ampt[a], Martin Jaeger[b], Pepijn E. T. J. Geutjes[b], Sybren S. Wijmenga[a] and Maarten Honing[b]

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Fluorine containing compounds are becoming increasingly widespread in pharmaceutical and agricultural industry, since a fluorine substituent exercises a favourable influence on the chemical and physical properties of the compound, such as changing lipophilicity or altering the compounds metabolism.[1,2] In the structure elucidation of these compounds, NMR plays a pivotal role. Despite the favourable properties of the fluorine nuclei, such as sensitivity comparable to that of protons, and 100% natural abundance, large spectral dispersion, ^{19}F 2D NMR and certain ^{19}F 1D experiments, have not been exploited to their full potential. While the large spectral dispersion minimizes spectral overlap, fluorine NMR requires additional hardware such as probeheads and amplifiers and typically requires a reconfiguration of the spectrometer more commonly encountered with 'exotic' correlation spectroscopy.[3] On a Bruker DRX 400 FT-NMR equipped with a 5 mm QNP probehead, combined ^{1}H/^{19}F experiments were performed without changing the spectrometer configuration; for ^{19}F/^{13}C experiments the proton coil was detuned to fluorine and the preamplifier was bypassed.

Several types of ^{19}F NMR spectroscopy proved extremely useful in the structure elucidation of steroids especially when correlated to ^{1}H and ^{13}C as will be demonstrated in this study. In the examples chosen, ^{19}F correlation spectroscopy was used to assign diastereotopic fluorine nuclei as well as to determine the stereochemistry of a cyclopropyl ring fused to the A-ring of a steroid.

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P469

Interproton Distances from Nuclear Overhauser Effect (NOE) data: Rigid and Flexible Systems

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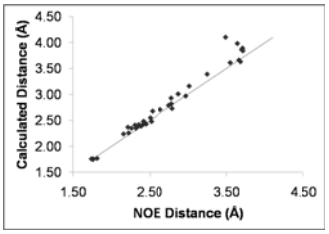
The determination of accurate interproton distances in solution using NOE data is an area of significant interest and complexity – the large majority of approaches rely on full relaxation matrix analysis of these data.[1,2] We present a much simpler method that can be used to derive accurate interproton distances from within rigid systems using 1D or 2D NOESY data. Strychnine is used as a model system to test the validity of this method. A comparison between the 1D NOE-derived distances and the best solvent-corrected gas-phase structure of strychnine[3] produces a mean absolute error of only 2.97% (0.09Å).



This technique is then applied to flexible molecules, where the possibility of more than one conformation contributing to the overall structure exists. 4-propylaniline is used as a model system, and we introduce results that suggest that NOE data can reliably predict average distances that compare very well with Boltzmann-averaged computational distances. Some ambiguities exist in literature over whether an r^{-6} or r^{-3} relationship should be used to describe internal motions faster than the overall tumbling of the molecule, and we demonstrate that the r^{-6} relationship holds in flexible, small molecules.

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P470

1D and 2D NMR tools. Quantitative ^{13}C and ^{31}P NMR of Castilla-La Mancha's Olive Oil

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Recently, NMR spectroscopy has been used to analyze the composition of foods. Due to its ability of simultaneously detecting a large number of organic compounds and to characterize them, it allows us to obtain the structural analysis using a swift and non invasive method. Different varieties of oils in our region were analyzed in order to distinguish its inborn characteristics by NMR spectroscopy.

^{1}H, ^{13}C, and 2D-NMR spectra allows us to distinguish between long-chain fatty acids (saturated and unsaturated) as free acid or as esterified with glycerol[1] and their distribution in the backbone of glycerin.[2] Minor compounds were easily detected too.

In addition, a methodology to quantify each of the components found in the different varieties of olive oil has been implemented, using an internal standard and/or a derivatiziting agent. Thus, the proportions of free fatty acids in the triglyceride form could be obtained by ^{13}C-NMR. For the quantification of the minor components, using a methodology by ^{31}P-NMR well established,[3] adding a derivatiziting agent, which contain ^{31}P in its structure, which reacts quantitatively with the free hydroxyl groups of the glycerine backbone.

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From 1D to 2D-NMR for metabolic profiling to large-scale annotations, and their approaches toward biomass engineering

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Since plants can fix carbon dioxide into useful biomass, studies of their metabolic systems may be an important field in the era of bio-refinery as an alternative to oil-refinery. The NMR-based metabolomics approach has much potential for not only basic science but also for applied science in plant systems. First, I will introduce techniques for the correlation of ^{1}H-NMR based metabolic profiling with genetic SNPs makers,[1] as well as plant hormone responses[2] in plant systems. Furthermore, stable isotope labelling with ^{13}C-carbon dioxide,[3] ^{13}C-glucose[4,5] or specific substrates[6] allows for the elucidation of metabolic pathways and movements by ^{1}H-^{13}C correlation NMR. In order to annotate a large number of metabolites from metabolite mixtures by 2D-NMR spectra, we have established a standard metabolite signal database and semi-automatic signal assignment software written in Java.[7,8] In particular, we have recently developed new statistical indices for large-scale annotations from a single 2D-NMR spectrum, enabling 211 plant metabolite annotations.[9] In addition to these metabolomics platform technologies, we have also introduced magic angle spinning (MAS) methods for characterization of low-solubility metabolites using intact plant tissues.[10] The potential for analyzing microbial metabolic dynamics[11] toward biomass engineering will be discussed in this presentation.

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P472

CIDNP study of peptides with S-containing amino acids

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Applying NMR spectroscopy to photoreactions often leads to line intensities different from thermal equilibrium, thus opening new ways to acquire information about structure of radical intermediates and reaction pathways. This method is known as chemically induced dynamic nuclear polarization (CIDNP). Typically the formation of a radical pair originating from a photo-excited dye and a quencher molecule, e.g. an amino acid residue, gives rise to polarization of the NMR spectrum. In a pulsed version time dependence measurements with microsecond resolution are possible allowing differentiation between geminate and bulk processes, so that reaction pathways and rate constants can be extracted. On the other hand field dependence of CIDNP allows determining magnetic resonance parameters (hyperfine coupling constants and g-factor) of elusive radicals.

The amino acid methionine is such a quencher because it is readily oxidized via electron transfer from the sulfur atom. Because such sulfur centered radical cations tend to stabilize themselves by forming a three electron bond between S and neighboring atoms with lone electron pairs, the reaction pathways depend on their distance.

While this had already been established for the free amino acid we extended this investigation to peptides and other S-containing biomolecules. We will present CIDNP results of a systematic study comparing peptides containing methionine and various co-residues (methionine, methylcysteine and glycine) in aqueous solution at ambient conditions. For studying the influence of geometric factors we performed measurements on linear and on cyclic structures in different enantiomeric forms. By comparing these peptides that differ in their sulfur-backbone-distances and number of thioether units differences in radical structure and reaction kinetics are shown and discussed. Here, primary and secondary reaction steps are differentiated and rate constants are determined.

P473

Structure Elucidation of Palau'amine Congeners

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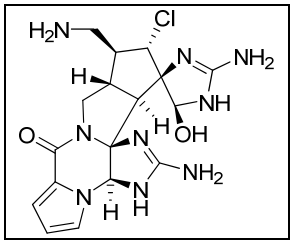
The determination of the relative and absolute configuration of natural products is essential to understand their biological activity and to allow their procurement through total synthesis. When crystalline products cannot be obtained, structural elucidations can be very difficult, especially when the targets do not lend themselves easily to standard 2D NMR techniques and may not easily be synthesized. The recent total synthesis of (±)-palau'amine[1] confirmed the structural revision of its relative configuration and that of its congeners.[2] The originally reported structure of palau'amine[3] served as a synthetic target for several groups worldwide for well over a decade.[4]



This work shows how effective NOESY/ROESY data in combination with computational methods can be used for the assignment of relative configurations. Since none of the palau'amine congeners have been crystallized, there is a special demand on NMR spectroscopy for this structurally very complex class of natural products. The investigated molecules have eight contiguous stereogenic centers, necessitating a method which allows a simultaneous determination of all unknown stereogenic centers. The determination of the relative configuration using ROESY data of axinellamine A and 3,7-*epi* massadine as well as macro-palau'amine (5 centers, a synthetic precursor of palau'amine) will be presented.

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P474

^{n}J(Se, H)-based conformational analysis of selenosugars: NMR and theoretical study

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Se-glycosides are important starting materials in synthetic carbohydrate chemistry playing a significant role in biological interactions as well. Both aspects are dependent on the conformation around the glycosidic bond. Se-glycosides were synthesized to study the conformational dependence, if any, of the $J(^{77}Se,^{1}H)$ spin-spin couplings.

Experimental values of J(Se, H) were determined by 1D and/or 2D ^{77}Se-^{1}H CPMG-HSQMBC[1] pulse schemes. The CPMG cycle applied at reduced power level circumvents the undesired heating of the sample, allowing accurate measurement of heteronuclear couplings for the temperature sensitive nuclei, such as Se. Theoretical calculations were carried out at the Second-Order Polarization Propagator Approach (SOPPA) level[2] taking into account all four non-relativistic coupling contributions to the total coupling constant, as implemented in DALTON program code.[3] All calculations of spin-spin couplings were performed using equilibrium geometries of the true-minimum conformers localized at the MP2/6-31G** level taking into account solvent effects within the IEF-PCM approach using GAMESS code. It is very encouraging that the population-averaged calculated values of ^{2}J(Se,H) and ^{3}J(Se,H) couplings are in a good agreement with experiment indicating an adequate theoretical level of the performed calculations. Indeed, this is one of a very few examples when high-level non-empirical calculations of spin-spin coupling constants (especially those involving selenium) are performed for molecules of that size.

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P475

Optimization of INPHARMA by multiple ligands and molecular docking

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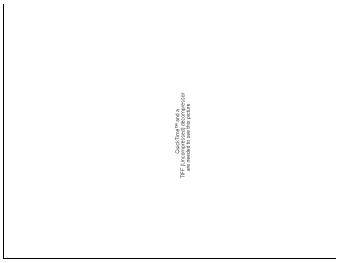
The INPHARMA method[1] uses a protein-mediated interligand NOE transfer between the protons of two ligands, which are competitively targeting a binding pocket. The method can be used in structure-based drug design to obtain the relative and even the absolute binding mode of the ligands. The presented idea is to use not only one combination, but three or more pairwise combinations of ligands to receive more information. It can be shown that this will lead to better and more reliable results than just one combination. Four indazole-based ligands, which bind weakly to PKA, were chosen and the NOESY spectra of the six possible double combinations recorded at different mixing times. Interligand peaks mediated by the protein were clearly observed and integrated. For every ligand 1000 molecular docking poses were created using PLANTS[2] and clustered by RMSD. For every clustered pose the theoretical interligand NOEs were back-calculated using the full-relaxation matrix approach[3] with our software ALICE. Comparison of experimental and calculated values together with the additional information of multiple combinations can rule out most wrong poses and lead towards correct binding modes known from crystal structure. The method shows a promising way towards the selection of molecular docking results which helps to speed up the development of new drugs.



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P476

Size of Molecular Clusters of Ethanol by Diffusion Measurements and Hydrodynamic Calculations

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Molecules of simple alcohols as well as water form hydrogen bonded clusters that are subject to fast reorganization in liquid state. The properties such as the cluster size, structure, lifetime, the free energy of its formation are necessary for explanation of often non-ideal macroscopical thermodynamical characteristics of the respective liquids.

We measured the temperature and concentration dependences of the translational diffusion coefficients of ethanol dissolved in a non-polar solvent (hexane). The typical cluster geometries obtained by DFT quantum chemical calculations were further used for theoretical prediction of the diffusion coefficients by means of hydrodynamic calculations (HydroNMR).[1] For the purpose of an application to small molecules, the typical settings of HydroNMR program were recalibrated by means of a model system of tetramethylsilane solution in hexane.

We determined the mean size of ethanol hydrogen bonded clusters present under different conditions. The clusters consisting of several units are present at decreased temperatures. The pure monomer was found at temperatures above 315 K in 0.16 mM ethanol solution. Its hydrodynamic radius was correctly predicted by the calculations.

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P477

Elucidation of Reaction Mechanism Providing Rapid Access to Spiro-indolin-3-one

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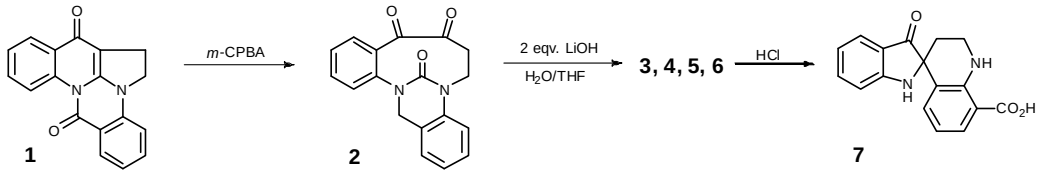
The heterocycle **1** subjected to an oxidative fragmentation reaction affords N-acyl cyclic urea **2**.[1,2] Whilst exploring the chemical reactivity of **2** by using a range of nucleophiles that may induce rearrangement, it was found that treatment of **2** with lithium hydroxide afforded the spiro-indolinone **7** after acidification. Following this reaction by ^{1}H NMR disclosed four intermediates (**3**, **4**, **5** and **6**). Structure elucidation of those intermediates was hampered by lack of hydrogen atoms in the cores of molecules where the structures were altered. However, dramatic differences in chemical shifts of aromatic protons were observed. These were caused by changes in both 2D and 3D structure of molecules and allowed us to identify all intermediates using combined approach of NMR spectroscopy techniques and molecular modelling at B3LYP/6-31G** level of theory.



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P478

Binding studies using an integrated dissolution DNP NMR spectrometer

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Recently it was demonstrated that large nuclear spin polarisation in liquid state samples can be generated by first increasing the polarisation of the nuclear spin system in solid state using dynamic nuclear polarisation (DNP) at low temperature followed by a fast dissolution step.[1]

A two-centre, integrated 3.4 T DNP polariser and 9.4 T liquid state NMR spectrometer has previously been presented.[2] Due to the proximity of the two magnetic centres in such a system, the polarised sample can be rapidly transferred and, furthermore, this can be done in the solid state followed by subsequent dissolution immediately above the NMR centre. This significantly reduces T_1 relaxation loss, as well as eliminating cross-relaxation that can arise when liquid-state samples are shuttled through a varying magnetic field. Consequently it is possible to observe signals from very short T_1 species in both natural abundance ^{13}C spectroscopy and low concentration ^{1}H spectroscopy.[3]

The DNP strategy is now being applied to study molecular binding. Specifically, of current interest is a system comprising Ala-D-γ-Glu-Lys-D-Ala-D-Ala pentapeptide binding with vancomycin.

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Multinuclear Solution and Solid-state NMR Investigation of Heterocycles Incorporating a B-N Bond

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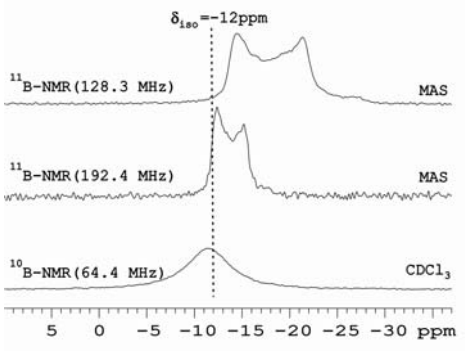
The incorporation of a BH_2 moiety into tetrazole–containing heterocyclic systems was studied by multinuclear (^{1}H, ^{13}C, ^{10}B, ^{11}B, ^{15}N) solution and solid-state NMR spectroscopy. The appearance of two diastereotopic BH protons and the *J*–coupling patterns of methylene protons in the ^{1}H NMR spectra obtained in solution state suggested intramolecular ring formation *via* the boron and one of the tetrazole nitrogen atoms, producing azaborinine derivatives. To support the proposed B–N bond formation, ^{10}B spectra in chloroform were also recorded.



In addition, solid–state NMR spectroscopy was applied to provide a link between the solution NMR data and the single crystal X–ray diffraction structure. Significant magnetic field dependence of the solid–state ^{11}B–MAS NMR spectra was observed. The isotropic chemical shift (δ_{iso}) obtained from ^{11}B–MAS was in good agreement with the ^{10}B chemical shift measured in chloroform.

We concluded that the comparison of solid–state and solution NMR spectra of azaborinine derivatives provides a feasible strategy for establishing the existance of the B–N bond.

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EPR Studies on Iron Nitrosyl Complexes

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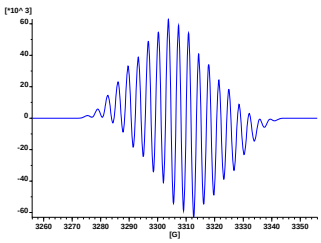
The recent realization that nitric oxide is a biological messenger in many physiological processes has brought about a renewed interest in its chemistry, particularly its iron complexes that are central to the role of nitric oxide in the body. Spectroscopic evidence would appear to implicate species of "$Fe(NO)_2^{+}$" type in a variety of processes ranging from polymerization, carcinogenesis, to nitric oxide stores. Our research aims at isolation and structural studies of biomimic iron nitrosyl complexes that are either coordinated to nitrogen or sulfur motif. We have shown that reactions between $Fe(NO)_2(CO)_2$ and a series of imidazoles generated new non-heme iron nitrosyls of the form $Fe(NO)_2(L)_2$ or $[Fe(NO)_2(Im\text{-}H)]_4$, which are closely related to the g = 2.03 species in biological systems. In this work, we will describe the EPR studies on two types of iron nitrosyl complexes: the dimeric Roussin's red salt esters $[Fe(\mu\text{-}RS)(NO)_2]_2$ (R = n-Pr, t-Bu, 6-methyl-2-pyridyl and 4,6-dimethyl-2-pyrimidyl) and $[Fe(NO)_2(L)]$, (L = 2,2'-bipyridine, 2,2',2"-terpyridine and 1,10-phenathroline). The former showed an isotropic g value of close to 2.000, while the latter exhibited a very complicated pattern in the 2.03 region as shown in the figure. The difference between the g values is explained by of the difference in unpaired electron distributions between the two types of complexes based on DFT calculations. This provides the theoretical bases for the use of g value as a spectroscopic tool to differentiate these biologically active complexes. Other spectroscopic evidences will also be discussed.



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P481

NMR Study of Some Organometallic Chalcogenides

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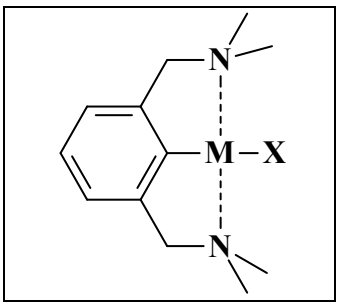
Multinuclear NMR spectra of organometallic chalcogenides (sulfides, selenides and tellurides) and analogous compounds of organotin, organoantimony and organobismuth will be presented. The common feature of these compounds is the presence of the N,C,N chelating ligands (2,6 – $(Me_2NCH_2)_2C_6H_3$ in the structure of the compounds[1] stabilising low valent metal centers (see Figure).



X-ray data proved the structures of compounds undoubtedly. Multinuclear NMR spectra were used to study the constitutions of products in solutions.

The values of appropriate chemical shift ranges as well the absolute values of various coupling constants will be presented.

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Acknowledgments: The authors thank the Czech Science Foundation for financial support (grant No. 207/10/0130).

P482

An NMR study on conformational preferences of diindolyl(thio)urea anion receptors

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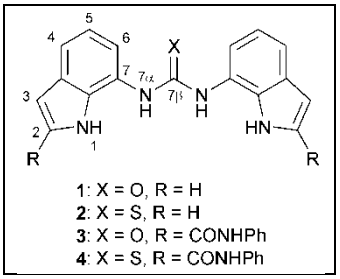
We have recently analyzed conformational preferences of several 2,7-disubstituted indoles with amide substituents at C2 and urea substituents at C7, which showed presence of distinct conformers in the presence and in the absence of anions.[1,2] Stimulated by these results, the conformational preorganization of diindolyl(thio)urea receptors **1-4** as well as their conformational changes upon binding of chloride and several oxoanions were studied by the means of NMR spectroscopy and augmented with energetic preferences established by *ab initio* calculations.



All receptors exhibit conformational preorganization in DMSO solutions, where *anti* orientation across both C7-N7α bonds is highly predominant. Anion-receptor interactions have been assessed through ^{1}H and ^{15}N chemical shift changes. NOE enhancements in the presence of oxoanions revealed that anion-receptor complexes favor the *syn* conformation along C7-N7α bonds.

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Acknowledgments: We would like to thank Professor Philip A. Gale from School of Chemistry, University of Southampton for providing the anion receptors.

P483

2D TR-NOESY Experiments Interrogate and Rank Ligand–Receptor Interactions in Living Human Cancer Cells

Silvia Mari[a], Chiara Invernizzi[b], Andrea Spitaleri[a], Luca Alberici[b], Michela Ghitti[a], Claudio Bordignon[b], Catia Traversari[b], Gian-Paolo Rizzardi[b] and Giovanna Musco[a]

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Integrins, the major class of heterodimeric transmembrane glycoprotein receptors, and the membrane-spanning surface protein aminopeptidase N (CD13) are two of the major membrane bound receptors highly expressed on the surface of tumour cells during angiogenesis, gaining importance as drug targets in cancer therapy. Recently it has been shown that deamidation of NGR sequence gives rise to isoDGR, a new αvβ3-binding motif.[1] Here we show that it is possible to apply 2D-TR-NOE techniques directly on human cancer cells to prove selective binding receptors such as αvβ3 and CD13.[2] We investigated the binding of a small library of cyclopeptides onto two human cancer cell lines differently expressing αvβ3 and CD13. Receptors can be also silenced with siRNA techniques to prove recognition specificity, and competition experiments can be applied to rank different ligands' affinity in a physiological context. In transferred NOE experiments, where the ligand molecule is in fast exchange between its free and receptor bound state, the averaged cross-relaxation rate $<\sigma ij>$ between two nuclei is described by $<\sigma ij> = N^F\sigma_F + N^B\sigma_B$ whereby N^F and N^B are the free and bound populations respectively and σ_F and σ_B are the cross-relaxation rates of free and bound ligand. Here we estimate a value for σ_F and σ_B for the case of a ligand interacting with a receptor localized on the membrane surface of living cells. We observe that σ_B can easily out-weigh the small value of σ_F. It is theretofore possible to detect binding by observing change in the sign of the ligand NOE cross-peak, even if it is in large excess (10^4-10^5 molar ratio) with respect to the receptor.

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P484

Fast 2D ^{1}H INADEQUATE NMR: a tool for precise quantitative analysis of metabolic mixtures

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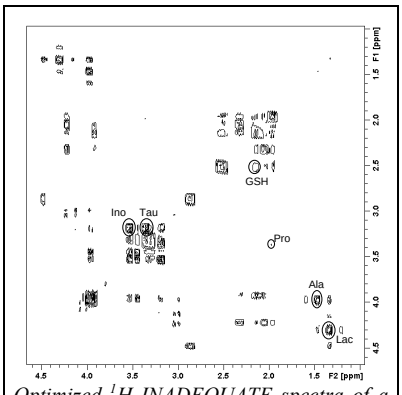
Quantitative analysis of metabolic mixtures by 1D ^{1}H NMR is a limited tool for precise quantification of biomarkers because of strong peak overlap. 2D NMR presents a high potential to unambiguously analyse metabolite contributions, however it suffers from long experiment durations. This drawback can be really prohibitive during establishment of calibration curves, a procedure which is necessary to obtain accurate and precise quantitative measurements. Moreover, long experiments are more sensitive to spectrometer temporal instabilities, leading to a degradation of precision.[1]

We have recently optimized a 2D ^{1}H INADEQUATE NMR method for a fast determination of metabolite concentrations in complex mixtures.[2] Acquisition and processing parameters have been carefully optimized to obtain the best precision in the shortest time possible. This protocol is evaluated in terms of precision and linearity on metabolite mixtures with concentrations as small as 0.1 mM. Quantitative ^{1}H INADEQUATE 2D spectra are obtained in 7 minutes with a repeatability better than 2 %. Moreover, the excellent linearity proves that our 2D ^{1}H INADEQUATE NMR protocol is a promising tool for metabonomic studies. Its potentialities for studying complex metabolic samples have been evaluated on breast cancer cell extracts.



Optimized ^{1}H INADEQUATE spectra of a model metabolic mixtures, obtained in 7 minutes.

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P485

Following the production of biolubricants by NMR

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With the aim to obtain more green products, the reaction of castor oil or castor oil biodiesel with fatty acids to produce biodegradable lubricants was studied and followed by ^{1}H and ^{13}C high resolution NMR. The spectra were acquired in a Varian MR-400 (9.4T) spectrometer, at ambient temperature. For the ^{13}C spectra a 40% solution of the sample in $CDCl_3$, 90º pulses, pulse interval of 10s, 2048 scans and the decoupler in the gated mode was used to assure quantitative conditions. The ^{1}H spectra were obtained using 5% solutions in $CDCl_3$, 45° pulses, 1.0s of pulse delay, 2.0s of acquisition time and 128 transients. The formation of estolides (oligomeric polyesters) due to the reaction of the carboxyl function of the fatty acid with the hydroxyl group present in the chain of castor oil or castor oil biodiesel was confirmed by HMQC. The reaction was followed mainly using the quintet at 4.87ppm of methinic hydrogen (H_m) at the ^{1}H spectra and the peak at 73.5ppm (methinic carbon C_m) at the ^{13}C spectra (Figure 1).[1] It was also possible to calculate the estolide number (EN) which is directly related with the size or molecular weight of the estolide obtained through the area ratio of appropriate peaks on both spectra.[1,2] The EN obtained by both methods are consistent and are also comparable with Size Exclusion Chromatography (SEC).

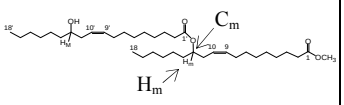

Figure 1. Structure of ricinoleic acid dimer.

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P486

PGSE NMR Diffusion and Overhauser Studies on a Variety of Transition Metal, Inorganic and Organic Salts: An Overview of Ion Pairing in Dichloromethane Solution

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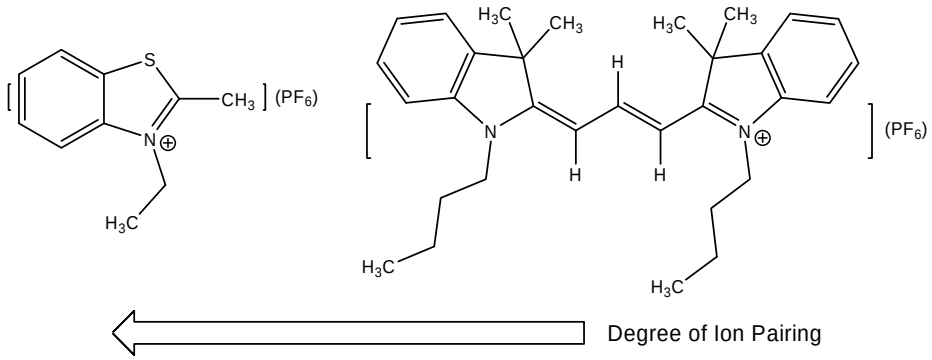

PGSE diffusion and ^{1}H, ^{19}F Overhauser studies on a variety of Transition Metal, Inorganic and Organic Salts are reported. These solution NMR results show that the charge distribution and the ability of the anion to approach the positively charged positions (steric effects due to molecular shape) are the determining factors in deciding the degree of Ion Pairing.[1]

References:
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P487

Rapid Quantitative Determination by ^{13}C-NMR of the Composition of Acetylglicerol Mixtures as Byproduct in Biodiesel Synthesis

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Commercially available partly acetylated glycerols (mono and diacetins) are a mixture of glycerol, 1- and 2-acetylglycerol, 1,2- and 1,3-diacetylglycerol, and triacetin. These byproducts of the biodiesel production are considered contaminants, and they alter the physical-chemical properties of the final product and can create engine problems such as engine deposits, corrosion, and failure. Usually, primary analytical methods involve chromatography (HPLC, GC), spectroscopy (MS, NIR), and wet chemical techniques (potentiometric, iodometric titration) which are often time-consuming due to sample preparation, extended analysis time, and/or complicated data analysis.

In this work, a complete ^{13}C chemical shift data for all five components allow for the identification of the components in the mixture and thus the determination of the composition is developed. This experimental protocol allows for rapid analysis of biodiesel mixtures of alcohols, glycerol, and mono-, di- and trisubstituted glycerides. Characteristic chemical shift ranges were developed with model compounds and used to fully characterize the conversion of triglyceride samples to biodiesel for two commercial production processes.

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Acknowledgments: Financial Support from the Consejería de Educación y Ciencia JCCM through project PBI08-0259-7224, and from FEDER projects UNCM05-23-039 and UNCM05-23-082 is gratefully acknowledged. CLTP also thanks the University of Castilla-La Mancha (Vicerrectorado de Investigación) for her Initiation to Research Grant.

P488

Characterization of Manchego Cheese by Nuclear Magnetic Resonance

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NMR spectroscopy is emerging as an alternative analytical tool in a number of applied fields, including Food Science. It is possible to identify many compounds in a complex mixture simultaneously and nondestructively.

"Manchego", a popular Spanish cheese, is produced from pasteurized or raw dairy milk according to a regulation approved by the European Union. The cheese has a protected denomination of origin (PDO) mark, which strictly defines the geographical area of its production. Cheese is a complex matrix which can change very much depending upon its origin, conditions of the manufacturing processing, microbial flora, enzyme activities, freshness, or ripening time.

A fast and reproducible extraction of the organic fraction[1] of Manchego cheese has allowed us to study lipid content, specially, conjugated linoleic acids which are really interesting for its antitumoral, immunomodulating and antidiabetic activities. Focusing on the ripening time, samples have been analyzed by ^{1}H, ^{13}C and ^{31}P-NMR upon derivatization of hydroxyl and carboxyl groups with a phosphorous reagent. On the other hand, the water-soluble metabolites, specifically, aminoacids content[2] of Manchego cheese has been related to ripening by the use of ^{13}C-NMR spectroscopy. The amino acid profile confirms the significant variations, as expected in ripening, giving an index of the proteolytic process.

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Acknowledgments: Financial Support from the Consejería de Educación y Ciencia JCCM through project PBI08-0259-7224, and from FEDER projects UNCM05-23-039 and UNCM05-23-082. We also thank the Consejeria de Educación y Ciencia for a predoctoral grant for María Moreno Peréz.

P489

Conformational isomerisation of Pt^{II}-coordinated 1,3,5-triazine in solution and solid state

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Platinum complexes bearing *N*-heterocyclic ligands have very promising biological and physical properties. Pt^{II}-coordinated 4-imino-6,6-dimethyl-1,4,5,6-tetrahydro-[1,3,5]triazine-2-ylamine was synthesized by amination of corresponding cyanoguanidine ligands followed by their intramolecular cyclization.[1] The structure of the complex obtained was studied by ^{1}H NMR spectroscopy in DMSO-d_6 and acetone-d_6, ^{13}C and ^{15}N NMR spectroscopies in solid state, X-ray diffraction and DFT calculations. It was shown that both *E,Z*- and *Z,Z*-configurations of *bis*(4-imino-1,3,5-triazine)Pt^{II} are present in solution. Furthermore, ^{1}H NMR titration evidences for increase of *E,Z*-conformer content by addition of solvent with low polarity. Investigation of complex precipitated from Et_2O by solid state NMR spectroscopy is in favor of two configurations of ligands. However, acetone-toluene (2:1) solution gave solid *E,E*-conformer as confirmed by X-ray analysis. According to DFT calculations of (1,3,5-triazine)Pt^{II}-complex isomeric forms the energetic stability of conformers decreases in a range: *Z,Z – E,Z – E,E*. Therefore, conformational isomerisation of a complex determined by Solid State technique can be accounted for intermolecular interactions and solvent nature.

References:
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Acknowledgments: This work was supported by grants of the President RF (MK 634.2009.3) and RFBR (grant 09-03-00065-a).

P490 (*)

Ex-situ DNP and water soluble perchlorinated trityl radicals: A flourishing match

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Dynamic Nuclear Polarization (DNP) -based on the transfer of non equilibrium nuclear polarization from electron spin polarization by microwave irradiation- is a potent and promising method to enhance the sensitivity of NMR signals in selected applications. Herein we report on the use of perchlorinated trityl radicals **1)** and **2)**, in Fig. 1, in *ex-situ* DNP experiments.[1] These stable and persistent water soluble radicals substantially differ from the commercially available and commonly used polarizing agents for the presence of chlorine nuclei. These radicals showed rather good efficiencies as polarizing agents of small molecules.[2] Moreover the sign of the DNP enhancement of radical **2** is substrate dependent, evidencing the fundamental role of intermolecular interactions.[2] The novel structure of these radicals prompted us to address the role played by chlorine nuclei on the DNP mechanism. DFT calculations suggest that in contrast to other trityl radicals, the polarization mechanism differs from the classical solid effect and support the hypothesis that polarization is transferred from the unpaired electron to chlorine nuclei and from these to carbon by spin diffusion.[3]

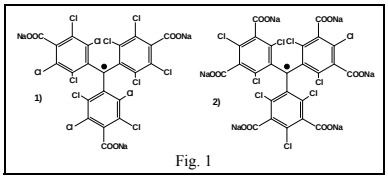
Fig. 1

References:
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Acknowledgments: M. Oliveros and Dr. N. Roques are gratefully acknowledged for the synthesis of the radicals used in this work.

P491

Metabolic Fingerprint of Latex by ^{1}H-NMR: Distinction between High and Low Production Clones

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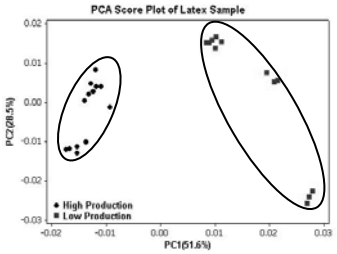
Latex, natural rubber, is composed for the most part by poly(cis-1,4-isoprene) polymer and minor compounds such as: proteins, carbohydrates, lipids, and minerals. Poly(cis-1,4-isoprene) has peculiar physical-chemistry properties and very often can not be replaced by synthetic rubber (poly(styrene-butadiene)).[1] ^{1}H-NMR spectroscopy is an important tool for organic compounds identification and has been applied with successful in the identification of animals and plant metabolites. Since the number of information provided by ^{1}H-NMR spectrum is wide the use of statistical tools is essential. Principal Component Analysis (PCA) has been shown positive results at the interpretation of ^{1}H-NMR data.[2]



The aim of this work was to evaluate the use of ^{1}H-NMR to distinguish between latex samples of *Hevea brasilienses* which shown high and low production of poly(cis-1,4-isoprene) by using PCA analysis.

The figure shows the PCA score plot of the latex samples. According to this was possible to differentiate samples between high and low production. The PCA loading plot (not shown) shows that the variables responsible for the distinction between the clusters are the compounds: acetate, acetoacetate, succinate, citrate, aconitic acid, choline, betaine, quebrachitol, ascorbate, and formate. The metabolite quebrachitol is a polyol present in high concentration in latex sample from *Hevea* and is related to the biosynthesis of poly(cis-1,4-isoprene).[3]

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P492

NMR of Na^+, glycine and HDO in isotropic and anisotropic carrageenan gels

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Chiral gels that become anisotropic when stretched or compressed differ from liquid crystals in that there is a continuum of anisotropy that can be obtained: the transition from isotropic to slightly anisotropic to more anisotropic states can be controlled and reversibly changed. In the last few years we introduced a simple device that achieves this by using gelatine as the chiral alignment gel.[1,2] Recently we described two new polysaccharide based gels that become anisotropic by stretching and can be reversibly adjusted just like gelatin, but at much lower gel concentrations, and can be used at 37 °C.[3] Chemically alike, ι- and κ-carrageenane gels yield quite different alignment properties for small chiral and prochiral solutes.[3] This finding implies structural differences for ι- and κ-carrageenan gels, and raises the question of the necessary structural elements for (carbohydrate) gels to become anisotropic when stretched. Here we discuss the isotropic and anisotropic states of ι- and κ-carrageenan gels as detected by Na^+ ions, glycine and monodeuterated water and monitored by solution ^{1}H, ^{2}H, and ^{23}Na NMR spectroscopy.[4] Anisotropy was introduced by stretching the polysaccharide gels, and the degree of structural alignment depended on the extent of stretching as well as gel and salt concentration, and the nature of cation and anion. For $^{23}Na^+$ (NaCl) a strong binding component of the anisotropy in ι- and less in κ-carrageenan gels was found, in contrast to a partial binding of glycine, and a spatial and a gel-concentration-dependent anisotropic effect for deuterated water (HDO). This finding is explained by the electrostatic interactions between Na^+ and ionic sulphate groups in the carrageenan polymer; HDO probably only interacts via hydrogen bonding; while glycine presumably interacts by both means.[4] The new methodology is ripe for spectral analysis of chiral mixtures.

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P493

Ligand Exchange Reactions in Cu(III) complexes: Mechanistic Insights by Combined NMR and DFT Studies

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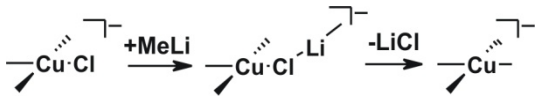

Regio- and diasteroselective C-C bond formation is one of the most important tools in organic synthesis. Thus organocopper reagents are frequently used in cross coupling reactions with alkyl halides or addition reactions with Michael acceptors.

In the past few years, high-resolution NMR studies revealed the experimental evidence for Cu(III) intermediates in conjugate addition reactions[1] as well as S_N2' and S_N2-type cross coupling reactions[2-4] of organocuprates, which had been proposed for years in theoretical studies.[5] In these NMR investigations not only the mechanistically expected Cu(III) intermediates but also tetraalkyl Cu(III)-species were detected.[3,4,6] Besides, the formation of several trialkyl Cu(III)-complexes with electron donating hetero-ligands was demonstrated.[7] These additional Cu(III) complexes hint at ligand exchange reactions in Cu(III) complexes.

Therefore, in this contribution possible intra- and intermolecular ligand exchange processes in Cu(III) intermediates are investigated by NMR and DFT calculations.[8]

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P494

NMR Structural Studies of the transient interaction between DC-SIGN and oligosaccharides and mimetics

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DC-SIGN (Dendritic Cell-Specific ICAM-3 Grabbing Non-integrin) is a C-type lectin presenting a Carbohydrate Recognition Domain (CRD) at the C-terminus, that recognizes specifically high glycosylated structures present at the surface of several pathogens such as viruses (HIV, SIV, Hepatitis C), bacteria, yeasts and parasites, and plays a key role in the infection process by interactions between carbohydrates from pathogens glycoproteins (gp120, GP1, etc.). A structural study of the transient interaction between DC-SIGN (ECD) and simple oligosaccharides or glycomimetics containing mannose or fucose has been performed by NMR. The interaction with the lectin was analyzed mainly by STD-NMR spectroscopy.[1] Using full matrix relaxation calculations employing CORCEMA-ST,[2] we investigate the effect that the existence of multiple modes of ligand binding,[3] and analyze the interaction of saccharides to DC-SIGN, comparing mannose with fucose based models and mimetics as well as multivalent analogues.

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Acknowledgments: We thanks the EU (PITN-GA-2008-213592, CARMUSYS) and MICINN (CTQ2009-07168).) financial support. J.A. holds M.E.C-Ramón y Cajal felowship.

P495

Advantages and Drawbacks to use Gradient Coherence Selection in Selective 1D and 2D HOESY Experiments

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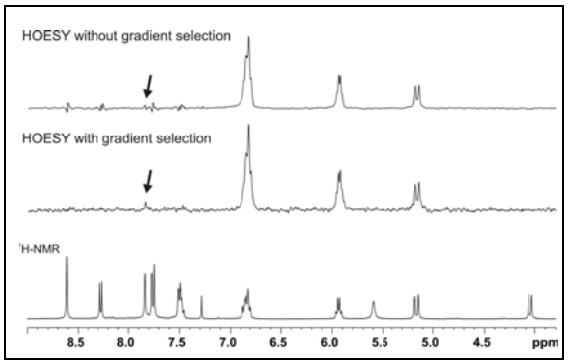
The use of gradient coherence selection is discussed in ^{1}H-X HOESY experiments. The use of a refocusing gradient for coherence selection affords ultra-clean HOESY spectra without need of phase cycle and free of artefacts from which tiny heteronuclear NOEs can be clearly observed. In addition to possible diffusion losses and an inherent sensitivity penalty, undesired convection effects can also be deleterious when working on non-viscous solvents. The use of sample rotation to minimize such convection effects is exemplified on ^{31}P and ^{19}F-containing compounds. The main advantages of the 1D version over the most time-consuming 2D counterpart are also presented and discussed.



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Acknowledgments: Financial support for this research provided by MICINN (projects CTQ2009-08328 and Consolider Ingenio-2010 CSD2007-00006) and Universitat Autònoma de Barcelona is gratefully acknowledged.

P496

A new promising class of paramagnetic labels: clathrochelate complexes with an encapsulated cobalt(II) ion

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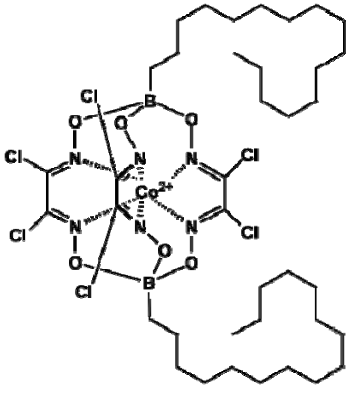
Hyperfine shifts in NMR spectra of paramagnetic complexes provide a wealth of information about structure and dynamics of investigated molecules.[1] The paramagnetic tags containing lanthanide or *d*-group metal ions are nowadays broadly used for structural characterization of various biological systems. Cage complexes with an encapsulated cobalt(II) ion[2] are perspective paramagnetic labels owing to the complete isolation of the encapsulated paramagnetic ion from the environment, the resulting stability of the complex and independence of its magnetic properties from the medium. The ^{1}H NMR spectra of the $C_{16}H_{33}$-substituted cobalt(II) clathrochelate provide an example of significant pseudocontact shifts leading to a complete resolution of the fifteen signals of methylene protons, otherwise heavily overlapped.



The possible functionalization by six ribbed and two apical substituents allows fine-tuning the properties of a complex to achieve the desired magnetic characteristics of an encapsulated ion. In this context, the spin-transition behaviour, which is observed for some complexes, is of a great interest.

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P497

Novel Simple Protocol for "in Tube" Derivatization and NMR Analysis of Chiral Alcohols and Amines

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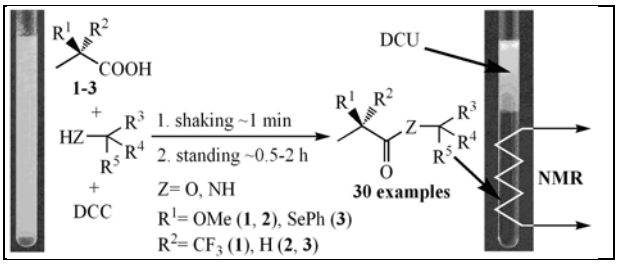
Determination of enantiomeric purity and absolute configuration of chiral molecules is an important task in modern asymmetric synthesis and catalysis as well as in drug design and investigation of natural compounds.[1] Nowadays NMR spectroscopy has become a powerfool tool for analysis of chirality. Though enantiomers cannot be distinguished by NMR spectroscopy itself, they can be converted into magnetically nonequivalent diastereomers by means of chiral derivatizing agents (CDAs), followed by reliable spectral analysis.[2] Recently we have developed a simple procedure of derivatization of chiral alcohols and amines in NMR tube suitable for direct NMR measurements without purification or isolation steps. Novel Selenium-based CDA **3** allows to use ^{77}Se NMR spectroscopy which offers superior discrimination of chiral compounds within the structure of the diastereomers compared to ^{1}H и ^{13}C NMR.[3]



Developed procedure of derivatization of chiral alcohols and amines with CDAs **1-3** in the NMR tube and results of NMR measurements will be presented.

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Acknowledgments: The work was supported by the Research Grant MK-1434.2010.3.

P498

Selective 1D HCH vs Selective 1D TOCSY: Complementary Tools for fast correlation of inter- and intra-residues ^{1}H-^{1}H connectivities

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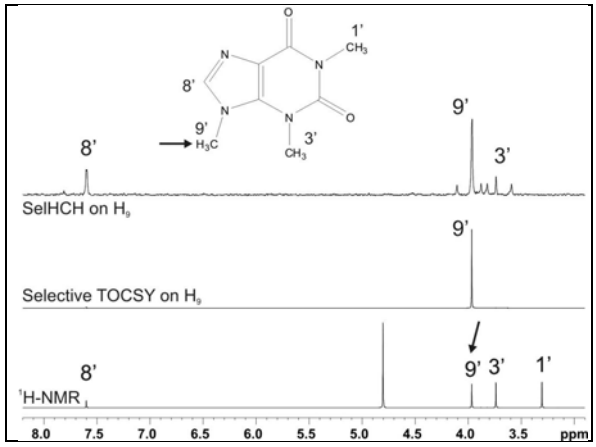
A selective 1D version of the HCH experiment (selHCH) is proposed for the fast and efficient connectivity between protons belonging to different proton spin systems. As any conventional selective 1D proton experiment, the selHCH experiment is simply based on the successful application of a frequency-selective 180º pulse on a well isolated proton. The clean final 1D proton spectrum probes to be an excellent complement to the conventional selective 1D TOCSY experiment to trace out inter-residue proton-proton connectivities through quaternary carbons or heteroatom centers. The experiment is based on a doubly-selective J_{CH} + J_{CH} coupling pathway and protons connected up to six bonds away can be observed. Experimental details and several examples will be discussed.[1]



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P499

Application of NMR-spectroscopy for keto-enol tautomerism observation in adamantane derivatives of 1,4-dihydroxynaphtholine

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While investigating reactions of adamantilation various derivatives of dihydroxynaphthalene and determined structure of synthesized compounds with using ^{1}H and ^{13}C NMR-spectroscopy, we have found out some features in a case of adamantilation of 1,4-dihydroxynaphthalene (**I**). Interaction **I** with adamantanole-1 in trifluoroacetic acid has led to formation 2-(1-adamantile)-2,3-dihydronaphthoquinone-1,4 (**II**), that is diketone form of 2-(1-adamantile)-1,4-dihydroxynaphthalene. In similar conditions diketone forms formation was observed at the interaction of compound **I** with tertiary butyl and amyl alcohols.

In presence of morpholine, compound **II** turns into phenolic form of 2-(1-adamantile)-1,4-dihydroxynaphthalene (**III**). This isomerization reaction took place for three days in a sealed NMR tube, from which oxygen was preliminary removed. During these days ^{1}H and ^{13}C NMR spectra were read every three hours; COSY and DEPT experiments were made. Concentration of keton and enol forms were calculated using integrated intensity of corresponding signals of proton spectra. After finishing the isomerization, NMR tube was depressurized and fast oxidation of compound **III** in 2-(1-adamantile)-naphthoquinone-1,4 (**IV**) was observed.

Compounds **II-IV** are an attractive and promising starting material for organic synthesis and hypothetically can possess useful biological activity.[1,2]

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P500

Colloidal behavior of wine tannins

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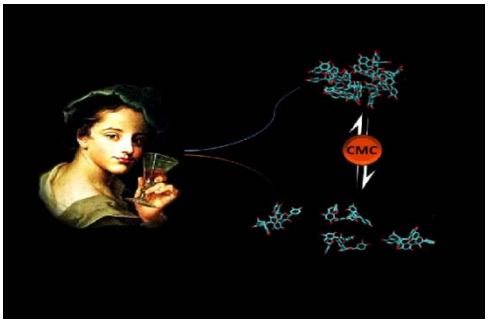
Tannins are one of the fourth keys components determining wine quality. They play an important gustative role since they are responsible for wine astringency and/or bitterness. They also act as structuring components directly linked to red wine turbidity and stability. If this later role is the direct consequence of their colloidal behavior, a recent study we performed shows that the colloidal state in which tannins are, can also influence their way to interact with proteins and thus their taste. So that, we were intested by studying the colloidal behavior of different synthesized procyanidins, galloylated or not, in order to get an insight in their specific self-association process by taking into account their specific 3D Structure. For this purpose, the colloidal behavior of 11different procyanidins (4 monomers, 6 dimers and 1 trimer) has been investigated using DOSY NMR Spectroscopy and Molecular Dynamics simulations. The analysis of all the collected data permits (i) to confirm that procyanidin tannins self-associate in a non specific way making "micelles" of polydisperse size (ii) to evaluate the average "micelles" size, close to 20Å, that corresponds to an aggregation of ten or so tannins molecules (iii) to determine the concentration above which tannins preferentially exist upon a micellar state (CMC) and (iv) to underline differences in behavior related to their specific 3D Structure. These differences, that greatly influence their way to interact with saliva proteins, are probably not involved alone to contribute to wine turbidity due to the nanometer size range of the formed "micelles".



P501

Multinuclear NMR and X-ray Diffraction Analysis of Some Thiosemicarbazone Derivatives

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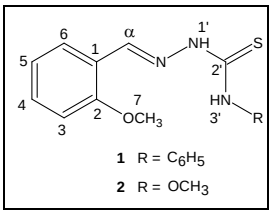
Thiosemicarbazones and their corresponding metal complexes are widely known compounds possessing diverse pharmacological activities, such as antitumor, antivirial, antibacterial, antifungal, antimalarial, *etc*. Bioactivity is closely related to molecular conformation which can significantly be affected by the presence of intra- and inter-molecular hydrogen bonds. Salicylaldehyde thiosemicarbazones can exist in several tautomeric forms with both intra- and inter-molecular hydrogen bonds. An intra-molecular O—H···N H-bond between the hydroxyl group and the azomethine N atom has been found in salicylaldehyde thiosemicarbazone family (resonance assisted H-bond). Additionally, an intramolecular N—H ···N H-bond between the thiourea NH group and the azomethine N atom was found in few salicylaldehyde thiosemicarbazones.



The aim of this research is to investigate the influence of substituents and solvents (different polarities, *i. e.* of different proton donor and acceptor abilities) on molecular conformation, tautomerism and structure of H-bonds in salicylaldehyde thiosemicarbazone derivatives. We present here a part of our study regarding the effect of substituting OH with OMe group in salycilaldehyde residue (therefore, breaking the O—H···N H-bond) on the overall structure and thione-thiol tautomerism. Solid state structures of two thiosemicarbazone derivatives **1** and **2** (two polymorphs) were characterized by high resolution ^{15}N and ^{13}C solid-state NMR spectroscopy and X-ray diffraction. The results were compared with those obtained for $CDCl_3$ and DMSO solutions. Possible tautomeric equilibrium changes were probed by multinuclear temperature dependent NMR experiments.

P502

The chemical shift prediction in tertiary amines and their *N*-oxides

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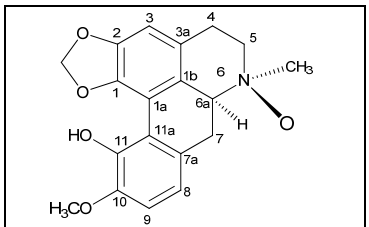
Amine *N*-oxides bearing different substituents on nitrogen atom are chiral compound with *N*-central chirality. We are interested in determination of the configuration of such chiral nitrogen by comparison of experimental and calculated NMR parameters, mainly isotropic chemical shifts.

Experimental NMR data of amines were obtained by measuring of ^{1}H and ^{13}C NMR spectra in $CDCl_3$. Subsequent *in situ* oxidation of starting amines by MCPBA in NMR tube and recording of the NMR spectra provided data for oxidized counterparts.



	H-6a	CH3	C-5	C-6a	CH3
obs	4.13	3.45	63.8	71.4	57.5
calc	4.25	2.97	64.2	70.1	57.1

Theoretical calculations were performed using *ab initio* (HF, MP2) and DFT methods on different levels of theory. In DFT, we have compared the influence of density functional, solvation and basis set. The testing of the calculation method was performed on set of achiral model compound including acyclic, cyclic and heterocyclic tertiary amines. It was found that geometry optimization including solvation (PCM) improved chemical shifts calculated afterwards. Also OPBE density functional was advantageous over other DFT functionals in prediction of NMR parameters.

Results of theoretical calculation screening were applied to chiral amine *N*-oxides, namely agroclavine-*N*-oxide[1] and bulbocapnine-*β*-*N*-oxide.[2] It was found that the experiment/calculation comparison approach could be successfully used in the prediction of chiral *N*-oxide configuration.

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Acknowledgments: The work is supported by the Czech Science Foundation (Grant No. 203/09/1919).

P503

Structure and Dynamics of N-terminal Sequences of Dermorphin Neuropeptide in the Solid State - NMR Spectroscopy Versus X-ray Crystallography

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In 70-ties of XX centaury the first reports showing that peptide have similar biological properties and functions as opiates were reported. The enkephalins (Tyr-Gly-Gly-Phe-Leu (Lenk) and Tyr-Gly-Gly-Phe-Met (Menk)), the first endogenous opioid peptides, were isolated and identified from pig brain in 1975. Other opioid peptides coming from natural sources, *e.g*. deltorphin I (Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH_2) and dermorphin (Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2) were found in the skin of South American frogs. The presence of D amino acid is crucial for biological activity. The synthetic analogs of given *supra* heptapeptides consisting of L-alanine are not biologically active.

Our interest is focused on influence of the alanine stereochemistry on crystal molecular packing and internal dynamics in the solid state of amino acids in sequences Tyr-Ala-Phe and Tyr-Ala-Phe-Gly. In the first part of the talk the power of NMR spectroscopy as tool for searching of different polymorphs of tri- and tetrapeptides containing L-alanine in sequence will be shown. NMR results will be compared with X-ray data of single crystals. The influence of stereochemistry of alanine on molecular packing in term of week C-H…π interactions will be presented. In the second part of the talk distinct molecular dynamics of polymorphs of tripeptides and tetrapeptides containing L- and D-alanine will be discussed. Applicability of ^{1}H -^{13}C PILGRIM, ^{1}H-^{13}C PISEMAMAS and ^{2}H NMR static measurement for analysis of molecular motion will be shown. Finally problem of bioactive conformation of native and fully ^{13}C labeled tetrapeptide containing D-Ala embedded to phospholipid DMPC/DMPG layers, usefulness of ^{1}H RF Driven NOESY and variable temperature ^{13}C NMR measurements, relation of X-ray structure to structure found in physiological environment will be discussed.

P504

Relaxation dynamics of ionic liquids in the glassy state: a Pulsed EPR study

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Low melting organic salts or ionic liquids (ILs) have been frequently studied in the past decade. These investigations have focused on their ability to serve as solvents in green chemistry, batteries, their potential as phase-transfer catalysts, etc. Moreover, ILs are interesting materials from a physical point of view as they allow us to modify the interactions between the molecular ions as well the interaction with other materials by chemically modifying the individual ionic molecules. Although crucial for understanding their properties in applications, very little is known about on their basic physical properties, especially concerning their glassy, amorphous states[1]. As molecular liquids, ILs are good glass-formers. Non-Debye and non-Arrhenius slow relaxation of the molecular ions is expected in the glassy amorphous states and has, indeed, been verified by a quasi-elastic neutron scattering study[2]. In order to access the dynamic features of the glassy amorphous states, EPR spin probes have been employed. The measured spin-lattice (T_1) and phase memory (T_m) relaxation times of spin probes (TEMPO, TEMPOL, ATEMPO) imbedded in three typical solid IL-matrices (bmimPF6, bmimBF4, emimBF4**)** show complex temperature behavior in the monitored low temperature interval (6 K - 80 K). The obtained data have been described in terms of relaxation processes in the molecular glass[3]: the direct relaxation process, the local mode relaxation process and the Raman relaxation process. The local mode with activation energy 5 meV is associated with anion dynamics. On the other hand, the effective Debye temperature is associated with dynamic modes of the cations in the ILs matrices.

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P505

SAR by ILOEs-based design of compounds targeting pro- and anti-apoptotic members of the Bcl-2 family of proteins

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NMR spectroscopy is a valuable method to characterize protein-protein and protein-ligand interactions. Important aspects of the use of NMR in drug discovery are its high sensitivity and its ability to identify fragments that bind in spatial proximity on the surface of a given protein, therefore providing the opportunity of linking them covalently to afford higher affinity bi-dentate compounds. We have recently reported on an NMR-based approach named SAR by ILOEs (*s*tructure *a*ctivity *r*elationships by *i*nter*l*igand nuclear *O*verhauser *e*ffect), that makes use of protein mediated ligand-ligand NOEs (ILOEs),[1,2] molecular modeling, and synthetic chemistry to identify initial weak hits and convert them into bi-dentate compounds with higher affinity. When this approach was used to screen for new inhibitors of Bid, a pro-apoptotic member of the Bcl-2 family, it allowed us to obtain two novel compounds, that bind to a deep hydrophobic crevice on the surface of the protein.[3] *In vitro* and cell-based assays proved that both bi-dentate derivatives are able to prevent Bid-mediated Smac release from mitochondria isolated from HeLa cells and cell death. Encouraged from these positive results, this time we have used the same approach to screen for new inhibitors of Bcl-xl, an anti-apoptotic member of the Bcl-2 family of proteins.

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P506

Lectin-based Drug Design: Combined Strategy for Lead compound Optimization

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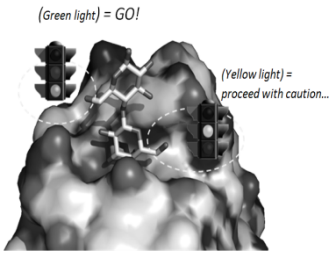
The growing awareness of the sugar code—i.e. the biological functionality of glycans—is leading to increased interest in lectins as drug targets. The aim of this study was to establish a strategic combination of screening procedures with increased biorelevance. As a model, we used a potent plant toxin (viscumin) and lactosides synthetically modified at the C6/C6' positions and the reducing end aglycan. Changes in the saturation transfer difference (STD) in NMR spectroscopy, applied in inhibition assays, yielded evidence for ligand activity and affinity differences. Inhibitory potency was confirmed by the blocking of lectin binding to a glycoprotein-bearing matrix. In cell-based assays, iodo/azido-substituted lactose derivatives were comparatively active. Interestingly, cell-type dependence was observed, indicating the potential of synthetic carbohydrate derivative to interact with lectins in a cell-type (glycan profile)-specific manner. These results are relevant to research into human lectins, glycosciences, and beyond.



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P507

Study of Beer Aging through NMR-based Methods-an Interdisciplinary Approach

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The ability of maintaining beer chemical and organoleptic properties, while achieving better understanding of beer chemistry, has been a major concern of the brewing industry.[1] High resolution Nuclear Magnetic Resonance (NMR) spectroscopy is a well recognized method for rapid compositional profiling of liquid foods, being increasingly used in tandem with multivariate analysis to aid rapid handling of large sample numbers. Examples of this in food analysis are the study of beers produced in different countries[2] or the predictive study of aging effects in vinegars.[3] In the present work, the thermally-induced aging of lager beer has been investigated using a range of complementary analytical methods. NMR/PCA (Principal Component Analysis) and NMR/PLS-DA (Partial Least Squares-Discriminant Analysis) regression models were developed to better understand the changes occurring in beer during degradation. A clear trend was observed, as a function of degradation extent, comprising variations in organic acids, dextrins and amino acids. 5-hydroxymethylfurfural (5-HMF), a marker of thermal degradation, was also identified as increasing. Due to the inherent low sensitivity of NMR, the same beer samples were also analysed by Gas Chromatography-Mass Spectrometry and by a specialised sensorial panel. The information obtained by the different analytical approaches was then inter-correlated, in order to probe new aging biomarkers and identify consistent relationships between the major compounds (viewed by NMR) and the lower content compounds (by GC-MS), many of which known degradation markers, thus contributing for a fuller chemical picture of beer aging. In addition, correlations were sought between sensorial properties and analytical data. The development of potential new rapid methods for beer analysis is discussed.

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P508

Dual Mode X-band Electron Paramagnetic Resonance of 4f-3d Dimers

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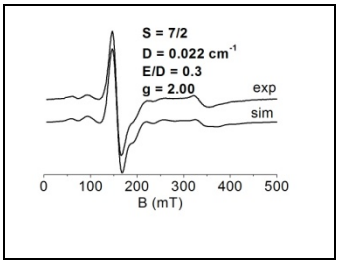
During the last years, mixed metal complexes involving lanthanide (4f) and transition metal (3d) ions have attracted the interest of researchers in the field of Molecular Magnetism in an effort to synthesize molecular materials with specific magnetic properties. In this class of complexes belong the 4f-3d dimers $[M^{II}Gd^{III}\{pyCO(OEt)pyC(OH)(OEt)py\}_3](ClO_4)_2 \cdot EtOH$ [M^{II} = Cu^{II} (**1**), Mn^{II} (**2**), Ni^{II} (**3**), pyCOpyCOpy: di-2,6-(2-pyridylcarbonyl)pyridine].[1]



The magnetic properties of these clusters are described by the spin Hamiltonian

$$H_{ex} = \vec{S}_M \tilde{J} \vec{S}_{Gd} + \vec{S}_M \tilde{g}_M \vec{B} + \vec{S}_{Gd} \tilde{g}_{Gd} \vec{B} + H_{ZFS,M} + H_{ZFS,Gd} \quad [1]$$

with S_M = 1/2, 5/2, and 1 for **(1)**, **(2),** and **(3)** respectively and S_{Gd} = 7/2. $H_{ZFS,M}$ and $H_{ZFS,Gd}$ are the zero field splitting terms of the 3d and 4f ion respectively. Magnetic susceptibility measurements indicate that the magnetic interactions are relatively weak; ferromagnetic for **(1)** and antiferromagnetic for **(2)** and **(3)**[1]. In general, bulk magnetic susceptibility measurements are not sensitive on possible anisotropy of the exchange coupling, the zero field splitting terms and the anisotropies of the *g* - tensors. A useful approach is to compare the properties of these dimers with isostructural ones where either paramagnetic 4f or 3d sites have been replaced by diamagnetic ions. For these reasons the Zn-Gd **(4)** and Cu-La **(5)**, Mn-La **(6)** complexes were prepared. In the present work we present dual mode X-band EPR studies at liquid helium temperatures for complexes **(1)** - **(6)**. A characteristic spectrum for **(4),** recorded in parallel mode is shown in the figure. Analysis of these spectra allows for the evaluation of the contributions of each term in equation [1] in the magnetic properties of the exchange coupled dimers.

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P509

The Influence of Ethylene Oxide Number on the Distribution between Oil and Water for the Igepal CA-520 Oligomers Revealed by PGSTE NMR

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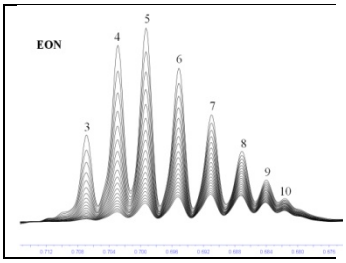
Igepal CA-520 (5 polyoxyethylene iso-octylphenyl ether) is a nonionic surfactant commercially available as a mixture of oligomers, the ethylene oxide number (EON) of which varies in agreement with a Poisson distribution.[1] It determines the partitioning of the surfactants between oil and water in inverse microemulsion systems, which behave as shift reagents. The signal separation, absent in pure organic medium, is noted in the basic and neutral microemulsions and more enhanced in the acidic one. Thus, by using the cyclohexane-igepal-HCl 0.1 M microemulsion we identified the resonance of each oligomer present in the mixture and its preferential localization. The study was conducted by means of ^{1}H, ^{13}C and pulsed field gradient stimulated echo (PGSTE) NMR.



Noteworthy is the multiplicity, due to different EON, of the proton signal of the *tert*-butyl, located right at the opposite end of the molecule with respect to the hydrophilic head (Figure). The selective partitioning was determined by diffusion coefficients. They increase for the oligomers with lower EON for two reasons: the lower molecular weight and the preference for the organic phase, where their diffusion takes place as monomeric species. The molar partition coefficient in the aqueous phase (p) for each oligomer and the relevant equilibrium constant (K_c) were calculated from the diffusion coefficients.[2] The results indicate that oligomers with EON> 5 have preference for the aqueous while those with EON≤ 5 for the organic phase.

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P510

NMR Spectroscopic Investigations on Enamine Intermediates in Organocatalysis

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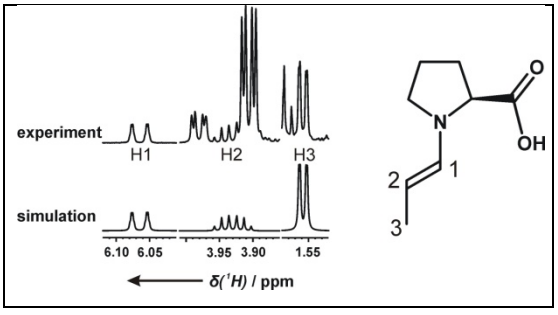
The detection and characterization of intermediates in organic reactions is crucial for the understanding of mechanisms and the rational optimization of reaction conditions. However, especially in the rapidly expanding field of organocatalysis, mechanistic studies are still scarce as compared to new synthetic applications. E.g. for the proline-catalyzed aldol reaction, one of the basic roots of the concept of enamine catalysis, the in situ observation of the enamine key intermediates has been missing for years.



Here we present our NMR investigations on the proline-catalyzed self-aldolization of aldehydes.[1] We succeeded in detecting the crucial and for a long time elusive enamine intermediate and could accomplish its structural characterization as an s-trans-E-enamine with the help of one- and two-dimensional NMR spectroscopy. The position of its equilibrium with the previously reported oxazolidinones was shown to be independent of the amount of catalyst and water in the sample. Moreover, our EXSY analyses reveal the direct formation of the enamines from the oxazolidinones in polar aprotic solvents. In addition, trends towards the stabilization of enamine intermediates will be presented. Alkyl substitution of the aldehyde in beta-position causes enamine stabilization whereas alpha-substitution decreases the detectable amount of enamine. Enamines are furthermore stabilized by solvents with strong and exclusive hydrogen bond acceptor properties such as DMSO or DMF.

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P511

On the Way to the Absolute Configuration of Mefloquine Hydrochloride Using NMR, DFT and Chiroptical Methods

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Mefloquine Hydrochloride (MQ·HCl) is the active ingredient of the major antimalarial drug known under the trade name Lariam®. Although the drug contains the racemic mixture of the (*R,S*) and (*S,R*) enantiomer the accurate knowledge of the absolute configuration of pharmaceutical compounds is important. The absolute configuration of (–)-MQ·HCl was assigned by anomalous X-ray diffraction by Karle et al.[1] to be 11*R*,12*S*. It was questioned in 2008 when Xie et al.[2] published that (–)-MQ·HCl was 11*S*,12*R* based on stereocontrolled asymmetric synthesis. The question to be answered - what is MQ·HCl's correct absolute configuration? To resolve this stereochemical problem we use the power of nuclear magnetic resonance (NMR) in combination with Density Functional Theory (DFT) calculations and chiroptical methods.

In addition to the conventional NMR parameter we also measured residual dipolar couplings (RDC's) using achiral and chiral alignment media developed in our laboratory. Using the NMR parameters, we created an ensemble of structures of MQ·HCl which was DFT optimized. Based on that ensemble we calculated ORD values and CD spectra. Finally, the comparison of these calculated ORD values and CD spectra with the experimental ones for both enantiomers of MQ·HCl leads to a clear confirmation that Karle et al. were right with assigning (–)-MQ·HCl to be 11*S*,12*R*. Results on enantiodiscrimination with chiral gels will also be presented.

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P512

The RDC module of hotFCHT for the analysis of flexible small organic molecules

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The three dimensional structure of conformationally flexible small organic molecules can be difficult to determine by NMR due to averaging of the usually observed ^3J couplings, NOEs and projection angles from cross correlated relaxation data. In contrast to these local parameters residual dipolar couplings (RDCs) are global parameters and provide complementary angular and distance information.[1,2]

Two examples of the use of RDCs for structure determination of flexible small organic molecules are presented: an α-methylene-γ-butyrolactone with unknown relative configuration[3] and a photoswitchable organocatalyst showing unsual residual catalytic activity.[4] The RDC module of hotFCHT[5] allows for a comprehensive analysis of the conformational flexibility in these systems. Using Eckart superposition[5,6] of multiple structure proposals the RDC data can be used for the calculation of the order tensor. Depending on the interconversion rates between the different conformations either the approximation of a common order tensor or the more general multiple order tensor approach is applied.

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P513

Signs of Small ^{29}Si-^{13}C Coupling Constants from a New 1D Experiment

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The SQSQc 2D experiment,[1] which uses ^{13}C detection in DQ/ZQ experiment for determination of the signs of small $J(^{29}Si$-$^{13}C)$ couplings, is decimated to a 1D experiment serving the same purpose. The reduced dimensionality leads to a considerable saving in measuring time.

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P514

The Extraction Procedure in the Metabolomic Analysis of Coffee and Tea Samples

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In metabolomics the aim is to detect and quantify all metabolites present in a certain organism, tissue or cell at a specific moment. One of the major problems in the analysis is the incomplete extraction[1]. In NMR based metabolomics generally aqueous solutions are used. In studies with humans or animals, where the samples are generally derived from body fluids this seems logic, but in plants a much larger variety of metabolites is encountered and the metabolites are obtained through the extraction of tissues. For the analysis of samples of tea and coffee the extraction procedure was investigated. Direct extraction with commonly used NMR solvents, did show that no single solvent provided a complete profile, but the best results were obtained with an extraction with a two-phase system consisting of water and chloroform. Water was found to be essential to obtain a good extraction of the apolar constituents with chloroform. The direct use of chloroform on dried plant material yielded incomplete extractions.

The application of the developed protocol on the coffee and tea samples permitted an accurate quantification of the caffeine content and the detection of specific metabolites in different types of coffee and tea.

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P515

ERETIC: A NMR quantitative tool for the pharmaceutical industry

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NMR spectroscopy is a powerful tool for the quantification of molecules in solution, since the area under a given resonance signal is proportional to the number of moles of nuclei responsible for that signal. On this principle, the use of a known concentration of an internal reference allows the direct quantification of the molecule of interest by calculating areas ratio of a specific resonance of the standard and of the molecule to quantify. This method is accurate and efficient but has some drawbacks. For example, the internal standard must be chemically inert towards the sample, soluble in the NMR solvent, compatible with the temperature, the pH and stable during the analysis. Moreover, it must not overlap resonances of the sample and must have preferentially a small relaxation time. The ERETIC (Electronic Reference To access In vivo Concentrations) method described by Akoka's team in 1995 was a major technological solution as instead of using an internal chemical reference, a radio frequency (RF) electronic reference is used for the quantification.

The ERETIC signal is an exponential decay generated on a free channel of the spectrometer and sent to the unused coil of the probe or directly routed from the transmitter to the receiver. It is then combined with the free induction decay (fid) and after Fourier transform, a spectrum of the sample with the additional ERETIC peak is obtained. The initial calibration of the ERETIC signal against a standard of known concentration allows the use of this peak for molecule quantification. Using this adjustable ERETIC signal, an internal chemical reference is not needed anymore which eliminates the previously mentioned drawbacks.

We will demonstrate and illustrate here that this ERETIC quantitative tool has a wide range of pharmaceutical applications, especially for vaccines characterization and control. It can be applied on the quantification of small molecules used, for example, as raw material to the quantification of large biomolecules, such as the bacterial polysaccharides used as active substances of some vaccines.

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P516

HPLC (Ultra Performance Liquid Chromatography) - NMR (Nuclear Magnetic Resonance spectroscopy) biased Hemodialysis analysis

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Hemodialysis is one of the most common renal replacement therapy which is used to provide an artificial replacement due to renal failure.

We have assessed the used dialysate samples, an obvious clinical utility for metabolites (the end products of cellular regulatory processes[1]) that help to diagnose renal failure at an early stage and to monitor treatment response, were collected from dialysis patients and measured by 800 MHz NMR spectroscopy and HPLC. NMR spectroscopy is very powerful tool as the high information content of the resulting spectra, the relative stability of chemical shifts, the ease of quantification and the lack of any need to pre-select the conditions employed for the analysis. Additionally HPLC has made reduced spectral overlap and higher sensitivity.[2] So we demonstrate the method on dialysis sample using HPLC coupled to NMR for get the high quantitative results.

Our results confirm that HPLC-NMR-based metabolites such as hippuric acid, indoxyl sulphate and creatinine can successfully be identified. We discuss also impact of advanced PCA tools in screening biomarkers in body fluids and detecting relevant "macroscopic" symptoms.

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P517

HRMAS NMR–based Metabolomics for the diagnosis of thyroid tumors

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Although thyroid nodules are common, 10-15% are malignant and require surgical treatment. A systematic approach to their evaluation is important to avoid unnecessary surgery. Fine-needle aspiration biopsy has resulted in substantial improvements in diagnostic accuracy. However, despite FNA effectiveness, about 10% of all specimens are classified as "indeterminate" (mainly follicular lesions). In those cases, clinicians usually recommend surgical excision for a definitive diagnosis. Because only 15% to 20% of these lesions are malignant, up to 85% of the patients in this subgroup may undergo unnecessary surgery. The approach we want to develop is based on the premise that metabolic variations/changes will pre-empt the development of morphologic modifications associated with malignancy. Consequently, the development of a HRMAS NMR-based technique that evaluates metabolic criteria represents a candidate approach for the diagnosis of "indeterminate". Here, we will show that this technique is suitable for highlight metabolic differences between benign and malignant thyroid tissues.

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P518

NMR investigation of guest-host complex between chloroform and cryptophane c

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Cryptophanes are cage-like molecules with remarkable possibility of binding small organic molecules (e.g. chloroform or dichloromethane) as a guest inside their cavity. In our work, cryptophane-C in complex with chloroform was investigated. Cryptophane-C possesses two non-equivalent cups, one of them with missing methoxy groups.

The kinetics of the chloroform exchanging between the free and the bound state was investigated by ^{1}H exchange spectroscopy. Moreover, the preferential orientation of the bound chloroform molecule with respect to the cryptophane molecule was investigated by means of NOESY and ROESY experiment. These experiments provide information about the spatial relation between specific protons in the guest and in the host. The experimental results were compared with quantum chemical calculations.

P519

Simulation of ^{129}Xe NMR shift of Xe dissolved in benzene

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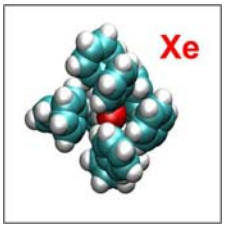
Inert Xe atom is an excellent NMR probe. The sensitivity of the ^{129}Xe NMR chemical shift to the environment of the Xe atom provides an excellent non-invasive tool for studying different materials, electronic and solvent effects, microscopic biological processes, etc. Computational studies help to predict the ^{129}Xe NMR parameters and are very useful in interpretations of the experimental data.[1]



In this pilot study, our goal was to demonstrate how the dynamic effects arising from intermolecular interactions of xenon atom with surrounding solvent (here benzene) influence the ^{129}Xe NMR chemical shift. We have performed 1000 ps run of NPT molecular dynamics (MD) simulation of a Xe atom dissolved in a box of benzene molecules using the Amber software. Subsequently, we used snapshots from the resulting trajectory for calculations of the ^{129}Xe NMR shift at the DFT nonrelativistic level of theory (BHandHLYP functional) keeping only the first solvation shell. The calculated dynamically averaged value of nonrelativistic ^{129}Xe chemical shift of 175±3 ppm is about 20 ppm below the experimental value of 195 ppm. This is partially attributed to the missing relativistic effects[1] in the shielding calculations. The calculations of relativistic corrections are currently in progress. We find out that the ^{129}Xe NMR shifts are strongly influenced by the benzene solvent molecules. MD simulation in combination with the BHandHLYP calculations of the snapshots provides affordable results that can be used for supporting the experiment or serve as a model in more complicated computational studies.

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Acknowledgments: Academy of Sciences of the Czech Republic (Z04 055 905) and the Czech Science Foundation (grant no. 203/09/2037).

P520

The relationship between spin probe dynamics and free volume in a series of small molecular and polymer glass-forming systems

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We report the combined micro-structure and local-dynamics investigations in a series of small molecular van der Waals (vdW) – or hydrogen (H) – bonded type as well as polymer glass-forming systems by using two different, i.e., atomistic and molecular probe methods. The small molecular systems included: *dietyl phtalate (DEP), diglycidyl ether bisphenol A (DGEBA), meta-tri-cresyl phosphate* (*m-TCP*), *propylene carbonate (Pc), m-toluidine (m-TOL) glycerol (GL)* and a series of *propylene glycols* from monomer *propylene glycol (PG)* up to *poly (propylene glycol) (PPG).* The polymeric systems contained those of diene type: *cis-trans-1,4-poly(butadiene)* (*c-t-1,4-PBD*), of vinylidene type: *poly(isobutylene) (PIB)* and finally, of vinyl type: *poly (vinylmethyl ether) (PVME).*

The primary information about the reorientation dynamics of molecular spin probe *2,2,6,6-tetramethyl-1-piperidinyloxy* (*TEMPO*) from electron spin resonance (ESR) is related to the annihilation behavior of the atomistic - ortho-positronium (o-Ps) probe (V^w = 170 Å^3) as measured by positron annihilation lifetime spectroscopy (PALS). It was found that a transition of the spin probe mobility from slow regime to rapid one at the operationally defined spectral temperature parameter, T_{50G}, characterized by the o-Ps lifetime, τ_3 (T_{50G}) = 2.25 ± 0.15 ns. Then, within the free-volume concept of o-Ps annihilation, the crossover of the spin probe *TEMPO* mobility between these motional regimes is connected with the occurrence of the mean free volume hole, V_h = 122 ± 15 Å^3 in the glass-formers almost independent of the chemical and topological (small molecular or polymer) character of matrices as well as of the type and extent of intermolecular (H- or vdW- bonding) interactions between their constituents.

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P521

Beyond biomolecular applications of Saturation Transfer Difference NMR

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We demonstrate the successful application of Saturation Transfer Difference NMR[1] to study interactions between organic pigment or latex particles and dispersant molecules, systems of interest for example in inkjet ink-formulations. In these systems we selectively irradiate particles, which assume the role of the 'protein'. These organic nanoparticles provide a dense network of tightly coupled proton spins enabling efficient transfer of saturation via spin diffusion. Subsequently, the saturation is transferred to any dispersant molecule that adsorbs onto the pigment or latex surface under the fast exchange conditions, but not to other non-ligand molecules in the mixture. While the STD response for these organic nanoparticle dispersions appears to behave quite similarly to the protein-ligand case, considerable differences are evident. First, binding involves a surface rather than a well defined binding site. Second, ligand concentrations are similar or even lower than the number of available interaction sites, i.e. we are not working under the conditions of large ligand excess typically used for biological applications. Starting from low dispersant to particle ratios, we show that the normalized STD intensity initially rises unexpectedly as dispersant is added, which is then followed by the more common decay at higher dispersant concentration. By exploring ligand to protein ratios varying from 0.1 to 10 in the phenylalanine-BSA system, we are able to reproduce this same trend, which indicates that it originates from the experimental conditions rather than from the nature of the particles involved. Finally, we demonstrate that STD NMR is a powerful tool to monitor molecular rearrangement at the pigment surface. In the case of a flat-on deposition, the STD amplification factors are similar for each proton within a dispersant molecule. As molecules rearrange adopting more vertical orientation, STD amplification factors differentiate and become the largest for protons directly attached to the surface.[2]

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P522

Multi-Dimensional NMR Study of Ethyl Substitution in Ethylcellulose

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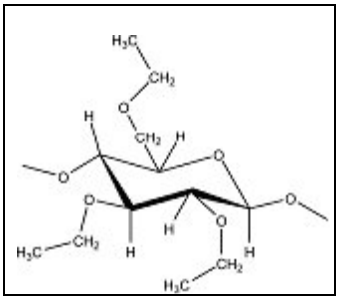

Ethylcellulose is a commercial thermoplastic synthesised by a reaction of wood pulp to form the ethyl ether derivative of cellulose. The nature of commercially manufacture ethyl cellulose is defined by the manufacturing process, rather than the sites of substitution around the cellulose glucose rings. In the solid state directed substitution has the possibility of disrupting intra-chain cellulose hydrogen bonding and therefore modulating the flexibility of the cellulose backbone. Here we report results from multi-dimensional solution NMR experiments used to determine both the qualitative nature and quantitative degree of ethylation for cellulose in solution. A combination of solvent manipulation together with 2D-correlation spectra (COSY, HSQC, HMBC) allowed the assignment of the ^{1}H and ^{13}C NMR spectra.

P523

NMR investigations of the interactions in dendrimer-copper salts systems

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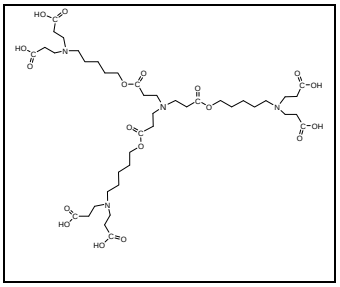
Dendrimers are uniform, spherical polymers capable of accommodating guest molecules on their surface or within large interior cavities. They have recently been shown to act as effective drug delivery vehicles for a range of organic compounds. We are interested in the potential use of dendrimers as delivery vehicles for transition metal-based drugs. There is also considerable advantage in having the ability to transport charged but chemically inert drugs without the need to synthetically add extra functional groups for conjugation to neutral dendrimers.



In such context, NMR measurements have been performed on newly synthesized acid-terminated poly(amino)ester dendrimers, which have already been reported as good siRNA transporters.[1,2] We are reporting here preliminary NMR results as regards to the complexation process between these dendrimers and cooper salts. Specifically, 1D and 2D NMR data are combined to evidence and characterize the strong interaction involved in these systems.

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P524

Solvation of Strong H-Bonds by Polar Aprotic Solvent. One-sample low-temperature NMR/UV-Vis study

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Geometries of H-bonds in solutions are often fluxional due to the high nuclear polarizability of the bridging proton. A small change in the local electric fields can induce a large change in the H-bond geometry. Electric fields come mainly from the solvation shell, which might include a counterion for a charged system. A set of possible configurations of the solvation shell ("solvatomers") creates an effective bridging particle distribution function. Lifetimes of individual solvatomers are limited by the reorientation time of the solvent molecules (nano- to microseconds). Thus, the signals of solvatomers are averaged in NMR spectra and some information about the proton distribution is lost. To overcome this problem we propose to use combined NMR/UV-Vis method (UVNMR), in which NMR and UV-Vis spectra are measured for the same sample within the magnet of the NMR spectrometer.[1]

We present a UVNMR study of intermolecular anionic complexes with strong OHO bonds between nitrophenols and carboxylates. We discuss the spectral features which reflect the actual distribution of H-bond geometries and the temperature effects on it. For example, in order to separate the effects of the solvent ordering from the effects of the counterion we propose to fix the latter intramolecularly. As a suitable object we have studied complexes of betaines with carboxylic acids dissolved in CDF_3/CDF_2Cl. As an outlook we present the modification of the detection probe which would allow one to measure not only NMR and UV-Vis but also Raman spectra inside the NMR magnet. The latter could provide further insight into the inhomogeneous proton distribution caused by the ensemble of solvatomers.

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Acknowledgments: This work has benefitted from financial support by the Russian Foundation of Basic Research and Deutsche Forschungsgemeinschaft.

P525

Structural basis for methylarginine recognition by the Tudor family of proteins

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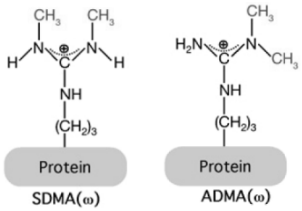
Arginine methylation has long been observed in RNA binding proteins and histones, but only recently it has been implicated in a variety of cellular processes, including pre-mRNA splicing, transcriptional regulation, trafficking, signal transduction, and spermatogenesis. In contrast to the wealth of data showing how effector modules target histone lysine methylation, knowledge on the recognition of methylarginine has arguably lagged behind, as no structures of a reader bound to these methylation marks is yet known. Ample evidence suggests Tudor-mediated assembly of piRNPs and hnRNPs in a methylarginine-dependent manner. Previous studies in our lab have demonstrated that the Tudor domain of the SMN protein specifically recognises methylarginine motifs. However, only recently we were able to overcome technical difficulties and obtain high quality NMR data on these interactions.



In this study we present the first structures of Tudor domains complexed with symmetrical (sDMA) and asymmetrical (aDMA) dimethylated arginines (Figure). The structures reveal the fine details effecting the state-specific readout of the methylation marks, supporting our obervations by isothermal titration calorimetry (ITC) that sDMA is a better Tudor substrate than aDMA. A series of binding site mutations substantiates our structural findings. Our data offer the mechanistic framework for the recruitment of methylated protein components to cellular locales, like Cajal-bodies (biogenesis of hnRNPs) and nuage granules (piRNA pathway), by Tudor proteins. Impaired assembly of hnRNP subunits of the spliceosome seem to generate the motor-neuron phenotype of Spinal Muscular Atrophy and piRNP compartmentalization is critical to germ cell preservation.

P526

Spatial Structure and Backbone Dynamics of Asymmetric Dimer of β-Hairpin Antimicrobial Peptide Arenicin-2 in Membrane Mimicking Environment

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Membrane-active cationic antimicrobial peptide arenicin-2 (AR-2, 21 a. a.) was isolated from marine polychaeta *Arenicola marina*. Previously it was shown that in aqueous solution the peptide adopts significantly twisted β-hairpin conformation. Spatial structure and backbone dynamics of AR-2 was studied by ^{1}H, ^{13}C, ^{15}N NMR spectroscopy in membrane mimicking environment of DPC micelles. It was shown that the peptide forms asymmetric dimers by parallel association between N-terminal β-strands of two β-hairpins (CN↑↑NC). The dimer structure is stabilized by eight intermonomer hydrogen bonds, and the monomers are slightly shifted from each other along direction of β-strands. The right-handed twist of β-hairpin significantly decreases upon interaction with DPC micelle and dimerization. Backbone dynamics of the peptide monomer in aqueous solution and the peptide dimer in DPC micelle were characterized by ^{15}N relaxation data. It was shown that interaction with DPC micelle lead to stabilization of the peptide's backbone. At the same time μs-ms conformational fluctuations of Gly12 residue situated in the β-turn region of the hairpin were observed in both media. The effective τ_R (~ 12.5 ns at 47°C) of AR-2 in complex with DPC micelle corresponds to spherical particle with hydrodynamic radius ~ 28 Å. The topology of AR-2/DPC complex was determined using water-soluble and lipid-soluble paramagnetic probes (Mn^{2+} and 16-doxylstearate). Results indicate that the AR-2 dimer has transmembrane arrangement within the DPC micelle with N- and C-termini and β-turn fragments of both peptides are located above the hydrophobic region of the micelle. These findings support the model in which antimicrobial activity of AR-2 is conditioned by formation of toroidal pores assembled from β-structural peptide oligomers and lipid molecules.

Acknowledgments: This work was supported by Russian Foundation for Basic Research, Grant from the President of Russian Federation and by Federal Target Program "Scientific and Science-Educational Personnel of Innovative Russia".

P527

Synthesis and NMR Characterization of Triptycene Containing Polystyrene

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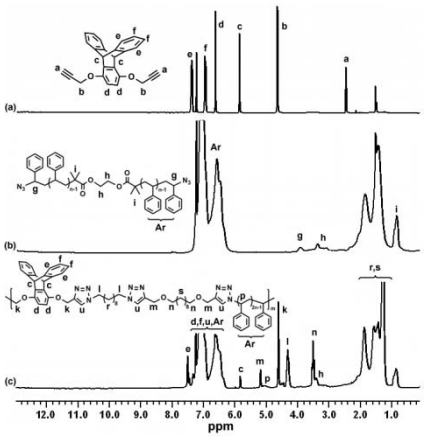
Polystyrenes with triptcyene units were synthesized by combination of Atom Transfer Radical Polymerization (ATRP) and "Click" chemistry processes. The click reaction between diazido compounds, namely diazido polystyrene, diazidodecane and dialkyne compounds, dipropargyloxytriptycene and dipropargyloxydecane resulted in the formation polystyrene with triptycene units in the main chain. Characterization of the intermediates and the target polystyrene was done by means of ^{1}H-NMR FT-IR and spectral analysis, and GPC, DSC and TGA studies. While alkyne protons of triptycene (a protons in Figure 1a) and end group protons of azide terminated polystyrene (g protons in Figure 1b) completely disappeared, the new signal corresponding to methylene and methine protons (p,m,l,k protons in Figure 1c) linked to triazole rings were observed at between 5.18 and 4.31 ppm. The characteristic bridgehead protons of triptycene observed at 5.87 ppm (c protons in Figure 1a) were detectable in the corresponding copolymer spectrum at 5.82 ppm (c protons in Figure 1c). Although the sequences of the aliphatic and aromatic propargyl molecules are irregular, these results indicate that composition of the resulting polymer is in agreement with the feed ratio of the click components in coupling process.



^{1}H-NMR spectra of dipropargyloxy triptycene (DPT) (a), azide terminated bifunctional polystyrene (N_3-PS-N_3) (b), and triptycene containing copolymer (CP1) (c) in $CDCl_3$.

P528

Classical and Alternative Approach for Configuration Assignment on the example of model pyrrolidine

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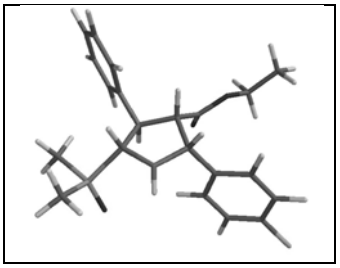
Assignment of the relative configuration of the four chiral atoms in a model pyrrolidine derivative has been achieved through the classical approach – J-based configuration analysis (proton, carbon, phosphorus), NOE measurements and computational methods.[1]



The model molecule has been aligned in two anisotropic media - cross-linked polydimethylsiloxane, PDMS[2] and poly-γ-benzyl-L-glutamate, PBLG[3] Configuration assignment based on the alternative approach[4] - RDC measurement in the alignment media, followed by fitting of PM3 optimized geometries with the programs PALES[5] and Mspin[6] has been probed.

Pro's and contra's of both methods for the case of a five-membered ring are discussed.

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P529

Octasolketal-substituted phthalocyanines: synthesis and systematic study of metal effect and substitution pattern on ^{13}C NMR

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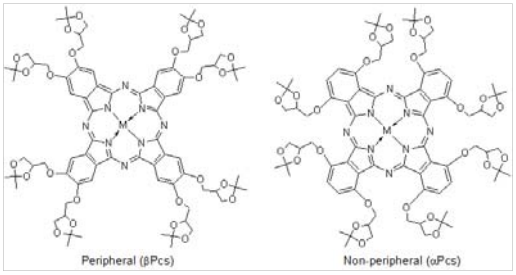
A complete series of solketal octasubstituted phthalocyanines have been synthesized, with peripheral (β) or non-peripheral (α) substitution pattern.[1,2] Their ^{13}C NMR properties are compared relatively to this substitution pattern or the nature of the central metal (Ni, Zn, Pt or H_2).[1,2] During the syntheses of the solketal-substituted Pcs, the value of an extensive study of the metal and peripheral effect became apparent, especially from the ^{13}C NMR.



By synthesizing eight octasubstituted Pcs, we had the opportunity to make such comparisons Pcs, divided into two groups according to their substitution pattern: peripheral (βPcs) and non-peripheral (αPcs).

This is the first systematic study of the influence of the nature of the metal and of the substitution pattern on ^{13}C NMR of phthalocyanines. DEPT (Distortionless Enhancement by Polarization Transfer), HSQC (Hetero Single Quantum Correlation) and HMBC (Heteronuclear Multiple Bond Correlation) have been used for a complete attribution of all the carbons of the phthalocyanine macrocycle and the substituents, allowing subsequent analyses of the metal and substitution pattern on ^{13}C chemical shifts.

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Acknowledgments: The financial support of the Turkish National Council of Research and Science TUBITAK (project 106T376) is gratefully acknowledged.

P530

fac-$\{Ru(CO)_3\}^{2+}$ selectively targets the His pair of the β amyloid peptide. Can ruthenium complexes be considered as possible candidates for Alzheimer Disease treatments?

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The amyloid β-peptide (Aβ), in the form of amyloid fibrils, is the major component of the extracellular deposits of Alzheimer's disease (AD). No curative AD treatments have been developed so far. It was recently shown that targeting His-13 and His-14 of β-amyloid peptides may result into a dramatic decrease of its cytotoxicity and a few platinum compounds were successfully exploited *in vitro* for this purpose.[1] In this work, the reaction taking place between the novel ruthenium(II) complex fac-$[Ru(CO)_3Cl_2(N1\text{-thz})]$, recently synthesized and characterized by some of us,[2] and the $A\beta_{28}$ peptide was investigated by a variety of biophysical methods.[3] ^1H NMR titrations highlighted a selective interaction of $\{Ru(CO)3\}^{2+}$ with $A\beta_{28}$ histidine residues; CD revealed the occurrence of a substantial conformational rearrangement of $A\beta_{28}$; ESI-MS suggested a prevalent 1:1 metal-peptide stoichiometry. For a better description of such interaction, other ligands were used as well, such as $A\beta_{28}$ protected at N-terminus ($AcA\beta_{28}$), the rat $A\beta_{28}$ ($rA\beta_{28}$), L-His, GlyGlyHis (GGH) and the reduced glutathione (GSH).

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P531

Investigation of the base-pair opening dynamics of a DNA:DNA duplex containing a T.T mismatch

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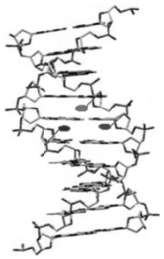
There is considerable interest in the development of artificial catalysts that mimic enzymatic activity. One approach in this challenging area of research is the use of nucleic acid building blocks, whose base-pairs are equipped with functional groups mimicking peptide chains.[1] Our system of interest consists of a 14mer DNA duplex in which a catalytic triad resembling the active site of α-chymotrypsin is introduced. The approach requires the introduction of a T.T mismatch that can destabilize the duplex and influence its dynamics. In addition, the effects caused by modifying the nucleotide bases on these parameters have yet to be explored. Using NMR spectroscopy, we report on the stability and and base-pair opening dynamics of the non-modified mismatched duplex, shown below, and compare it to a first generation of triad mimicking duplexes. In addition, the use of salt up to 100 mM is shown to have limited impact, at least in the systems studied here.



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P532

Dynamic NMR and DFT Studies of Catalytically Relevant NHC Pd(II) Complexes

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Catalytically relevant[1] Pd(II) complexes involving N-heterocyclic carbenes (NHCs) and bidentate N- and P-donor ligands (shown on figure) were synthesised and characterised. Their fluxional behaviour was studied by ^{1}H, ^{13}C and ^{31}P NMR in $CDCl_3$ solution (from 213K to 323K) and by DFT calculations at B3LYP/CEP-121G level of theory. The variable-temperature ^{1}H and ^{13}C NMR spectra of Pd(II) complex involving bidentate N-donor ligand show two almost equally populated conformers. The rate constants were calculated from CLSA of ^{1}H VT NMR spectra and 2D EXSY experiments. The rotational barrier is $\Delta G^{\neq} \sim 15$ kcal/mol using Eyring equation. The DFT calculations reveal the exchange process as restricted rotation around C-N bond with rotational barrier of $\Delta G^{\neq} = 13.7$ kcal/mol, while the rotational barrier around Pd-C bond was estimated to be $\Delta G^{\neq} = 25.6$ kcal/mol.

The ^{1}H, ^{13}C and ^{31}P VT NMR spectra of Pd(II) complex involving bidentate P-donor ligand show four none equally populated conformers. The rate constants were calculated from CLSA of ^{31}P 1D NMR spectra and ^{31}P 2D EXSY experiments.

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Acknowledgments: Financial support by the National Research Fund of Bulgaria (UNA-17/2005 and TK-X-1606/06), the Sofia University Research Fund and COST action CM0802 ‘‘PhoSciNet” is gratefully acknowledged.

P533
Let's DENs

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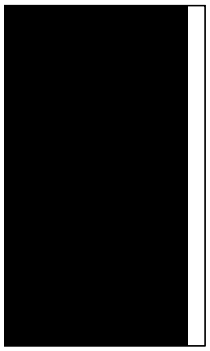
High-resolution solution-state NMR spectroscopy has been used to characterize the structure of Pd dendrimer-encapsulated nanoparticles (DENs). First,[1] full analysis was done of the homo- and heteronuclear 1D and 2D NMR data of the fourth-generation hydroxyl-terminated poly(amidoamine), PAMAM, dendrimer (G4-OH), which is a 15 kDa macromolecule containing over a 1000 protons that show severely overlapping signals due to the high (pseudo-)symmetry. Comparison of the NMR data without (G4-OH) and with (G4-OH(Pd_{55})) nanoparticles unambiguously demonstrates that single nanoparticles are encapsulated within individual dendrimers. DOSY experiments prove the absence of aggregate formation, as well as furnish the hydrodynamic radius of the dendrimers. Illustrative and corroborative experiments were also performed with the extraction of DENs using alkanethiols. Consecutively,[2] simple 1D 1H-NMR data of Pd nanoparticles encapsulated within sixth-generation hydroxyl-terminated PAMAM (a 60 kDa dendrimer) allowed determination of the size of Pd DENs (G6-OH(Pd_x)) ranging from 55, 147, 200 to 250 atoms. Therefore, solution-state 1H NMR data provide a straightforward tool to characterize nanometer-size nanoparticles, where otherwise advanced and less accessible techniques, e.g. TEM, have to be used, that moreover only provide sampled and ex-situ data.



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P534
Automated identification of phenolic compounds

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Polyphenolic compounds are abundantly found in nature and are very diverse in structure due to a large number of possible substitution patterns. Therefore, the identification of these compounds cannot be done on the sole basis of mass spectrometry data. The identification process can be accelerated when MS (MS/MS) and high quality ^{1}H-NMR data are combined. Although ^{1}H-NMR spectra are relatively fast and easy to obtain (provided there is a good sample), the existing databases contain only a limited number of polyphenolic compounds. Here, we present a database of experimental and predicted ^{1}H-NMR spectra of phenolic compounds based on high quality data. The predicted ^{1}H-NMR spectra were generated from 3D chemical structures using the PERCH NMR Software trained on a large set of experimental NMR spectra. By incorporating the stereochemistry, intra-molecular interactions, and solvent effects into the mathematical model, ^{1}H chemical shifts and ^{1}H-^{1}H couplings were predicted with high accuracy. The predictive model was used to extend the database with NMR spectra of about 3000 phenolic compounds available from public resources. The 3D structures were generated using a set of fragments with the correct stereochemistry, which is especially important for compounds containing sugar moieties. The ^{1}H-NMR spectra were automatically annotated with the atom labels generally accepted in the literature. The spectrum querying was done in combination with the mass using a list of chemical shifts with or without integral values. The hit list was sorted according to the match between the query and the database spectra. When the correct compound is present in the database it is very easily distinguished from false positives. For example, there are 15 compounds with chemical formula of C15H10O7. When querying the list of experimental chemical shifts of quercetin against the database, only one hit gave a good match. This possible quandidate was confirmed by an automated iterative fit of the experimental and theoretical 1H-NMR data. The other 14 hits failed in this automated fitting procedure. The 3D Mol files and the NMR predictions (in a binary format) are available for download.

P535
Let's be SMARD ☺!

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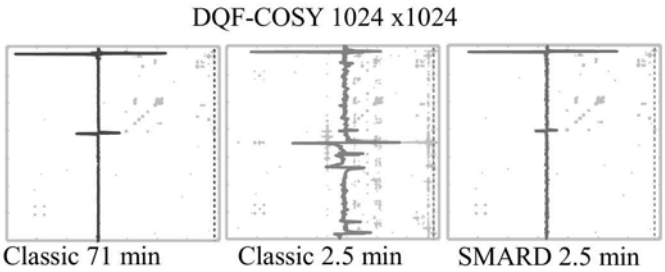
A simple way to reduce the experimental time required for classical 2D experiments can be achieved by reducing the recovery delay t_{RD} between consecutive increments. The general rule-of-thumb consisting in waiting longer than the longitudinal relaxation time T_1 need not be respected. One can often afford the concomitant reduction of the signal-to-noise ratio, but the major drawback comes from an increase of artefacts in the spectra. These are due to interference of transverse and longitudinal magnetisation components from one scan to the next. A suitable choice of nearly-orthogonal pulsed field gradients (PFG's) generated by triple gradients can suppress unwanted coherence transfer pathways. In order to prevent accidental refocusing, the direction of the PFG's must be changed from scan to scan. SMAll Recovery Delay (SMARD) allows one to reduce the experimental time by as much as an order of magnitude.



P536
Anodic oxidation of selenadiazoloquinolones in alkaline media (EPR study)

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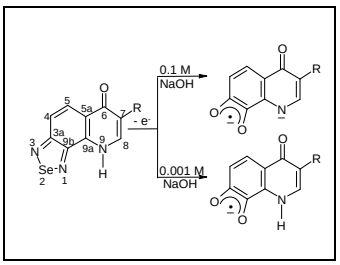
Newly synthesized derivatives of 6-oxo-6,9-dihydro[1,2,5]selenadiazolo[3,4-h]quinoline variously substituted at position 7 (R_7 = H, COOH, $COCH_3$, CN, $COOC_2H_5$ and $COOCH_3$,) are established in strongly alkaline aqueous solutions (0.1 M NaOH; pH ~ 13) in the N_9-deprotonated and in less alkaline (0.001 M NaOH; pH ~ 11) in the protonated oxo tautomeric forms. Upon their anodic oxidation in alkaline solutions the selenadiazolo ring is replaced by two oxide anions forming consecutively the paramagnetic species analogous to the ortho semiquinone radical anions, as evidenced by EPR experiments in partially labelled $H_2{}^{17}O$. Additionally, the identity of the individual R_7 substituents was found to be clearly reflected in the hyperfine coupling pattern of detected EPR spectra.



Acknowledgments: This work was supported by grants: APVT-0055-07, VEGA/1/0018/09 and 1/0225/08, 2003SP200280203 and SK-AT-0016-08. MZ acknowledges the support of the Jubilee Fellowship for Post-Doctoral Candidates (SK - AT, Action Austria-Slovakia).

7.5 Theory & Methods

Posters

P537
Structural dynamics at multiple time scales

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Spin relaxation measurements in liquids provide most valuable information on internal dynamics of proteins, which is a key factor of their functions. It is therefore important to provide physical models that can serve as a basis for the interpretation of the protein motions. Assuming that these can be related to the structure of the molecule, we recently introduced a model based on a *Network of Coupled Rotators* to describe internal dynamics of proteins from the knowledge of their three-dimensional structures. ^{15}N NMR relaxation rates can thus be predicted and conformational entropies of bond vector calculated. This approach also illustrates the absence of one-to-one relationships between order parameters and *conformational entropies*, and therefore explains the difficulty to relate both quantities from experimental data.[1] Further theoretical developments in the study of protein dynamics demonstrated the presence of multi-scale *fast* internal motions, and this complex behaviour was shown to be accounted for by a simple model based on *fractional Brownian dynamics*.[2] Structural dynamics also involves *microsecond time scale dynamics*, as shown by chemical shift modulation measurements of backbone C'N coherences.[3] These experiments suggest the unusual view of a protein where *slow motions* are present *across the entire backbone, and with characteristics* that can be related to the secondary structure elements. This is clearly in contrast with the conventional picture usually provided by ^{15}N NMR relaxation.

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P538
Analysis of CPMG Sequences with Low Refocusing Flip Angles

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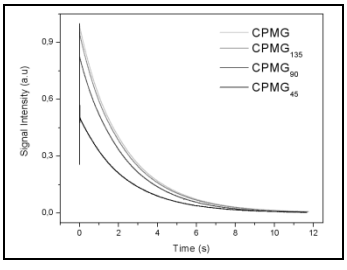
The Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence has been used in many applications. Recently, CPMG has been used in *on-line* measurements[1] where it was applied by long periods. In this case, 180° refocusing pulse can cause undesirable sample heating and equipment overload, which can reduce their durability and erroneous results. Thus, the purpose of this work is the experimental and theoretical analysis of CPMG sequence with low refocusing pulse flip angles (LRFA) using Bloch equation and the effect on the T_2 value under the parameters as magnetic field inhomogeneity, common problems occurred in low magnetic field, in order to use it in NMR *on-line* measurements. This Figure shows the experimental CPMG signals of water obtained on-resonance with refocusing flip angles of 180° (CPMG), 135° ($CPMG_{135}$), 90° ($CPMG_{90}$) and 45° ($CPMG_{45}$) in a less homogeneous field (FWHM=100Hz). However, CPMG signals with LRFA obtained in more homogeneous condition (FWHM=15Hz) were similar for all flip angle studied for experimental and simulated results showing the robustness of CPMG sequence. With this information it is possible to determine a limit to use different flip angles depending on conditions of B_0 inhomogeneous field used, which it can reduce power by more than 75% in *on-line* measurements.



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Acknowledgments: FAPESP, EMBRAPA Agricultural Instrumentation.

P539

Towards the integration of radiofrequency coils within high-frequency, single-mode EPR resonators: First W-band ENDOR results

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In the framework of the development of innovative resonant structures for high-field applications, a single-mode dielectric resonator with intra-cavity rf coil has been proposed for dynamic nuclear polarization studies at 3.4 T;[1] it demonstrated unexpectedly high NMR signal enhancements.[2] The high conversion factor of this device for both its rf and mw response is promising also for ENDOR applications. This contribution presents the first W-band ENDOR results of a single-mode mw resonator with integrated rf coil, which represents a simplified variant of the structure proposed in[1]. The employed mw resonator is a metallic non-radiative structure,[3] loaded by a standard W-band sample holder on which a coaxial hairpin coil has been attached. The room temperature pulsed Mims and Davies ENDOR signals of bis-diphenylene-phenyl-allyl radical dissolved in polystyrene, obtained with this structure, has been compared with the analogous signals obtained using external Helmholtz-like coils. The intra-cavity coil leads to a signal gain of about 4.7 with respect to the external coils. The analysis of the spectra suggests that in the investigated structure the thermal effects due to the high rf currents are negligible, while some mechanical effects cannot be excluded. The details of the measurements will be illustrated, together with an improved design of the intra-cavity coil that should eliminate the possible mechanical effects.

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P540

NVR-BIP: A tool for NMR Structure-Based Assignments

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An important bottleneck in NMR protein structure determination is the assignment of NMR peaks to the corresponding nuclei. Structure-based assignment (SBA) aims to solve this problem with the help of a template protein which is homologous to the target and has applications in the study of structure-activity relationship, protein-protein and protein-ligand interactions. We formulate SBA as a linear assignment problem with additional Nuclear Overhauser Effect constraints, which we solve within Nuclear Vector Replacement's (NVR)[1] framework using binary integer programming (BIP). We extend NVR to accept CH and NH RDCs, and test our technique on NVR's data set, as well as four additional proteins. Our results are comparable to NVR's assignment accuracy on NVR's test set, but higher on novel proteins.[2] We then test the effect of incorporating additional chemical shifts and using CRAACK[3] for amino acid typing.[4] Additionally, we employ machine learning techniques to view the assignments as a classification problem[5] and combine the terms in NVR's scoring function using support vector machines and boosting.

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P541

Electron Magnetic Resonance in Nanoparticles: Quantal Effects and Giant-Spin Description

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In order to better understand the transition from quantum to classical behavior in spin systems, the electron magnetic resonance (EMR) spectra of the γ-Fe_2O_3 and Fe_3O_4 nanoparticles embedded into non-magnetic matrices are studied. Common peculiarity of the spectra is a narrow spectral component superimposed on the broad one; the intensity of the narrow feature was found to decrease upon cooling by activation law. Besides, additional weak lines are discovered in low magnetic fields corresponding to the multiple-quantum resonances with k=2, 3, and 4. The spectra are interpreted in terms of a quantal model where a superparamagnetic particle is considered as a large exchange-coupled cluster with the giant total spin S~10^3. In this approach, the EMR spectrum is described as the sum of quantum transitions between the magnetic sublevels E_m corresponding to the lowest spin multiplet of the cluster, with account made for magnetic anisotropy, transition probabilities, and equilibrium populations. The narrow spectral feature is assigned to the allowed (Δm=±1) transitions between the upper levels with low $|m|$ values. The multiple-quantum resonances are related to "forbidden" transitions (Δm=±k) which become partly allowed due to the state mixing caused by either the single-particle anisotropy or inter-particle dipolar interactions. The role of the dipolar interactions has been studied on the Fe_3O_4 nanoparticles arranged into linear chains inside the parallel nanoscaled channels penetrating the alumina membrane. The observed anisotropy of the EMR spectum caused by the inter-particle dipolar interactions was used to determine the effective dipolar field and then to calculate the probabilities of the multiple-quantum transitions with k=2. It is found that the quantal description agree well with the experimental data both in functional form of the angular dependencies and the absolute values of the double-quantum intensity. Thus, the EMR studies in superparamagnetic nanoparticles can provide an effective bridge between relatively small spin clusters (including molecular magnets) described with quantal approach, and bulk ferromagnets commonly considered from the classical point of view.

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P542

Pharmacophore Mapping via Cross-Relaxation during Adiabatic Fast Passage

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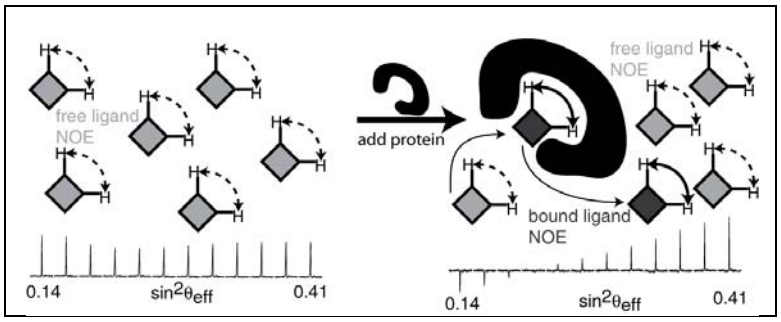

A novel NMR method is demonstrated for the investigation of protein ligand interactions. In this approach an adiabatic fast passage pulse, i.e. a long, weak pulse with a linear frequency sweep, is used to probe ^{1}H-^{1}H NOEs. During the adiabatic fast passage the effective rotating-frame NOE is a weighted average of transverse and longitudinal cross-relaxation contributions that can be tuned by pulse power and frequency sweep rate. It is demonstrated that the occurrence of spin diffusion processes leads to sizeable deviations from the theoretical relationship between effective relaxation rate and effective tilt angle in the spin lock frame and can be used to probe protein-ligand binding. This methodology comprises high sensitivity and ease of implementation. The feasibility of this technique is demonstrated with two protein complexes, vanillic acid bound to the quail lipocalin Q83 and NAD^+ and AMP binding to alcohol dehydrogenase (ADH).

P543

HIFI-NMR, a method for efficiently combining adaptive protein NMR data collection, assignment and validation

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HIFI-PINE represents a novel, fully-automated method for optimal and adaptive data collection and analysis in protein NMR spectroscopy. It represents another breakthrough step toward a new paradigm of NMR data collection and analysis that relies on feedback across various modules in order to provide faster, more robust, and highly reliable results in NMR structural biology. In a process that does not involve any manual intervention and normally takes less than a day, HIFI-PINE identifies peaks from all necessary 3D NMR experiments and provides complete chemical shift assignments, secondary structure determination, and assignment validation. The automated probabilistic data analysis, including spectral processing, peak picking, and assignment, is executed "on the fly", i.e. while spectra are being collected. The feedback mechanism from the assignment module guides data collection and ensures that sufficient information about peak lists and spin systems has been acquired to support robust resonance assignments and secondary structure determination. HIFI-PINE has been tested on [^{13}C, ^{15}N]-labeled small and medium-sized proteins. In all cases, the assignment quality was sufficient to support the final steps of structure determination.

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P544

Transverse Relaxation Rates in Homonuclear J-coupled Spin Systems

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The modulation of spin echoes by homonuclear scalar couplings renders the determination of transverse relaxation rates (R_2) of individual spins difficult for molecules that are isotopically enriched in ^{13}C or ^{15}N, and of course for scalar-coupled ^{1}H spins. To avoid modulations, most studies using refocusing pulses have so far been restricted to isolated or selectively labeled ^{13}C or ^{15}N spins. Two established strategies to avoid modulations use either fast pulse repetition rates[1,2] or selective refocusing pulses.[3] It has been shown recently, using refocusing pulses of moderate strength with the radio-frequency (*rf*) carrier on-resonance for a spin I ($\Omega_I = 0$) under investigation,[4] that cumulative effects of non-ideal pulses with 'tilted' effective field can quench modulations in multiple-refocusing schemes $\pi/2$-$[\tau$-π-τ-$]_{2n}$ provided $\nu_{rep} = 1/(2\tau+\tau_\pi) \neq \Omega_S/(2k\pi)$, where k is an integer and Ω_S is the offset of the main coupling partner S.[5] Pulse repetition rates $\nu_{rep} < \Omega_S/(2\pi)$, allow one to extract "apparent" rates R_2. Earlier work on systems comprising only two ^{15}N spins[4] or two ^{13}C spins[5,6] has now been generalized to ^{1}H spins[7] in polypeptides and proteins. Variation of the carrier frequency offers an additional way to obtain modulation-free decays. A lack of resolution can be overcome by INEPT or heteronuclear correlations.

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P545
NUS of SOFAST-based Multi-Dimensional Experiments

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Non-uniform sampling (NUS) of multi-dimensional data reduces the overhead of increments acquired for indirect dimensions resulting in greatly reduced measuring times relative to fully sampled data.[1] Similarly, the measuring times of multi-dimensional data can be minimized by the incorporation of the SOFAST-based acquisition scheme which allows for rapid pulsing.[2] Here we show that a combination of the two approaches in multi-dimensional experiments can further reduce measuring times by a factor greater than 4 relative to the application of either NUS or SOFAST-based acquisition individually. The ability of this combined approach to obtain high-resolution multi-dimensional data on the order of 1-3 minutes is illustrated for 13C/15N-ubiquitin using a QCI cryoprobe and its benefit to measuring fast processes for biomolecules is discussed.

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P546
Quantitative Aspects of PGSE Experiments

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Diffusion NMR experiments are a major tool for characterizing the structure and dynamics of complex mixtures.[1,2] Combined with proper data processing schemes, these experiments allow, in favorable cases, the ^{1}H spectra of the components of a mixture to be extracted. However, because they intrinsically rely on spin or stimulated echoes, magnetic relaxation phenomena lead to non-quantitative results. To circumvent this difficulty, one method called quantitative DECRA (qDECRA) has recently been proposed by Antalek,[3] which consists of recording several PGSE experiments to normalize signal attenuation due to magnetic relaxation through a clever linear regression of the data.

In this context, we propose an alternative strategy that aims at renormalizing PGSE data simply by using the magnetic relaxation time values of all the NMR signals. The problem then reduces to measuring T_1 and/or T_2 and, most importantly, to estimating the precision required for providing proper data renormalization and hence precise quantification. By analyzing simple model mixtures with one of the most frequently used pulse sequence, the so-called BPP-LED,[4] for which magnetic relaxation is primarily longitudinal, we show that the knowledge of both D and T_1 is sufficient for achieving accurate quantification for small- and medium-sized molecules. In the case of slowly tumbling molecules, which typically have short T_2 values, the above mentioned PGSE pulse sequence can be combined with a simple CPMG pulse train,[5] hereby allowing T_2 to be roughly estimated. Interestingly, while the precision of the so-obtained T_2 values appears quite low, the relatively weak influence of T_2 relaxation in data renormalization makes it sufficient for providing accurate quantification. In this case, however, spectral overlap seriously complicates the analysis, especially for homonuclear coupled spin systems due to strong *J*-modulation artifacts.

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P547

^{15}N- and ^{13}C group-selective STD NMR techniques for sensitive binding studies

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Saturation transfer difference (STD) is a valuable tool to study the binding of small molecules to large biomolecules and to obtain detailed information on the binding epitopes. We demonstrate that the proposed ^{15}N/^{13}C variants of group-selective, 'GS-STD' experiments[1] provide a powerful approach even in the presence of internal motions of the target protein. The STD spectra obtained in four different experimental setups (conventional ^{1}H STD, ^{15}N GS-STD, $^{13}C_{Ar}$ and $^{13}C_{aliphatic}$ GS-STD) revealed that the signal intensity pattern of the difference spectra is affected by the type and the spatial distribution of the excited 'transmitter' atoms and by the efficiency of the spin-diffusion. The performance of the experiments is demonstrated on a system using a lectin (galectin-1) and its carbohydrate ligand (lactose).

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P548

Theoretical modeling of the metal ion effects on NMR parameters in nucleic acid backbone

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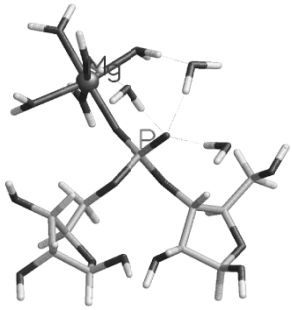
The negatively charged phosphate group of nucleic acid backbone represents one of the most important solvation sites in nucleic acids. An impressive amount of work has been done on characterizing the structure of the solvation shell of canonical DNA as well as of RNA backbone patterns. The analysis of X-ray structural data revealed well localized water molecules in the closest vicinity of the phosphate group. The presence of physiological monovalent and divalent cations in the first solvation shell of the phosphate was also confirmed.[1]



The X-ray identification of biologically essential Na^{+} and Mg^{2+} metal ions is not a straightforward task since these ions and the water molecule possess the same number of electrons. In many cases, methods of molecular spectroscopy can be used for the metal ion recognition.[2] We investigated the possibility of characterizing the specific interactions of metal cations with the phosphate group by NMR spectroscopy. On the basis of molecular dynamics simulations and ab-initio calculations of chemical shift tensors and indirect spin-spin coupling constants we propose several options for monitoring the metallation of the nucleic acid phosphate.

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P549

Structural Assembly of Molecular Complexes Using Residual Dipolar Couplings

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We present and evaluate a rigid-body molecular docking method, called PATIDOCK,[1] that relies solely on the three-dimensional structure of the individual components and the experimentally derived residual dipolar couplings (RDC) for the complex. We show that, given an accurate *ab initio* predictor of the alignment tensor from a protein structure, it is possible to accurately assemble a protein-protein complex by utilizing the RDC's sensitivity to molecular shape to guide the docking. To achieve this, we developed a computationally efficient method, called PATI,[2] for predicting the alignment tensor of a protein directly from its three-dimensional structure, provided the alignment is caused by planar steric obstacles (e.g., bicelles, PEG/hexanol). The proposed molecular docking method is computationally efficient and robust against experimental errors in the RDCs or binding-induced structural rearrangements in the individual components. We analyze the accuracy and efficiency of the RDC-guided docking method using experimental or synthetic RDC data for several proteins, as well as synthetic data for a large variety of protein-protein complexes. We also test our method on two protein systems for which the structure of the complex and steric-alignment data are available (Lys48- linked di-ubiquitin and a complex of ubiquitin and a ubiquitin-associated domain) and analyze the effect of flexible unstructured tails on the outcome of docking. The results demonstrate that it is fundamentally possible to assemble a protein-protein complex based solely on experimental RDC data and the prediction of the alignment tensor from three-dimensional structures. The ability to assemble a molecular complex using RDCs is remarkable, because it shows that despite the purely angular nature of residual dipolar couplings, they can be translated into distance/translational constraints. This is due to RDC's sensitivity to molecular shape and reflects the fact that it is the shape of the molecule that causes its steric alignment. Additionally, we developed a method for combining RDCs with other experimental data, such as ambiguous constraints from interface mapping, which further improves structure characterization of the protein complexes.

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P550

Coupling *in situ* NMR and X-Rays Absorption at high temperature with Molecular Dynamics to describe the speciation in molten zirconium fluorides

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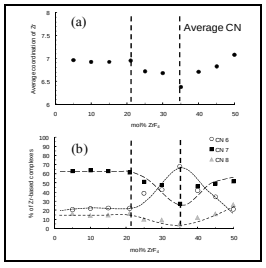
We investigated the local structure around zirconium and fluorine atoms in molten mixtures of alkali fluorides and zirconium tetrafluoride by the combination of high temperature NMR and EXAFS experiments up to 1500K.[1] The ^{91}Zr HT NMR signal evolution can be interpreted with an average coordination number evolving non-monotoneously between 6 and 7 for the zirconium over all the domain of composition, as confirmed by EXAFS experiments at the Zr K-edge. Molecular dynamics (MD) has shown the coexistence of 3 different complexes in the melt: $[ZrF_6]^{2-}$, $[ZrF_7]^{3-}$and $[ZrF_8]^{4-}$, with proportions depending on the composition and on the nature of the alkali. In agreement with ^{19}F HT NMR data, MD calculations highlight that the number of bridging fluorines between zirconium fluorides complexes increases with the ZrF_4 contentration and inversely the number of free fluorines decreases. These structural informations combined with dynamical approach given by diffusion coefficient measurements are crucial for the fluoro-acidity determination, one of the hot topics for molten salts applications.



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P551

Examples of Cultural Heritage Analyzed by Mobile NMR

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A compact and mobile, single-sided ^{1}H NMR sensor, the NMR-MOUSE®, has been employed to characterize non-invasively the layer structure of historic walls,[1] bones, and paintings.[2] Following laboratory tests on a mock-up hidden painting, paint and mortar layers were studied at the Papyrus Villa and the House with the Black Room in Herculaneum. The effects of different conservation procedures on the frescoes were studied in the black room, and two types of mortar layer structures were identified in the papyrus villa from moisture profiles. Such profiles were also recorded through the Mosaic of Neptune and Amphitrite in Herculaneum, revealing large differences in moisture content of the tesserae and the same moisture content in the supporting mortar. Ancient bones were studied by NMR and micro CT to investigate NMR as a tool to determine bone density. The oil-on-canvas self-portrait of Rembrandt from ca. 1668 has been analyzed in the Wallraf-Richartz Museum in Cologne to characterize the different paint layers in view of a possible restoration. These investigations demonstrate the use of the portable and single-sided NMR technology for non-invasive studies of objects of cultural heritage.

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P552 (*)

Does radical pair recombination act as a quantum measurement?

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Spin-correlated radical pairs occur in various biological processes and are well studied.[1] The theoretical treatment of the evolution of their density operator has been established more than 30 years ago.[2] Very recently, it has been proposed that the fundamental treatment requires quantum measurement theory.[3] Within short time two different equations of motion for the propagation of the density operator under the reaction term have been published.[3,4] The experimental validation for both treatments, however, is lacking and it is still unclear which differences could be observed experimentally.[4]

As an example for a radical pair recombination, we investigate here the spin-dynamics of the solid-state photo-CIDNP effect.[5] This effect occurs in photosynthetic reaction centers as a consequence of the spin dynamics in spin-correlated radical pairs. The extension of the theoretical treatment to calculate the ground state nuclear polarization allows for a simulation-based exploration of possible solid-state photo-CIDNP ^{13}C MAS NMR experiments. First results indicating the occurrence of a quantum Zeno effect will be presented.

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P553 (*)

Towards Real-Time NMR Studies of Biological NanoMachines

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NMR spectroscopy offers a near unique ability to monitor structural and dynamic changes in real-time and at atomic resolution. Historically, the application of fast real-time 2D NMR techniques has been limited to the study of small proteins (<20 kDa) on the second to minute time-scale. We have recently established that, with a combination of innovative isotope labelling schemes[1-2] and optimized NMR spectroscopy,[3] similar real-time NMR studies can also be applied to much larger macromolecular assemblies. We have developed several new strategies for the production of perdeuterated proteins with specific [^{1}H,^{13}C]-labelling of isoleucine γ_2- or alanine β-methyl groups[1], or stereo-specific labelling of prochiral methyl groups.[2] As these labelling approaches are entirely co-compatible, protein samples can be prepared with multiple combinations of methyl labelling patterns. We have also exploited the residual level of protonation in perdeuterated proteins – usually seen as an artefact of isotope labelling schemes – to speed-up the acquisition of NMR data by an order of magnitude.[3] This approach has enabled the acquisition of high quality 2D [^{1}H,^{13}C]-methyl spectra of 500 kD protein assemblies in less than one second.[3] By combining these sensitivity-optimised labelling protocols, fast NMR experiments and automated molecular approaches[4] we have been able to develop a robust and systematic strategy for the rapid and cost-effective resonance assignment of every labelled methyl group in such large protein assembly.

With these widely applicable tools it is now possible to observe and characterise the structural and dynamic events that govern biomolecular function in large protein assemblies. The use of such techniques can allow simultaneous atomic- and time-resolved investigations into the folding, self-assembly and structural rearrangement processes occurring in biological nanoparticles. The utility of these new methods will be highlighted using TET2, an homododecameric nanomachine of 468 kDa involved in protein quality control.

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P554

Insensitive: a free application to visualize spin dynamics for education

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The theory of NMR differs from that of other methods in spectroscopy in the way the manipulations of the system are described by quantum mechanics. It is not uncommon that not all of the necessary quantum mechanics is included in the chemistry curriculum. Learning and teaching NMR dynamics beyond simple spectrum analysis is therefore not an easy venture.

There are three ways in which the mathematics is commonly formulated: The vector model, the density matrix approach and the product operator formalism.[1] Especially the transition from the geometrically intuitive but incomplete vector model to the more consistent product operator formalism demands a high degree of abstraction. While the density matrix approach is again less intuitive and becomes complicated very fast with an increasing number of different spins, a basic understanding of it is necessary to ably work with product operators.

In this work in progress a new, free computer program is presented. It is intended to be used for education and self-education as a companion to established textbooks.[2,3] Based on the density matrix approach, it displays all three formulations of a spin system alongside each other. It easily visualizes possible manipulations such as arbitrary rf-pulses, chemical shifts and scalar coupling on an ensemble of up to four different spins-1/2 and up to two spin types (I and S) in solution. This makes the limitations of each of the models easily recognizable to students who are new to spin physics.

The application is written in C and Objective-C. The number of spins is easily extensible, sufficient computational power provided.

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P555
Multispectral filters for quantitative MRI

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Reconstructing a parametric map from MR images consists in estimating NMR parameters from a vector of noisy observations. To separate the various parameters of interest, it is necessary to vary their respective influences in the acquisition dataset. To this end, several images of the same object are acquired with different weightings (e.g. different characteristics of diffusion-sensitized gradients for quantitative diffusion mapping). A multispectral image - a set of N frames - is then systematically available, the useful signal in each voxel being a signature vector of size N. Filtering the multispectral dataset is a generic preprocessing step that helps to reduce noise propagated in the quantitative maps. For this purpose, new multispectral filters were introduced based on the nonlocal means algorithm (NLM, 1). An important concept in NLM is to consider that the restored intensity obtained in a given voxel is a weighted average of all voxel intensities in the image. Calculations of both the weight $w(i,j)$, which quantifies the similarity between voxels i and j, and the restored intensities are revisited here to take into account multispectral properties of data and a general statistical model for the MR signal (i.e. a noncentral chi-squared distribution). The index of similarity has to be robust to prevent averaging over regions presenting edge or small structures. Buades et al.[1] advocate computing $w(i,j)$ between two voxels by comparing the intensities of their neighborhoods, making use of similar patches over the image. Because this redundancy is infrequent in MR images, we propose changing this subset-based similarity by making use of the multispectral context. Thus vector-valued intensities between voxels i and j are compared by computing Bayes probabilities of having the same true amplitude in each frame and then merging these probabilities in a scalar weight $w(i,j)$ using different operators.[2] The final step of the filter is a weighted estimator that is unbiased for observations following noncentral chi-squared distribution. The denoising performance obtained with these new variants of NLM is illustrated on both numerical and real datasets. These filters are the first schemes based on the nonlocal principle dedicated to multispectral MRI and can be applied to regularizing well-registered datasets before parametric reconstruction.

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P556
Enantiodiscrimination in oriented solutions of PBLG: where does it come from?

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During the last decade, it was successfully demonstrated that enantiomeric pairs of low molar mass compounds yield slightly different NMR spectra when dissolved in a liquid crystalline (LC) mixture containing poly-γ-benzyl-*L*-glutamate (PBLG) and an organic co-solvent.[1,2] This effect is due to different averaging of magnetic tensors for the enantiomers, in this partially ordered chiral environment. Spectral enantiodiscriminations using deuterium or proton coupled carbon-13 NMR spectroscopy has been successfully observed for a wide range of organic molecules, but the underlying mechanism remains obscure. To shed light on this issue we have undertaken Molecular Dynamics simulations for selected chiral and prochiral solutes[2,3] in the presence of an α-helical oligomer of PBLG. We have used the OPLS force-field,[4] as implemented in GROMACS.[5] Orientational order parameters of solutes, which are proportional to NMR quadupolar and dipolar splittings, have been calculated by averaging over trajectories. The order parameters in these systems are very small, therefore long trajectories are needed for convergence; alternatively, *ad-hoc* strategies can be adopted for a more efficient sampling. For polar solutes, we have found a clear indication that enantiodiscrimination originates from their interactions with the ester groups of PBLG side chains. Interestingly, the effect persisted, although weaker and in different form, after switching off the charges. Here the first results are discussed and compared with experimental data.

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P557

Zero-quantum suppressed homonuclear correlations

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The high resolution 2D TOCSY, NOESY and ROESY spectra often contain crosspeaks containing alternating phase elements added to the theoretically pure-absorption-phase signals as if a phase-sensitive-COSY-like spectrum were superponed to the expected spectrum. This effect is due to the zero-quantum coherences which give rise to anti-phase dispersive elements in the spectra. There are several suggestions in the early literature (phase cycling, gradient pulses, trim pulses and combinations of these) to suppress or filter these contributions but none of them have proved entirely satisfactory. J. Keeler and M. J. Thrippleton suggested a method for suppressing zero-quantum coherences from COSY, TOCSY and NOESY experiments.[1] The method of their zero-quantum suppression involves applying simultaneously a swept-frequency 180° adiabatic pulse and a mild gradient pulse. The method was applied with minor modifications (sine-bell shape instead of rectangular gradients, 'gp' syntax instead of 'gron-groff' syntax), tested for COSY, TOCSY and NOESY and has been found to be better than the TOCSY and NOESY pulse programs available in the Bruker pulse program library. Their method can't be expanded for ROESY because the magnetization is not in the z axis during the mixing time of the ROESY. C. Thiele et al. suggested a new ROESY variant (Efficient Adiabatic Symmetrized ROESY; EASY-ROESY) with off-resonance spinlock and adiabatic half-Gauss ramps before and after the spinlock.[2]

The gradient & adiabatic pulse pair has been built into the new ROESY version in order to improve the suppression of the coupling-origin alternating-phase elements of the signals. The advantages - the better signal shapes - of the methods are demonstrated on examples using srtychnine as model compound. The selective 1D versions are also available. The zqs-TOCSY and -NOESY versions have been used as routine measurements in our laboratory since August 2007 as well as the zqs-easy-ROESY since the autumn of 2009.

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P558

Applicability of the Parahydrogen Induced Polarization Technique (PHIP) on Acetylene Dicarboxylic Acid Dimethylester

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In this work, we present experiments demonstrating significant NMR signal enhancement during the parahydrogenation of acetylene dicarboxylic acid dimethylester. One feature of the acetylene dicarboxylic acid, the acetylene dicarboxylic acid dimethylester and their resulting products are their symmetric structures. Due to this symmetry, the two inserted parahydrogen protons are magnetically equivalent in the molecule. Thus, they should not result in any hyperpolarized signal because their emission and absorption part of the antiphase signals exhibit the same chemical shift and therefore should cancel out. However, it was shown that this is not always the case and that hyperpolarized signals from symmetric molecules can be observed.[1] The results will be presented along with a series of simulation attempting to provide a better understanding of the PHIP signal pattern of symmetric molecules, based on the latest theoretical developments.[2-4]

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P559

High-resolution ^{1}H NMR Spectra in Inhomogeneous Field via Dipolar Interactions between Proton and Quadrupolar Spins

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Intermolecular multiple-quantum coherences (iMQCs), arising from intermolecular dipolar interactions, have been widely used in high-resolution NMR spectroscopy in inhomogeneous fields.[1] In this report, two new pulse sequences based on intermolecular double- and single-quantum coherences (iDQCs and iSQCs) between proton and quadrupolar nuclei were proposed for fast acquisition of high-resolution ^{1}H NMR spectra in inhomogeneous fields. Taking the *IS* (I=1/2, S=1) spin system as an example, the efficiency of the new pulse sequences was studied. For the two sequences, it is the range of magnetic field inhomogeneity rather than chemical shift that is sampled in the indirect dimension, which enables a great reduction in acquisition time and amount of data. The information of chemical shifts, relative peak areas and multiplet patterns is retained in the 1D projection of resulting 2D spectra acquired in inhomogeneous fields. The apparent J coupling constants are magnified. For the iSQC spectra, the magnification factor is 1.5. For the iDQC spectra, the magnification factor depends on the gyromagnetic ratio of proton and quadrupolar spin. Analytical signal expressions were derived based on product operator formalism. Experimental observations are in good agreement with theoretical analysis. These pulse sequences are applicable to both isolated and J-coupled spin systems in liquid. Compared to the sequences proposed previously,[2] these two sequences are more suitable for moderate or relative large inhomogeneous fields.

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P560

Multiple time scale analysis of fast internal dynamics in proteins from NMR relaxation data

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NMR relaxation experiments of ^{15}N in isotopically labeled proteins represent the most widespread probes of internal protein dynamics. Relaxation rates obtained from these experiments can be analyzed to derive amplitudes and time scales of internal motions, as well as thermodynamic information about the populations of conformers. However, their analysis in terms of dynamical parameters is not straightforward. We will present a novel analysis of NMR relaxation rates based on a multiscale description given by the *fractional Ornstein-Uhlenbeck (FOU) process*, which, in a different context, has been shown to adequately describe the internal dynamics of proteins over a broad range of time scales, ranging from picoeconds to seconds.[1,2] In order to investigate its relevance for the analysis of NMR relaxation data, our approach was tested on *synthetic* relaxation rates obtained by *molecular dynamics (MD) simulations*. The analysis of R_1, R_2 and NOE rates calculated from MD simulations of two proteins with significantly different overall tumbling times (ubiquitin with 76 residues and 6-phosphogluconolactonase with 266 residues) was undertaken in terms of Mittag-Leffler functions, which are related to the fractional Ornstein-Uhlenbeck process that takes into account the presence of multiple scales in internal dynamics. The results obtained for these proteins allowed us to discuss the advantages and limitations of this approach.

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P561

Ultrafast Intermolecular Zero-Quantum Coherence NMR Spectroscopy Based on Spatially Encoding Technique

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Spatial encoding ultrafast technique enables a 2D NMR spectrum to be obtained in a single scan.[1,2] On the other hand, intermolecular multiple-quantum coherences (iMQCs) can be used to obtain high-resolution NMR spectra in inhomogeneous fields.[3] In this report, we utilized spatial encoding technique to speed up the acquisition of 2D intermolecular zero-quantum coherence (iZQC) spectra. Distant dipolar field (DDF) was generated by both the spatial encoding gradients and coherence selection gradient. The gradient-driven decoding technique was adopted to acquire the iZQC signals. Theoretical expressions for the signals from the proposed iZQC sequence were derived according to the DDF treatment combined with product operator formalism. Experimental results verify our theoretical analysis and the feasibility of the method. The signals disappear when the DDF is along the magic-angle direction, implying that the signals are indeed from the iZQCs. One-dimensional high-resolution spectra can be extracted from the projection of 2D decoupled iZQC spectra from the fields with inhomogeneity severe enough to completely erase all spectral features in conventional spectra. Moreover, the proposed iZQC sequence can effectively suppress residual solvent signals. This study opens a way to improve the acquisition efficiency of iMQC methods.

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P562

Validation by QM method of the solution structure of MT-II triazolyl cyclopeptides

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MT-II is a potent super-agonist of melanocortin receptors, characterized by lactam bridge in the bioactive sequence (His6-D-Phe7-Arg8-Trp9).[1] In our previous work we designed and studied a new intramolecular side chain-to-side chain [1,2,3]triazolyl modification bioisosteric to the lactam.[2] In current study we applied this strategy on MT-II sequence, stabilizing the β-turn conformation which was proved essential conformational feature for the bioactivity.[3] The fully elucidated solution-structures of peptide have been obtained by 2D-NMR spectroscopy. The NMR spectra of the peptide showed a double pattern of resonances due to conformational equilibrium. In order to validate the solution NMR structure we performed a DFT conformational analysis regarding the two possible conformations simulating the presence of solvent (IEF-PCM, DMSO),[4] and we simulated the NMR spectra by means of QM calculations. Simulation of 2D NMR spectra of molecules with increasing molecular weight is indeed here suggested as a tool in their structure elucidation.

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P563

Microstructure of Simple Electrolyte Solutions as Studied by NMR-Relaxation Method and Quantum-Chemical Calculations

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Electrolyte solutions attract the attention of many scientists due to their important role in various physical, chemical, biological, and technological processes. The investigation of ion salvation-shell regularity is very complicated because of the lack of suitable research methods. Solving the problem becomes especially hard in the case of labile complexes characterized by fast exchange of ligands. A NMR-relaxation method for the investigation of aqueous salt solutions was earlier developed.[1] Complementary investigations, using two independent methods, provide more extensive and reliable information about the microstructure.

It has been shown that the nearest vicinity of many ions is constant over a wide temperature range but some ions (Li^+, Cl^-, Br^-) change the microstructure of their hydration shells in the range of 30-40 ^oC. Probably the effect of the change of the coordination number of the Cl^- anion can be responsible for the thermoregulation of warm-blooded animals.

In order to conciliate the data obtained from proton and deuteron resonances the electric field gradients and the QCC of deuterons from different molecular complexes were estimated from quantum-chemical calculations. All calculations were carried out using GAMESS 2003, GAUSSIAN98, GAUSSIAN03 programmes. The DFT method with hybrid functional B3LYP was chosen to take into consideration non-local electronic correlation. Flexible basis sets (6-31++G**, 6-311++G**, aug-cc-pVDZ, aug-cc-pVTZ and aug-cc-pVQZ) with diffuse functions were used, that is specially important for molecular systems with hydrogen bonds.

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P564

Shuttling Device for Field Cycling Experiment on NMR Relaxation Study

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Dipolar-dipolar (DD) interaction and chemical shift anisotropy (CSA) effect are two major sources of nuclear magnetic relaxation at high field. Conventionally molecular motions are extracted from analysis of longitudinal relaxation rate (R_1), transverse relaxation rate (R_2) and Nuclear Overhauser effect (NOE) measured at a fixed field which cannot distinguish the contribution from DD interaction and CSA effect. Since CSA contribution is magnetic field dependent whilst the dipolar interaction is field-independent at the high field range one can separate these two contributions from relaxation rates measured at multiple fields. We have built a field cycling apparatus based on a Bruker AVANCE600 NMR spectrometer. By shuttling samples vertically to the desired heights inside the magnet for relaxation and back to the central position for detection, one can measure relaxation at lower fields with high resolution (from 0.04 Tesla to 14.1 Tesla). The round trip shuttling time from the central position to the top of magnet is about 0.16s, which is suitable for measuring relaxation rate in the range up to $10s^{-1}$. In this poster we will present the details of field cycling apparatus design and some preliminary results of the dynamics of single amino acids and a di-peptide based on field dependent ^{13}C T_1 measured with the shuttling device.

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P565

Design and Implementation of 2D DESIRE Experiments

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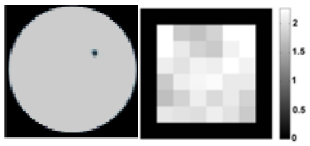
An alternative to the conventional Fourier encoding techniques is the DESIRE (Diffusion Enhancement of SIgnal and REsolution) method. This real-space method promises not only to increase the SNR but to also reveal new, diffusion-based, contrast[1,2] which will nevertheless further extend the applicability of MRM to the study of single biological cells. Images based on the DESIRE effect in one dimension have previously been obtained.[3] We present here the design and implementation of DESIRE in two dimensions and report the first 2D DESIRE image.

Conceptually, a DESIRE experiment consists of three steps. The first step is to achieve saturation in a well defined location. In order to do so we followed the "k-space" approach first proposed by Pauly.[4] We used a single shot spiral out k-space trajectory for excitation and computed RF pulses necessary to saturate the spins inside infinitely-long square prisms (100 μm side). A saturation pulse and the necessary power to obtain a 90° flip angle were computed for every saturation location. In order to correct for distortions due to off-resonance effects we incorporated a field map in the design of the RF pulses. Fig. **A** shows the saturation profile, a 100 x 100 μm^2 square cross-section prism, obtained inside a 1.8 mm radius cylinder filled with water. In step two we successively saturated 36 different locations and acquired the NMR signal. In the final step we subtracted these signals from the reference signal (same slice, no saturation) and assigned the result to the directly-saturated pixel. The 2D DESIRE image thus obtained, a 600 x 600 μm^2 square centered at the center of the sample, is shown in Fig. **B** (100 x 100 μm^2 in plane resolution, slice thickness 1mm). One possible cause for the inhomogeneity observed in the image (9.6%) can be the inhomogeneity in the B1 profile and should be eliminated by incorporating B1 field maps in the computation of the RF pulses.



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P566

Use of Filter Diagonalization Method (FDM) to process high resolution steady state free precession (SSFP) signal with strong Fid-Echo overlap

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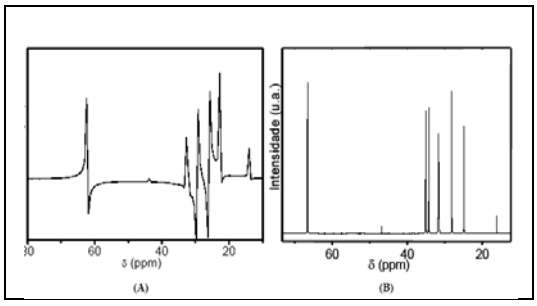
The Steady-State Free Precession (SSFP) sequence is not routinely used to enhance signal to noise ratio in high resolution NMR spectroscopy because it introduces severe spectral anomalies. These anomalies are due to the presence of an echo in the SSFP time domain signal. The Fourier transformed spectra of SSFP signals show phase distortions, truncation artifacts and poor digital resolution. FDM can deal very efficiently with truncated signals and has become a promising technique to complement the already established Fourier Transform formalism. No less important is the FDM ability of separate corrupting or uninteresting signals from complex NMR spectra, without disturbing overlapping or nearby signals. Therefore, by using the FDM it is possible to separate the overlapped FID and echo signals. In this paper we use FDM to processes ^{13}C NMR spectrum acquired with SSFP sequence with strong FID-echo overlap, with 30 ms pulse rate. Figure (A) shows the Fourier transformed spectrum of 1-octanol, obtained with SSFP sequence with 30 ms pulse rate and Figure (B), the FDM spectrum of the same NMR data. With this result we can conclude that FDM can be a powerful tool to process high resolution ^{13}C signals obtained by SSFP sequence, without phase distortions, poor digital resolution and truncation artifacts as observed in figure 1A.



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P567

SEC-Low Field NMR: Online Detection of Mass-separated Polymers by NMR-Spectroscopy

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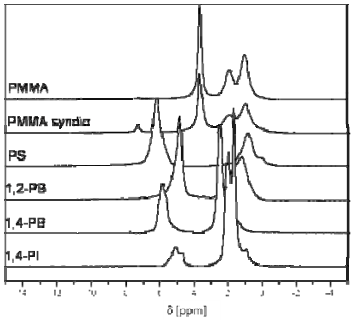
A prototype of a 20 MHz bench-top medium resolution (MR) FT-NMR (Fourier Transform-Nuclear Magnetic Resonance) spectrometer has been adapted for online coupling with Size Exclusion Chromatography (SEC). An integral part of the design is the use of permanent magnets, which, compared to high-resolution NMR, keeps investment and running costs comparatively low. Sensitivity and selectivity are sufficient for polymer characterization. As polymers are investigated in solution in the coupled experiment, an essential problem is suppression of the predominant solvent signal. Among other schemes, T_1 relaxation differences can be exploited for our purpose. Additionally, mathematical data treatment allows a significant selective enhancement of the polymer signal. First online spectra and chromatograms will be shown.



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Diagonal-Free 3D/4D HN,HN TROSY-NOESY-TROSY

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Structure elucidation by NMR spectroscopy relies mostly on measuring inter-proton distances via NOE cross signals in NOESY spectra. In proteins, the subset of H^N-H'^N NOE contacts is most important for deriving initial structural models, and for spectral assignment (verification) by 'nOe walking'. Yet, unambiguous assignment of H^N-H'^N NOE contacts essentially requires editing via the associated ^{15}N dimensions that offer spectral dispersion superior to H^N, particularly in large, helical or unfolded proteins. ^{15}N dimensions also show maximal TROSY effect[1] for resolution and sensitivity enhancement, favouring the 3D(4D) (H)N,HN TROSY-NOESY-TROSY implementation[2] with evolution of both ^{15}N dimensions. The principle underlying TROSY, i.e. strict separation of spin state selective (**S**^{3}) magnetisation pathways, furthermore opens an elegant way to suppress the intense, uninformative diagonal signals that are the major source of spectral overlap, baseline distortion and diverse artifacts in NOESY spectra: since spin states represent magnetically distinct sub-populations, filtering for different spin states before and after NOE mixing suppresses the conserved diagonal signals, and selects only the stochastic cross relaxation pathway that leads to NOE cross signals.[3] This suppression scheme is, however, voided if both S^3 magnetisation pathways are mixed again after NOE evolution, as happens with conventional subsequent H→N INEPT transfer. Using a modified ST2-PT module instead, we show how S^3 pathways are rigorously kept apart also in the (H)N,HN TROSY-NOESY-TROSY sequence.[4] Thus, diagonal suppression is achieved with virtually no additional losses in NOE intensity, as shown for the 40 kDa Maltose Binding Protein.

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P569

Hyperpolarized and radical-free solutes via Dynamic Nuclear Polarization utilizing thermoresponsive, spin-labeled Hydrogels

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NMR and related techniques have become indispensable tools with innumerable applications in chemistry, physics, biology and medicine. One of the main obstacles in NMR is its notorious lack of sensitivity. This obstacle could be overcome by in vitro hyperpolarization of molecules via Dynamic Nuclear Polarization (DNP). Although various DNP methods have found important applications in chemistry, biology and medicine, one severe problem remains: stable and mostly toxic radicals have to be admixed to the target molecules. The admixed radicals cause NMR line broadening and even more fundamentally they lead to fast T_1 relaxation immediately after the polarization step is completed. This severely limits the time frame during which the accomplished hyperpolarization can be used. Hence, fast and reliable separation of radicals and polarized material remains an important issue for improving applicability of DNP. Here, we introduce the use of thermoresponsive, spin-labeled hydrophilic polymer networks (SL-hydrogels)[1] for DNP, and demonstrate that they allow fast and simple radical - solute separation.[2] Heating of the swollen hydrogel to the critical temperature T_C induces a reversible, fast (≤ 1s) and dramatic volume decrease (≥ 500 volume%) thereby expelling hyperpolarized water and other target molecules from the radical-bearing polymer network. Two different spin-labeled thermoresponsive hydrogels (T_C = 63°C) were synthesized and investigated with regard to their applicability for Overhauser DNP. The obvious benefits are the prolonged T_1 after the collapse, the *radical-free* and *non-toxic* solute containing the hyperpolarized biomolecules allowing for biomedical applications.

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P570

Analysis of the Inverse Halogen Dependence of Nuclear Magnetic Shield in Trihalophosphanes

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Inverse halogen dependence (IHD) is an increase of the chemical shift on going from chlorine to iodine, which is often observed for early transition metal in higher oxidation states and in the main group for p block compounds in the lower oxidation states.[1] Decomposition of ^{31}P shielding tensor obtained from SO-ZORA Hamiltonian at BP86/TZ2P level (Fig. 1) showed that the paramagnetic term (σ_{PARA}) is the dominant contribution to shielding tensor behaviour and the spin-orbit term (σ_{SO}) is the secondary one.[2] The decrease of energy gap between ^{31}P lone pairs and $\sigma^*_{P\text{-}X}$ and s-σ character of P-X bonds reinforce the discrete increase of SO/FC term along the series.[3,4]

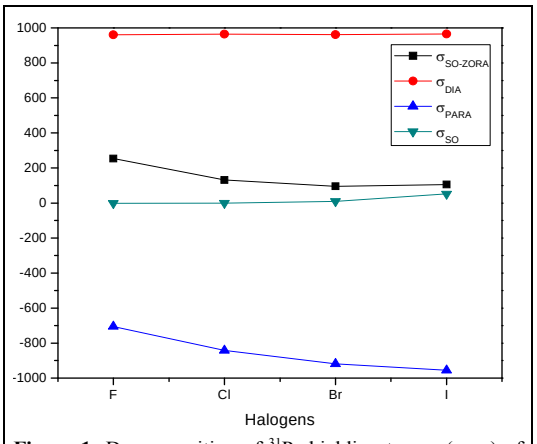

Figure 1: Decomposition of ^{31}P shielding tensor (ppm) of trihalophosphanes PX_3.

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P571
Solvent models for calculations of NMR parameters

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A universal solvent approach is difficult for the nuclear magnetic resonance (NMR) shielding and spin-spin coupling constants that in part result from collective delocalized properties of the solute and the environment. In this work bulk and specific solvent effects are discussed on experimental and theoretical model systems comprising solvated alanine zwitterion, cation and anion,[1] and chloroform[2] molecules. Density functional theory (DFT) computations performed on larger clusters indicate that standard dielectric continuum solvent models may not be sufficiently accurate. In some cases, more reasonable NMR parameters were even obtained by approximation of the solvent with partial atomic charges. Combined cluster/continuum models yielded the most reasonable values of the spectroscopic parameters, provided that they were dynamically averaged. The roles of solvent polarizability, solvent shell structure, and bulk permeability were investigated. NMR shielding values caused by the macroscopic solvent magnetizability exhibited the slowest convergence with respect to the cluster size. For practical computations, however, inclusion of the first solvation sphere provided satisfactory corrections of the vacuum values. The simulations of alanine and chloroform chemical shifts and *J*-coupling constants were found to be very sensitive to the molecular dynamics model used to generate the cluster geometries. The results show that computationally efficient solvent modeling is possible and can reveal fine details of molecular structure, solvation, and dynamics.

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Acknowledgments: Financial support from the Grant Agency of the Academy of Sciences of the Czech Republic through Project KJB400550903 is acknowledged.

P572
Ab initio simulation of spin diffusion in spinning powders

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Experimentally, fully quantitative methods have been developed to detect spin diffusion, which is driven by dipolar couplings between spins and thus depends directly on molecular geometry. However, because of the many-body nature of the problem, modelling spin diffusion is anything but simple and is now one of the key barriers to accurate NMR-driven structure determination of solid materials.

We first show that the coherent time-evolution of experimental observables for large spin systems can be simulated accurately within reduced Liouville spaces in the experimentally relevant case of powdered systems under magic-angle spinning.[1] We are able to simulate polarisation transfer in systems of more than 100 protons; this constitutes an order-of-magnitude increase in the number of spins for which such dynamics have been simulated.

We then show that *ab initio* simulation in reduced Liouville spaces can quantitatively reproduce experimental multi-spin polarisation transfer curves measured from spin diffusion experiments among protons (PSD) in powdered organic solids under magic-angle spinning, using a network of dipolar couplings derived only from geometry, and with no adjustable parameters.[2] These simulations are shown to capture the full dynamics of spin diffusion observed experimentally, at short, medium and long time scales, which no other model has been able to do previously.

Building on the ability to simulate the behaviour of a "bath" of strongly-coupled protons, we finally investigate the possibility to improve the accuracy of semi-classical models for the simulation of spin diffusion among carbons under the influence of protons, proton-driven spin diffusion (PDSD).

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P573

Hyperpolarized Xenon-129 as an NMR Probe in Chemical Reactions

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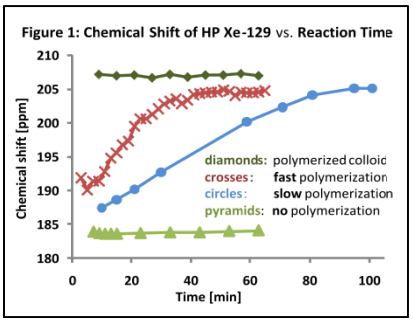
Xenon atoms have two useful properties for NMR spectroscopy: They are very sensitive to their environment due to their highly polarizable electron cloud even without the need of covalent bonds and they are able to be hyperpolarized (HP), overcoming the problem of the low SNR of thermally polarized Xenon. It is therefore favorable to use hyperpolarized Xenon as an NMR probe in dynamic processes like chemical reactions.

The polarized gas can be dissolved continuously into a reaction mixture by the use of hydrophobic hollow-fiber membranes, allowing for the molecular dissolution of hyperpolarized Xenon and the continuous replacement of depolarized gas. Repeated spectroscopic Xe-129 NMR measurements can show e.g. a polymerization process with good time-resolution.



Here, HP Xe-129 NMR is used for the online monitoring of miniemulsion polymerizations. It is known that the chemical shift of Xe-129 reflects the composition of the reaction mixture.[1] The application of the described technique to miniemulsion polymerization reactions gives an easy method for the analysis of these reactions. Kinetic data for different reaction conditions (e.g., initiator, reaction temperatures) has been determined from the chemical shift of the dissolved Xenon [Figure 1]. Comparable kinetic data can be obtained by calorimetry,[2] allowing for the verification of the method.

The application of the Xe-129 NMR method to thermoneutral reactions (e.g., enzymatic reactions) should allow for the determination of kinetic data for systems immeasurable by calorimetry.

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P574

Heterogeneity of segmental dynamics of filled EPDM rubber by ^{1}H Hahn Echo Measurements

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Second van Vleck moment M_2 and correlation time distributions were determined at low magnetic fields from the Hahn echo measurements for a series of unfilled and filled EPDM samples as a function of filler type and filler content. The fillers are of carbon black, silane based and calcium-carbonate type. A simple filter was applied to eliminate the contribution of bound rubber. A theory for the dependence of the Hahn echo decay,[1,2] valid for the entire echo time range, was developed to describe the dynamics of mobile polymer chain segments. First the experimental data were fitted with the theoretical expression by considering the average values of the ^{1}H residual second van Vleck moment $\langle M_2 \rangle$ and correlation time $\langle \tau_c \rangle$. Then, using the average value of $\langle M_2 \rangle$ the distribution of the correlation times was obtained. The experimental results confirm the use of a log-Gauss function for the distribution of τ_c. Finally, using an average value of the correlation time, the distributions of second van Vleck moment f(M_2) were determined for the entire series of samples. These distributions were obtained using a fast, Laplace-like inversion procedure.[3] The unfilled EPDM sample yields a bimodal distribution of second van Vleck moments. The two modes are associated with the mobile and dangling chain-end polymer segments. By restraining the mobility of the bound rubber segments, the fillers enhance the dynamic heterogeneity of the mobile polymer segments. This is observed from changes in the distributions of mobile and free-end polymer segments but also by the apparition of a new and distinct mobile peak associated with *bound-mobile interface* polymer segments.

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P575 (*)

Characterization of Picomole Amounts of Oligosaccharides from Glycoproteins by ^{1}H NMR Spectroscopy

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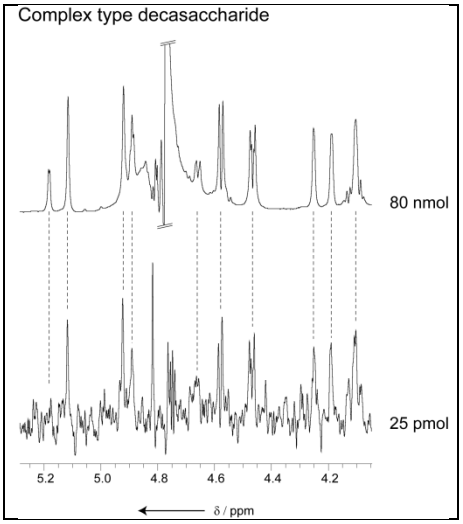
The use of NMR is limited if only small quantities of the sample are available. Detection limits are given in the range of nanomoles of sample for commercially available probes.[1] We demonstrate spectra of sucrose and a complex *N*-type decasaccharide, measured in amounts of just a few picomoles (down to 15 pmol). Special precautions for sample preparation and instrument setup have to be used to record spectra at such low quantities. Water suppression by a factor of 500000 has been essential to observe signals close to the solvent.[2] The figure on the right shows the comparison of two spectra of an *N*-type decasaccharide measured in amounts of 80 nmol (top) or 25 pmol (bottom), respectively. The signals of the structural reporter groups (e.g. anomeric protons of *N*-*a*cetylglucosamines, mannoses, fucose) are sufficient to identify the glycan structure. Therefore, it is possible to obtain structural information of compounds available in only minute quantities of a few nanograms.



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P576

Iron Oxide Magnetic Nanoparticles and Molecular Nanomagnets: a comparative EMR study

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Electron Magnetic Resonance (EMR) is a powerful tool to investigate the magnetic properties of molecular nanomagnets (MNMs) and it provides useful information in the analysis of magnetic nanoparticles (MNPs). These two systems have been usually treated using two different approaches: quantum mechanics for MNMs and classical mechanics for MNPs. The developments in the synthetic techniques are now providing objects of the same size for the two classes of systems, generating the need of a unified approach, to which EMR can offer a fundamental contribution.[1]

For this purpose we compare here the EMR results obtained on an iron(III) cluster made up of 19 metal ions, Fe_{19},[2] and on magnetite/maghemite nanoparticles mineralized in the Dps protein from the bacterium *Listeria innocua.*[3]

Powder spectra analysis pointed out similarities between the two systems. In particular, forbidden transitions can be observed in the spectra, indicating the presence of discrete levels also for MNPs. On the other side, Fe_{19} spectra show a temperature dependence similar to that of MNPs. The single-crystal W-band study of Fe_{19} evidenced that, although the fine structure is not well resolved in all the spectra, at particular temperatures and orientations, the presence of single transitions within a high spin multiplet can be distinguished.

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P577

Automated Assignment using Multi-Way Decomposition

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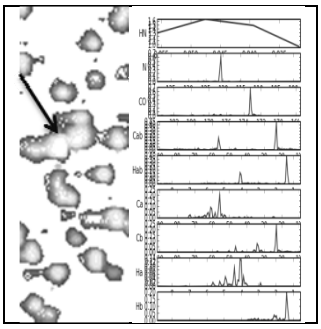
Multi-way decomposition of projections of high-dimensional spectra offers a variety of possibilities for comprehensive characterisations of proteins: complete resonance assignment, 3D structure and more. All recorded projections are two-dimensional and have the chemical shift of HN along the directly detected dimension. The other dimension corresponds to a linear combination of several chemical shifts. The *joint* multi-way decomposition of all spectra results in descriptions of spin systems, called components, which each consist of all nuclei in a $C\beta H_n$–$C\alpha H$–C'–NH–$C\alpha H$–$C\beta H_n$ fragment that stretches over two adjacent residues.[1] A component describes each nucleus of the fragment with one shape (see right side of figure).



With a combination of multi-way decomposition and automated assignment the time for characterization of a protein is greatly reduced with respect to traditional approaches.

Here we present the automated assignment combining projection experiments with multi-way decomposition of three proteins of varying size: ubiquitin, azurin and mmp20. The algorithm for resonance assignment (SHABBA) combines correlation among shapes, peak picking, chemical shift statistics and matching of shapes from adjacent components. The figure illustrates the decomposition of a fragment centred on the HN of Glu 2. The input projection (left) demonstrates significant overlap, while the shapes of the resulting component (right) allow unambiguous determination of chemical shifts with visible noise in only two shapes.

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Acknowledgments: EU project *Extend-NMR*; CCPN; Bruker Biospin.

P578

Entanglement dynamics of spin systems in pure states

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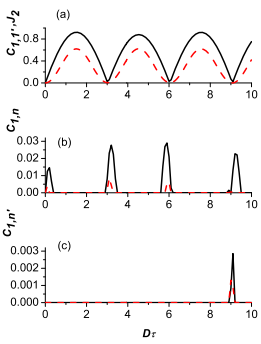
In the present paper we consider an application of the MQ NMR technique for creation of entanglement in systems initially prepared in the pure state. Results of computer simulations of entanglement dynamics are presented for real structures which were used in MQ NMR experiments. In the state populations of all the quantum states except one of them are the same but nonzero which can be represented in the following form $\rho_{ps} = \frac{1-\alpha}{2^N} \cdot \hat{1} + \alpha \cdot \rho_p$, for ρ_p a pure. Here $\hat{1}$ is the identity operator and α is parameter which depends on experimental conditions and number of spins *N*. The behavior of a MQ coherence in the pseudopure state is exactly the same as the behavior of the pure one because they differ only on the scaled unit matrix which does not contribute to observables and it is not changed by unitary evolution transformations. At the same time, entanglement depends on whether the state of the spin system is pure,α=1 in Eq. (1), or pseudopure.[1,2] The numerical experiments with cyclopentane molecules revealed the close connection between the intensity of MQ coherences of the second order and concurrence between the between closest spins (see Fig (a)). As a result, the 2Q intensity can be used as entanglement witnesses for such systems.



Dynamics of the concurrences C_{mn} and the intensities of the MQ coherences J_2 vs. time in the cyclopentane starting with the pure initial state.

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P579 (*)

Lanthanide chelates as relaxation switches for brute force polarisation

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There is intensive interest in the development of NMR hyperpolarisation techniques, with many potential applications in vivo and in vitro. One approach is to polarise nuclei by pre-exposure to high magnetic fields at ultra-low temperatures. However, this 'brute-force' approach requires a relaxation switch, in order to speed up the rates of polarisation during the cooling process without causing undue losses in polarisation during the subsequent warming and measurement periods. We anticipated that a class of paramagnetics - namely those lanthanides which have very short electron spin relaxation times at room temperature - might act as a relaxation switch. Such paramagnetics cause only small amounts of nuclear relaxation at room temperature, but can be expected to enhance relaxation during the cooling process. Of particular interest are lanthanide chelates such as dysprosium-DTPA, because they can be expected to have low toxicity.

In initial experiments on water/glycerol (1:1) solutions of 2 molar 1-^{13}C sodium acetate at 1.6K and 3.35T, we compared the relaxivity of six different lanthanide-DTPA chelates. Of the lanthanides tested, holmium proved to be the strongest relaxation agent, followed by dysprosium. The ^{1}H T1 values measured in the presence of the dysprosium and holmium agents were approximately 30-fold to 60-fold shorter than the corresponding ^{13}C relaxation times. In further experiments, we have used another spectrometer that is coupled to a dilution refrigerator. As an indication of our results, the ^{1}H T1 for a water/glycerol (1:1) solution of 2 molar 1-^{13}C sodium acetate, doped with 2mM holmium-DTPA, was about 280 minutes at 620mK and 3.4T. We have also carried out successful low-field thermal mixing experiments by ramping the field from 3.4T through zero to -3.4T, and in so doing achieved ^{13}C polarisations that are approximately 1,000-fold greater than the equilibrium polarisation commonly obtained in clinical spectroscopy. Taken together with additional room temperature studies that we have carried out following rapid dissolution, our results suggest that these lanthanide chelates might act as effective relaxation switches for brute force polarisation.

P580

Effect of RF phase shift on the Third Spin Assisted Recoupling in Solid-state NMR

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We introduce the concept of phase shift in the context of the Third Spin Assisted Recoupling (TSAR) mechanism and demonstrate its potential for detecting long-distance transfer in biomolecular systems. The modified pulse sequences of PAR[1] and PAIN-CP[2] still rely on cross-terms between heteronuclear dipolar couplings involving assisting protons ^{1}H in order to mediate zero- and double-quantum recoupling (^{13}C-^{13}C, ^{15}N-^{15}N, ^{15}N-^{13}C polarization transfer).

Using average Hamiltonian theory we demonstrate that the phase shift compensates off-resonance contributions yielding improved polarization transfer and substantial broadening of the matching conditions. We use numerical simulations to explain the fine structure of the TSAR optimization maps. This constitutes a major improvement in the context of the TSAR based methods since it alleviates the main drawback of the method: i.e. the sensitivity of the transfer with respect to precise rf settings. The potential of this new concept for biomolecular NMR is illustrated with 2D correlation experiments on a 19.6 kDa protein (U-[^{15}N, ^{13}C]-YajG) at high magnetic fields (up to 900 MHz ^{1}H frequency) and fast sample spinning (up to 65 kHz MAS frequency).

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P581

IPAP-HSQMBC: Measurement of Long-Range Proton-Carbon Coupling Constants from spin-state-selective Multiplets

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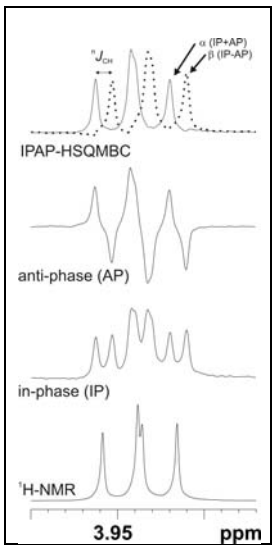
A new method is proposed for the precise and direct measurement of long-range proton-carbon coupling constants ($^nJ_{CH}$) in natural abundance molecules. Two complementary in-phase (IP) and anti-phase (AP) data are separately recorded from a modified HSQMBC experiment. Time-domain IP and AP data are finally added/subtracted to provide spin-state-selective HSQMBC spectra. In contrast to the conventional HSQMBC experiment in which a fitting processing is necessary to analyze the anti-phase coupling pattern, the value of $^nJ_{CH}$ can be extracted by simple analysis of relative displacement of cross-peaks. The robustness of this IPAP-HSQMBC experiment is evaluated experimentally and by simulation using a variety of different samples and experimental NMR conditions.



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P582

J-edited Spectroscopy Along the NMR Sample: Combining Selective Refocusing Experiment and Parallel Acquisition through Spatial Encoding

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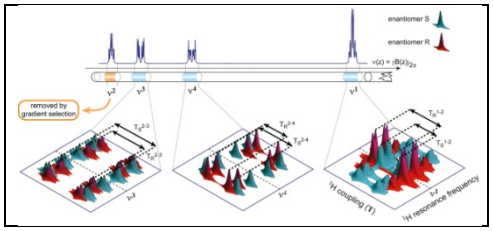
Extraction of spin-spin NMR couplings can be a hard and time-consuming task for chemists, although it opens the way to informations that are essential to the characterization of a solute, and notably to the determination of its tridimensional geometry. Actually there is currently no general NMR experiment that can give access, on a single spectrum, to a fully resolved – and assignable- measurement of a whole coupling network.



We propose to develop NMR sequences based on a frequency sample spatial encoding, that allow to run simultaneously different selective experiments in different parts of the sample. We apply this approach to the implementation of a Gradient-encoded SElective ReFocusing sequence (G-SERF).[1] The combination of homonuclear selective refocusing techniques and pulsed field gradients leads to the edition, within one single spectrum, of every couplings which are experienced by a given proton, leading to real phased *J*-resolved spectroscopy. The analytical potential of this technique will be demonstrated for the analysis of solutes in isotropic media, as well as mixtures of enantiomers dissolved in chiral liquid crystalline solvents: the characterization of each stereoisomer is then made possible, through a straightforward assignment and measurement of each homonuclear coupling network, using this new pulse sequence.

References:
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P583

High temperature PFG NMR for industrial concerns: understanding solvation and transport properties in cryolitic melts

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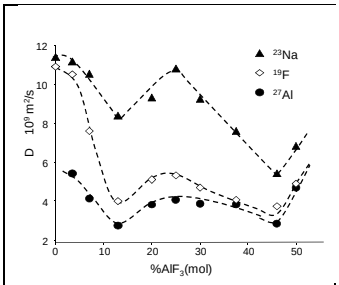
The energy requirements for the electrolytic production of aluminium are economical and environmental issues for the industry. The composition of electrolytic baths (mainly containing cryolite Na_3AlF_6, alumina Al_2O_3 and fluorides additives) is thus widely investigated in order to optimise working temperature, dissolution of alumina and electrical conductivity. However, due to the high temperature (up to 1000°C) and the corrosiveness of the cryolite-based melts involved in this process, only few experimental techniques are suitable for the determination of the physicochemical properties of such reactive media.

In this context, we have used high temperature NMR with boron nitride crucibles and CO_2 laser heating system to determine in molten NaF-AlF_3 binary mixtures the anionic fractions of the different fluorine-containing species: F^-, AlF_4^-, AlF_5^{2-} and AlF_6^{3-}.[1]

Moreover, thanks to a newly developed Pulsed Field Gradient NMR device,[2,3] we are now able to measure self-diffusion coefficients up to 1500K for numerous nuclei, like ^{27}Al, ^{23}Na, ^{7}Li and ^{19}F in NaF-AlF_3 and LiF-AlF_3 cryolitic melts. The transport properties characterised in both systems depending on the temperature and the composition provide a more precise description of the ionic species and clarify their respective roles in the electrical conductivity of the systems.



Self-diffusion coefficients as function of composition in NaF-AlF_3 binary mixture

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P584

Computer-aided mixture design for NMR-based fragment screening

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Fragment-based drug discovery is widely applied in industrial and academic screening programs.[1] Several screening techniques rely on NMR to detect fragment binding. NMR-based methods are among the most sensitive techniques[2] and have the further advantage of yielding a low rate of false positives and negatives. However, NMR is intrinsically slow compared to other screening techniques which requires screening of fragment mixtures to increase throughput. Here we present a fast and straightforward computer-aided method to design mixtures from a fragment library with minimized signal overlap. This approach allows the direct identification of one or several active compounds without the need for deconvolution. Our approach was accomplished by converting NMR spectra to "fingerprints", a meaningful computer-readable format, and minimizing the global signal overlap with a Monte Carlo algorithm. The scoring function used favors a homogenous distribution of the global signal overlap. The method does not require additional experimental work since the only data required are NMR spectra, which are generally recorded for each single compound as quality control before introduction into the library. For a library of 340 compound mixtures with a size of 5 were calculated. Our method achieved an average global signal overlap of 2% compared to 44 % for randomly generated mixtures. We confirm that the mixtures generated *in silico* coincide with *in vitro* mixtures and no significant signal shift occurs. Finally, we generated virtual libraries and demonstrated that this method is applicable regardless of the mixture, library size or the characteristics of the library.

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P585

Application of a DOQ-008-CGCRE INMETRO – Analytical Validation Methods using quantitative ^{1}H NMR spectroscopy

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The orientations from the DOQ-CGCRE-008 INMETRO were applied for determination biodiesel content (% v/v) in commercials diesel by quantitative ^{1}H NMR and the results compared with IR (NBR 15568/EN 14078) spectroscopy.

A calibration curve (2.0 to 6.5, % v/v) was prepared using known amounts of a biodiesel (B100) considered to present nearly 100% of the fatty acid methyl esters (FAME). All the samples were solved in a commercial diesel from a Brazilian refinery (D441). We analyzed a Diesel from an Interlaboratorial Program (IP) (PI19), containing biodiesel, to validate the calibration curve and measure the recovery as well. All ^{1}H NMR experiments were acquired on a VARIAN Mercury 200- with a 5 mm probe. The spectra were obtained at 200.026 MHz for ^{1}H, using 5% of sample v/v in $CDCl_3$ as solvent, and TMS as internal reference. The pulse used was the 45°. Thirty two pulses were employed to the acquisition spectra with acquisition time of 1.992 and relaxation delay of 20 s. Spectra were processed with 12000 data points using an exponential weighing factor corresponding to a line broadening of 0.5Hz. The Linearity was evaluated with a calibration curve obtained plotting the integrated area in 3.65 ppm versus concentration and showed satisfactory characteristics: R^2 = 0.9926, linear and angular coefficients statistically significant ($p < 0.003$) and the prediction error for all points was below 4%. The Recovery (accuracy) was evaluated using tree points between 3.2 and 5.6 (% v/v) and showed results from 95 and 97% that is a good recovery for analytical method. The Intermediate Precision was evaluated with the diesel from the IP whose result for the percentage of biodiesel by volume was 4.7 by IR spectroscopy with a standard deviation of 0.3. The reported result by the laboratory with the same method was 4.7 (% v/v) and the ^{1}H NMR result was 4.5 (% v/v), with a deviation of 0.3 and confidence interval 4.2 to 4.7 (95%). The data presented here show that the NMR method was comparable with the IR within the acceptable precision in determining the content of Biodiesel in commercial Diesel.

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P586

Distance measurements in peptides using Gd^{3+} spin labeling and DEER at W-band

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Here we present Gd^{3+} (S=7/2) spin labeling as a new alternative to nitroxide spin labeling for investigations of peptides conformation in solution. These are based on distance measurements using double electron-electron resonance (DEER). In analogy to conventional site directed spin labeling using nitroxides, Gd^{3+} tags that are derivatives of dipicolinic acid (4MMDPA)[1] were covalently attached to two cysteine thiol groups introduced into the peptide amino acid sequence at specific positions. The motivation for using this new class of spin labels for distance measurements is sensitivity improvement offered by high fields, particularly W-band (95 GHz, ~3.5 T). The high field EPR spectrum of Gd^{3+} is characterized by an intense central transitions due to the $|-1/2> \rightarrow |1/2>$ transition that is superimposed on a broad background due to all other transitions. The line width of the central transition sub-spectrum is broad enough to afford DEER measurements and has an isotropic character, namely independent on where in the spectrum the DEER pulses are situated, all possible orientations of the inter-spin vector **r** contribute to the DEER effect and there is no orientation selection. In contrast, at high field the g anisotropy of the standard nitroxide spin labels become well resolved and consequently the DEER measurements may exhibit orientation selection which complicates data analysis significantly.

This work demonstrates the feasibility of such distance measurements on melittin, a common model for anti microbial peptides. We have introduced cysteines in positions 15 and 27 and then prepared two types of peptides, one labeled with two nitroxide spin labels the other with two 4MMDPA-Gd^{3+} labels, and carried out comparative W-band DEER measurements. In the case of Gd^{3+} we explored the effect of the ratio of [Gd^{3+}] and [4MMDPA] in the labeled peptide, the measurement temperature that affects the relative intensity of the central transition, and the DEER acquisition time that is affected by spectral diffusion. All these were found to have a significant effect on the DEER modulation depth and should therefore be optimized.

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P587

Automated assignments of backbone and methyl resonances for proteins up to at least 25 kDa: Application to structural characterization of protein-ligand interactions

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NMR is widely applied to characterize interactions of proteins with ligands. For structural characterization of such interactions, resonance assignments are needed. In particular for industrial laboratories, standard methods for obtaining them are too labor intensive, and automatic procedures only work in a robust manner for small proteins.

We have developed a procedure, with which we have doubled the size limit of automated projection spectroscopy (APSY) based automated assignments to 25 kDa. We obtain assignments of the entire backbone (H^N, N, C', C^α, C^β) and of all side chain methyls, except for methionine εCH_3. The procedure consists of three 4D and 5D APSY NMR experiments (including the new 4D APSY HNCACB) that are recorded within 5 days - with a single protein sample with random fractional deuteration. Random fractional deuteration was chosen because (1) it is inexpensive and simple to implement, and because (2) all methyl groups remain largely protonated and can therefore be assigned. With this labeling pattern, however, signals from methyl groups split into CH_xD_y isotopomer multiplets. This additional complication can be elegantly overcome by an optimized projection reconstruction routine.

The assignments obtained in this way enable chemical shift mapping with amide and methyl moieties, and they are the basis for detailed structural characterization of protein-ligand interactions by intermolecular NOEs. Several examples from our work on interactions of proteins with drug candidates (*i.e.* organic compounds, peptides, antibodies, RNA) based on automated assignments are shown.

P588

^{129}Xe Signal Amplification by Gas Extraction and Compression for Remote Detection of a Xenon Biosensor

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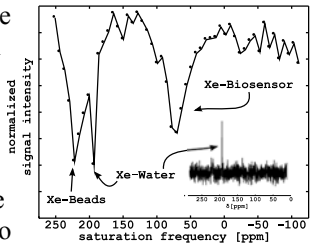

^{129}Xe is an attractive nucleus for use as a molecular contrast agent due to its acute chemical sensitivity, its large nuclear polarization attainable by spin-exchange optical pumping, and the lack of natural background signal in the body. With chemical exchange amplification provided by Hyper-CEST,[1] cryptophane-based molecular sensors have been detected with hyperpolarized xenon at sub-picomolar concentrations.[2] Traditionally this is done by monitoring the aqueous xenon peak while saturating at the xenon biosensor resonance frequency. In certain situations, the concentration and/or polarization of the aqueous xenon might be too low to be detected directly. In such cases, it is advantageous to extract the dissolved xenon into the gas phase where the concentration can be greatly increased through compression or liquefaction.[3] The figure to the right shows a single-scan saturation transfer spectrum of xenon dissolved in polystyrene beads, water, and a cryptophane biosensor. The spectrum was encoded with dissolved xenon, which was then extracted and compressed for detection in the gas phase. The directly detected spectrum of the dissolved xenon, averaged 16 times, is inset for comparison.

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P589

Shuttle DNP Spectrometer with a Two-Center Magnet

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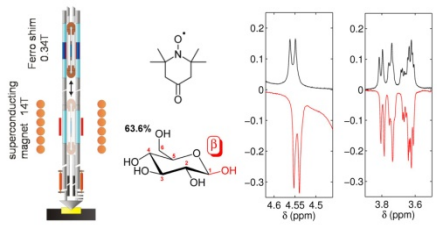

A DNP set-up is described where a liquid sample is hyperpolarized by the electron-nucleus Overhauser effect in a field of 0.34 T and transferred to a field of 14.09 T for NMR detection.[1] In contrast to a previous set-up,[2] using two dedicated magnets for polarization and detection, a dedicated ferroshim system was inserted into the bore of a 14.09 T shielded cryomagnet to provide a homogeneous low-field region in the stray field above the magnetic center. After polarization in the low-field the sample is transferred to the high-field magnetic center within 40 ms by a pneumatic shuttle system. In our set-up a standard high-resolution inverse $^{1}H/^{13}C$ selective probe was used for NMR detection and a homebuilt EPR cavity, operating in the TM_{110} mode was used for polarization. First experimental data are presented. We observed a maximum proton Overhauser enhancement of up to ε^{HF} = -3.7 in the high-field position for a 5 mM 4-oxo-TEMPO-D,^{15}N (TEMPONE)/H_2O sample. While this reproduces the DNP enhancement observed also in the old set-up, with the new set-up we observe enhancement on larger molecules that were impossible to enhance in the old set-up. Therefore, we can demonstrate for the first time Overhauser enhanced high resolution proton spectra of glucose and 2,2-dimethyl-2-silapentane-5-sulfonic acid sodium salt (DSS) in D_2O, where the high resolution spectrum was aquired in the high-field position after polarizing the sample in the low-field.

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P590

Epr in the characterization of paramagnetic species in bio-oil by-products of ligneous-cellulosic biomass

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Paramagnetic species was characterized by Electron Paramagnetic Resonance (EPR) in bio-oil produced from the pyirolysis of ligneous-cellulosic biomass; rice-peel, peach-stone and mixture of husk and bagasse of sugar-cane with grass. The *g*-factor and the peak-to-peak linewidth (ΔH_{pp}) were determined for the organic free radical signal detected in the bio-oils.

Bio-oil	ΔH_{pp} (±0.1G)	g-factor (±0.002)
Husk/bagasse of sugar-cane with Grass (BPBG)	1.2	2.0022
Rice-peel (BCA)	5.2	2.0031
Peach-stone (BCP)	7.0	2.0033

The results relative to *g*-factor for EPR signal were 2.0022; 2.0031 and 2.0033, respectively for BPBG, BCA and BCP, which indicates more tenor of aromatic compounds in the bio-oil obtained from the biomass mixture. The free radicals detected in the bio-oils are delocalized in aromatic chemical structures contained besides carbon and hydrogen, heteroatoms as nitrogen and oxygen. The *g*-factor will be higher, how much higher the tenor of heteroatoms.

The linewidths (ΔH_{pp}) relative to 5.2 and 7.0 characterize free radicals in medium of high viscosity and with higher dipolar interaction of the spins.

Bio-oils of ligneous-cellulosic compound have structures of asphaltenes and malthenes in their compounds, which explains the similarity with the values of *g* and ΔH_{pp} in petroleum.

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P591

Reaction Monitoring by Low Field NMR Spectroscopy

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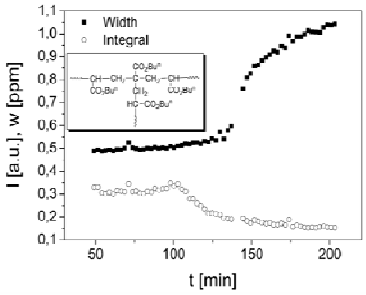
Online reaction monitoring has a large impact on optimization of environmental and time resources in process analytics. Low field NMR spectroscopy has been adapted to the needs of online monitoring of reactants. The instrument was equipped with 12+1 shims and lock functionality. Two examples are presented: A heterogeneous catalytic reaction of toluene to methyl-cyclohexane was monitored by 20 MHz spectroscopy and compared with offline gas chromatography.[1] Classic data analysis as well as a PLS approach were applied to the data. Second, the emulsion polymerization of butyl-acrylate could be followed by 1H-NMR,[2] kinetic parameters could be extracted from the analysis of integral and width (see figure).



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P592

Robust new solver for Stochastic Liouville equation in Langevin form: Scalability and application to transition metal EPR line shape

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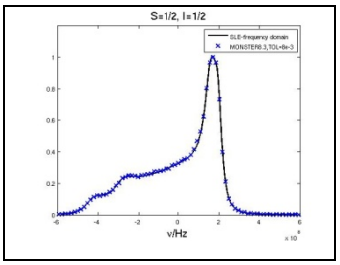
We present an efficient adaptive solver (MONSTER) for direct numerical solution of the Stochastic Liouville equation (SLE) in the time domain exemplified with EPR-line shape calculation. Recent work on the slow motion problem showed that the SLE solved in the time domain with detailed molecular dynamics[1] is an interesting complement to the commonly used frequency domain approach.[2] Although theory is readily available for arbitrary problems, time domain simulations have so far been restricted mainly to smaller basis sizes relevant for an EPR nitroxide spin label and simplified by using high field approximation. In this work we propose novel variance reduction methods that addresses the numerical challenges associated with the control of temporal discretization and statistical sampling errors. The results presented in the figure shows that we reproduce the frequency domain absorption line shape without employing commonly used approximations. Further we consider the "inversion" of dynamic/magnetic parameters from Cu(II)-porphyrin EPR X-band spectra at room temperature by solving the 419904 dimensional SLE using constrained Brownian Dynamics trajectories. More specifically we work with SLE to circumvent the requirement to use an uncertainty model in form of A-strain otherwise needed to analyse frozen spectra[3] and in addition we get biologically relevant dynamical information at physiologically meaningful temperatures.



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P593

Mobility of a lanthanide tag investigated - towards structure determination of "invisible" protein conformations by paramagnetic NMR

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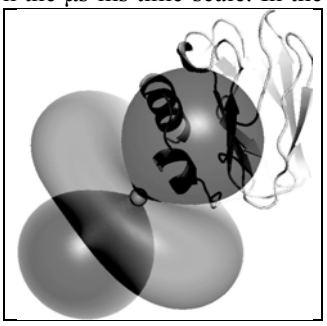
Lanthanide tags can introduce paramagnetic effects in proteins, among them pseudocontact shifts (PCS). The introduction of large PCSs in a protein enables a novel way of studying protein dynamics on the µs-ms time-scale. In the presence of PCSs, protein movements give rise to paramagnetically induced exchange broadening. This paramagnetic exchange broadening can be measured and quantified using relaxation dispersion techniques.[1] The aim is to extract PCSs of "invisible" lowly populated protein conformations, and from these PCSs determine their structure. However, tag mobility may give rise to undesired relaxation dispersion effects. Therefore the requirements on the rigidity of the tag are rigorous, more rigorous than for the purpose of measuring directly PCSs of the ground state. The recently developed CLaNP-5 tag has previously been shown to have a highly restricted mobility.[2] With the aim of using this tag to study protein dynamics via paramagnetic exchange broadening we have investigated the mobility of the CLaNP-5 tag attached to different sites of a rigid protein. ^{1}H CPMG relaxation dispersion methods are used to test for tag movements. Fore some attachment sites no significant relaxation dispersion is observed, suggesting the tag is rigid on the µs-ms time-scale. However, for other sites large dispersions effects are observed, which show that the tag is mobile in certain cases. We conclude that the CLaNP-5 mobility depends on the geometry and dynamics of the attachment site and origin of this dynamics is investigated. The results may improve the design of lanthanide binding tags and lead to better criteria for choosing the sites for tag attachment.



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P594

DOTA-M8 - a highly rigid lanthanide chelating tag, inducing very large pseudo-contact shifts in proteins

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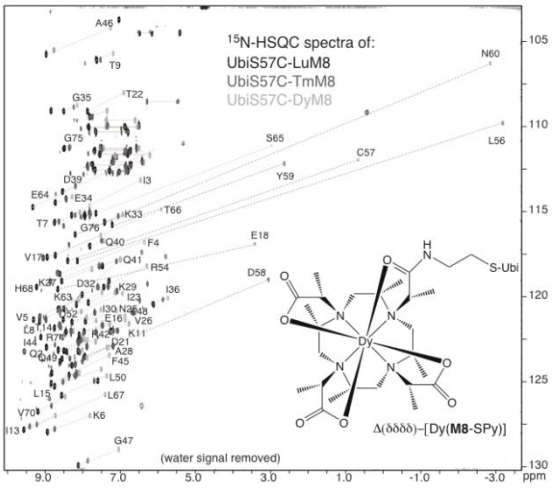
Lanthanide chelating tags (lct) that are site-specifically attached to a protein induce pseudo-contact shifts (pcs) in the nuclei of the protein. This very long-ranging effect (>50Å) can be used as a powerful tool in structural biology.[1] We have recently developed[2] an unusually rigid, high affinity chelating tag based on an eight-fold methylated DOTA framework.[3] With suitable protein/lanthanide combinations we achieved pcs exceeding 10 ppm in ^{1}H and ^{15}N chemical shifts for backbone amide resonances. The effects are detectable beyond 50Å and can be precisely described by determining the anisotropy parameters of the magnetic susceptibility tensor, the position of the metal and the orientation of the tag relative to the protein.



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P595 (*)
Rapid 3D MAS NMR at Critical Sensitivity

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The application of MAS NMR to large molecules is currently limited primarily by the signal-to-noise ratio (S/N) in the multidimensional spectra required for adequate resolution. Thus, the most pressing need in MAS NMR is arguably for more efficient data acquisition. In solution NMR, more efficient acquisition has relied on non-uniform sampling (NUS). Although extending NUS to MAS NMR would be of enormous practical importance, the application of conventional NUS methods to MAS NMR has been limited by the specific problem of accurately modeling weak signals in noisy spectra, in addition to the general problems of quantitative spectral reconstruction and slow computation. The lower sensitivity in MAS NMR experiments requires an unprecedented robustness of any NUS scheme in order to minimize artifacts.

Here, we address these challenges with SIFT (Spectroscopy by Integration of Frequency and Time domain information), a rapid and model-free method for computing a NMR spectrum from a NUS time domain dataset.[1] SIFT works by replacing missing information in the time domain with a priori knowledge of "dark" regions in the frequency domain, i.e. those regions known to contain no NMR signals. The frequency domain information, assimilated by a very rapid computational process, obviates some time-domain sampling with no sacrifice in resolution and no modeling bias.

Dark regions in MAS NMR commonly result from bandwidths set by the need for rotor-synchronized sampling in the indirect evolution dimensions. We demonstrate the use of this definitive frequency domain information, to expedite a 3D MAS NMR experiment 3.4-fold without loss of sensitivity, resolution or spectral accuracy. Thus SIFT is a powerful tool for quantitative structural and dynamical investigations of demanding solid-state samples.

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P596
Ensemble calculations of unstructured proteins constrained by RDC and PRE data: a case study of urea-denatured ubiquitin

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The detailed, quantitative characterization of unfolded proteins is a largely unresolved task due to the enormous experimental and theoretical difficulties in describing the highly dimensional space of their conformational ensembles. Recently, residual dipolar coupling (RDC) and paramagnetic relaxation enhancement (PRE) data have provided large numbers of experimental parameters on unfolded states. To obtain a minimal quantitative model of the unfolded state according to such data we have developed new modules[1] for the use of steric alignment RDCs and PREs as constraints in ensemble structure calculations by the program XPLOR-NIH. As an example, ensemble calculations were carried out on urea-denatured ubiquitin using a total of 419 previously obtained RDCs[2] and 253 newly determined PREs from eight cysteine mutants coupled to MTSL. The results show that only a small number of about eight conformers is necessary to fully reproduce the experimental RDCs, PREs and average radius of gyration. C^{α} contacts determined on a large set (500) of eight-conformer ensembles show significant (10-20 %) populations of conformations that are similar to ubiquitin's A-state, i.e. corresponding to an intact native first β-hairpin and α-helix as well as non-native α-helical conformations in the C-terminal half. Thus methanol/acid (A-state) and urea denaturation lead to similar low energy states of the protein ensemble, presumably due to the weakening of the hydrophobic core. Similar contacts are obtained in calculations using solely RDCs or PREs. The sampling statistics of the C^{α} contacts in the ensembles follow a simple binomial distribution. It follows that the present RDC, PRE and computational methods allow the statistically significant detection of subconformations in the unfolded ensemble at population levels of a few percent.

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P597

A consistent and systematic approach to the design, fabrication and testing of permanent magnets applied to single-sided NMR

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Permanent magnet-based NMR has been a continuously developing field in the past 15 years due to its attractive portability, lack of maintenance need and lower cost. One major challenge of such magnets is the achievement of high field homogeneity. While several prototypes have recently been reported to achieve sub-ppm homogeneity, the volume of interest has always remained small compared to the magnet size. In addition, few systematic approaches to the design of such magnets have been proposed. We have introduced in the past an analytical method for the design, fabrication, characterization and shimming of permanent magnets,[1,2] based on the well-known technique of spherical harmonics expansion of the magnetic potential and of the field components. This framework can be applied to in situ and ex situ magnets to realize desired field profiles such as highly homogeneous fields, or strong constant gradient. We concentrate here on the design and realization of single-sided magnets. We will give a theoretical analysis of the problems of the remote homogeneous field and of the remote constant gradient, along with the issues of high precision field measurements in strong gradients. A first prototype generating a gradient of 3.3 T m^{-1} with a field of 0.33 T (^{1}H frequency of 14 MHz), 2 cm away from the surface of the magnet has been fabricated and tested. The diameter of the magnet is 20 cm with a height of 12 cm, for a weight of about 40 kg. We experimentally achieved variations of less than 100 ppm in planes parallel to the surface of the magnet in a region of 8 mm diameter and 6 mm height. This corresponds to a 1D resolution better than 10 µm in this entire volume. We will also propose a new RF surface coil design generating a field parallel to the coil plane with optimal sensitivity at a given penetration depth. Using these coils and the magnet prototype, we were able to record NMR spectra for the purpose of relaxation measurements and 1D tomography.

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P598

Suppression of line broadening due to Bloch-Siegert shift in ^{19}F solid-state NMR under ^{1}H decoupling

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The Bloch-Siegert shift (BS shift) due to off-resonance RF irradiation causes broadening of the resonance line in the presence of B_1 inhomogeneity. In this work, the idea of BS shift compensation, originally demonstrated in homonuclear decoupling in a liquid sample,[1,2] is applied to suppress line broadening in ^{19}F solid-state NMR under ^{1}H decoupling.

In the pulse sequence shown in Fig.1, BS shift due to ^{1}H decoupling is compensated by additional off-resonance irradiation on the other side of the peak through the observing ^{19}F channel during the evolution period. ^{19}F spin evolution free from the BS shift during the indirect dimension is demonstrated in a 2D spectrum in Fig. 2.

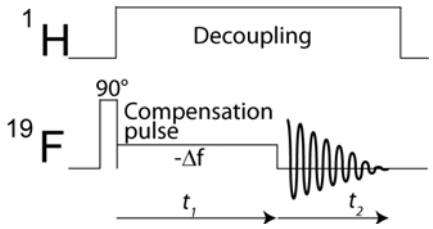

Fig. 1. A scheme for suppression of line broadening due to BS shift in ^{19}F solid state NMR under ^{1}H decoupling.

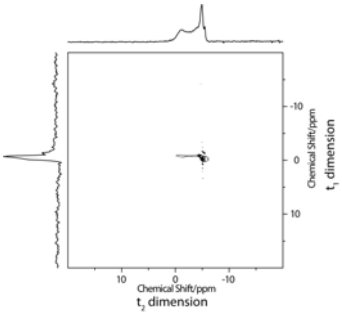

Fig 2. 2D spectrum of 1-fluoroadamantane. The reference frequency is isotropic shift of 1-fluoroadamantane without ^{1}H decoupling. Carrier frequencies are 299.5199 MHz for ^{1}H and 281.7903 MHz for ^{19}F. Proton decoupling strength is 236 kHz. Strength and offset frequency of compensation pulse are 23 kHz and -200 kHz.

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P599
^{13}C shielding scale for MAS NMR spectra

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Routine ab initio calculations available with commercial computational packages are frequently used as an additional tool in the analysis of ^{13}C NMR spectra. The calculations are usually performed for sample and reference molecules at the same level of approximation and converted into NMR chemical shifts. Such theoretical shifts can be useful for the trial assignment of spectral signals but this procedure is not always reliable if the appropriate shielding constants remain unknown. However, if the absolute shielding of a given nucleus in one reference molecule is established, the shielding scale is known and the experimental chemical shifts can easily be converted into shielding constants.[1] For ^{13}C NMR spectra the carbon shielding in a CO molecule is known with accuracy ±0.9 ppm and can be transferred to liquid TMS.[2] Recently we have presented a new general method of shielding measurements available for isotropic species on a standard NMR spectrometer.[3] In the present study we have applied this method for the measurements of ^{13}C shielding constants for three reference samples in spherical ampoules: pure liquid TMS, 1% liquid solution of TMS in $CDCl_3$ and solid fullerene (C_{60}). Next the ^{13}C MAS NMR spectra were obtained for the solution of TMS in $CDCl_3$, C_{60} and additionally for 2 powdered solids: glycine and hexamethylbenzene. It enables us to set up the ^{13}C shielding scale for carbon compounds obtained in the MAS NMR experiments, i.e. free from bulk susceptibility effects. We found the following ^{13}C magnetic shielding constants at temperature 297 K: pure liquid TMS + 183.94 ppm, 1% liquid solution of TMS in $CDCl_3$ +183.20 ppm, liquid $CDCl_3$ (with 1% TMS) + 106.19 ppm, solid C_{60} + 40.24 ppm, solid glycine + 140.24 and + 6.53 ppm for the $-CH_2-$ and –COOH signals, solid hexamethylbenzene + 166.78 and + 51.90 ppm for the methyl and aromatic ^{13}C signals, respectively. The experiments with spherical ampoules were performed on a Varian INOVA-500 MHz spectrometer and the MAS spectra were obtained at spinning speed of 5 kHz using a Bruker AVANCE II 500 MHz NMR spectrometer.

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P600
NMR structure of the protein NP_247299.1: Comparison with the crystal structure

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The NMR structure of the protein NP_247299.1 in solution at 313 K has been determined and is compared with the X-ray crystal structure that was also solved in the Joint Center for Structural Genomics (JCSG) at 100 K and at 1.7 Å resolution.[1] Both structures were obtained with the current, largely automated methods used by the JCSG. This work aims for quantitative statements about the location of structure variations that may arise from either one of the methods used, or from the different environments in solution and in the crystal. To evaluate possible impact of the different software used for the crystallographic and the NMR structure determinations and analysis, respectively, we introduce the concept of reference structures, which are computed using the NMR software with input of upper-limit distance constraints derived from the molecular models representing the results of the two structure determinations. We use this new approach to quantify global differences that arise from the different methods of structure determination and analysis versus those that represent interesting local variations or dynamics. Near-identity of the protein core in the NMR and crystal structures thus provided a basis for identification of complementary information from the two different methods. It was thus observed that locally increased crystallographic B-values correlate with dynamic structural polymorphisms in solution.

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P601
Cutoff-free Traveling Wave NMR

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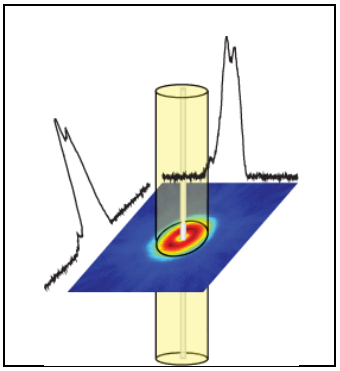
In recent articles Traveling-Wave MRI/NMR Brunner et al.[1] fascinated the community with the demonstration of MRI images acquired through travelling rf waves in the magnet bore of an MRI scanner. This approach has a significant limitation in that each bore has a specific cutoff frequency, which is very high (in the case of Brunner et al. the cutoff frequency was very close to the operating frequency with a bore diameter of 58 cm). The smaller the bore, the higher the cutoff frequency. With a 51 mm (standard bore) magnet bore one would obtain 3.45 GHz, too large to be useful.



We overcome this limitation by turning the magnet bore into a transmission line (TL). TLs allow the propagation of TEM modes without a cutoff frequency,[2] and thus allow broadband propagation of waves through the sample.

NMR spectra and images acquired with such an arrangement will be shown (example in the Figure), and genuine travelling wave behavior will be demonstrated.

In addition to facilitating NMR spectroscopy and imaging in smaller bores, this approach will also allow one to easily perform heteronuclear travelling wave experiments, and the study of samples in unusual geometries (e.g. microfluidics) and with relatively inaccessible samples. Furthermore, this arrangement allows testing general traveling wave concepts.

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P602 (∗)
Simultaneous acquisition of pulse EPR orientation selective spectra

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High resolution pulse EPR methods are usually applied to resolve weak magnetic electron-nuclear or electron-electron interactions that are otherwise not resolved in the EPR spectrum. Full information regarding different magnetic interactions, namely, the principal components and orientation of principal axis system with respect to the molecular frame, can be derived from orientation selective pulsed EPR spectra that are acquired at different magnetic field positions within the inhomogeneously broadened EPR spectrum. These experiments are usually carried out consecutively, namely a particular field position is chosen, data is accumulated until the S/N is satisfactory, and then the next field position is chosen.

Here we present a new approach for data acquisition of orientation selective pulse EPR experiments, referred to as parallel acquisition, which can speed up such a set of experiments considerably. In this approach several orientation selective pulse EPR measurements are performed in parallel during the waiting (repetition) time between consecutive pulse sequences that is required due to the finite spin lattice relaxation time. This is achieved by rapidly changing the main magnetic field B_0 to different positions along the EPR spectrum, performing the same experiment on the otherwise idle spins. This scheme represents an efficient utilization of the spectrometer time and will provide the same spectral information in shorter time. This approach is demonstrated on orientation selective ENDOR (electron-nuclear double resonance), ESEEM (electron spin echo envelope modulation), ELDOR (electron-electron double resonance) –detected NMR and DEER (double electron-electron resonance), of nitroxides carried out on a W-band spectrometer. We show that a factor of 3-6 reduction in total acquisition time can be obtained, depending on the experiment applied.

P603

Field-induced alignment of liquid crystal director

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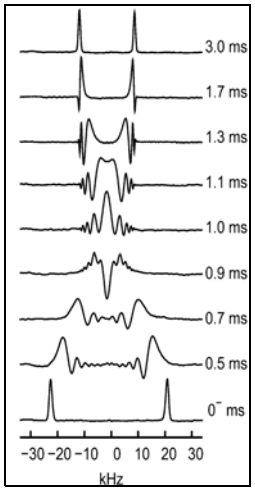
One of the key quantities controlling the response times for liquid crystal displays is the rotational viscosity for the motion of the nematic director. There is, therefore, considerable interest in measuring this quantity for nematic liquid crystals and a variety of techniques have been developed to achieve this. Time-resolved deuterium NMR spectroscopy is a powerful method to investigate field-induced rotation of the director in a nematic liquid crystal, but the method requires that the director does not rotate significantly during the acquisition of the NMR spectrum. We have extended the method to systems where this is not the case and the observed NMR spectra are now found to contain novel oscillatory features. To understand these oscillations we have developed a model combining both director and spin dynamics. In addition to increasing the information content of the time-resolved NMR spectra it also proves possible to determine the field-induced relaxation time from a single spectrum instead of series of spectra, as has been the case with previous studies.



We report the results of experiments designed to demonstrate the oscillations in the deuterium NMR spectra for 5CB-d_2 and the striking form that they adopt. The validity of the theory is confirmed by the good agreement found between the simulated and experimental deuterium NMR spectra. This combination of experiments and simulations also allowed us to determine the director relaxation time.

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P604

Non-uniform frequency domain for optimal exploitation of non-uniform sampling

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Random sampling of NMR signal, not limited by Nyquist Theorem, yields up to thousands-fold gain in the experiment time required to obtain desired spectral resolution. Discrete Fourier Transform (DFT), that can be used for processing of randomly sampled datasets, provides rarely exploited possibility to introduce irregular frequency domain. Here we demonstrate how this feature opens an avenue to NMR techniques of ultra-high resolution and dimensionality. We present application of high resolution four- and five-dimensional experiments dedicated for protein backbone assignment and measurements of coupling constants using the high-dimensional E.COSY multiplets. Improved digital resolution together with ultra-narrow linewidths allow to reach the precision of frequency determination that was impossible for high-dimensional spectra before. Spectral data acquired with the use of proposed techniques allow easy assignment of protein backbone resonances and precise determination of coupling constants.

P605

Generation of ^{1}H and ^{13}C hyperpolarized *N*-vinyl-2-pyrrolidone

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Nuclear Magnetic Resonance is a rather insensitive technique caused by the low gyromagnetic ratio of the nuclear spins. Therefore, the lack of sensitivity of NMR renders hyperpolarization methods like Dynamic Nuclear Polarization (DNP)[1] or Parahydrogen Induced Polarization (PHIP) very important.[2,3] PHIP is based on a homogeneously catalyzed hydrogenation of an unsaturated precursor with parahydrogen. The inserted protons populate only specific Zeeman energy levels due to their antisymmetric spins and exhibit a polarization far above the Boltzmann polarization. Polymerizable monomers like *N*-vinyl-2-pyrrolidone (NVP) are interesting substrates for PHIP experiments.

In order to obtain the possibility to investigate a polymerization in real time, it is necessary to start the parahydrogenation with a precursor molecule containing a triple bond to afford hyperpolarized NVP. The preparation of the model compound *N*-ethinyl-2-pyrrolidone was achieved by a copper(II)-catalyzed coupling reaction of 1-brom-2-(triisopropylsilyl)acetylene and 2-pyrrolidinone with following removal of the TIPS-protecting group.[4] After reduction of the triple bond using parahydrogen, the resulting double bond of NVP exhibits significant signal enhancements for protons as well as for carbon-atoms enabling the online monitoring of the subsequent polymerization reaction.

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P606

Using multi-frequency NMR relaxation for probing wettability in multimodal porous rocks

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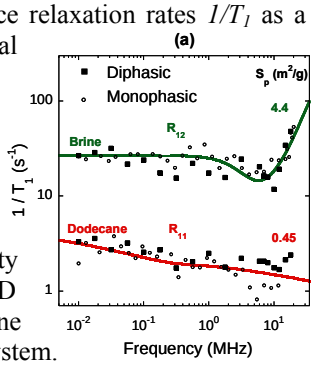
We present a new and non invasive method for probing wettability of rock/oil/brine systems using nuclear magnetic relaxation dispersion (NMRD).[1] This technique measures the variation of proton spin-lattice relaxation rates $1/T_1$ as a function of magnetic field strength or nuclear Larmor frequency. Unlike conventional transverse relaxation studies, this approach gives a direct probe of the dynamical surface affinity of fluids, thus allowing the separation of wetting from non-wetting fluids through their typical NMRD features (Fig.1). To quantify these features we introduce a microscopic dynamical surface affinity index which measures the dynamical correlation (*i.e.* microscopic wettability) between a diffusive fluid and fixed Mn^{2+} paramagnetic relaxation sources at the pore surfaces.



For the first time, we apply this technique to carbonate reservoir rocks of bimodal porosity saturated with a mixture of dodecane and 50 kppm NaCl (Fig. 1). The experimental NMRD results obtained on carbonate core plugs of bimodal porosity saturated with this oil/brine mixture ($S_{w,irr,}$ Fig. 1) clearly discriminate the wetting behavior of the fluids in the pore system. The data have been processed using a proposed model (continuous lines in Fig.1) that clearly reveals the pore size dependence of wettability. Here the typical shapes of the two separated NMRD profiles allows us to conclude that water stays in the small pores while oil is on the large ones. This proves the existence of a flow path between the large pores that does not involve the small ones.

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P607 (*)

Parahydrogen-Induced Polarization in Heterogeneous Hydrogenation: an Aqueous Phase, MOF and SILP catalysts

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Parahydrogen-induced polarization (PHIP) is a powerful tool for studying homogeneous catalytic hydrogenation reactions because it gives a dramatic NMR signal enhancement and thus can provide information about reaction products and intermediates even if they are present at low concentrations. Obviously, heterogeneous catalysts are much easier to separate from a reaction mixture than the homogeneous ones. Therefore, the use of heterogeneous catalysts in hydrogenation reactions could be an alternative route to produce catalyst-free hyperpolarized fluids.[1] PHIP has been observed, for the first time, in the heterogeneous hydrogenation of the double bonds of unsaturated amides and ethers in aqueous solution using supported metal catalysts. Until now, PHIP in heterogeneous hydrogenation reactions was reported only for gas phase or organic-liquid phase processes, limiting a wider practical utilization of the polarization effect. Therefore, the first observation of PHIP produced with supported metal catalysts in an aqueous phase heterogeneous hydrogenation is very important for future MRI applications and for the verification of reaction mechanisms of aqueous phase heterogeneous catalytic reactions.

PHIP was also observed in the gas phase heterogeneous hydrogenation of propyne catalyzed by Pd nanoparticles embedded in an ionic liquid phase supported on activated carbon fibers (Pd/SILP/ACF) and the Au(III) Schiff base complex attached to the metal-organic framework (MOF) material.

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P608

Development of NMR Methods and New Protocols for Rapid Backbone Assignment: Implication to Structural and Functional Proteomics

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New NMR methods and protocols have been designed for rapid assignment of backbone resonances ($^{1}H^{N}$, ^{15}N, $^{13}C^{\alpha}$, and $^{13}C'$), secondary structure identification, and in favorable cases the tertiary structural fold determination of proteins. The assignment protocols described here are based on a few check points (glycines, alanines, and serines/threonines) in the HSQC spectrum derived from specific experimental techniques and the sequential amide $^{1}H^{N}$ and ^{15}N connectivities in a set of spectra. These protocols are most helpful to re-establish sequential assignments of target proteins perturbed by binding of ligands/drugs and thus have the implication to protein-protein interaction studies and structure-based drug design programs. Alternatively, the signals of peptide ligands can also be traced in studies of structure-function relationships by NMR. Moreover, the approach described here has also great prospects for (a) NMR structural investigations of unstable proteins and the proteins which tend to precipitate in solution in a matter of days, (b) in in-cell NMR studies, and (c) NMR based protein-folding studies where structural and dynamics features of each equilibrium transition state created by various means (either by temperature, pressure, or chemical denaturants) are required to be characterized. The whole assignment procedure has also been automated and the algorithm has been named as **AUTOBA**. We have also designed a web-based server for the same (http://www.tifr.res.in/~hosur/autoba). We believe that the approach would be of immense value for routine use in protein NMR.

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P609

Use of nearest neighbour Heisenberg XY interaction in NMR Quantum Information Processing: Creation of Bell States on end Qubits and a W State in a linear chain of 3-qubits

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NMR quantum computations so far has been mainly carried out using qubits, all of which are coupled to each other with unequal couplings so that all transitions are resolved, allowing access to the full Hilbert space. However, such systems are non-scalable and possible only for a small number of qubits. It is therefore important to develop protocols which use near neighbour (nearest and next-to-nearest neighbour) interactions for Quantum Information Processing (QIP).

There have been theoretical proposals for quantum state transfer using nearest neighbour Heisenberg-XY interactions in a linear spin chain and more complex spin networks.[1,2] Few of these proposals have also been realized experimentally by simulating the evolution caused by the Heisenberg-XY interactions in a nuclear magnetic resonance quantum computer.[3]

In our first work in this direction, we demonstrate here, the use of nearest neighbour Heisenberg-XY interactions for creating multi-particle entangled states in a linear chain of nuclear spin qubits. Bell states on end qubits and W-state have been created experimentally on a 3-qubit NMR quantum computer. Tomography of each of these has been performed to confirm the creation of such states. These results will be presented.

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P610

Comparison of the occupation numbers n_d in $La_{2-x}Sr_xCuO_4$ estimated by NMR/NQR and ARPES probes

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The analysis of electric-field-gradients (EFG's) measured[1] in $La_{2-x}Sr_xCuO_4$ is performed to estimate the intracell distribution of charge among one copper ion and two oxygen ions in the conducting planes of the high-T_c cuprates. Using the essentially model independent expression for EFG's, with the lattice contributions to EFG's treated self-consistently,[2] we obtain that in the $x=0$ parent compound the average number of holes on Cu is $n_d \approx 0.72$. It changes to $n_d \approx 0.75$ in the $x=0.3$ compound, with the linear dependence on the hole doping δ, $n_d(\delta) \approx n_d(0) + (d\, n_d/d\, \delta)\, \delta$, in the metallic phase.

The occupation numbers n_d are then calculated using the Emery three-band model for conduction electrons, with two first-neighbour hopping integrals (t_{pd}, between two Cu-O neighbours, and t_{pp}, between two O-O neighbours) and the Cu-O splitting energy Δ_{pd}. For the values of these parameters estimated from ARPES and optical conductivity measurements,[3] we obtain that the calculated n_d's are about 20 percent smaller than that estimated by the EFG analysis. It is possible to overcome this discrepancy by including next-to-nearest hopping integrals, which contribute to the Fermi surface curvature, but do not result in the substantial intracell charge redistributions. Alternatively, such a large difference between two estimations of n_d may be due to the omission of incoherent contributions to n_d.

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P611 (*)
Analytical derivatives of spin dynamics simulations

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We report analytical equations for the derivatives of spin dynamics simulations with respect to pulse sequence and spin system parameters (couplings, shieldings, tensor orientations, pulse widths, phase shifts *etc*). The resulting derivatives may be used in fitting, optimization, evaluation and stability analysis of spin dynamics simulations and experiments.

Importantly, the derivatives in question often cannot be obtained numerically: modern large-scale simulation algorithms have multiple dynamic cut-offs and tolerances (in orthogonalization, matrix inversion, singular value decomposition, *etc*) meaning that a small perturbation in a parameter may trigger a step change in the simulation result. In other words, many algorithms are not numerically differentiable with respect to their parameters. They may also display high levels of numerical noise due to the finite precision of machine arithmetic. Even when they are reasonably accurate, numerical derivatives have a high computational cost in such large-scale simulations: typically between two and four separate simulations per parameter. The equations reported are significantly faster, much more accurate, and, importantly, much more reliable than finite difference approximations.

Three methods are offered for the calculation of the derivatives in question

- Time co-propagation in Hilbert or Liouville space – the derivative simulation is propagated alongside the main simulation.
- Derivative superoperator in Liouville space – a superoperator is generated, which transforms the density matrix into the derivative with respect to the parameter chosen.
- Eigensystem differentiation for time-independent Hamiltonians in Hilbert and Liouville space – eigenvalue and eigenvector derivatives are used in a diagonalized representation.

The algorithms above have been implemented into the development version of the *Spinach* library (production version to be released on 1 Oct 2010). Matlab source code is available from the authors upon request.

P612
Carbon and proton shielding tensors in methyl halides

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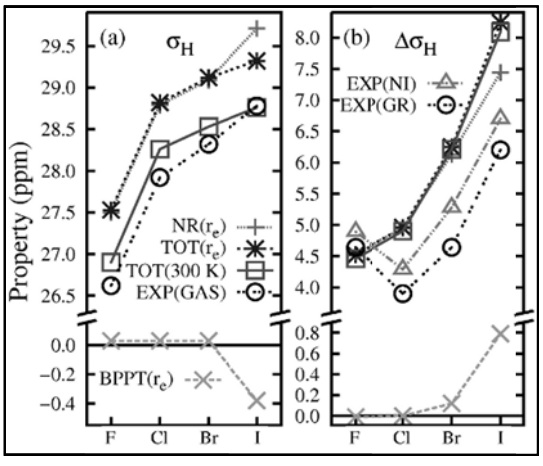
The series of methyl halides, CH_3X (X = F, Cl, Br, and I), is prototypic for demonstrating the s.c. normal halogen dependence of light-atom nuclear magnetic resonance shielding constants in the presence of halogen atoms of varying electronegativity. We report a systematic experimental and first-principles theoretical study of the ^{13}C and ^{1}H shielding tensors in this series.[1] The experimental shielding constants were obtained from gas-phase NMR experiments and the anisotropies were determined using liquid crystal NMR spectroscopy. Quantum chemical calculations were carried out at ab initio and density functional theory levels, involving relativistic corrections taken into account at the leading-order Breit-Pauli perturbation level. Anharmonic and harmonic vibrational corrections were performed on both the experimental and computational data. The main trends of the shielding constants and anisotropies of the nearby light ^{13}C and ^{1}H nuclei as functions of the halogen mass, were confirmed to be mainly due to relativistic effects. Overall, the current experimental and theoretical results are in excellent agreement for all the shielding parameters, setting a standard for further investigations of normal halogen dependence.



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P613

Characterization of the Unpaired Electron Spin Distribution in Paramagnetic Metalloproteins by Natural Bond Orbitals

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A natural bond orbital (NBO) modeling of unpaired electron spin density in metalloproteins is derived, which aims at an easy and fast calculation of accurate paramagnetic NMR parameters of nuclei in the immediate vicinity of paramagnetic centers where the point dipole approximation breaks down. The NBO description thereby facilitates restrained molecular dynamics simulations of metalloproteins by allowing an easy inclusion of paramagnetic restrains of nuclei close to paramagnetic centers.

The NBOs are two-center eigenvectors of the electron density matrix and assemble chemical bonding in a natural way. The singly occupied molecular orbitals that give raise to paramagnetic interactions consist primarily of valence orbitals, which make the NBOs a good and transferable basis for the unpaired electron spin density. Metal–ligand NBOs account for approximately 90% of the unpaired electron spin density in model complexes of the metalloproteins rubredoxin and plastocyanin, thus forecasting that paramagnetic NMR parameters can be calculated from a small number of spin density building blocks that reflect the chemical bonding environment. We show that accurate paramagnetic relaxation rates are calculated for ^{1}H nuclei in the immediate vicinity of the paramagnetic site when the unpaired electron spin density is modeled by metal-ligand NBOs, whereas the paramagnetic relaxation and hyperfine Fermi contact shift of ^{13}C and ^{15}N cannot be calculated from the metal–ligand NBO density alone. Yet, accurate paramagnetic ^{15}N NMR parameters are calculated within the NBO formalism by including local ^{15}N centered NBOs that contribute to the paramagnetic ^{15}N NMR parameters. Overall, the NBOs provide a simple and accurate description of the unpaired electron spin density that will allow restrained molecular dynamics simulations and geometric structure determinations of metal sites in metalloproteins.

P614

Comparison of Ring Current Methods for Use in Molecular Modeling Interactions of NMR Three-Dimensional structures

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The characterization of the structure and the dynamic of proteins is one of the major current challenges in biology. In Nuclear Magnetic Resonance spectroscopy (NMR), chemical shifts provide measurable and highly sensitive probes for the study of molecular structures. In the field of structural biology, chemical shifts of ^{1}H, ^{13}C, ^{15}N, nuclei can be used to improve the quality of structures of nucleic acids and proteins. Aromatic rings and their associated ring currents can affect the chemical shifts of nearby nuclei. The aromatic ring contribution of the chemical shift, if high enough, may provide very accurate information about the protein/nucleic acid interface. In this poster we applied the comparison and calibration of different methods commonly used to estimate ring current effects on chemical shifts: Biot-Savart law,[1] Classical Point-Dipole,[2-4] Johnson-Bovey,[5] and Haigh-Mallion.[6] These models were used to estimate the ring current contribution to the chemical shift of neighboring protons and then implemented in home-made software dedicated to analyze protein-DNA/RNA complex. The use of the Haigh-Mallion model, on a specific example of protein/RNA interaction, is described and its usefulness discussed.

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P615

Double Electron-Electron Resonance based distance measurements in Gd^{3+}-nitroxide radical spin pairs

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Double Electron-Electron Resonance (DEER) has become an important method to study the structure of macromolecules, due to its ability to provide distance constraints in the range of 1.5-6 nm. In contrast to the conventional application of distance measurements by DEER,[1] in which the molecule is doubly labeled with nitroxide radicals, in this study we use chelate complexes of Gd^{3+} as one moiety of the spin pair.

To show the performance of the method two systems with either a well defined distance or a broader distribution of Gd^{3+}-nitroxide radical distances were studied.

The most efficient experimental scheme turns out to be the one with detection on the maximum of the EPR spectrum of Gd^{3+} and with the pump pulse on the peak of the nitroxide spectrum. The sensitivity increases substantially by performing the experiment at Q-band instead of X-band.

Transverse relaxation of Gd^{3+} is not monoexponential, nevertheless echo decay relevant for DEER can be characterized by a decay time of approx. 4 µs, which is in the same range as T_2 times of nitroxide radicals. Furthermore, the DEER measurement in this arrangement can be done at lower temperature with shorter repetition time, which should provide better sensitivity or access to longer distances as compared to the nitroxide-nitroxide spin pair.

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P616 (*)

(Solvent) PRE-assisted structural analysis of large protein complexes

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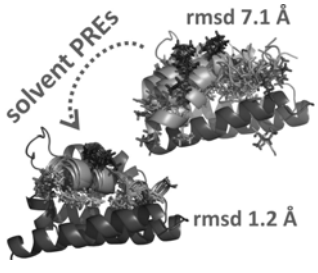
Determining structure and architecture of multi-component proteins and their complexes is essential for understanding cellular signaling that involves communication between the domains or subunits. Here we present approaches for structural analysis of large protein complexes. We combine the efficacy of paramagnetic relaxation enhancements (PREs) for the detection of long-range distance information with the favourable sensitivity and resolution of ^{13}C direct-detected experiments in an efficient structure calculation protocol.[1,2] This allows accurate definition of interfaces in proteins and protein complexes and is especially useful for studies of high-molecular weight perdeuterated molecules. PREs by soluble paramagnetic agents (i.e. Gd(DTPA-BMA)) provide a rich source of structural/dynamic information and constitute an attractive and complementary alternative to covalent spin labels.[3] The strength of this methodology is that it is non-invasive (i.e. no covalent modifications needed), an excellent indicator of (transient) interactions and local structure and easily applicable. We found that solvent PREs are particularly helpful for structural refinement and docking of large protein complexes. Motivated by understanding the general mechanisms behind nuclear export of cargos in the ternary 150 kDa nuclear export complex we show that accuracy and convergence of conventional docking calculations can be significantly improved by a limited set of experimental solvent PRE data, which can be readily obtained. While conventional (restraint-driven) docking programs show poor convergence when only sparse data are available, our approach promises significant time-savings and improved accuracy of docking especially for large and protein complexes.



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P617
De-noising Protocol using Random additions

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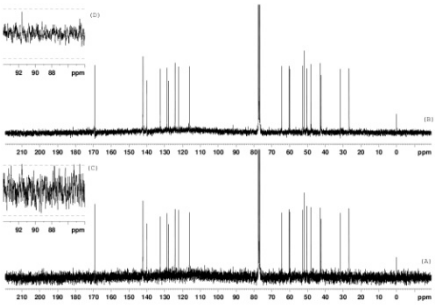
In this work the authors expect to prove the existence of a new protocol used to improve the signal to noise ratio by a factor up to 40% in FT NMR based spectroscopy. This work will also show one case where the use of this protocol could produce near of 3 times in timing saving on the FT NMR measurements.



Our protocol is based on Bootstrap[1] approach and was applied at Strychnine sample in solution NMR, the MCM-41 Zeolite (SSNMR) as a test and implementation of protocol, finally in a real case sample we applied the protocol in one sample of nanoparticules of SnO_2 dopped with Europium in a Porous Vycor Glass.

Figure 1 (right) shows the $^{13}C\{^1H\}$ solution NMR spectra (1024 scans) of Strychnine sample (10mg/ml) using a normal acquisition and process (down) and the same spectra using of this sample generated by 1024 individual scans processed with the present protocol (up), at left side of this figure the magnification of the noise for both spectra was used to show by visual inspection the efficiency of the implemented protocol. All spectra were acquired in a narrow bore Bruker Avance 300MHz spectrometer. The protocol was applied using a very simple home made C routine.

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Acknowledgments: IQ-UNICAMP, FAPESP, INCT-Bioanalitica and Prof. Dr. Italo Odone Mazali and M.Sci. Mathias Strauss for the nanoparticles sample.

P618
A neural network approach to the rapid analysis of FDMPO spin adducts kinetics from fast isotropic ESR spectra

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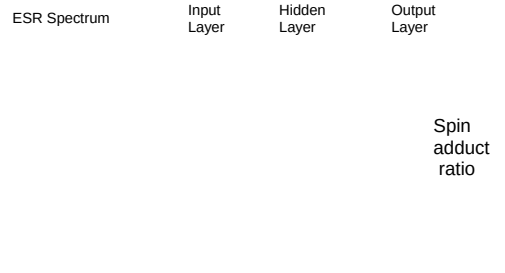
The technique of spin trapping has gained wide acceptance as a method for detecting shortlived radicals. When the free radicals react with a spin trap, they produce paramagnetic, and thus ESR-visible, adducts with higher stability than that of the primary radicals. The spin trapping technique is used to study the kinetics of the reaction. Such measurements result in a large number of multi component spectra where the ratio between spin adduct components is time dependent.



Feed forward artificial neural networks were applied for extracting the ratio of the components from ESR spectra of FDMPO spin adducts. A simulation based fitting procedure was performed to extract the parameters of each spin adduct formed in the system under study. The extracted parameters were used to create a training set of ESR spectra with different ratio of spin adduct components. Subsequently, the radial basis neural network was learned to associate a multi component spectrum with the desired ratio of the spin adduct components. The radial basis network trains rapidly and performs well both on simulated and experimental spectra as long as the signal-to-noise ratio is larger than approximately 500. Both iterative fitting and neural networks approaches were used for analysis of FDMPO spin adduct kinetics in the Fenton reaction in DMSO, ethanol and glycerol. The use of neural networks, separately trained for each system, increased the speed of analysis by 100 times. These findings suggest that neural networks offer a promising approach for rapidly extracting ratio parameter without the need for iterative simulations.

P619

A version of ARIA adapted to the grid computing and its performances on CASD-NMR targets

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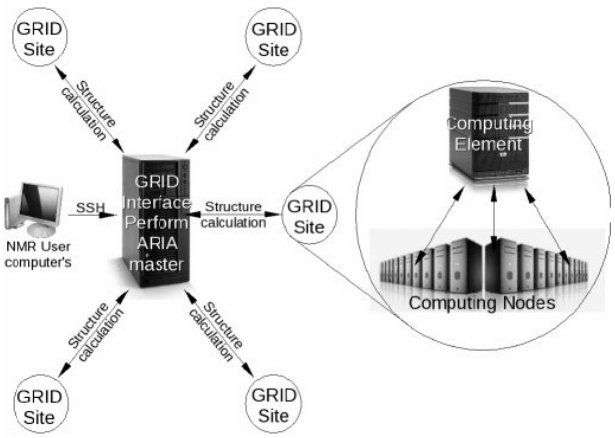
The procedure ARIA (Ambiguous Restraints for Iterative Assignment) for the NMR structure calculation and NOEs automatic assignment requires more and more computing resources, to be able to handle low resolution data obtained.

Thus, new developments on ARIA have been made to adapt it to a grid computing infrastructure. A grid is a set of distributed computing resources (storage, CPUs, ...) working together. As such infrastructure is distributed among several sites, some security and software considerations could raise new constraints about the runtime environment of ARIA. One of them is that there is no shared filesystem between the grid nodes.



The ARIA version for grid computing will be deployed on the French RENABI GRISBI interface and allow everyone (with valid certificate) to run ARIA calculations with these NMR data. In this version, the GRID interface performs the master program ARIA who dispatches cns jobs of structures calculation on the computing elements and retrieves them when finished. The performances of ARIA on the grid were tested on the nine blind targets proposed for the CASD-NMR (Critical Assessment of automated Structure Determination by NMR) in 2009-2010.

P620 (*)

Balanced Triple Resonance Probe for Cryogenic MAS NMR and Dynamic Nuclear Polarization at 700 MHz

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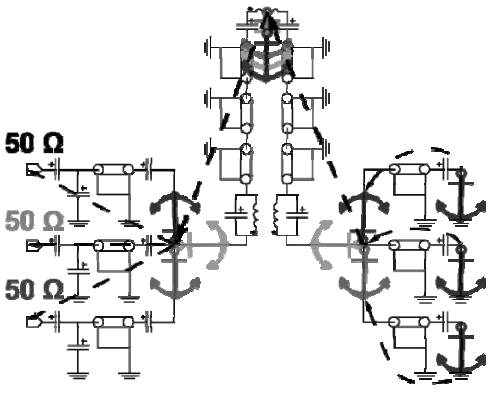
High-field Dynamic Nuclear Polarization (DNP) combined with MAS NMR is an emerging technique for structural and functional investigations of biological systems, such as membrane proteins and amyloid fibrils.[1,2] In addition to the NMR spectrometer, this technique also requires a high power microwave source and a special DNP MAS NMR probe.[3] Hu et al.[4,5] have recently introduced a new efficient RF circuit design for multi resonant SSNMR probes.[6]

We utilized a similar RF circuit in our design of a cryogenic MAS NMR probe for DNP experiments at 700 MHz / 460 GHz. The schematic on the right illustrates the essential idea of this strategy, dubbed "back propagation of a common impedance node." The focus of this communication is the implementation of this novel RF circuit in the high-field DNP probe.



Schematic showing back propagation of a common impedance node through the RF circuit.

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P621
Out-of-Phase PELDOR

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Pulsed Electron-electron Double Resonance (PELDOR) is a method frequently used to determine the structure of bio-macromolecule on nanometer scale.[1] With this technique distances between native paramagnetic centers or introduced spin labels and their mutual orientation can be extracted from the experimental data.[2] Usually PELDOR experiments are carried out in the high temperature limit, i. e., when the Bolzmann populations of spins oriented parallel and antiparallel to external magnetic field are almost equal. There are also well-developed theories describing PELDOR in this case. However, the high temperature limit conditions are no more fulfilled in the experiments done in high magnetic field (above 6 Tesla) at low temperature (below 5 K), when the Zeeman interaction energy of an electron spin becomes comparable with the thermal energy k_BT.[3] In this work we demonstrate that the PELDOR signals measured at low temperature and high field deviate from the signals measured in the high temperature limit. The signals recorded at low temperature contain standard *in-phase* component which is usually observed in PELDOR and additional *out-of-phase* component that disappears by increasing the temperature.[4] In the rotating coordinate system, it means that we observe not only the modulation of the refocused transversal magnetization along a single axis but rather its precession in the *x-y* plane with dipolar frequency. For this effect we provide a qualitative explanation as well as a detailed analysis based on the density matrix formalism. We have also shown that the ratio of *out-of-phase* signal, which contains the same intermolecular relaxation term as *in-phase* signal, to *in-phase* signal can be utilized to determine distance distribution function without background correction of PELDOR time trace.

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P622
Solution NMR strategies that reveal the chemistry of colloidal nanoparticle dispersions: from quantum dots to organic pigments

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Small organic ligands or macromolecules that interact with nanoparticles are key to create, stabilize and manipulate colloidal dispersions of nanoparticles, yet the details of the interaction chemistry *in situ* at the nanoparticle surface remain scant and qualitative at best. In an effort to improve this situation, we present a variety of NMR based strategies that enable a detailed understanding of the chemistry of the nanoparticle-ligand interface under non-perturbing conditions.[1-4] Depending on the chemical exchange regime in the ligand to nanoparticle equilibrium (slow vs. fast exchange) and the nature (organic vs. inorganic) and size (a few nm up to tens of nm), different solution NMR techniques can be combined. These include classical solution techniques (1D ^{1}H, ^{1}H-^{13}C HSQC) diffusion ordered spectroscopy (DOSY), but also transfer NOESY[2] and Saturation Transfer Difference[3] spectroscopy, techniques that are typically associated with protein-ligand studies but can be adapted to reveal more about ligand binding, surface coverage and organisation. Combined with ligand titrations, dilution studies or ligand exchange an in depth-view on the chemistry at the ligand-nanoparticle interface will be presented on selected systems (PbSe and CdSe with oleic acid, ZnO and CdTe with aliphatic amines, quinacridone pigments with SDS) and will illustrate the generic character of the approach.[1-4]

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P623

Dynamic Nuclear Polarization-Enhanced Solid-State NMR at 14.1T

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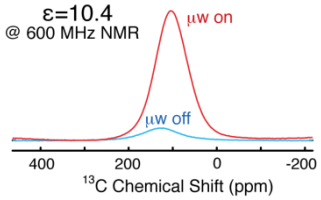
We report a solid-state CW DNP/NMR experiment under a high external field condition of 14.1 T (600 MHz for ^{1}H frequency), which would allow increased resolution in the NMR spectra.[1] To perform the experiments, we have combined a commercial high-resolution solid-state NMR spectrometer and a wide-bore magnet with a 395-GHz gyrotron oscillator FU CW II, sub-millimeter (sub-mm) wave transmission and low-temperature gas supplier systems that we developed. The gyrotron generated the sum-mm wave with power output of about 40W in the second harmonic TE_{06} mode. Sufficient amount of power for DNP (0.5-3W) was transmitted to the sample in a low-temperature DNP/NMR probe using a smooth-wall circular waveguide system. DNP enhancements of 10 and 4 were obtained at 90 K for ^{13}C-glucose in the presence of TOTAPOL and TEMPO/BDPA, respectively. The DNP due to the Cross Effect was suggested from the static magnetic field dependence of the enhancement. Possible improvements for the high-field DNP will be discussed.



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P624

Photo-CIDNP MAS NMR

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In photosynthetic reaction centers (RCs) under illumination, photochemically induced dynamic nuclear polarization (photo-CIDNP) can be observed by ^{13}C and ^{15}N MAS NMR as dramatic increase of signal intensity [for reviews, see 1,2]. The solid-state photo-CIDNP effect leads to signal enhancement of a factor of more than 10,000 and allows therefore to study the photochemically active machinery of RCs directly in membranes and cells in great detail. The occurrence of the effect has been predicted on the basis of enhanced electron polarization detected by EPR and was discovered in 1994 by McDermott's group.[3] Based on field-dependent[4] and time-resolved experiments,[5] the origin of the effect is now understood. Photo-CIDNP-MAS NMR is applied as analytical tool to study the photochemical machinery of an increasing number of RCs as for example bacterial[6,7] and plant[8,9] RCs. It appears that the solid-state photo-CIDNP effect is an intrinsic property of natural photosynthetic RCs.[10]

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P625

A smoothing monotonic optimal control approach for design of magnetic resonance experiments

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Optimal control theory is a very powerful area of applied mathematics which has gained recent interests for the applications to the quantum coherent systems. Currently, the most popular OC method which applied in the nuclear magnetic resonance (NMR) pulse sequence design is based on the gradient approaches known as gradient ascent pulse engineering (GRAPE).[1] Later methods based on the monotonic convergence algorithms using the Krotov approach have emerged.[2] Despite an increasing use of optimal control approaches in NMR community, the question which optimal control method is best remains open provides an interesting challenge to the researchers. The answer may depend on the constraints put on the optimization in terms of chemical shift compensation, powder averaging, coupling selectivity, hardware constrains etc. Another issue of importance is the simplicity of the method in numerical implementations, speed, accuracy, and robust convergence properties. A third issue is to the design of pulse sequences with smooth rf variation to facilitate implementation on standard NMR instrumentation. In this presentation, we describe the development of an improved monotonic convergent algorithm that addresses most criteria above. The algorithm is demonstrated by representative NMR examples also providing comparison to results obtained using previous GRAPE/Krotov approaches.

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P626

Increasing the efficiency of the macromolecular NMR spectroscopy

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The non-uniform sampling (NUS) schemes allow large flexibility for optimized experiment design, which so far has not been fully exploited. Optimization of the sampling schedule is possible based on a prior knowledge about the signal. Thus, random sampling pattern can be considered as a special case, when nothing is known about the frequencies and line shapes. In many practical situations, however, this information is available for one or several spectral dimensions. Recently, we suggested targeted acquisition (TA) approach for data collection. TA is based on incremental NUS and concurrent spectra processing and evaluation. Quality of the spectrum gradually improves as more data is collected. Now we elucidate possibility to use information about the signal obtained at *i*-th step of the TA for predicting optimal sampling schedule for the next step. Thus, experimental design optimized for maximal sensitivity for selected acquisition times is achieved in the iterative procedure.

Several examples of the new approach are presented, including 3D and 4D spectra of intrinsically disordered proteins. We demonstrate iterative interleaved acquisition of a set of triple resonance experiments with concurrent processing by Multi-Dimensional Decomposition (MDD) and automated backbone assignment. The approach is general and can be used for all systems amenable for modern NMR spectroscopy in liquids.

P627

Towards direct refinement of structures against INPHARMA data

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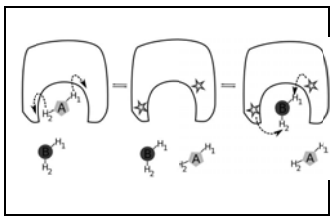
The INPHARMA[1] method relies on observation of transferred NOEs between two different ligands, which are binding competitively to the same binding site. The method provides the orientation of two competitive ligands, which can serve as a template for development of high-affinity drug candidates from the studies of weakly binding compounds.

The quantitative evaluation of INPHARMA peaks relies on back-calculation of interligand NOEs from calculated sets of protein-ligand complex structures in pairwise manner. This approach requires substantial amount of computer time. On the other hand, the quality of the final answer is limited by the structure calculation protocol.



In order to overcome these issues, the methodology employing direct refinement techniques are under development. Recently, INPHARMA restraints have been implemented in XPLOR-NIH program as additional pseudoenergy term, which enables performing energy minimization and molecular dynamics simulations. The results of energy minimization and simulated annealing protocols for protein kinase A (PKA) and small-molecule ligands will be presented.

The other approach involves evaluation of interligand NOEs directly during docking simulations. Since, most of the docking software rely heavily on large number of energy evaluations a simplified distance-based energy function is required.

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P628

Spin Relaxation of a Short-Lived Radical in Zero Magnetic Field

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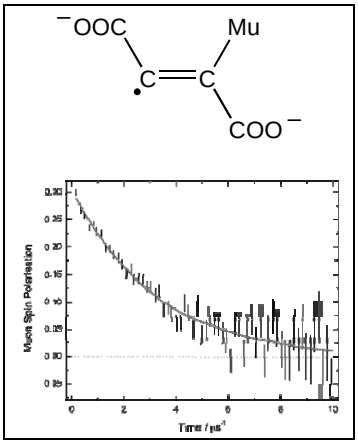
A short-lived free radical containing only one $I = ½$ nucleus, the muoniated 1,2-dicarboxyvinyl radical dianion, was produced in an aqueous solution by the reaction of muonium with the dicarboxyacetylene dianion. The identity of the radical was confirmed by transverse field muon spin rotation spectroscopy and DFT calculations. The muon spin relaxation rate of this radical was measured as a function of temperature in zero magnetic field by the zero field muon spin relaxation technique. The results have been interpreted using the theoretical model of Fedin et al.[1] The muon spin polarization decreases exponentially with time after muon implantation and the temperature dependence of the spin relaxation rate indicates that the dominant relaxation mechanism is the modulation of the anisotropic hyperfine interaction due to molecular rotation. The effective radius of the radical in solution was determined to be 1.12±0.04 nm from the dependence of the muon spin relaxation rate on the temperature and viscosity of the solution, and is approximately 3.6 times larger than the value obtained from DFT calculations.



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P629

A new approach combining different MRI methods to improve understanding of the dynamic processes occurring during xanthan tablet swelling

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Hydrophilic matrix tablets are widely used for controlled delivery of drugs. On contact with water or body fluids the outer surface of these tablets hydrate and swells, forming a hydrogel coat around the dry central core. The key element in drug release from hydrophilic matrix tablets is the gel layer that regulates the penetration of water and controls drug dissolution and diffusion. The central dry core is a glassy state polymer. As medium penetrates into the tablet, a penetration front between the dry glassy and the hydrated glassy polymer appears. As the proportion of medium increases the hydrated glassy polymer is progressively transformed to a rubbery state (gel). The interface between the glassy and rubbery states is called the swelling front. The polymer chains swell as they hydrate, and the eroding front appears as an interface between the swollen tablet and the bulk medium.

A new method utilizing combination of SPI, multi-echo MRI and T_2 mapping was developed for accurately determining moving fronts during the swelling of xanthan tablets: the penetration, swelling and erosion front. This method eliminated the limitations of standard MRI methods used in previous studies and improved current understanding of the dynamic processes involved in xanthan swelling. Hydration of xanthan tablets was studied in six media, differing in pH and ionic strength. All six media penetrate through the whole tablet in 4 h ± 0.3 h, but formation of the gel layer is significantly delayed. The position of the swelling front was the same, independently of the different xanthan gel structures formed under different conditions of pH and ionic strength. The position of the erosion front, on the other hand, is strongly dependent on pH and ionic strength, as reflected in different thicknesses of the gel layers. Moreover, experiments simulating physiological conditions showed that changes of media influence xanthan gel structure relatively quickly, and consequently the drug release kinetics.

P630

3-cm and 2-mm Band ESR Spectroscopy of Free-Radical and Multispin Systems, Exhibiting Extremal Reactivity at Low Temperature

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The chemical generation of radicals (without γ-, β-, UV-irradiation) was observed at temperatures 50-200K and lower in reactions of direct halogenations due to extremal reactivity in polymers, monomers and nanomaterials. The models of multicentered synchronous reactions in intermediate polymolecular complexes between hydrocarbon molecules and halogen ones are considered. Reactions do not practically need the activation energy; they occur in nanodiffusion regime and are limited by molecular mobility of halogen. At the first time the free-radical mechanism of polymolecular complexes conversion was shown, as well as the possibility of determination of their initial composition in accordance with structure of radical intermediates formed. The radicals obtained may be used in halogenation, polymerization, grafting, oxidation and other processes. Using such methods it is possible to reach radical concentration about 10^{18}-10^{19} spin/g, close to concentration of radicals formed during γ-irradiation by doses equal to 10 (and even 100) Mrad.

The spin dynamics and saturation effects in molecular crystals and polymer systems were studied via 3 cm EPR spectroscopy. Specific effects of spin dynamics - joint multispin effects at molecular paramagnetic domains and at conduction electrons in multijoint systems were discovered and observed.

High-conjugated systems of lignocellulosic complex and nanocristalline cellulose were studied vie unique 2-mm ESR spectroscopy method (f=0.15 THz, H=5 Tl). In this method g-factor has high resolution (10^{-5}-10^{-6}).

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P631 (*)
Solid-State Quantum Gates based on Hybrid Electron-Nuclear Spin Systems

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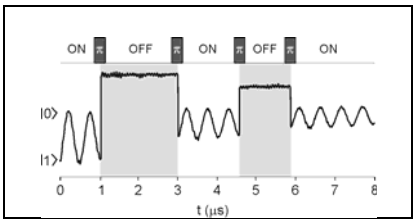

Electron and nuclear spins are ideal elements for quantum bits (qubits) because they are natural two-state systems with relatively long decoherence times. A fundamental challenge in the realization of a solid state quantum computer is the construction of fast and reliable two-qubit quantum gates. Of particular interest in this direction are hybrid systems of electron and nuclear spins, where the two qubits are coupled through the hyperfine interaction. However, the significantly different gyromagnetic ratios of electron and nuclear spins do not allow for their coherent manipulation at the same time scale. While this difference can been utilized for the construction of quantum memories, the slow inversion of nuclear spins using rf pulses (typical period ~ 10 μs) can be a severe obstacle for the efficient function of a two-qubit quantum gate.

Recently,[1] we showed that under certain conditions (i.e. exact cancellation) it is possible to invert the nuclear spin polarization within sub-microsecond time intervals and also to toggle active and passive state dynamics using only mw pulses. This approach overcomes the asymmetry in relaxation times which is an inherent property of hybrid electron-nuclear spin systems and thus gives new perspective by considering them not only for performing quantum memories but also for building solid-state quantum gates. Here we examine possible pulse sequences that are based on this concept of fast nuclear spin manipulation without rf pulses in order to construct a complete set of universal quantum gates. The theoretical fidelities of important two-qubit quantum operations like for instance the controlled-NOT or the SWAP gate are analysed by means of numerical simulations. In addition, some technical issues that might be crucial for the physical realization of these gates using model systems are also discussed.

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P632
Diffusion spectrum of water

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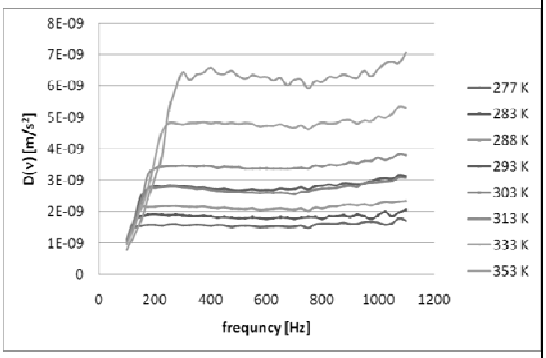
Velocity autocorrelation spectra of bulk water at different temperatures were measured by modulated gradient spin echo method – CPMG in high gradient field.[1,2] The spectra were measured in relevant frequency range of 0-1 kHz. The results show that a simple model of Brownian self-diffusion is not applicable and the diffusion dynamics of water molecules can be described with slow chain-like dynamics in water caused by coupling of diffusing molecules to broken bonds in hydrogen bond network:

$$D(\omega) = D_0 \frac{1+\frac{D_\infty}{D_0}\omega^2\tau^2}{1+\omega^2\tau^2},$$

where D_0 is the bulk self-diffusion constant as measured with PGSE. D_∞ is the self-diffusion spectrum limit at high frequencies. The parameter τ corresponds to the coupling of single water molecules to the hydrogen-bond network and the fits show that the coupling decreases with temperature.



Measured velocity correlation spectra of water at different temperatures. The sharp drop at low frequencies is induced by hardware.

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P633

Heterogeneity of nano-filled EPDM elastomers investigated by inverse Laplace transform ^{1}H NMR relaxometry

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The effects of nano-filler particles on a series of reinforced EPDM elastomers were characterized by low field NMR with measurements of ^{1}H transverse (T_2), longitudinal (T_1) and longitudinal in rotating frame ($T_{1\rho}$) relaxation times.[1] The complex polymer-filler interactions result in heterogeneous polymer chain dynamics. A broad variety of fillers N121, N683, N990 and Ecorax® 1720 carbon black, Ultrasil® 7000 GR, Ultrasil® 7000 GR +Si69, Coupsil® 8113 silane based, and Precarb® 400 calcium-carbonate based reinforcing fillers was studied at different content (20 phr to 70 phr). These filler are characterized by a range of particle dimensions, surface area and physico-chemical activity. The measured NMR relaxation curves were inverted into distributions of relaxation times by one-dimensional Laplace transformation.[2] The distributions identify multimodal polymer network dynamics. The T_2 and $T_{1\rho}$ distributions are the NMR parameters most sensitive to fillers type, concentrations, and EPDM chain dynamics. The heterogeneity in the relaxation NMR parameters T_2, T_1, and $T_{1\rho}$ originates from the distribution of the correlation times which characterizes the polymer chain motions. Correlations between the filler content (phr) and the transverse relaxation time distributions were established for all eight filler types. Moreover, the combined analysis of relaxation time distributions and 2D T_1–T_2 distributions leads to the identification of multiple dynamic components of polymer chain segments that result from the interaction of the polymer matrix and the filler. Correlations between microscopic parameters and mechanically parameters were established.[3]

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P634

Multiple Echoes in Hyperpolarized Liquid Solutions of ^{129}Xe and of ^{3}He

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Many experiments have been performed on thermally polarized solvents for observing multiple spin echoes due to distant dipolar fields in liquids,[1] and have been increasingly popularized by the CRAZED experiment.[2] We report our experimental results and simulations of multiple spin echoes in hyperpolarized ^{129}Xe dissolved in cyclohexane and in hyperpolarized ^{3}He dissolved in liquid ^{4}He. This set of experiments and simulations allows the investigation of echoes in liquids due to distant dipolar fields at very low spin temperatures (down to 10 μK). In the case of xenon, we discuss the agreement between experiments and simulations and the application of our methods to further enhance the study of multiple NMR-masers.[3] The observed agreement between simulations and experiments shows that in the case of homogeneous evolution of the magnetization (where all spins experience the same longitudinal relaxation), even at very low spin temperatures, the observed echo decays follow predictions from the classical average dipolar field and quantum mechanical density operator formalisms. In the case of helium, we provide new experimental and simulation results to help explain the observation of multiple echoes using a 90°-180° pulse sequence,[4] which have not previously been explained.

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P635

Simulation of Spin Relaxation and Chemical Exchange in Solid-State NMR

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A general theoretical description for dynamic effects in NMR studies of isolated and coupled spin systems is presented.[1,2] The approach is valid for arbitrary spin systems (I = 1/2, 1, 3/2, 2, 5/2,..), and applicable for the description of both NMR lineshape effects and spin-spin relaxation phenomena.

The programme features simulations of dynamic NMR experiments on stationary samples (powders and single crystals) with spin systems that might undergo various internal magnetic interactions, such as chemical shift, first and second order quadrupolar interactions as well as dipolar and indirect spin-spin couplings, with arbitrary interaction tensor orientations. In principle, any type of stationary 1D and 2D solid-state NMR pulse experiment can be computed, including several R.F. channels, finite pulse widths with arbitrary phases and R.F. offsets, phase cycling, and different relaxation time-intervals. In the case of quadrupolar nuclei with non-integer spins, the central and satellite transitions can be considered individually or collectively.

Simulations can be carried out for rigid samples, and for samples with inherent molecular dynamics. Here, spin-spin relaxation and line shape effects are caused by the consideration of various (superimposed) jump and diffusion processes, reflecting internal and overall motions. Model simulations show the influence of the magnetic interactions and motional characteristics on the resulting NMR line shapes and relaxation data.

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P636

Structure of the pore-forming subunit of the twin arginine translocase determined using a combined approach of liquid- and solid-state NMR

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Besides the sec protein export system, bacteria have evolved the twin-arginine translocase (tat) system to transport fully folded proteins across the cytoplasmic membrane. In *Bacillus subtilis* the tat system consists of TatC, which is believed to recognize the signaling sequence, and TatA, which is hypothesized to asssemble into a pore composed of variable multimer.

Here, we report the structure of monomeric TatA, which was solved through a combination of liquid and solid state NMR.[1] The extent of secondary structure was determined in SDS detergent micelles. Monomeric TatA consists of a transmembrane α-helix connected by a short hinge region to an adjacent amphiphilic α-helix and a non-structured C-terminal tail. NOE contacts across the hinge region allowed us to partially restrain the mutual orientation of the two helices, but did not yield a precise single structure. Since detergent micelles may influence structural details especially of pliant segments we further measured ^{15}N-PISEMA spectra in magnetically oriented lipid bicelles to derive orientational constraints to position the two α-helices relative to each other and with respect to the membrane normal. Both liquid and solid state NMR restraints were combined to restrain the structure in molecular dynamics simulation in the COSMOS force field. Time averages of the NMR parameters were computed to also take into account the molecular mobility.

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P637

Angular Dependence of P-31 Chemical Shielding in Nucleic Acid Backbone: A Combined MD-DFT Study

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We present a comprehensive quantum chemical analysis of backbone torsion-angle influence on P-31 chemical shielding in nucleic acids. Snapshots obtained from the molecular dynamics simulation of $[d(CGCGAATTCGCG)]_2$ were used to construct geometries of hydrated dimethyl phosphate (DMP). Subsequent DFT calculations for the hydrated DMP demonstrate that P-31chemical shift is dominated by the torsion angles α and ζ. The functional dependence is modulated by the adjacent torsion angles β and ε. The inclusion of solvent leads to an additive upfiel shift of 2-3 ppm and a damping of the α, ζ dependences.

P-31 is a sine function of the torsion angle ζ (populated between 120° and 315°) with a maximum at 180° and a minimum at 270°. For the torsion angle α populated between 250° and 315°, the chemical shift decreases with increasing α. The decrease is linear to quadratic depending on the average value of β. The difference between P-31 shifts of the B_I and B_{II} substates (crucial for structural interpretation of NMR data) by MD-DFT is 2.1 and 1.6 ppm for two DNA residues of interest, in accord with 1.6 ppm inferred from experimental data. Likewise, a more negative P-31 chemical shift for a residue in pure B_I conformation compared to residues in mixed B_I/B_{II} conformation is given by MD-DFT, in agreement with experiment. Reproducing the very small differences between P-31 in two mixed B_I/B_{II} conformation turnes out more difficult.

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Oxidation of $[PtCl_4]^{2-}$ with H_2O_2 in acidic solution: a re-investigation with ^{195}Pt NMR

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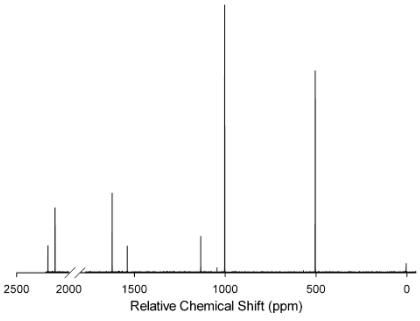
Oxidation of $[PtCl_4]^{2-}$ in water with hydrogen peroxide (H_2O_2) results in the *trans*-$[PtCl_4(OH)_2]^{2-}$ species exclusively.[1] We have found that this oxidation if carried out in acidic solution (1M $HClO_4$) results in many species, contrary to previous reports. These have been assigned to $[PtCl_{6-n}(H_2O)_n]^{n-2}$ (n = 0-4) complexes by means of ^{195}Pt NMR (Figure), with *trans*-$[PtCl_4(H_2O)_2]$ present in only low concentrations relative to the *cis*-$[PtCl_4(H_2O)_2]$ stereoisomer and $[PtCl_5(H_2O)]^-$. This unexpected result could be ascribed to various factors including *i*) ionic strength effects, *ii*) anation/aquation reactions, *iii*) ClO_4^- reduction or *iv*) Pt(II) assisted ligand exchange. Our results suggest that although *trans*-$[PtCl_4(H_2O)_2]$ is the main oxidation product in acidic solution, this rapidly scrambles into a distribution of species during or subsequent to oxidation partly as a result of Pt(II) catalyzed ligand exchange (Figure). In view of the complicated mechanistic implications for these reactions, we artificially mimicked the likely conditions during and after oxidation, to show that the same species distribution is obtained as for the oxidation of $[PtCl_4]^{2-}$. In this regard Pt(II) assisted ligand "catalysis" seems to play a significant role in determining the overall species distribution following oxidation of $[PtCl_4]^{2-}$.



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P639

Signal Enhancement in Protein NMR using the Spin-Noise Tuning Optimum

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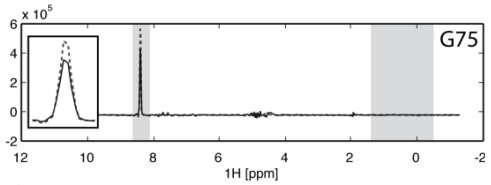
We present the benefit of an alternative tuning strategy based on the spin-noise response[1] for application in common high-resolution multi-dimensional biomolecular NMR experiments with water signal suppression. Using this tuning approach (determination of the spin-noise tuning optimum SNTO[2,3]), depending on the particular probe and pulse sequence used, signal-to-noise ratio improved up to 30% with only a marginal decrease in pulse performance.



$^{1}H^{15}N$ HNCO signal of G75 (Ubiquitin in 50 mM ammonium acetate, pH 4.5), Bruker Avance 500 (11.7 T), TXI probe. Dotted line at SNTO ($\Delta\omega_c$= -165 kHz).

According to theory,[1] a symmetrical "dip" in the thermal noise would be observed at the rf-circuit's resonance frequency (i.e. $\Delta\omega_c$= 0), in practise this lineshape may be found at a significant tuning offset $\Delta\omega_c$, and may provide a considerable improvement in signal-to-noise ratio of multi-dimensional NMR experiments. Once the SNTO condition is found for a specific setup, and the pulses are calibrated at this new tuning condition (they are slightly longer than for conventional tuning) one may use the benefit of sensitivity enhancement without any further modification and only an acceptable increase in pulse length.

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P640

Exploring the limits to spatially resolved NMR in the presence of fast-diffusion liquid phases

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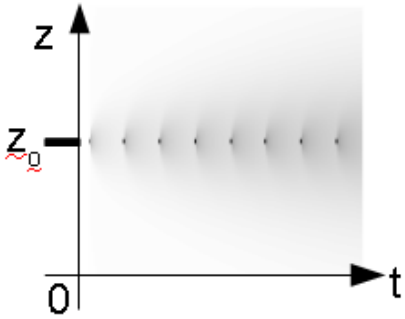
Recent advances in MRI have demonstrated spatial resolutions down to 1 µm. Magnetic resonance force microscopy has the potential to reach sensitivity for single nuclear spins. Given these numbers, in vivo imaging of single cells or even biomacromolecules may seem possible. However, for in vivo applications, there are fundamental differences in the contrast mechanisms compared to MRI at macroscopic scales as the length scale of molecular self-diffusion exceeds that of the spatial resolution on the NMR time scale.[1] Those effects - which are fundamentally different from the echo attenuation in field gradient NMR - even may lead to general limitations on the spatial resolution achievable in aqueous systems with high water content. In our contribution, we explore those effects on a model system in a high-resolution stray-field imaging setup in which the effects of periodic excitation of a thin layer of water inside a large liquid cell were studied. In addition to experimental results, simulations based on the Bloch-Torrey equation will be presented. Last but not least, implications of the findings with respect to possible contrast mechanisms and limitations in spatially selective NMR of biological specimens at ultrahigh resolution will be discussed.



Spatiotemporal spreading of NMR excitation (dark greyshades) by diffusion from a thin, periodically excited layer located at z_o.

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P641

Changes in high-impact polystyrene (HIPS) during simulated recycling runs studied by NMR relaxation and other techniques

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High impact polystyrene was subjected to various simulated recycling runs and studied by a range of different wet-chemical, mechanical and spectroscopic (FTIR, Raman, TD-NMR) characterization techniques in order to identify changes in the material's performance and the underlying structure-property relations. Simulated recycling conditions applied were (i) repetitive extrusion runs on the same material and (ii) thermooxidative ageing at 90 °C under forced ventilation.

Of the characterization techniques applied, TD-NMR is the most simple approach with respect to sample preparation. The NMR results are compared with those obtained by the other methods. In the case of thermooxidative ageing, NMR, FTIR and Raman produced quite parallel results while mechanics showed a different trend with a sharp decrease during the first days of ageing. This strong initial decrease in mechanical performance can be attributed to a chemical degradation of the rubber due to chain scissions. As the spectroscopic techniques show this process still goes on during further ageing. The NMR result is especially interesting as it shows a strong increase in the more mobile fraction of the rubber signal due to increasing numbers of free chain ends. At the same time, also increases in cross-link-density can be observed that lead to decreasing relaxation times of both rubber signal components. In the reprocessing series, several parallel effects leading to a degradation of the matrix and the rubber particles were found. All spectroscopic techniques decently correlate with both the mechanics and the wet chemistry. Wet chemistry seems to be the most precise approach to track down the degradation effects in this case.

P642

Quantitative 2D and 3D Γ-HCP Experiments for the Determination of the Angles α and ζ in the Phosphodiester Backbone of Oligonucleotides

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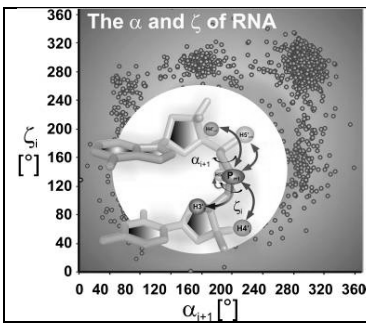
A novel heteronuclear NMR pulse sequence, the quantitative Γ(HCP) experiment, for the determination of the RNA backbone angles α($O3'_{i-1}$-P_i-$O5'_i$-$C5'_i$) and ζ($C3'_i$-$O3'_i$-P_{i+1}-$O5'_{i+1}$) in ^{13}C labeled RNA is introduced.[1] The experiment relies on the interaction between the CH-bond vector dipole and the ^{31}P chemical shift anisotropy (CSA) which affects the relaxation of the ^{13}C,^{31}P-double and zero quantum coherence and thus the intensity of the detectable magnetization.[2] Two versions of the pulse sequence optimized for the CH and CH_2 groups are introduced and demonstrated for the 14mer cUUCGg-tetraloop model system RNA and for a 27mer RNA with previously unknown structure.[3, 4] The restraints were incorporated into the structure calculation of a very high resolution structure of the model system RNA.[3] Comparison with the X-ray structure of the cUUCGg tetraloop confirms the high quality of the data and suggests that the method can significantly improve the quality of RNA structure determination.



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P643

Use of SSFP ^{13}C NMR to monitor *in situ* electrochemical reaction in spectroelectrochemical cell

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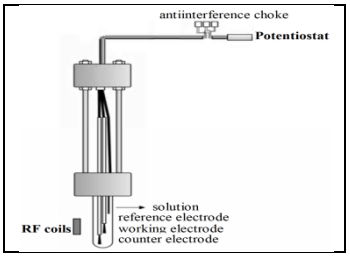
The main advantage *in situ* measurements, which couple, electrochemistry techniques (EC) and nuclear magnetic resonance spectroscopy (NMR) is obtain information in real time about electrogenerated species, in solution. Most EC-NMR studies uses the ^{1}H NMR detection to monitor the electrochemical processes due to ^{1}H high sensitivity and fast data acquisition.[1,2] To obtain ^{13}C spectra faster spectrum than conventional ^{13}C NMR sequence to monitor *in situ* the electrolysis's reaction (organochloride reduction) we examined the application of ^{13}C Steady State Free Precession sequence (SSFP). Figure 1 shows the diagram of EC-NMR cell assembled in a 10 mm NMR tube. The spectroelectrochemical cell contains the three electrodes, the reference, working and counter electrodes. The *in situ* electrochemical reaction was performed with potentiostat coupled in the cell placed inside the high-field NMR spectrometer. The ^{13}C SSFP measurements were performed for 10 minutes during the electrochemical reaction. The signal to noise enhanced provided by SSFP sequence demonstrates by first time the possibility of *in situ* monitoring of ^{13}C NMR in spectroeletrochemical study.



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P644

Assignment of the Proton and Carbon-13 Resonances of an unsymmetrical beta-Cyclodextrin Derivative

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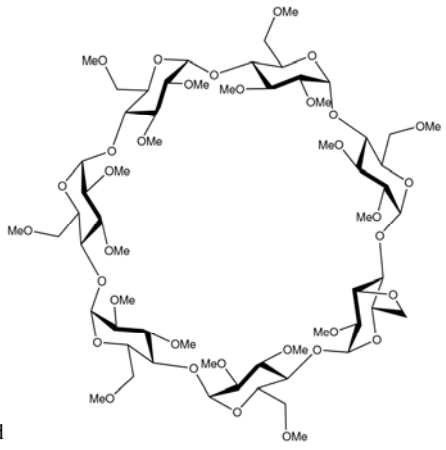
Compound **1** is a starting material in the synthesis of chiral stationary phases for gas chromatography.[1] The assignment of its ^{1}H and ^{13}C sugar resonances was achieved by means of new and conventional pulse sequences.

The sequential assignment of the sugar units was obtained using a F_1 decoupled F_1 band-selective 2D TOCSY – ROESY experiment. The ^{1}H and ^{13}C resonances in each sugar unit were assigned by means of sensitivity optimized 3D TOCSY – DQFCOSY and TOCSY – HSQC spectra, of F_1 band-selective 2D HSQC – RELAY and of aliased 2D HSQC – TOCSY[2] spectra.



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P645

Speeding up the NMR of disordered proteins by an order of magnitude, using paramagnetic relaxation and projection-reconstruction NMR

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NMR spectroscopy is the most suitable technique to obtain insight into the details of protein conformational disorder. However, NMR is a time-consuming technique because most of the experimental time is necessarily wasted due to the slow recovery of magnetization from one scan to another.

Here we report the very rapid recording of NMR data for unfolded proteins in solution. Using the neutral organometallic complex Ni(DO2A), the average proton relaxation rate for the intrinsically disordered protein alpha-synuclein increases from 1.76 s-1 to 5.76 s-1 at 30 mM concentration of the paramagnetic agent, with negligible line broadening for the protein. Due to this advantage, sensitive 2D spectra could be recorded in as little as 30 seconds, an order of magnitude faster than in the absence of the agent (to achieve the same sensitivity). Also, a high quality 3D HNCO of alpha synuclein was recorded in as little as 15 minutes by employing projection-reconstruction techniques, in addition. Our study shows that the neutral paramagnetic agent Ni(DO2A) is accessible to the entire disordered polypeptide chain, which offers a significant advantage over its application to folded proteins.[1]

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P646

Efficiency of dynamic nuclear polarization for varying ^{1}H and ^{13}C nuclei concentration

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Solid-state dynamic nuclear polarisation (DNP) in conjunction with fast dissolution is a new method for enhancing the polarisation of nuclear spins in liquid-state NMR experiments.[1] Molecules of interest are polarised at low temperature using a dedicated 3.35T magnet. The frozen sample is dissolved rapidly using hot solvent and shuttled pneumatically to a high resolution spectrometer where it is injected into an NMR tube.[2]

Here we present results of DNP studies on ^{1}H and ^{13}C nuclei using TEMPO and trityl free radicals in water/glycerol solutions. The scope of the study was solid state polarisation parameters dependence on sample composition. The concentration of nuclei of interest participating in the DNP (either ^{1}H or ^{13}C) was increased in a set of experiments. A set of solid state characteristics including enhancement as a function of irradiation frequency, relaxation time constant and polarisation time constant were measured for all the samples. An attempt was made to explain the solid state polarisation dependence by a theoretical model. Additionally dissolution experiments were performed and liquid state enhancements measured at the ambient temperature.

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Unified framework for comparison of performance of libraries of NMR experiments in applications to challenging proteins

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Systematic benchmarking of multidimensional protein NMR experiments is a critical prerequisite for optimal allocation of NMR resources for structural analysis of challenging proteins, *e.g.* large proteins with limited solubility or proteins prone to aggregation. A typical problem is selection between TROSY and none-TROSY versions of triple resonance experiments, which is to be made at rather early stages of NMR work. We created a unified framework[1] including rigorous mathematical description of NMR experiments and a set of benchmarking parameters such as, expected signal-to-noise ratio in resulting fully acquired nD spectra, resolution, detailed propagation of density operator for magnetization transfer pathways analysis and spectral artifacts. This framework is essentially a software solution capable of encapsulating of NMR pulse sequences and associated setup algorithms in a wiki-like web-accessed database interfaced to the Spinach library (*http://spindynamics.org*), which enables accurate simulation and benchmarking of NMR experiments on large spin systems. A key feature is the ability to use a single user-specified spin system to simulate the majority of deposited solution state NMR experiments, thus providing the (hitherto unavailable) unified framework for pulse sequence evaluation. This development enables predicting relative sensitivity of deposited implementations of NMR experiments, thus providing a basis for comparison, optimization and, eventually, automation of NMR analysis. The benchmarking is demonstrated with two proteins, of 170 amino acids aX-I domain of Integrin and 440 amino acids NS3 helicase.

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P648

Optimization of the Overhauser Effect with nitroxide radicals at high magnetic fields using a 260 GHz high-power gyrotron

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Dynamic nuclear polarization (DNP) is an important technique to enhance sensitivity of NMR signals. In the liquid state the operative DNP mechanism is the Overhauser Effect and is achieved by driving EPR transitions into saturation by microwave pumping. In this new microwave driven equilibrium state, cross relaxation between unpaired electrons and nuclear spins can significantly enhance the polarization of nuclear spins.[1] Previously, unexpectedly high DNP enhancements of more than -10 on water protons have been achieved in an aqueous solution of Fremy's Salt at magnetic fields of 9.2 T (corresponding to 400 MHz 1H NMR frequency and 260 GHz EPR frequency) using a low-power solid-state microwave source (max. power of 45 mW).[2] However, we did not achieve maximum saturation of the EPR transitions with this source. Recently, a DNP enhancement of -29 has been achieved on a similar sample using a high-power gyrotron microwave source (max. power 20 W).[3] The degree of saturation can be modeled using a semiclassical relaxation theory including electron coherences.[4] These experimentally observed DNP enhancements, which exceed the predicted values extrapolated from low-field DNP experiments, demonstrate the potential of DNP for liquid-state samples at high magnetic fields.

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P649

Flow Regime Analyzer Based on Low-Field Nuclear Magnetic Resonance and Halbach-Type Magnet Arrangements

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Many industrial applications require exact characterization of the individual phase velocities and fluid fractions of multi-phase flow. In this work we present a system that enables direct, real-time determination of velocity and fluid fractions of three-phase flow by means of low-field Nuclear Magnetic Resonance.[1] The apparatus includes a main Halbach magnet of 60 cm in length with a cylindrical region of interest (ROI) of 10 cm in diammeter and 10 cm in length, as well as two prepolarization Halbach magnet sections. The prepolarization magnets are arranged on either side of the main magnet to enable the measurement of bi-directional flow. Using a recently developed method for characterizing three-phase flow based on analysing the early behavior of the echo amplitudes of a CPMG sequence, flow velocity and fluid fractions of oil/water mixtures were determined for flow-rates between 5 and 60 m^3/h without the need of any static or pulsed magnetic field gradients. The experimental results verify the theoretical framework. In addition, a contrast in signal intensity originating from fluid phases with different longitudinal relaxation times, T_1, was created by changing the effective length of the prepolarization magnetic field using rotateable stacks of Halbach magnets. In addition to measuring flow velocity and fluid fractions, the system enables the acquisition of two-dimensional magnetic resonance images of the cross-section of the ROI. Imaging different phantoms filled with $CuSO_4$-doped water established a spatial imaging resolution of approximately 1 cm. Magnetic Resonance Imaging of the cross-section of a flow conduit has the potential to significantly aid the interpretation of complex flow conditions.

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Solid-state NMR and Dynamic Nuclear Polarisation on Membrane Proteins

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We describe first dynamic nuclear polarisation experiments on model compounds and membrane proteins acquired on a new, purpose built spectrometer. The hardware consists of a high power 258GHz gyrotron (Gycom, Nizhny Novgorod, Russia) connected via corrugated waveguides to a specially modified Bruker 3.2mm cryo-MAS probehead operating at 100K at a 393MHz Bruker Avance II spectrometer. Signal enhancement and long-term stability of this system will be demonstrated on model compounds dispersed in glycerol-water mixtures together with various mono and multi-radicals. Furthermore, we will report a systematic screen of optimised sample preparation conditions allowing the best possible compromise between magnetisation transfer while maintaining lipid bilayer integrity. We will illustrate the use of our DNP setup on selectively and uniformly labelled samples of the integral membrane proteins proteorhodopsin, EmrE and LmrA. Linewidths and signal enhancement in dependence of sample preparation conditions will be discussed.

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PHIP in symmetrical systems: effect of isotopic enrichment and hydrogenation reaction intermediates

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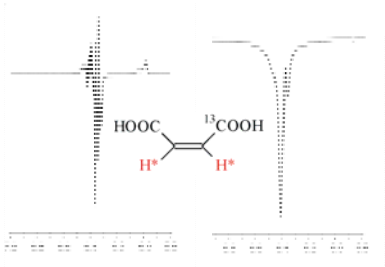
Hyperpolarization can be obtained from parahydrogenation reactions providing that the symmetry of the hydrogen molecule is broken in the products. The addition of the two protons to chemically equivalent positions may also yield to strong heteronuclear (^{13}C) hyperpolarization[1] due to asymmetrical coupling of the two protons with the heteroatom. Polarization intensity is related to the fact that the singlet state is maintained during the hydrogenation reaction and in the ^{1}H-NMR spectrum an antiphase hyperpolarization pattern is expected.

It has also been shown that ParaHydrogen Induced Polarization (PHIP) can be observed on symmetrical molecules (A_2 spin sytem)[2] due to the occurrence of asymmetrical relaxation processes at the hydrogenation intermediates.

Herein we report our recent observations on the parahydrogenation of symmetrical molecules catalysed by homogeneous Rh(I) catalysts aimed at getting more insight into the factors determining the occurrence of the above referred polarization effects. Hyperpolarization can be observed as an antiphase or an emission signal in the same hydrogenation product (see figure) depending on the stabilization effects played by the solvent on the hydrogenation intermediates. DFT calculations, employed with the aim to elucidate the hydrogenation mechanism, show that the solvent is coordinated to the metal and stabilizes the intermediate species.



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P652

Low temperature probe for NMR and longitudinal detection of EPR

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Longitudinal detection (LOD) has long been used as an alternative detection method for EPR. Its benefits compared with transverse detection include a greater robustness, no need for a microwave resonator in which to place the sample, and detection during high-power microwave irradiation.[1]

We have designed and built a low temperature probe to measure LOD EPR spectra and study Dynamic Nuclear Polarisation (DNP) processes in the same sample. The probe head has a cylindrical geometry. It consists of a coil former designed to hold two orthogonal saddle coils for NMR applications and a solenoid for LOD EPR. The first of the saddle coils is used for proton NMR excitation and detection. It forms part of a resonant circuit tuned to the ^{1}H frequency of 144 MHz. The second saddle coil is part of an untuned circuit and is used for broadband excitation. The sample holder, with a height of 17.5 mm and an internal radius of 4 mm, is designed for large volume samples.

The probe is designed to be used in a Krymov W-band setup: it is inserted into the external part of a probe described by Gromov et al.[2] For both NMR and EPR experiments, the probe head can be immersed in liquid helium and cooled to 1.3 K. A Krymov microwave bridge[2] is used to irradiate the sample, but alternatively a lower cost unstabilised microwave source (ELVA-1, St. Petersburg) can be used.[3]

We will present details of the design of the probe and probe head, as well as the implementation of the software to control the experiments. Results of both initial NMR and LOD EPR experiments will be shown.

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P653

Measuring self-diffusion up to 1500K: issues and challenges

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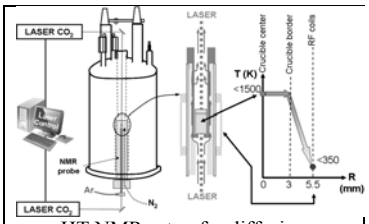
The study of high temperature liquids is always a technical challenge in many fields of fundamental or industrial research such as geology (magma), metallurgy (metals and electrolytes), glass, nuclear waste recycling or energy (battery, fuel cell, molten salt reactor)… Hence, in contrast with room temperature liquids, high temperature liquids suffer from a lack of dynamical information due to the technical difficulties to deal with. However dynamics, and particularly self-diffusion description, is essential for a better understanding of their properties.



HT NMR setup for diffusion measurement

We have developed a new setup in order to achieve working temperature much higher than the previous setups (limit 700K). Our setup is based on Pulsed Field Gradient Nuclear Magnetic Resonance combined with CO_2 laser heating. All technical constraints have been controlled: temperature calibration, convection artefact, thermal protection of the NMR probe, sample time stability. In situ self-diffusion coefficients of several nuclei can now be reliably measured up to 1500K.[1] This new setup opens wide perspectives in the study of high temperature liquids.

To illustrate the potentialities of this method, we will present results on molten alkali fluoride systems[2] and confront them to the available literature data and to molecular dynamics simulation.

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P654

Constant ^{1}H and ^{13}C signal enhancement in NMR using hollow fiber membranes and parahydrogen

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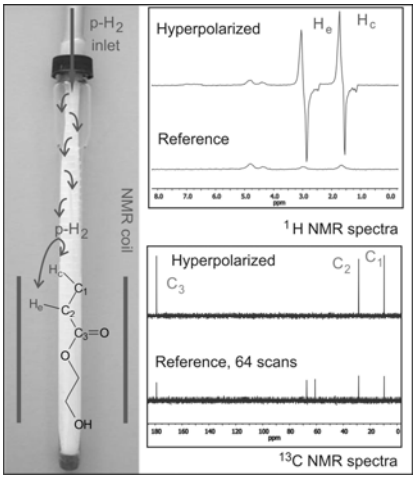
Enhancing the sensitivity of nuclear magnetic resonance via Parahydrogen Induced Polarization (PHIP) is of high interest for spectroscopic investigations. PHIP is a chemical method, which makes use of the correlation between nuclear spins in parahydrogen to create hyperpolarized molecules.[1] In order to achieve the highest possible sensitivity gain it is of great importance to optimize the reaction and measurement conditions of the parahydrogenation technique.



We optimized the conversion rate and established optimal NMR measurement conditions by utilizing hollow fiber membranes[2] for continuous parahydrogen delivery while performing PASADENA experiments. This new way of dissolving parahydrogen more efficiently into water without the occurrence of foam and bubbles offers the opportunity to implement continuous flow measurements under pressure, leading to higher conversion rates and higher polarization levels. Furthermore, this careful control of the parahydrogenation reaction generates a constant hyperpolarization of ^{1}H and ^{13}C over a certain time (several minutes) which enables us to perform 2D NMR experiments with very high sensitivity.

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P655

Structure of Potential Targets for Drug Rational Design against *M. Tuberculosis*

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One of the keys to *Mycobacterium tuberculosis* (*Mtb*) success as a pathogen is its ability to persist in its host organism in a latent state after infection. Controlling the entry and exit from dormancy is therefore important in the development of novel anti-tubercular therapies. RpfBc is a secreted protein that decreases the growth lag time when added to dormant cultures of *Mtb*. The structure[1] of its functional domain is a compact hybrid of the Soluble-Lytic-Transglycosidase and c-type lysozyme folds, both of which cleave peptidoglycan (PG). In response to its environment, *Mtb* modulates the expression of genes in order to promptly adjust to new conditions. Stimuli are transduced via sensor kinases present on the mycobacterial membrane. We present the solution structure of the extracellular domain of one of these kinases,[2] PknB, consisting in a repetition of four PASTA domains. We also identified and solved the structure of two protein substrates of PknB. OdhI is a Krebs cycle key regulator in *C. glutamicum*, almost constituted of a single FHA domain. The solution structures of both phosphorylated and unphosphorylated isoforms[3] revealed a major conformation change and the first autoinhibition mechanism for an FHA domain protein. Rv2175c from *Mtb* is a protein of unknown function: its solution structure[4] shows an original winged HTH motif, indicative of a DNA-binding protein. All these proteins represent attractive targets for the development of drugs against *Mtb*, and the NMR structures described here offers valuable templates for their rational design.

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P656

LESR and TR-ESR characterization of fluorene-based polymers for photovoltaic applications

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Spectroscopic characterization by conventional continuous wave light induced ESR (LESR) and Transient ESR (TR-ESR) with time resolution in the range of nanoseconds has been performed on some conjugated polymers including fluorene units and co-monomers such as benzothiadiazole and arylamines. Laser irradiation at 532 nm and 355 nm have been used for photoexcitation. The polymers have been examined both in frozen solution and in thin film form and either in the absence or in the presence of the acceptor 1-(3-methoxycarbonyl)-propyl-1-phenyl-(6,6)C61 (PCBM). The results have been compared with those obtained on two commercial polymers: poly-3-hexyl-thiophene, (P3HT) and poly[2-methoxy-5-(3',7'-dimethyloctyloxy)-1,4-phenylen]-alt-(vinylene) (MDMO-PPV).

In films of the pure polymers, a very weak LESR signal was observed, assigned to S=1/2 charged species $P^{+\bullet}$ and $P^{-\bullet}$, whereas a strong TR-ESR single line with decay time of about 1 μs was observed. The latter signal was assigned to mobile triplet species with a motionally averaged lineshape. On the contrary in frozen polymer solutions, localized triplet state TR-ESR spectra were observed.

In all of the polymer/PCBM films, TR-ESR showed the presence of a strong signal of PCBM triplet state, whose spectral shape is significantly varied with respect to the lineshape due to triplet population by inter system crossing. This difference suggests that the PCBM triplet generation in our polymer/PCBM films is due also to charge carriers recombination.

This work provides information about the photophysics and the relative energies of the excited states involved in both the charge carrier photogeneration and recombination processes in these materials.

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A Unified Representation of Protein Dynamics in Solution

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NMR Residual Dipolar Couplings (RDCs) provide uniquely informative probes of biologically relevant motions as they are sensitive to dynamics occurring on timescales up to the millisecond. However dynamically averaged couplings report on both global alignment properties of the protein in an anisotropic medium and the local dynamic fluctuations, so that both contributions have to be correctly estimated in order to accurately extract the biologically relevant motions. Here, we develop a robust structure-free approach to the elucidation of local motions from RDCs measured in the protein Ubiquitin.[1] This analysis allows us to determine for each peptide plane the average orientation and the local conformational dynamics, using the GAF (Gaussian Axial Fluctuation) model. Firstly, all alignment tensors are quantitatively and simultaneously determined, using appropriate dynamic descriptions. An accurate picture of the local motion is then determined, using robust statistical testing to ensure that only dynamic models with the appropriate level of complexity are invoked. Results are extensively cross-validated against 'free' data sets. A complementary approach, called Accelerated Molecular Dynamics (AMD), was applied to the same system.[2] This restraint-free method characterizes the conformational landscape probed during increasing timescales. Experimental RDCs and J couplings were used to identify appropriate statistical mechanical sampling and results were compared to the GAF approach. The two vastly different methods converge to very similar results, substantiating the validity of the approaches, and providing a unified, self-consistent representation of protein dynamics in solution. In Ubiquitin the presence of motion on the nano-second to millisecond range is mainly restricted to surface loops. This accurate estimation of the dynamics present in globular proteins at timescales up to the millisecond provides fascinating insight into the conformational basis of protein flexibility and into the forces governing molecular recognition and function.

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P658

Heteronuclear Double-Resonance methods in NMR as probes for fast dynamics in biomolecules

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Biological processes are often governed by local dynamics occurring on µs-ms timescales, which can result in chemical-exchange contributions to relaxation. In particular, chemical-exchange-induced cross-relaxation between multiple-quantum (MQ) coherences can yield plenty of information about fast local dynamics (exchange rates) as well as thermodynamical (populations and equilibrium constants) and structural (chemical shifts) parameters.[1] Furthermore, the study of the relaxation of MQ coherences allows the characterization of conformational motions that affect two spins simultaneously. We have designed new Heteronuclear Double-Resonance (HDR) methods[2,3] based on well-known decoupling schemes, applied simultaneously to two scalar-coupled spins, to preserve MQ coherences, so that the interconversion between them (e.g. $H_xN_x \rightarrow H_yN_y$) can occur only through cross-relaxation. These methods enable the investigation of conformational dynamics, which are faster than those accessible to MQ CPMG experiments, much like single-quantum (SQ) spin-locking methods give access to faster time-scales than SQ CPMG methods. A remarkably compact analytical expression for the MQ cross-relaxation rate under HDR irradiation was obtained inspired by the approach of Podkorytov and Skrynnikov.[4] Experiments carried out on proteins have led to the characterization of fast exchange processes occurring on a timescale of ~40 µs, in agreement with earlier works.[1]

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P659

Multinuclear insight into the dynamics of inorganic melts: from simple to network-like liquids

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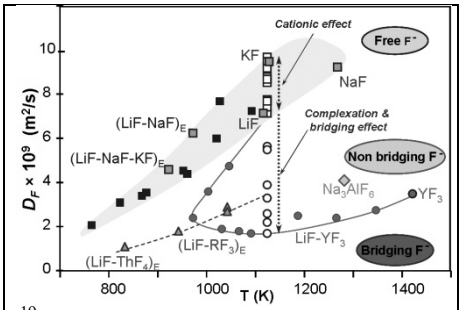
Molten salts constitute an interesting class of high temperature liquids because of the predominance of short-range Coulomb forces which tend to reduce the ionic mobility by preventing ions from escaping out of their counterion shell. They also present a wide range of different structural behaviours at atomic scale. Their liquid structure can be very simple and seen as an ideal bath of polarisable hard spheres.[1] It can also be structured as an assembly of atoms forming long-lived ionic units called 'complexes'. When these complexes are connected to each other via bridges, a network-like liquid can even be formed.[2] Actually the dynamics of liquids is intimately related to their own structure. The self-diffusion coefficient (D) is a particularly important parameter as it is involved in every model describing dynamical systems (macroscopic properties – viscosity, electrical and thermal conductivities), and at the same time it is a signature of the melt structure. Here we present self-diffusion measurements obtained by high temperature PFG-NMR in several molten fluorides systems (alkaline fluoride mixtures, alkali-rare earth fluorides mixtures, cryolitic systems). The multinuclear (^{19}F, ^{7}Li, ^{23}Na, ^{27}Al) approach provided by NMR is a relevant point of view of the relation between the structure and the dynamics of liquids. The effects of temperature and composition in the different systems are clearly shown.



^{19}F self-diffusion coefficients in molten fluorides.

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P660

Noise Signals in Solid State NMR

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Even at equilibrium an ensemble of spins gives rise to a small fluctuating signal. This phenomenon was predicted by Bloch[1] in 1946, and was later detected under a variety of conditions for liquid ^{1}H samples.[2-5].NMR noise can also be used as an alternative tuning indicator, leading to the so called spin noise tuning optimum (SNTO).[5,6]

In this work, we give the first examples of the detection of NMR-noise in solid state NMR. Experimental results are shown for static and MAS conditions. We show that the line shape of the spin noise signal is not only tuning but also matching dependent. The tuning and matching position to get the dip line shape not only varies considerably between different probes but also between different preamplifiers (also the shape of the wobble curve changes much with different preamplifiers). A negative noise signal (absorbed circuit noise)[7] can be obtained for high spin densities under MAS conditions. The spin noise tuning procedure can also be used to optimize signal-to-noise ratios in ^{1}H-MAS experiments.

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P661

Quasi-equilibrium in liquid crystal ^{1}H spins via eigen-selective decoherence

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A quasi-equilibrium (QE) state is a stage of the spin dynamics, that can be characterized by a spin-temperature, that is, by a diagonal density operator in a timescale much shorter than relaxation towards thermal equilibrium with the lattice. This state can be observed in solids and nematic liquid crystals (LC) by NMR Jeener-Broekaert[1] (JB) experiment, for example. The existence of QE is controversial: it is claimed that in solids dephasing of a huge amount of spin states over a short time, enables the use of a diagonal density matrix when calculating the observables.[2] However, in a LC the number of effectively interacting spins (8 spins in PAA_{d6}) seems too small in this view. A full-quantum (FQ) theoretical approach justifying the QE in LC is presented.[3] Spins are treated as an open quantum system, where mechanical molecular operators are included in the dipolar Hamiltonian, together with the spin operators. FQ description, allows to disentangle different timescales in the dynamics: Liouvillian evolution of a closed spin system, reversible adiabatic quantum decoherence, irreversible quantum decoherence and relaxation.

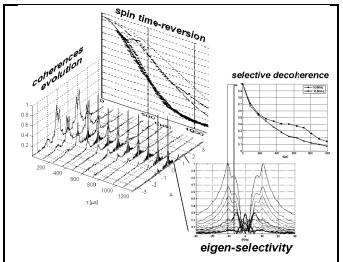

fig.1: eigen-selelective decoherence

Our theoretical approach predicts the occurrence of a decay process we called eigen-selectivity. We present an experiment which clearly shows this effect on the multiple quantum coherences spectra (fig. 1). Experiments showing the occurrence of an irreversible trend towards QE are presented: time reversal of the spin dynamics with MREV8 (fig. 1) and magic echo pulse sequences starting from the JB initial condition. Numerical calculation of the dipolar signal on a LC molecule (fig. 2 and Ref. 4) supports this conclusion.

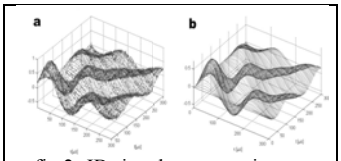

fig.2: JB signals. **a**: experiment. **b**: a single molecule calculation

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P662

NMR study on slow crystallization and plural crystalline phases of room temperature ionic liquid, 1-butyl-3-methylimidazolium hexafluorophosphate

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We measured ^{1}H-T_1, T_2 of 1-butyl-3-methylimidazolium hexafluorophosphate $[C_4mim]PF_6$ as a function of temperature in the range from 203 to 403 K. There was no discontinuous change of ^{1}H-T_1, T_2 in the cooling and heating process. However, after solidification by liquid nitrogen, the ^{1}H-T_1, T_2 trace changed discontinuously at 233 and 253 K in the heating process (Fig.). First discontinuous change of ^{1}H-T_1, T_2 values at 233 K is crystallization from the glass and second change is phase transition between crystalline phases. Furthermore, the sample turn to the third phase transition during one night keeping in the crystalline state in a refrigerator. These three crystal phases correspond to the crystal phases of α, β and γ, which reported as the results of calorimetric and Raman spectroscopic studies by Endo *et al.*[1]

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P663

High Frequency Modulated Gradient Spin Echo Diffusion Measurements with Chemical Shift Resolution

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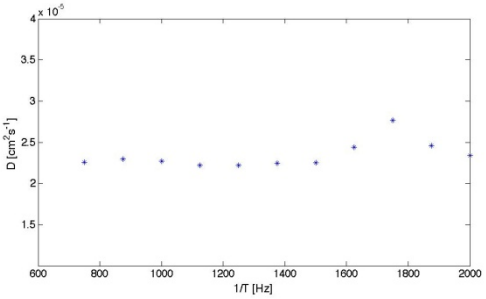
The use of the Modulated Gradient Spin-Echo (MGSE) technique[1] enables self-diffusion measurements at short displacements. The oscillating phase factor produced by the modulated gradients results in a signal decay that depends on the Velocity Auto-correlation Function of the molecules. The diffusion dependent signal attenuation can then be accumulated over several rapidly oscillating cycles of the modulated gradients, making the measurement sensitive shorter time scales than what is achieved in the Pulsed Gradient Spin Echo experiment. The motion is described in the form of a Displacement Power Spectrum or a diffusion spectrum. We present a new MGSE pulse sequence based on CPMG-refocusing in a constant gradient, that enables diffusion measurements with chemical shift resolution in the obtained spectra, and with higher modulation frequencies than previously obtained.[2] To avoid effects from gradient-slicing and unwanted coherences[1], while maintaining high chemical shift resolution, the samples were prepared in a shigemi tube (0.5 mm sample hight). The figure shows the obtained diffusion coefficients as a function of the modulation frequency (diffusion spectrum) in a water sample. The measurements are stable up to 1.6 KHz. Examples from applications of the method in micro-emulsions will also be presented.



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P664

Shuttling and mixing of hyperpolarized solutions in dissolution DNP NMR experiments

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DNP at cryogenic temperatures in conjunction with sample dissolution can provide a signal enhancement of a factor of 10^4 for single-scan liquid-state NMR spectroscopy.[1] The drawback is that the high polarization decays after dissolution with the longitudinal relaxation time constant $T1$ which limits the time period during which the signal can be detected with an appreciable enhancement. On the other hand the strong signal enhancement allows to overcome the sensitivity limitation of conventional NMR and makes it possible to directly observe low concentrated heteronuclei in a single acquisition. Dissolution DNP-NMR seems ideally suited for studies of the dynamics of unstable or quickly changing molecular systems, such as ligand molecules binding to receptor proteins.[2]

Here, we demonstrate practical solutions for fast and robust sample shuttling to a high field magnet after dissolution from a standalone polarizer.[3] To extend dissolution DNP spectroscopy to the study of dynamical changes on the molecular level it is frequently necessary to rapidly mix two liquids immediately before the NMR signal is acquired. While the first liquid contains the highly polarized spin system, which may be a ligand or a protein, the other solution could contain either receptor molecules or small molecules that trigger a particular dynamical process of the hyperpolarised molecule. We demonstrate here our implementation of a fast mixing strategy and apply it to a simple protein ligand system.

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P665

A Comparison of the PGSE and MGSE Pulse Sequence in Measurements of Diffusion Spectra

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Diffusion spectrum measurements are a powerful tool in studies of restricted diffusion and with it related properties of matter. A diffusion spectrum $D(\omega)$ is equal to the Fourier transform of the velocity autocorrelation function and therefore holds information on molecular displacements in different time scales. While unrestricted diffusion can be measured by virtually any pulse sequence that includes magnetic field gradients diffusion spectra can only be measured by pulse sequences that have a distinct single peak in the frequency spectrum of effective magnetic field gradients divided by frequency squared.[1] An example of these is the modulated gradient spin-echo sequence (MGSE), which consists of a constant magnetic field gradient and the CPMG RF pulse train that causes alternation of the effective magnetic field gradient. The MGSE sequence results in the echo signal attenuation with the attenuation exponent proportional to the diffusion spectrum at the gradient modulation frequency. The entire diffusion spectrum is then measured by repeated MGSE experiments with changing gradient modulation frequency. Most commonly used pulse sequence in diffusion measurements, the pulsed field gradient spin-echo sequence (PGSE), has an effective gradient of which frequency spectrum divided by frequency squared has a broad distribution extending from zero frequency to $2\pi/\Delta$ (where Δ is the time interval between both magnetic field gradient pulses of the PGSE sequence) and is therefore not suitable for measurements of diffusion spectra. However, this broad distribution can be narrowed significantly if signals of two PGSE sequences with slightly different Δ are subtracted. Therefore, PGSE sequences with incrementing Δ intervals also enable measurements of diffusion spectra. However, there remain problems in the proposed MGSE subtraction method that are associated with decreased sensitivity and contamination due to higher harmonics in the effective gradient spectrum.

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P666

Simulation and fitting of ESR spectra from macromolecules undergoing global and internal dynamics using stochastic trajectories

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The global and internal motions of proteins occur on similar time scales. In addition, intra- and inter-domain dynamics of multi-domain proteins tend to be coupled during functionally relevant conformational transitions. Identifying and characterizing correlated global and internal motions using magnetic resonance spectroscopy is notoriously hard. In quantitative analysis of electron spin resonance (ESR) spectra one typically attempts to simulate the spectrum from first principles starting with a prospective model of the conformational transition. A flexible computational scheme for simulating ESR spectra of both diffusive and jump-like motional models from stochastic realizations of their trajectories was recently developed.[1] Thanks to its modular nature the approach easily handles complex dynamical models with motional coupling. Here, the formalism is extended along five different lines: (1) Nitroxide spin labels containing both ^{14}N and ^{15}N are treated. (2) Spectra at several different frequencies are simulated simultaneously for almost no additional computational cost. (3) Forbidden transitions occurring at high magnetic fields are treated. (4) Spectra of two dipolar-coupled spin labels subject to slow molecular motions are simulated. (5) Automated fitting to experimental spectra for some of the magnetic and motional parameters is implemented. With these additions the developed computational approach becomes a powerful interpretative tool for continuous-wave ESR spectroscopy of biomolecules containing a single or two dipolar-coupled spin labels.

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P667 (*)

Measurements of Quadrupolar Coupling Constants in Deuterium Labelled Ubiquitin

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Measurements of ^{2}H relaxation rates in deuterated proteins, particularly $R^Q(D_+)$ and $R^Q(D_z)$, have been used to characterise dynamic parameters in protein side chains and more recently backbone positions.[1,2] ^{2}H relaxation rates are dominated by the Quadrupolar Coupling Constant (QCC), which allows for ease of analysis in order parameter determination and model-free analysis. Conversely, if the dynamics are well characterised, through alternate relaxation analysis or molecular dynamics simulations, the QCC value can be determined through these measurements. Presented is the experimental determination of $R^Q(D_+)$ and $R^Q(D_z)$ rates for D^N and $D^{C\alpha}$ in ^{2}H labelled ubiquitin in order to directly determine QCC variability in these sites.[3] In addition, an indirect determination of the QCC values in the C_α position has been obtained through scalar coupling of the second kind. While a uniform QCC value has been established for methyl groups, QCC values for the D^N as well as the $D^{C\alpha}$ deuterons are correlated with the inverse cube of D···O=C distances and apparent hydrogen bonding.[4,5]

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P668

ESR-spectroscopy Investigation of Free-Radicals in Macromolecular Antimutagens Based on Chitosan

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In view of the deteriorating environmental radiation background, the development of approaches to the creation of highly effective antimutagenic systems (among them of polymeric nature) is currently an urgent problem.

This work is devoted to the development of new water-soluble macromolecular antimutagens based on non-toxic biodegradable polycation chitosan and plant antioxidants added to the side chain of polymer. For revealing of possible correlation between a number of hydroxyl groups in structure of a low molecular weight antioxidant and protective efficiency of macromolecular antimutagens**,** new water-soluble conjugates of chitosan were synthesised from plant antioxidants - gallic and syringic acid, accordingly. The row of antioxidant containing (in quantity from 1 to 3% of weights) water-soluble conjugates of chitosan was synthesised and characterised.

To realize the possible mechanism of such systems protective action, and also to compare the substance radiation resistance and its antimutagenic efficiency, the investigation of the free radicals generated at low-temperature (77K) radiolysis of polycations has been carried out via ESR spectroscopy method.

It has been shown that the structure of both the relative contribution of radical and anion-radical intermediates depends on the type of phenolic antioxidants in a side chain of polymer.

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P669

Heteronuclear relaxation measurements as a new method to study anisotropic supramolecular structures

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Heteronuclear $^{13}C^{\alpha}$ relaxation measurements are applied in a novel method to investigate the organization of a supramolecular assembly. The assembly of the pore forming cyclic lipodepsipeptide pseudodesmin A[1] into supramolecular structures of indefinite size in non-polar organic solvents will be reported. Based on the monomer conformation of this small peptide building block and diffusion data, a model was previously proposed for the pseudodesmin A self-assembly.[2] Here, heteronuclear $^{13}C^{\alpha}$ relaxation behaviour is exploited to validate this model.

The $^{13}C^{\alpha}$ relaxation rate constants, R_1 and R_2, are known to be very sensitive to the degree of anisotropy of the molecular object and to the orientation of the CH bond vector, as described by Woessner.[3] By confronting R_1/R_2 ratios with the CH bond vectors within the known monomer conformation, the orientation of the monomers within the supramolecular assemblies can be assessed. The rotational diffusion coefficients of the assemblies can be obtained from the data, leading to their average dimensions. In the case of pseudodesmin A, it is demonstrated that the length of the cylinder like structures increases with concentration, while the diameter remains constant. In addition, the orientation of the monomer molecules vs. the direction of growth reveals the surface area where the intermolecular contact takes place, which for pseudodesmin A is in agreement with an end-to-end helix stacking, validating the proposed model.

We demonstrate that this method can be used for the study of the self-assembly of small molecules into anisotropic supramolecular structures, provided they contain sufficient distinguishable CH (or NH) groups that sample various orientations. The main advantages of this technique are that it provides structural information about the orientation of the monomer molecule within the assembly and that it can be applied in the solution state.

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P670

Remote detection of a Xenon-based Molecular Sensor

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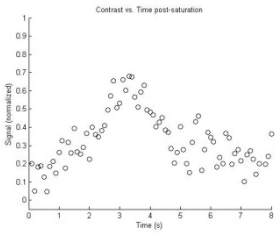
Xenon-based molecular sensors (sensors) are attractive molecular imaging contrast agents due to their sensitivity to their local environment. In combination with chemical exchange saturation transfer of hyperpolarized nuclei (Hyper-CEST)[1] and optimization of the detected signal via remote detection,[2] these sensors promise to be useful tools in both microfluidic-based chemical analysis and *in vitro* bioassays, where multiple microscale assays can be sensitively read out with a single detector. Depletion of MR signal by Hyper-CEST is achieved by pulsing at the sensor-associated ^{129}Xe resonance frequency. The exchange of saturated xenon out of the sensor, into the bulk pool, leads to a detectable decrease in the MR signal of free xenon in water. Here, a one second pulse was applied by a commercial 30 mm probe to depolarize sensor-associated hyperpolarized ^{129}Xe as water passed through a region containing the sensor (on). Immediately following application of the pulse, the xenon solution peak was stroboscopically read out via a home-built detection probe tuned to the xenon resonance frequency. The same sequence was then repeated with the saturation pulse applied equidistant downfield of the xenon solution peak in order to compensate for RF effects (off). The normalized contrast, (off – on)/ off, is shown in the figure to the right. We observe a clear region of saturation ~ 2.7 seconds following the onset of detection.



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P671
Validation of Quantitative NMR Analysis of Fatty Acid in Phamaceutical Excipients

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NMR is by definition a quantitative spectroscopic tool because the intensity of a resonance line is directly proportional to the number of resonant nuclei.[1] Quantitative NMR spectroscopic methods are widely used nowadays because they can be considered primary method of measurement considering the criteria in the CCQM definition.[2,3]

Taking advantage of metrological properties of quantitative NMR, in the present work experiments were performed for the determination of a known component in a mixture of fatty acids in commercial products which have castor oil as pharmaceutical excipient. The sample was used without previous sample preparation, fatty acid separation, purification or derivatization by traditional chromatographic methods.

^{1}H NMR spectroscopy was performed using standard reference which was used by a stem coaxial insert placed into the sample tube. The methodology was validated and accuracy, linearity (> 0.999%), limits of detection (0.13 %) and quantification (0.34%), and ruggedness were established. As a result it was found that the maximum combined measurement uncertainty is 1.6% for a confidence interval of 95%.

The methodology makes possible wide-spread application especially for complex mixtures of fatty acids in a better way than traditional analytical methods. No separation or sample preparation is necessary, in a short time of analysis with high precision and accuracy.

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P672
ISD: latest improvements

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Extracting the essential constraints that allow protein structures to be determined.

Protein Structure determination methods often use NOESY-derived distance restraints. This data can be both inconsistent and of varying quality depending on the position in the sequence of the involved residues. Here we use two tools, FIRST[1] and QUEEN,[2] to narrow down a set of constraints to it's essential constituents. Using the ISD software package,[3] we were able to show that convergence is maintained even after deleting 11 out of 12 restraints for some simulations, without substantial degradation of the main structural features. Suppressing these restraints also lowered the variance of the positions of the atoms and shows that these tools could help to make datasets more consistent.

Improvements of ISD's replica-exchange sampling scheme.

We implemented Tsallis sampling on the whole posterior probability function, asynchronous and all-pairs replica-exchange, and an optimization scheme that automatically adjusts the parameters to achieve even acceptance ratii.

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P673

SSA: An iterative algorithm for suppression of spectral artifacts in multidimensional spectra obtained by random sampling and non-uniform Fourier transform

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Owing to its resolving power, multidimensional NMR methods became a routine in biomolecular research. However, the classical approach to acquisition of ND spectra requires the sampling theorem to be fulfilled. As a consequence, maximum evolution times in indirectly detected dimensions are usually limited already for 3D experiments. In other words, one has to accept the trade-off between broad lineshapes and reasonable experiment duration.

Spectral resolution can be enhanced with the use of non-uniform sampling of evolution time space, accompanied with an appropriate processing. Non-uniform Fourier Transform (nuFT), being one of the most straightforward approaches, yields reliable estimates of spectra, and is capable of handling randomly sampled signals. Although this kind of analysis is sufficient for spectra containing a moderate number of peaks in small dynamic range, some applications (like NOESY) needs a more sophisticated treatment of acquired data.

Here we present an efficient algorithm for removal of artifacts in randomly sampled 3D and 4D spectra. The signal separation algorithm[1] (SSA) follows the CLEAN principle, which was previously adapted to NMR also by other authors.[2,3] It was shown that the algorithm preserves relative peak intensities,[1,4] and has various potential applications, including the most demanding NOESY spectra. It is also demonstrated that the results are competitive to those obtained by other popular methods, namely maximum entropy reconstruction and multidimensional decomposition.

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P674

^{1}H NMR spectroscopy as an alternative tool for the detection of γ-ray irradiated meat

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The effect of γ-ray irradiation on the fatty acid profile of chicken meat was examined at doses of 0.5, 2.5, 5.0, 7.5, 10.0 and 15.0 kGy by ^{1}H-NMR spectroscopy. A method based on the employment of integral intensities of the signals in specific spectral ranges was used to detect chemical changes in the lipid moiety of the food during the irradiation treatment. NMR spectral results revealed a clear dose-dependent effect of irradiation on the fatty acid profile. A trend toward an increase in the amount of saturated fatty acids and a decrease in the amount of polyunsaturated fatty acids in the triacylglycerol composition of the irradiated meat samples compared with the non-irradiated one was established with increasing the irradiation dose. The trend of decreasing polyunsaturated fatty acyl groups was associated with a decrease in oxidative stability of meat fat after application of γ-ray irradiation. The results of this study demonstrated that ^{1}H-NMR spectroscopy could be used as a simple, very fast and complementary alternative to the cumbersome and time consuming GC/MS analytical method EN 1785[1] or/and EN 1784,[2] respectively, due to its capability of immediate quantifying saturated, mono- and polyunsaturated fatty acids in irradiated meat without any sample work-up.

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P675

Cell-free expression and liquid state NMR Spectroscopy for structure determination

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Despite major technical advances in methodology structure determination of membrane proteins by NMR spectroscopy still poses a significant challenge. It was demonstrated that these problems can be overcome, allowing for the sequential backbone assignment of membrane proteins with the aid of cell-free expression systems. The spectral overlap often observed for helical MPs can be resolved using selective labelling strategies. Within the CF system efficient amino acid type selective labelling with almost all amino acid types is possible with minimal metabolic scrambling; whereas in bacteria this is restricted to certain amino acid types, or usage of auxotrophic strains is required. Given its open nature the CF system ensures complete control over the amino acid pool of the reaction, any non-labelled amino acid type can be exchanged by its labelled derivative which instantly ensures 100% label incorporation into the synthesized protein without any background labelling and loss in yield. Labelling strategies such as the transmembrane segment enhanced labelling relying on the use of six amino acids (AGLFIV) that predominantly cluster in the TM region and the combinatorial labelling have been developed. These strategies enable the unambiguous identification of consecutive amino acid pairs and/or stretches that can be subsequently used as anchor points for the backbone assignment and further structural analysis.

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Study of polymer translational dynamics by NMR modulated gradient spin echo

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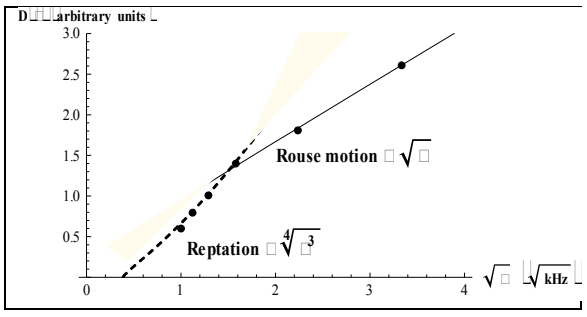
The dynamics of polymer chains in a melt is a complex multi-body problem that has to take into account the entanglements of the chains and their time fluctuations. Two of the most widely used theories for polymer melt dynamics reduce the problem to a single chain motion in an effective medium: the Rouse model for the simple case of unentangled chains (Rouse 1953) and the reptation model for entangled chains (de Gennes 1979, Doi and Edwards 1989). However, the experimental or computer-experimental evidence for the quantitative reliability of the models is not particularly strong. Time window of NMR pulse gradient spin echo, which is used to determine features of polymer dynamics by measuring self-diffusion motion, is limited to above 2 ms and cannot view the full range of segmental and reptation displacements in dense polymers. Novel technique of NMR modulated gradient spin echo method[1] is able to view faster molecular translations by providing the velocity autocorrelation spectrum of molecular motion, D(ν), to about 100 kHz (10 μs). Method can be used to measure D(ν) of polymer segmental motion . As shown in figure, the obtained experimental data for a melted polymer confirms theoretical prediction about the crossover from the Rouse to the reptation dynamics that appears, where D(ν) goes from $\nu^{3/4}$ (reptation) into $\nu^{1/2}$ (Rouse) dependence. Shape of spectrum gives the values of relevant dynamic parameters.



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P677

Relaxation Behavior of Hyperpolarized ^{83}Kr and ^{129}Xe in the Presence of Surfaces

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Longitudinal relaxation of noble gas isotope ^{83}Kr (I = 9/2) is primarily dominated by quadrupolar interactions during periods of surface adsorption. Relaxation behavior of ^{83}Kr has been previously studied under different temperatures,[1] surface-to-volume ratios,[2] surface hydration[3] and chemically modified surfaces[2,4] that were further utilized as an MRI contrast.[4] Utilizing a remote detection scheme,[1] whereby relaxation is allowed to occur in one location while being detected in a secondary location, longitudinal relaxation of hyperpolarized (hp) ^{83}Kr and ^{129}Xe was studied on surfaces at various magnetic field strengths. In addition this scheme was used to the gas-phase relaxation behavior of ^{83}Kr and ^{129}Xe in the presence of a breathable mixture of oxygen (~20%). Remarkably on a stainless steel surface, at any magnetic field strength, the longitudinal relaxation time for ^{83}Kr is longer than the longitudinal relaxation time for ^{129}Xe. This is attributed to the larger gyromagnetic ration of ^{129}Xe, making ^{129}Xe ~50 times more sensitive to paramagnetic species than ^{83}Kr. In the presence of a breathable mixture of oxygen the longitudinal relaxation time of ^{83}Kr is reduced by ~30% of the ^{83}Kr relaxation in the gas phase. Although reduced, this relaxation time is still significantly longer than when a metallic surface is present, suggesting that surface relaxation is still the dominant mechanism even in the presence of a paramagnetic species. This permits future *in vivo* lung studies where breathable amounts of oxygen can be present and still obtain surface dominated relaxation data.

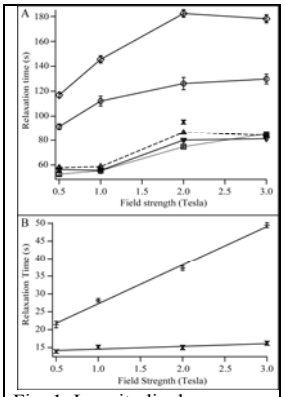

Fig. 1. Longitudinal relaxation times of hp ^{83}Kr (A) and ^{129}Xe (B) on different surfaces plotted against field strength. Signal intensity obtained by remote detection is fitted to a mono-exponential function to obtain T_1.

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P678

Multithreaded Simulation Of Solid State NMR Spectra on CUDA Enabled Video Cards

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Computer simulation of solid state NMR spectra can provide additional information on the structure of the material. It is possible to determine the shielding and coupling parameters by means of total lineshape analysis of a static or MAS spectra.[1] There are some programs for this purpose, for example the SIMPSON[1] simulation package and the Solids module of Bruker's Topsin program.[2]

These simulations can be time consuming calculations. The spreading of multicore processors gave the possibility to reduce runtime by distributing the calculation on multiple CPUs. Even faster calculations can be performed on GPGPU (General Purpose Graphic Processor Unit) based NVidia video cards implementing the CUDA programming language extension. Reports on very fast simulations can be found in the literature those are based on this parallel multiprocessor architecture[3] but these methods have not appeared on the field of solid state NMR spectrum simulations yet.

We have applied the CUDA technique to paralellize the solid state NMR spectrum simulations. The resonance frequency of many crystalline orientations are calculated by separate threads parallelly. The program can handle CSA, dipolar couplings, quadrupole interactions and J-coupling by means of perturbation theory.

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P679

Towards a High Temperature Superconducting NMR beyond 1GHz: Field Stabilization for Solid-state NMR in a 500MHz HTS NMR

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Achieving a higher magnetic field is important for higher sensitivity, better resolution and for solid-state NMR. However, a Low Temperature Superconductor (LTS) are incapable of generating in excess of 23.5 T. Our project replaces the innermost Nb_3Sn coil of the 920 MHz NMR with a Bi2223 High Temperature Superconductor (HTS) coil for 1.03 GHz NMR operation[1] within fiscal year 2010. As the first step, we have developed a 500 MHz NMR with a Bi-2223 HTS innermost coil and have resolved inherent difficulties such as (i) the coil winding using the fragile HTS tape conductor (ii) the magnetic field fluctuation by an external DC power supply because of the small residual resistance and joint resistance of the HTS coils, which prevent the persistent mode of operation and (iii) relaxation in the screening current induced in the HTS tape conductor. An external lock system has been developed for solid-state NMR to compensate these magnetic field fluctuations and it consists of an NMR microcoil and a frequency counter to measure the field fluctuations. The obtained ^{13}C NMR spectra of adamantane with this system shows that the field fluctuations are stabilized less than 0.04 ppm for more than 24 hours. The 2D-^{13}C solid-state NMR spectrum of isoleucine and ^{17}O NMR spectrum of tripeptide were also acquired. These results prove that HTS technology, potentially making magnets higher magnetic field and smaller size, will open up new horizons on the NMR world.

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P680

NMR Hydrogen Exchange Study of Tyrosine Hydroxyl and Cysteine Sulfhydryl Groups by Deuterium Isotope Shift Effects

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An NMR hydrogen exchange study is a powerful method for investigating protein dynamics. However, there are few corresponding studies on the side-chain hydroxyl (OH) and sulfhydryl (SH) groups, despite their structural and functional importance. The difficulty of an NMR study on the OH/SH groups arises from the fact that the NMR signals of the OH/SH protons are likely to undergo exchange-broadening, due to the faster exchange of the OH/SH protons with the solvent water, in comparison to that of the amide protons.

Here we present a new NMR method for investigating the hydrogen exchange rates of OH/SH groups. In our approach, the signals of carbon atoms directly attached to oxygen/sulfur atoms are observed, as an alternative to the OH/SH proton signals, by using proteins selectively labeled with a newly synthesized amino acid (i.e., ζ-SAIL Tyrosine and [3-^{13}C:3,3-$^{2}H_2$] Cysteine). In an H_2O/D_2O (1:1) solution, the carbon atoms give rise to signals split by a deuterium isotope shift effect with a size of ~0.1 ppm, if the exchange is slow (k_{ex} < ~10 s^{-1}); otherwise, they are observed as averaged signals. Furthermore, when the exchange rate is on the order of 0.1 to 10 s^{-1}, inter-isotopomer ^{13}C-exchange peaks can be observed, which enable its quantitative evaluation.[1] The peak volume ratio between two isotopomers also provides information on the proton/deuterium fractionation of OH/SH groups.[2] We will demonstrate the applicability of this method for the OH groups of Tyrosine and the SH groups of Cysteine in the 18.2 kDa *E. coli* peptidyl-prolyl *cis-trans* isomerase b.[1,2]

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P681

On the force acting on nuclei in a magnetically levitating body

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The subject matter of this work is to study the effect of nuclear magnetism to the balance of forces in magnetically levitating bodies. Levitation of water[1] is known to require vertical magnetic field gradient exceeding ca. ~1400 T/m^2, which is so strong that only limited institutes equipped with world-leading strong magnets can demonstrate the striking levitation experiments. On the other hand, paramagnetic oxygen assisted magnetic levitation, known as magneto-Archimedes levitation,[2] requires less strong field gradient and feasible with widespread superconducting magnets used for NMR spectroscopy. In order to manipulate the nuclear magnetization in a levitating water drop, an NMR probe was built with a sealed sample container, in which oxygen gas up to 1 MPa can be supplied and liquid water can be injected. The events inside the container can be monitored with a CCD camera placed inside the bore of the magnet. Magneto-Archimedes levitation was successful in the fringe field of a wide-bore 14 T superconducting magnet. Also, a tuned saddle-coil is placed inside the sealed container, so that rf irradiation can be applied to the proton spins. The plan of this project is to observe what happens to the levitating water when the proton magnetization is inverted.

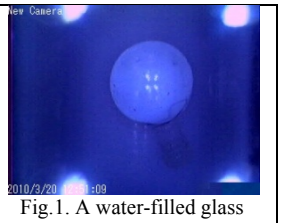

Fig.1. A water-filled glass sphere (5 mm diameter) levitating by the magneto-Archimedes effect.

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P682

Alignment induced TROSY shift changes applied to protein structure determination

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^{1}H-^{15}N TROSY signals of protein are changed from their isotropic positions, when it is placed in a weakly aligned state. The induced TROSY shift differences are determined by the combination of the residual dipolar couplings (RDC) and also the residual chemical shift anisotropies (RCSA). The alignment induced TROSY shift changes, thus, can be used to determine the alignment tensor for the protein.[1] Because TROSY spectrum gives ^{1}H-^{15}N correlation peaks in higher resolution relative to HSQC, the TROSY-based alignment tensor analysis allows us to obtain global structural information even for large protein to which the approach using RDCs cannot be readily applied. This approach allows us to determine the accurate domain orientations in proteins over the size limit in the conventional NMR spectroscopy.[2]

In spite of the avid advantages above, there are some practical drawbacks in this approach. One comes from the co-linearity between NH bond vector and the least shielded CSA principal axis, which attenuates the magnitude of the alignment induced TROSY shift changes. The other is from the variations in ^{15}N CSA values of amide groups, which have apparent dependency to local structures. In this presentation, I will demonstrate the drawbacks are practically overcome to allow the TROSY-based alignment analysis, by tuning the conditions for aligning proteins and the modification to the tensor calculation. The details in our remedy will be shown with some results obtained from the application to various proteins.

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P683 (*)

Relaxometry of singlet nuclear spin states

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Singlet states are identified with the term 'long-lived' nuclear spin order because their relaxation occurs *slowly* in comparison to T_1 and other constants. As symmetry dictates, the singlet decay constant – 'T_S' – of a spin-½ pair is insensitive to relaxation mechanisms perfectly correlated across the nuclei. Best regarded is the case of immunity to the intra-pair dipolar coupling. For proton systems, especially, where the singlet is excited between two geminal nuclei (a CH_2 pair), lifetimes up to $T_S/T_1 \sim 40$ can be observed. This is otherwise to say that singlet relaxation, 'rather unusually', is dominated by mechanisms *asymmetric* across the pair. In this light our presentation will show some examples of the information content accessible using T_S-*relaxometry*.

The attention will be given to proton relaxation mechanisms characteristic of >2-spin systems. Amongst these, 1) for proton singlet relaxation at the H_2C^{β} environment of amino acids and small peptides, the effect of out-of pair dipole couplings will be discussed as probes of local molecular conformation, particularly vicinal bond torsion angles; 2) we will quantitatively interpret paramagnetic-induced singlet relaxation caused by aqueous metal ions at μM concentration levels. Analytical rate formulae and symmetry properties of the relaxation will be given to emphasise the differences in T_S- and T_1-type information. We will outline the good application potential to infer about molecular conformation, binding, and dynamical processes in solution.

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P684

Characterization of high susceptibility porous systems by low-field 2D NMR relaxometry

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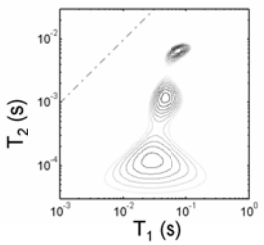
Natural and manufactured macroporous materials are often rich in magnetic impurities and, hence, characterized by T_1-T_2 asymmetries since T_2, unlike T_1, is proportional not only to pore-size, but also to internal susceptibility differences.[1,2] We use T_1-T_2 maps of pore-filling water as low-field NMR characterization of high-susceptibility porous media.[3] Clay-based materials were chosen as model specimens for this study, to properly modify their porous and susceptibility features by acting on time and temperature of sintering. The T_1-T_2 correlation maps furnish a detailed picture of the structural rearrangements induced by even small changes in the sintering parameters, so providing a NMR map for materials, and for related manufacturing processes as well. With this demonstration experiment we show the potential of T_1-T_2 correlation maps in the detection of porous and magnetic environments for water trapped in porous systems, so addressing the feasibility of this NMR method also for on site applications.



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P685

Single-Scan Multinuclear NMR at Earth's Field using Para-Hydrogen Induced Polarization

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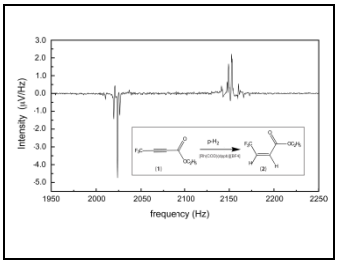
An approach to earth's field NMR of dilute samples, based on nuclear spin hyperpolarization by para-hydrogen induced polarization (PHIP)[1] is presented. Hyperpolarization of ^{1}H and ^{19}F nuclear spins was achieved by using an optimized adiabatic magnetic field cycling scheme[2] following para-hydrogenation of 310 µl fluorinated alkyne, 9 mM in deuterated acetone. Simultaneous NMR detection of ^{1}H and ^{19}F was realized at earth's field with just one single scan using a conventional inductive coil. Because of the small sample volume, the earth's field NMR spectrum could be acquired indoor, in close proximity to armed concrete floor and ceiling, with no appreciable degradation of the signal lineshape. A polarization enhancement of 10^{8} compared to thermal equilibrium was measured for both nuclei. Our results indicate that PHIP can be used to substantially lower the detection limits of earth's field NMR, extending the area of potential applications of this technique.[3]



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Hyphenated NMR Structure Calculation -fast structure calculations using composite protocols for monomers, homo dimers and homo multimers

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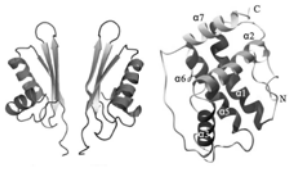
The calculation of structures from NMR restraints, often for molecules that cannot be crystallised, is still a strong driver in solution state NMR laboratories. However, we have found that achieving initial convergence in iterative assignment/structure calculations (in our case using ARIA2[1]) is often a distinct problem unless extreme care is taken with defining a set of clean set of nOe peaks and in many cases an initial set of definitive assignments for a subset of peaks.



Recently two protocols have been published that allow the calculation of backbone folds to be carried out with some rapidity with relatively poor nOe data (PASD/MARVIN[2]) or chemical shift data only (CS Rosetta[3]). Here we show that using these structure calculation methods to achieve initial folds (validated using residual dipolar couplings [RDCs]) while using ARIA2[1] as a refinement protocol allows the rapid calculation of complete nOe structures at high resolution with good speed. The results are especially interesting in the case of homo-dimers (and multimers) as initial backbone folds can be calculated using chemical shift based protocols without the need for the use of ambiguous distance restraints and symmetry restraints. These monomer structures can then be assessed for accuracy using RDCs, assembled using RDC based symmetry models and finally refined using combined nOe assignment/structure protocols to give a complete structure.

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P687

Spherical Tensor Selection in High Resolution Solution-State NMR

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In Nuclear Magnetic Resonance, the spin density operator is described by a superposition of orthogonal spin operators, these spin operators are usually differentiated using their *coherence* order. On the other hand, Spherical Tensor Selection can decompose the NMR signal into components passing through different spherical tensor operators.[1] We implemented Spherical Tensor Selection by exploiting the rotational symmetry of the density operator components in all three rotational directions, generating new types of phase cycle.

In this poster, two-dimensional correlation experiments that generate similar signals to double quantum-filtered COSY experiments will be presented. In addition, signals similar to triple-quantum-filtered COSY and E-COSY experiments will be discussed. Finally, the use of field gradient pulse for phase cycling simplification will also be explored.

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P688

Experimental aspects of orientation selective PELDOR in W-band

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For organic radicals, PELDOR experiments at high magnetic fields contain information not only about the distance between the paramagnetic species but also about their relative orientation. The three-dimensional biradical structure is encoded in a complex pattern of orientation selective PELDOR traces. This is crucial information to study conformational changes of labelled macromolecules. However, the execution of orientation selective PELDOR experiments, particularly in the case where the labels are not collinear, is aggravated by a narrow bandwidth of a single mode resonator. This was the main motivation to develop an experimental setup that allows performing PELDOR experiments with a variable separation of pump and detection frequencies up to 350 MHz. We present both the test experiments and the comparative PELDOR experiments performed with the use of the commercial spectrometer setup on the model biradical systems with non-collinear orientations of g-tensors. The experimental aspects of high-field PELDOR as well as some characteristic features of the observed PELDOR modulations are discussed.

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TR-EPR study on excited states and radicals photoproduced in conjugated oligomers

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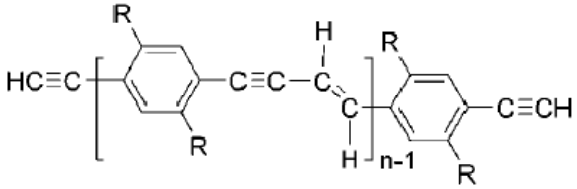
A fluorescent conjugated polymer, **1**, (E)-Dioctyloxy poly(p-phenylene-ethynylene-vinylene) (PPEV derivative) with low molecular weight (M_n~2840), has been recently synthesized and characterized by UV-visible, fluorescence, and NMR spectroscopies.[1]

1



R= OC_8H_{17}

In this study we present the further investigation on **1**, performed with conventional EPR, Time Resolved EPR (TR-EPR), and CV techniques. The polymer has been studied both in frozen solution and in form of thin solid film. UV-vis spectra and CV measurements have been employed for the determination of the polymer band gap in solution. TR-EPR spectroscopy with pulsed laser photoexcitation at 532 nm, has been used to produce and study the excited triplet state of the polymer. Blends (1:1) of **1** and PCBM have been prepared as thin solid films. The spin polarized paramagnetic species produced by laser irradiation in the pure polymer and in the blends have been observed and characterized with TR-EPR.

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P690

Improved approach for simultaneous collection of three-dimensional NOESY $^{13}C,^{15}N$–HSQC data

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We have revisited the pulse sequence for the simultaneous three-dimensional NOESY $^{13}C,^{15}N$–HSQC experiment. We report a modified approach that provides better water suppression, better spectral quality, and sensitivity comparable that achieved by acquiring the ^{13}C- and ^{15}N-NOESY spectra independently, but improved compared to previous simultaneous approaches. These improvements are accomplished by using Watergate to suppress the solvent peak while preserving water magnetization; the latter is critical for obtaining ^{15}N-NOESY sensitivity equivalent to that of an independently acquired spectrum. The only drawback of our approach is that the Watergate step wipes out resonances near the water, most notably from $^{13}C^{\alpha}$. Because of this, we optimize the carbon spectral window for maximal signal and resolution from methylene and especially methyl groups. Given these features, our simultaneous experiment is ideally suited for collecting NOESY spectra in water with samples of medium to large proteins that are uniformly ^{15}N labeled and protonated only at the methyl positions. For uniformly ^{13}C labeled protein samples, a second 3D experiment must be collected to get NOESY peaks from $^{13}C^{\alpha}$ groups, and for this, we propose using a ^{13}C-NOESY experiment based on the sensitivity-enhanced ^{13}C-HSQC pulse sequence, with the carbon window optimized for $^{13}C^{\alpha}$ groups. This approach yields good water suppression and good S/N for the $^{13}C^{\alpha}$ groups, and the data can be acquired in less time than for a regular full aliphatic carbon NOESY experiment.

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Optimization and Evaluation of Dynamic Nuclear Polarization in Aqueous Solution at 15 MHz/9.7 GHz

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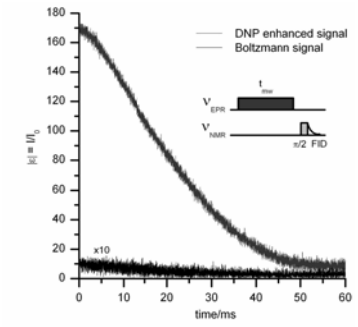

Dynamic nuclear polarization (DNP) is emerging as a potential tool to increase the sensitivity of NMR aiming at the detection of macromolecules in liquid solution. One possibility for such an experimental design is to perform the polarization step between electrons and nuclei at low magnetic fields and transfer the sample to a higher field for NMR detection.[1] We describe the optimization of a polarizer set up at 0.35 T (15 MHz ^{1}H NMR/9.7 GHz EPR) based on commercial hardware.[2] With the nitroxide radical TEMPONE-D,^{15}N in water a maximum DNP enhancement of – 170 on the water ^{1}H is observed at room temperature by irradiating on either one of the EPR lines.

To evaluate the Overhauser mechanism governing DNP in liquids, water ^{1}H relaxation rate measurements have been performed as a function of magnetic field from 0.00023 to 9.4 T.[3] The relaxation profiles were analyzed according to the full theory for dipolar and contact relaxation to estimate the coupling factor responsible for solution DNP effects. Additionally, the saturation level of the two hyperfine lines was investigated by pulsed ELDOR experiments. When fully saturating one line, the total saturation level was found to be 0.8 for 10 mM polarizer concentration – well consistent with the coupling and enhancement factors.

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P692

Characterization of correlated dynamics on fast timescales in biomolecules by Heteronuclear Double-Resonance methods

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In a heteronuclear two-spin system (e.g., $I = H^N$ and $S = {}^{15}N$) undergoing correlated chemical exchange the cross correlation of the fluctuations of the isotropic chemical shifts causes a differential relaxation of double- (DQ) and zero-quantum (ZQ) coherences that induces cross relaxation between multiple-quantum (MQ) coherences (e.g., $H_xN_x \rightarrow H_yN_y$).[1] The study of chemical-exchange-induced MQ cross relaxation can provide a wealth of information about the kinetics, structural changes and thermodynamics of correlated dynamic processes involving two-spin systems embedded in biomolecules.[2] We show here that the new Heteronuclear Double Resonance (HDR) methods that we have recently introduced[3,4] permit, through the observation of the cross relaxation of MQ coherences, a full characterization of correlated dynamic processes occurring in proteins on fast timescales (tens of μs), not accessible to other MQ NMR methods. Much like other relaxation dispersion methods, the chemical exchange contribution to cross relaxation is quenched by the applied *rf* fields. Inspired by earlier works,[5] we present a fully analytical model leading to a remarkably compact expression that describes the dependence of the MQ cross-relaxation rate on the *rf* amplitude. This expression has a similar structure as the relaxation dispersion for single-quantum (SQ) coherences under CW spin-locking, modified by a correction factor that takes into account the phase-alternating double-resonance features of our method.

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P693 (*)

The Haupt effect under static and magic-angle spinning conditions

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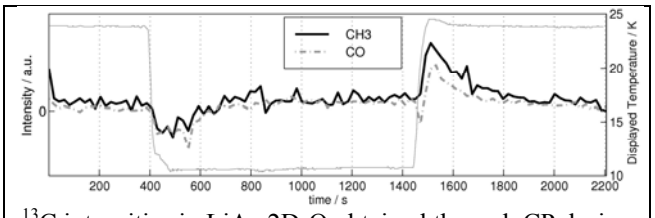
It has been shown that the Haupt effect can be used as a source of dynamic nuclear polarization (DNP) in solid-state NMR. We report on the progress in attempting to use this method under magic-angle-spinning conditions (MAS) and unravel the detailed mechanism of this complicated effect.



^{13}C intensities in LiAc.2D_2O obtained through CP during the Haupt experiment under MAS

In 1973 Haupt reported the observation of a strong increase in dipolar order in gamma-picoline after quickly raising the temperature from 4 to 50 K. Over many hundreds of seconds a dynamic NMR signal appeared that constituted an increase of dipolar order by a factor of some 10000. The effect was explained by Haupt by assyming a coupling of the librational state of the tunneling methyl group to the spin states, and a second-order phonon-driven transition that leaves the Zeeman order unaffected while populating levels that correspond to so-called dipolar order. Despite much experimental and theoretical work on spin conversion processes in methyl groups, much of the details of the Haupt effect remain poorly understood today.

Driven by recent interest in hyperpolarization methods, and encouraging results obtained in our group that showed that the Haupt dipolar order can be transferred to other nuclei, we have applied the Haupt effect under MAS conditions. Our results indicate that the effect works under MAS, but the efficiency is currently unclear. This, however, appears related to difficulties in converting the dipolar order into observable order under MAS, rather than changes to the spin conversion process. A detailed description of the generation of dipolar order under static and MAS conditions will be presented and compared to the Haupt order. Novel mechanistic aspects of the Haupt effect will be addressed using experiments performed under static conditions.

P694

^{1}H-DOSY NMR for the polymer mass distribution of aged phenolic resins

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^{1}H-DOSY NMR can be an attractive method for determining the polymer mass distribution of paint polymers. Size Exclusion Chromatography is the first choice for delivering Mn and Mw information. However, for some polymer types interaction occurs with the styrene-divinylbenzene particles of the column.

The ability of ^{1}H-DOSY NMR is tested for obtaining the polymer mass distribution of a polyphenolic resin that is suspected of interaction. At first the best ^{1}H-DOSY NMR method is set up by studying: (a) the best temperature domain for a Mw range up to 100,000 g/mol, (b) effect of spinning or non-spinning the NMR sample tube on the repeatability, (c) the maximum diffusion time that can be used in relation to T_1 relaxation, (d) the number of gradient steps for maximum reproducibility at low analysis time, (e) choice of solvent, (f) best CONTIN calculation or Filt method and (g) internal standard or not.

The best ^{1}H-DOSY NMR method we found uses: a temperature of 35°C, spins the NMR tube at 20Hz, 64 gradient steps with 32 scans for 2 mg/ml polymer solution, approx 200 ms as the maximum diffusion time, internal standard TMS, and Filt method is as good as CONTIN (RegMult 0.001, Simpson, DFmin 2, DISPmax -10.5).

This ^{1}H-DOSY NMR method is applied on several aged polyphenolic resin samples. The resins are dissolved in DMSO-d6 and a calibration curve of polyethylene glycol is used for calculating diffusion coefficient into log(M). The polymer mass distribution results show an increase in Mn and Mw when the solid content of the polymer solution is higher, storage time or temperature (RT-50°C) is increased. The results compare well with SEC.

P695

Shimming based on B_0 field mapping for spectroscopy experiments using multiple coils and receivers

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Dissolution DNP (Dynamic nuclear polarization) is a technique that provides high non-thermal polarisation for liquid state NMR experiments.[1] In conjunction with fast 2D spectroscopy methods[2] it is well suited to provide information about intra molecular connectivity. In order to test the idea of extending this technique, so it can also provide dynamical information about the sample, a probe that has the ability to acquire spectroscopic data from various locations of the sample was built.[3] With this probe head, comprising of two radio-frequency coils tuned to the ^{1}H frequency of 400 MHz, fast 2D COSY spectra from two positions of the sample were successfully acquired using a BRUKER AVIII spectrometer console with a dual receive and transmit setup.

A weakness that was identified in this method was the difficulty of achieving good shimming and hence narrow line widths for both coils at the same time. Thus a strategy for automated simultaneous shimming was developed and tested.

It is based on acquiring B_0 field maps from the two coils and determining the required shim currents by calculating a least squares fit to minimise the residual B_0 deviations.[4] Very good agreement between the predicted and the obtained shimmed field maps has been achieved. The line shape and signal intensity could be improved. This allows extending the multi coil method to samples where high resolution is needed. Further work is in progress to improve this strategy.

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P696

Assignment of protein resonances based on novel multidimensional techniques

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Employment of random sampling of evolution time space allows to increase the spectral resolution and dimensionality without increasing experimental time.[1] In resulting spectra peak overlap is remarkably reduced. A further improvement is the processing method – sparse multidimensional Fourier transform (SMFT). Basing on peak positions form a lower dimensional spectrum (e.g. 3D HNCO), only those 2D cross-sections of spectra are calculated, in which peaks appear. This significantly simplifies the spectra analysis and saves disk space.

In 4D HNCACO, 4D HNCACACB and 5D HN(CA)CONH assignment of H_N, N, C', C_a and C_b resonances is based on finding peaks of the same position in two 2D cross-sections, which indicates, that these cross-sections originate from adjoining amino acid residues. Thus, the cross-sections are sorted and finally arrays of cross-sections are obtained.[2] Assignment of the arrays to respective fragments of polypeptide chain is based on length of arrays and some amino acid specific information (e.g. reverse sign of glycine peaks in some spectra). Due to high resolution and dimensionality, ambiguities in interpretation of spectra are very rare, which makes the approach promising for automatic assignment procedures.

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P697

5D ^{13}C-detected NMR experiments for backbone assignment of unstructured proteins with a very low signal dispersion

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Two 5D NMR experiments for backbone resonances assignment of disordered proteins will be presented. The pulse sequences exploit relaxation properties of unstructured proteins and combine the advantages of 13C direct detection, non-uniform sampling, and longitudinal relaxation optimization to provide unambiguous assignment of unstructured proteins with highly repetitive sequences in favourable man and machine time. Each experiment provides resolution that allows for an unambiguous assignment from a single spectrum. The performance of the pulse sequences will be shown on an example of partially disordered delta subunit of RNA polymerase from *Bacillus subtilis*. The unstructured part of this 20 kDa protein consists of 81 amino acids with frequent sequential repeats. Backbone resonance frequencies of all nuclei were unambiguously assigned by each experiment.

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P698

NMR as a tool for reversible and irreversible protein heat denaturation studies

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Detailed description of heat denaturation is important for understanding protein function and for its modification by techniques of protein engineering. This presentation demonstrates that NMR can be used as a powerful tool for such studies. One-dimensional NMR spectra reflect presence of well-defined three-dimensional structure of the studied protein. Such spectra also offer certain selectivity – analysis of the methyl and aromatic regions provides insight into the packing of the hydrophobic core of the studied enzyme, while the amide region reflects changes of backbone conformation. As such, NMR represents a complementary approach to differential scanning calorimetry. A denaturation curve can be obtained by recording a series of spectra at gradually increasing temperature. We designed a protocol allowing to quantify the structural changes by calculating integral of absolute values of spectral differences.[1] We also developed numerical fitting procedures for both reversible and irreversible denaturation. The procedure allows to evaluate enthalpic and entropic contributions in a case of sufficiently sampled temperatures. The methodology is presented on examples of two proteins – delta subunit of RNA polymerase from *Bacillus subtilis* and non-specific lipid transfer protein 1from barley. The former protein exhibits a typical reversible denaturation, while the later one denaturates irreversibly at extremely high temperatures (approximately 110 °C). In order to monitor protein behaviour as such high temperatures, a special set-up (sealed tubes and a choice of suitable probe-head) was used.

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Acknowledgments: This work was supported by the Grants 1M0570 , MSM0021622413, and LC06030 of the Ministry of Education, Youth, and Physical Culture of the Czech Republic and by Grant AV0Z40310501 of the Academy of Sciences of the Czech Republic.

7.6 CERM – The Host Institution

Posters

P699

Before CERM…

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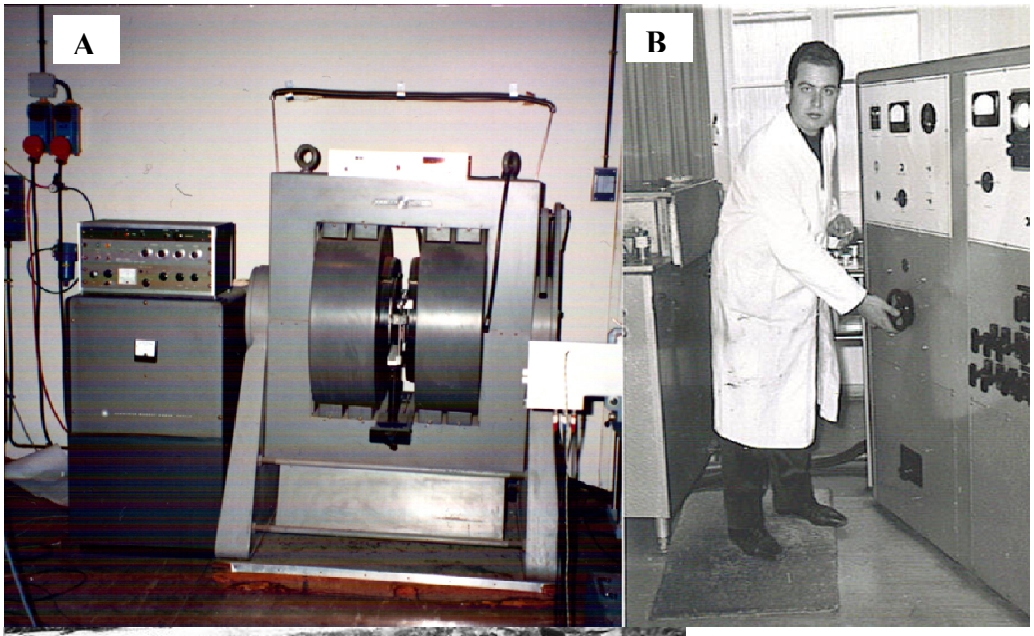
It was the year 1967…



-60

P700

NMR for the design of superoxide biosensors

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The effect of introducing positive charges (lysines/arginines) in human cytochrome *c* on the redox properties and reaction rates of this protein with superoxide radicals is studied.[1] The mutant proteins are investigated by NMR spectroscopy to obtain information on the protein fold and to characterize heme iron environment. Further, the mutants are studied using cyclic voltammetry and amperometry to get information on the electrochemical properties and the sensing characteristics for the detection of superoxide. Best behaving variants correspond to residues clustered in a specific protein surface area. NMR coupled to Energy Minimization calculations provides a rational for the higher sensitivity of some mutants with respect to the wild-type-based sensor.

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P701

Metals in Protein Structures: Classification and Functional Prediction

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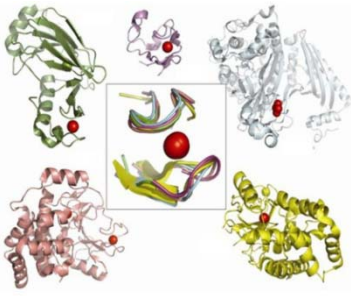
The PDB contains a large fraction of metal-binding structures reflecting the importance that metals play in proteins. Indeed, it was estimated that a large fraction of proteins encoded by living systems are metalloproteins[1] and about 40% of all structurally characterized enzymes perform metal-dependent reactions.[2] Notwithstanding, bioinformatics resources devoted to the study of metals in biological systems have been scarce and largely unsuccessful so far, most likely due to the difficulty to establish formal criteria to describe the exceptional variety of metalloproteins.



In this scenario, we proposed to describe metal sites as 3D models which include the coordination sphere and its close surroundings.[3] This representation allows metal sites to be compared in a systematic and largely automated fashion, thus allowing: (i) a formal classification of metal sites in structures providing an ideal framework for the development of a new database aimed at containing the available information on metalloproteins,[4] (ii) the design of new web tools for the study of metalloproteins[4] such as Metal-Finder, which is aimed at predicting the function of metal sites in new structures, as well as the presence of metal sites in new structures unloaded with their metals. This is especially helpful for Structural Genomics projects. Additionally, this approach constitutes a useful basis to perform detailed comparative analyses addressing issues that range from the structural properties to the evolution of metalloproteins.

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4. Project funded by the Italian Government (FIRB - "Futuro in Ricerca")

P702

Applications of Metabolomics in Cancer Research

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Metabolomics is the global quantitative assessment of endogenous metabolites within a biological system. This evolving field may have a multitude of uses in oncology, including the early detection and diagnosis of cancer and new insights on metabolic fluxes. No serum-based test is currently of sufficient sensitivity or specificity for widespread use in oncology. NMR-based metabolomics profiling paired with *ad hoc* statistical analysis, is used to provide a metabolomic picture of the colorectal and breast cancer pathology[1,2] and of treatment effects. Elucidation of cancer metabolism may provide essential insights into both the intercellular environment and host/tumour interaction, allowing recognition of new biomarkers for diagnosis and prediction of outcome, new therapy targets and novel approaches for monitoring response and toxicity. Our results show how metabolomics can single out a clear signature of these two type of cancers and provide a new analytical tool in the area of cancer biomarker discovery. Moreover, metabolomics has been used to identify a better characterization of the disease status as disease progression, metastasis localization, inflammatory process and enlighten not diagnosticable cases of micrometastasis.[3]

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P703

NMR-fingerprinting contribution to define Standard Operating Procedures for biobank samples

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Metabolomics is an emerging area of research focused on measuring the ensemble of small molecules (MW < 1500 Da) in biospecimens. NMR-based metabonomics, in its most popular version, consists of recording and analyzing 1D ^{1}H NMR profiles of biological fluids such as serum and urine. Metabolomics has established itself has a powerful tool for the high-throughput fingerprint of biofluids. It provides a biochemical snapshot from a human body fluid: changes from "normality" are detected and correlated to the presence of a disease, its progression or remission, drug toxicity, etc.[1] The reliability of the approach requires that the chemical nature and the relative concentration of the metabolites present in the biofluids are neither affected by the preanalytical conditions nor by the analytical methodology. In this sense, NMR of biofluids represents an election technique in order to define Standard Operative Procedures (SOP) for the handling of biological specimens. Possible critical points in the pre-analytical workflow of the various biospecimens (blood, urine and tissues) have been identified and used to assess sample quality upon identification of analytes able to provide the chemical signature of different degradation processes.[2-4]

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P704
Structural and functional characterization of S100 proteins

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The S100 family is a group of small, acidic, EF-hand calcium binding proteins that modulate various cellular functions like mobility, growth, differentiation and secretion.[1] S100s act intracellularly as Ca(II)-signalling and Ca(II)-buffering proteins and several members are secreted into the extracellular space where they interact with receptors on the cell surface.[2] These proteins have gained a growing interest due to their deregulated expression in cardiomyopathies, neurodegenerative and inflammatory disorders, and certain cancers.[1]

A large set of the S100s and their most relevant protein targets have been and expressed and characterized in order to clarify the molecular mechanisms by which these proteins regulate cell activity. The structural and dynamical features as well as the metal binding properties of S100A13,[3] S100A5,[4] and S100A16 have been already investigated by NMR, X-ray and ITC. The interactions of selected S100 proteins such as S100B, S100P and S100A5 with intra- and extra- cellular partners such as p53 and RAGE receptor are being investigated for a functional characterization of target-S100 recognition and binding. Mutation on calcium binding site have been designed to produce proteins that contain a high-affinity lanthanide-binding site for structure refinement and interaction studies.

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P705
Prediction and Analysis of Metalloproteomes

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Genome-wide studies are providing researchers with a potentially complete list of the molecular components present in living systems. However, there is currently a lack of well-established experimental methods to analyze the complete set of metalloproteins encoded by an organism (the metalloproteome). This information is essential for a comprehensive understanding of the processes occurring in living systems. Predictive tools must thus be applied to define metalloproteomes.

In this scenario, we developed bioinformatics methods[1], based solely on protein sequences, for the prediction of metalloproteins. With these methods, it is possible to scan entire proteomes for metalloproteins which are identified by the presence of specific metal-binding sites, metal-binding domains, or both. The predicted metalloproteins can be then analyzed to obtain information on their function and evolution. As case studies, we predicted the content of zinc, non-heme iron, and copper proteins in a representative set of organisms taken from the three domains of life. The zinc proteome represents about 9% of the entire proteome in eukaryotes, but it ranges from 5% to 6% in prokaryotes. In contrast, the number of non-heme iron proteins is relatively constant in eukaryotes and prokaryotes, and therefore their relative share diminishes in passing from archaea (about 7%), to bacteria (about 4%), to eukaryotes (about 1%). Copper proteins represent less than 1% of the proteomes in all the organisms studied. The programs used for these searches have been implemented in a single package.

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P706

Monitoring the oxidative protein folding in mitochondria by NMR

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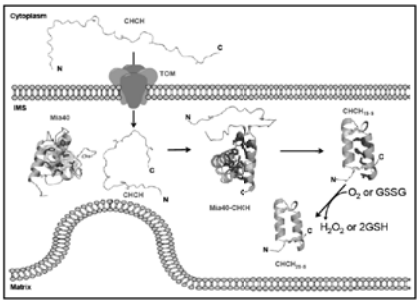
Protein folding occurs through a number of states that are often dependent on the cellular compartment and compartment-specific protein components that facilitate the folding process. This is the case of a large share of mitochondrial intermembrane space (IMS) proteins (CHCH), characterized by an alpha-helical hairpin structure bridged by two intramolecular disulphide bonds. Once imported in the IMS, CHCH folding process exploit an electron cascade pathway which involve two proteins, in human named Mia40[1] and ALR, responsible for catalyzing the disulphide bond formation. We describe, for the first time at the molecular level, the folding process of a CHCH protein starting from its unfolded state through all of the intermediate steps.[2] We found that a CHCH protein is largely unfolded in the cytoplasm and that Mia40 in the IMS induces a conformational transition in a specific targeting region of the CHCH protein,[3] from an unstructured to an α-helical state, upon the formation of an intermolecular disulphide bonded CHCH-Mia40 complex. We also reconstructed the molecular level the electron cascade mechanism involving Mia40 and ALR. In conclusion, we defined the electron transfer and molecular recognition processes at the basis of the cellular pathway responsible for the mitochondrial import of CHCH proteins.



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P707

Single Protein Labelling (SPL) for In Cell NMR Studies

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The in-cell NMR spectroscopy is one of the most powerful tools for the investigation of biomolecules in vivo. Our goal, together with a large number of researchers, is to be able to see and study a particular protein inside its own cell, especially inside human cells. Nowadays "in human cells NMR" remains a dream but big efforts are in progress in this field, focusing the research on some well known systems, such as *E.coli*. In-cell NMR is possible only when the protein of interest is strongly overexpressed in the bacterial cell.[1,2] Switching the culture medium from a unlabelled medium (biomass accumulation) to an isotopically labelled one, at the moment of protein induction, generate an excess of the target labelled protein inside the cells. In this way, also other components of *E.coli* (polymerases, antibiotic resistance, nucleic acids etc…..) are labelled as well. All those components together form a background of resonances that interferes with the protein signals. Thus when the expression level of the target protein is lower than hundreds of μM[2], the background can cover the protein's signals.

The single protein labelling (SPL) method described here is an alteration of SPP, single protein production,[3] method. The system is designed to obtain high quality bacterial sample for in-cell NMR where only the protein of interest is isotopically labelled. The induction of the MazF toxin, used to convert cells into a "living bioreactor",[4] is followed by the medium switch and by the induction of the target ACA-less gene. This procedure is able to generate the exclusive labelling of the biomolecule of interest.

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P708

Aspects of Mechanistic Systems Biology in copper homeostasis

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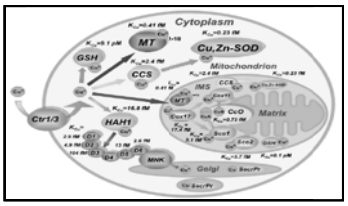

Mechanistic Systems Biology can be defined as an understanding the mechanisms of life at the molecular level and of modeling them in such a way to be able to predict outcomes when a given organism assimilates food, drugs, or other chemicals. That can be done only taking into acount of the dynamic structure of proteins, the specificity of protein-protein interactions and the resulting properties of molecular machines, pathways and entire networks taking place in the cell. Thus once copper is imported into the cell, several pathways involving a complex system of copper proteins is responsible for trafficking it specifically where it is required for cellular life, while minimizing toxicity. The factors driving the copper transfer between protein partners along cellular copper routes are, however, not fully rationalized.

In our recent study[1] we provide the thermodynamic basis for the kinetic processes that lead to the distribution of cellular copper. In this work we determine, through a unified electrospray ionization mass spectrometry (ESI-MS)-based strategy, in an environment that mimics the cellular redox milieu, the apparent Cu(I)-binding affinities for a representative set of intracellular copper proteins involved in enzymatic redox catalysis, in copper trafficking to and within various cellular compartments, and in copper storage. The resulting data show that copper is drawn to the enzymes by passing from one copper protein site to another, following the gradients of increasing copper-binding affinity. This result complements the finding that fast copper-transfer pathways require metal-mediated protein–protein interactions and therefore protein–protein specific recognition.[2]

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P709

Iron uptake by ferritin: the biomineral precursors

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Ferritin directs the reversible biomineralization of iron. X-ray crystallography has recently provided the structure of the iron ions bound at the catalytic ferroxidase site. Through a combination of solid state and solution ^{13}C-direct detection NMR experiments,[1,2] partial sequence specific assignment of resonances was achieved of the 480 kDa bullfrog ferritin, an homopolymer composed by 24 subunits with 4-helix bundle structure. Paramagnetic broadening in solution ^{13}C-^{13}C NOESY spectra, induced by iron(III) products emerging from the ferroxidase site, traced their progression to the biomineralization central cavity. Magnetic susceptibility measurements support the formation of ferric multimers as biomineral precursors. Non-native metal ions are able to bind ferritin, slowing down the oxidation process of iron at the ferroxidase site and may be used to shed further light on the metal ion trafficking pathways within this nanocage protein.

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P710

Solid-State NMR and In-Cell NMR Studies of SOD1 Folding and Amyloidogenesis

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One of the cellular defence systems from oxidative stress is the Cu,Zn-superoxide dismutase (SOD1), a 32 kDa dimeric protein containing one zinc and one copper ion in each of its subunit. The incorporation of metal ions is crucial for the structural stability and enzymatic activity of SOD1. *In vivo* these events are conducted under a complex mechanism. SOD1 has been genetically linked to the familial amyotrophic lateral sclerosis (fALS), a fatal neurodegenerative motor neuron disease. Massive researches on fALS support the popular amyloid hypothesis that the misaggregation of SOD1 is the hallmark of the disease onset. Recently, emerging evidences have raised a general SOD1 aggregation mechanism starting from wild-type (WT) or fALS-associated SOD1 mutants lacking metal ions.

In vitro solution NMR studies highlight a dramatic protein flexibility of apoWTSOD1 which makes accessible conformations prone to oligomerize, while the rigid structure of the metalated protein is unable to do it. In order to verify this behaviour in an environment approximating the intracellular one, we have performed *in-cell* NMR experiments on WTSOD1 in bacterial cells to obtain information on the SOD1 folding states in the cytoplasm.

Solid-state NMR (SSNMR) methods have been also applied to characterize the apoWTSOD1 amyloids. First, with the help from available solution and solid-state NMR assignments on SOD1 in different metallation states, over 90% residues were assigned with SSNMR spectra of [^{13}C, ^{15}N]-uniformly labelled apoSOD1 microcrystals. These data outline that the conformation of the long loops of apoWTSOD1 are restricted due to supramolecular packing. On the contrary, SSNMR spectra fingerprinting of apoWTSOD1 soluble oligomers and insoluble fibrils indicates that the protein experience significant structural changes with respect to the apoSOD1 microcrystals. In conclusion the results here are building up together a systematic, multiscopic view on the mechanism of SOD1 amyloidogenesis.

P711

The evolution of Structural Biology towards the philosophy of INSTRUCT

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Background: INSTRUCT (Integrated Structural Biology) is a European Research Infrastructure within the ESFRI (European Strategy Forum for Research Infrastructures) Roadmap,[1] and responds to the new perception that Structural Biology should employ an integrated approach among all possible technologies and not be based solely on solving the structures of single proteins or even complexes. The results are framed within a cellular context, thus allowing the characterization of cell components and molecules as well as functional processes at atomic resolution to obtain a complete description of the system. This novel Structural Biology approach has a central position within Mechanistic Systems Biology.

A Dream - Cell localization of metal ions and proteins: monitoring the functional states of proteins: Our expertise regards metal ions in cells, their uptake, traffic and excretion. For certain metal ions, metal trafficking occurs by exchange from a protein donor to a protein acceptor through a metal mediated protein-protein interaction. Some of these proteins are unfolded in certain compartments and fully mature in others where they exert their function. One perspective is to couple the above studies with in-cell NMR. Regarding the latter, some progress has been made in *E. coli*, while results in eukaryotes have been substantially less and more scattered. Over-expression methods could be developed in eukaryotic cells in order to permit NMR studies within organelles.

At present, the in-cell localization of metalloproteins through X-ray metal fluorescence suffers because there is either not enough resolution to distinguish the organelles, or there is not enough sensitivity due to low metal concentration. X-ray bioimaging at synchrotron facilities may allow further progress to overcome some of these problems.

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P712
Role of dynamics in MMPs activity

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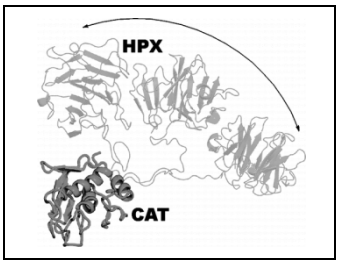
Matrix metalloproteinases are a class of proteolytic zinc enzymes involved in the degradation of several extracellular proteins, included extracellular matrix components and extracellular domains of cell-surface receptors. The uncontrolled or the pathology-driven activity of MMP is associated to a large set of diseases such as rheumatoid arthritis, autoimmune diseases and cancer. Over the last years we investigated the different steps of the hydrolytic process involving the catalytic domain for their obvious implications in drug design. We are now carrying out an extensive analysis of the structural and dynamical features of the full-length enzymes in order to clarify the mechanisms for substrate recognition before its hydrolysis. In particular, for MMP-1 and MMP-12, the role of the hemopexin domain (HPX) in substrate recognition has been investigated and the interdomain flexibility well established.[1-3] The NMR analysis of the interaction between MMP-1 and a triple helical peptide has provided details on the structural bases of type I collagen degradation by collagenases.



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P713
In vitro studies of SOD1 aggregation and its linkage with ALS

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that results in the death of motor neurons. An inherited form of ALS has been linked to mutations in the gene encoding for the Cu, Zn superoxide dismutase (SOD1). SOD1 is a dimeric metal binding enzyme; each subunit contains one zinc and one copper ion which are necessary respectively for the structural stability and activity of the protein. ALS-related defects in SOD1 result in a gain of toxic function that coincides with aberrant oligomerization. The presence of aggresomes rich in SOD1 protein in the neuronal tissues of ALS patients is one of the neuropathological hallmarks of ALS. However little is known about the origin and the architecture of these aggregates.

Previously we characterised human SOD1 with respect to its ability to form oligomers.[1] It was observed that WT SOD1 and various mutants, when lacking both metal ions, oligomerize under physiological conditions through the oxidation of free cysteines (Cys 6 and Cys 111), thus forming high molecular weight soluble oligomers which could constitute the toxic species existing prior to formation of the insoluble aggregates.

Now, in order to better elucidate the role of the two cysteines residues in the initial steps of the process, we are tracking the evolution during time of mixtures of the two mutants C6A and C111S by solution NMR and Mass spectrometry. Another target is to structurally characterize the dimeric apoSOD1 protein and the high molecular weight apoSOD1 assemblies by performing SSNMR. Microcrystals, oligomers and fibrils of apoSOD1 were investigated. Finally our studies focused on the search of potential inhibitors of the oligomerization process. Several compounds known to interact with thiol groups of cysteines were tested and we discovered that the anticancer drug cisplatin is inhibiting the SOD1 oligomerization *in vitro* by binding to Cys 111 as revealed by X-ray and NMR analyses.

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P714

The dual functions of the Sco protein family: copper chaperone and thioredoxins

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Sco is a family of proteins ubiquitous to all kingdoms of life. Ortholog and paralog genome browsing has shown that more than one representative of this class are often present in bacterial and eukaryotic genomes. Sco proteins have been first suggested to be involved in copper ion delivery to the cytochrome *c* oxidase. Accordingly, the structures of Sco proteins from bacteria and eukaryotes[1-3] revealed a copper binding site constituted by two Cys residues from a CXXXC motif and an additional conserved His residue. However, the fold of Sco proteins showed a structural similarity to thioredoxin family, suggesting that an oxidoreductase activity may be an important aspect of Sco protein function. The two cysteines of the CXXXC motif have been proposed to be the active-site residues of a thiol disulfide oxidoreductase function. This hypothesis has been supported by a structure of human Sco1 showing a metal ion bound by the two oxidized Cys residues of the CXXXC motif[2] and by simultaneous copper and electrons transfer events involving human Sco1.[4] Evidences of the thioredoxin function has been substantiated for bacterial Scos from *Thermus thermophilus*[5] and *Pseudomonas putida*.[6] All these results indicate that Sco proteins may exhibit more than one function that include copper transfer and/or redox activities. In this frame, specific sequence variations occurred during the evolution can result in fine structural changes influencing the reduction potentials of the CXXXC cysteines and their metal binding affinity.

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P715

Dynamical properties of extracellular signaling proteins: NK1 a case study

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Extracellular proteins regulate the cellular activity by interplaying with cell-surface proteins. NK1 is a splice variant of the multidomain growth factor HGF/SF, that consists of the N terminal (N) and the first kringle (K1) domains. HGF activity is involved in cell proliferation, survival, differentiation and motility[1] but its aberrant activation has been associated with tumor growth, invasion, and metastasis.[2]

The dynamical features of NK1 tandem construct have been investigated in solution on ^{15}N-^{13}C-enriched samples obtained from E. coli, using new high-yield expression protocols.[3] The reorientation of the backbone NH vectors with respect to the magnetic field occurs on a timescale that is faster than the rotational time of dimeric NK1 conformations, observed in X-ray structures, although NK1 construct exhibits a propensity to aggregate at sub-millimolar concentrations. The relaxation data suggest a degree of interdomain flexibility, without major contacts between the two domains. The present findings may provide new insight into molecular mechanism of HGF activity and relevant hints for drug design.

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P716

NMR to study weak metal-mediated protein-protein interactions

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Living organisms have developed specific processes to control metal transport and incorporation in the recipient proteins. In this way, it is made sure that the right metal is incorporated in the right protein. This is often accomplished through specific transport pathways where a metal ion, as it enters the cell, is coordinated by a specific protein that delivers it to a selected partner protein, either as a final metal recipient or as one of the steps in the metal transfer pathway. Metal transfer occurs through protein-protein interactions mediated by the presence of metal ions. This is particularly true in the case of copper(I),[1,2] for which several solution structures of metal-mediated complexes are available [3;4]. In particular, the metal-mediated interactions through which the copper(I) ion is transferred from an Atx1-like soluble copper(I) chaperone to one of the soluble domains of a membrane-bound ATPase have been extensively studied.[3;4]

Several proteins involved in different metal transport pathways feature similar metal-coordination sites. This in principle might lead to the coordination of the wrong metal ion. Indeed, some proteins exhibit a higher affinity *in vitro* for a non-physiological metal ion. Therefore, in addition to the affinity of the different proteins for the various metal ions, also the protein-protein interactions play an important role in controlling the transfer of the metal ions from one partner to another, by determining molecular recognition between the partners. The aforementioned solution structures of complexes together with model structures, which can be obtained by a combination of homology modelling, sequence conservation analysis and docking, allowed us to separate the energetics of interaction in terms of protein *vs* metal contributions.

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P717

Direct Detection in Paramagnetic Proteins: from evaluation of Residual Dipolar Couplings involving non Detected Protons to Measurements of C'N Multiple Quantum Relaxation Rates

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^{13}C direct detected *protonless* NMR experiments avoid losses of information caused by fast relaxation or exchange broadening of ^{1}H signals and may therefore offer better sensitivity, particularly in the presence of paramagnetic ions. Residual dipolar couplings (*rdc*) have been shown to be precious as structural restraints and as parameters to investigate dynamics. Pulse sequences have been developed to show that $H^{\alpha}C^{\alpha}$ and $H^{N}N$ *rdc* can be obtained through ^{13}C detection even when the proton lines are very broad, or even not detected.[1] Similarly, fast ^{1}H transverse relaxation may quench coherence transfer in many conventional NMR experiments used for dynamics studies. Indeed, direct detection of ^{13}C nuclei offers an alternative route to the measurement of relaxation rates of C'N multiple-quantum coherences, which may contribute to a more complete description of backbone dynamics in proteins as a complementary tool to conventional techniques that focus on the relaxation of isolated ^{15}N and ^{13}C nuclei.[2]

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P718

^{13}C direct detection in solution: a new chapter in biomolecular NMR

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The development of experimental approaches to overcome the limits of ^{13}C detection in solution in parallel to an increase in instrumental sensitivity has brought ^{13}C direct detection in the suitable range for biomolecular applications. A set of exclusively heteronuclear NMR experiments has recently been proposed and can be used to achieve complete sequence specific assignment of a protein in solution and to determine useful observables that contain structural and dynamic information. These NMR experiments provide additional, in some cases unique, information to that available through ^{1}H detected NMR experiments. Initially developed to study paramagnetic biomoluecules these experiments are generally applicable for the structural and dynamical characterization of proteins in solution. They result particularly effective for the study of intrinsically disordered proteins or proteins fragments opening the way for their characterization directly in-cell. In fact, ^{13}C NMR spectroscopy has opened a new chapter for biomolecular NMR.

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P719

Enhancing resolution and sensitivity for solid-state NMR of microcrystalline proteins

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In recent years the combined progress in sample preparation, probe and magnet technology, and experimental schemes has opened the way to the structural characterization of biological macromolecules by solid-state NMR. Human superoxide dismutase (SOD), a dimeric Cu(II) enzyme of 32 kDa, still represent a challenge due to the large size of the molecule and to the short relaxation times consequence of the hyperfine interaction between the nuclei and the slow-relaxing unpaired electrons of Cu(II).

The introduction of relaxation-optimized methods for ^{13}C-^{13}C spin-state selection allowed us to greatly increase the resolution in crowded spectral regions removing the broadening due to the ^{13}C-^{13}C J-couplings. The introduction of ultrafast (60 kHz) MAS in the characterization of SOD demonstrate how low-power irradiation schemes can considerably enhance the sensitivity of multidimensional and multinuclear experiments based on both scalar- and dipolar-based transfers. This should lead to the possibility of obtaining precious structural constraints for the full macromolecular structure determination of the protein in its microcrystalline state.

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P720

Putting Order into Conformational Disorder: Maximum Allowed Probability obtained from paramagnetic NMR and SAXS restraints

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Structure and dynamics of proteins are strictly intertwined in defining the function of the proteins themselves. When they sample a wide conformational space, it is intrinsically impossible to quantify the probability of each state. Small angle scattering (SAS) techniques and paramagnetic NMR spectroscopy in solution can provide experimental observables that are weighted averages of the values corresponding to all sampled conformations. A maximum allowed probability (MAP) is defined to score each and every conformation according to its largest weight possible in any ensemble in agreement with the experimental averaged NMR and SAS data. We present the results obtained for the two-domain protein calmodulin as a test case, and we show the protein conformations with largest MAP (equal to 0.33) as well as the MAP of selected conformations previously obtained in the solid state by X-ray, and of other conformations selected to explore the whole conformational space, using SAXS data and three sets of pseudocontact shifts and residual dipolar couplings obtained after substitution of a lanthanide ion (Tb^{3+}, Tm^{3+} or Dy^{3+}) to the second binding site in the N-terminal domain. The method is universally applicable as it only requires NMR data on paramagnetic derivatives of the protein (using native metal sites or lanthanide tagging) and possibly SAS measurements.

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P721

The eNMR platform for Structural Biology

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The e-NMR project is a European cooperation initiative that aims at providing the bio-NMR user community with a software platform integrating and streamlining the computational approaches necessary for the analysis of bio-NMR data. The e-NMR platform is based on a Grid computational infrastructure. A main focus of the current implementation of the e-NMR platform is on streamlining structure determination protocols. Indeed, to facilitate the use of NMR spectroscopy in the life sciences, the e-NMR consortium has set out to provide protocolized services through easy-to-use web interfaces, while still retaining sufficient flexibility to handle specific requests by expert users. Various programs relevant for structural biology applications are already available through the e-NMR portal. The implementation of these services, and in particular the distribution of calculations to the GRID infrastructure, has required the development of specific tools. However, the GRID infrastructure is maintained completely transparent to the users. With more than 150 registered users, e-NMR is currently the second largest European Virtual Organization in the life sciences. The Xplor-NIH and AMBER portals are shown in detail. Possible applications in other domains of science are also mentioned.

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P722

The Metabonomic Signature of Celiac Disease and of Potential Celiac Disease

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Celiac disease (CD) is a multifactorial disorder involving genetic and environmental factors, thus having great impact on metabolism. Application of metabolomics to the study of CD using NMR fingerprinting has been successfully exploited by our group,[1] providing significant information on the metabolic alteration involved in the pathology. Using a combination of statistical techniques we are able to discriminate CD patients from healthy controls with high accuracy (about 84%). Altered serum levels of glucose and ketonic bodies suggest alterations of energy metabolism, while the urine data point to alterations of gut microbiota. After 12 months of gluten free diet all but one patients were classified as healthy by the same statistical analysis, with the metabolic profile reverting to the normality. Potential CD patients are subjects who do not have a jejunal biopsy consistent with overt CD, and yet have immunological abnormalities similar to those found in CD patients. We have found that potential CD largely shares the metabonomic signature of overt CD, allowing us to hypothesize that CD exists as such before intestinal damage occurs, so if the metabolic changes are (at least partially) independent of the bowel malabsorption, a deeper analysis of this fingerprint can be helpful to infer more information on the biochemistry of the disease.

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P723

Bio-NMR, NMR for Structural Biology

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Under the Seventh Framework Programme (FP7, funding scheme CPCSA) the EC is funding Bio-NMR, a new project for supporting research infrastructures for providing access and technological advancements in bio-NMR.
Bio-NMR pools pan-European resources of the most relevant bio-NMR infrastructures. Eleven partners (Consorzio Interuniversitario Risonanze Magnetiche di Metalloproteine Paramagnetiche; Johann Wolfgang Goethe Universitaet Frankfurt am Main; Universiteit Utrecht; CNRS, Rhône-Alpes Large Scale Facility for NMR; Forschungsverbund Berlin e.V.; ETH-Zürich; Masarykova University Brno, National Centre for Biomolecular Research; Kemijski Institut, Slovenian NMR Centre of the National Institute of Chemistry; The Chancellors, Masters and Scholars of the University of Oxford; The University of Birmingham; Goeteborgs Universitet) will provide access to researchers involved in structural biology. The Consortium includes seven other excellent partners (Magyar Tudomanyos Akademia Szegedi Biologiai Kozpont Enzimologiai Intezet; Max-Planck-Gesellschaft zur Foerderung der Wissenschaften e.V.; Weizmann Institute of Science; University of Warsaw; University of Aarhus; Universitat de Barcelona and the leading NMR manufacturer Bruker). Jointly, they will develop methods aimed at pushing the frontiers of biological NMR and improving the quality of access to allow users to tackle ever more challenging goals in cellular structural biology. All nineteen partners (including the company Spronk-NMR Consultancy) are involved in the networking activities. These include (1) knowledge transfer among consortium members, Bio-NMR users and other NMR researchers, (2) the demonstration to biologists of the potential of structural biology with NMR, and lowering the barriers to their becoming users, (3) interactions with industrial and medical communities, and (4) raising awareness of the impact of the results achieved through Bio-NMR among society, financing and governing bodies with the final aim of developing a plan for future self-sustainability. The overall project and its management have been conceived in coordination with INSTRUCT (An Integrated Structural Biology Infrastructure for Europe), which will contribute to the cultural frame and networking activities of Bio-NMR.

P724

A molecular view of the accessory proteins involved in iron-sulphur cluster biogenesis

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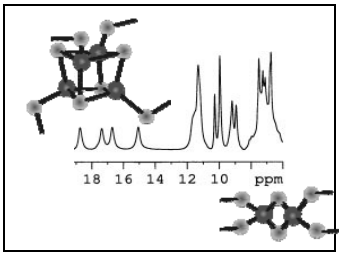
Iron is a necessary trace element found in all living organisms where it is used in the synthesis of heme, iron-sulphur (Fe/S) clusters and other cofactors. Irons in Fe/S cluster proteins are coordinated by four sulphur ions, either a thiolate group from cysteines or an inorganic sulphide ion. They have essential functions in metabolism, electron transport and regulation of gene expression. Numerous diseases have been associated with defects in Fe/S protein biogenesis. Despite the relative simplicity of Fe/S clusters in terms of structure and composition, their synthesis and assembly is a highly complex and coordinated process which yet has not been completely understood. Accordingly, the exact functions of the proteins involved in the maturation of Fe/S proteins are not known. In order to define their specific molecular roles we are structurally and biochemically characterizing these proteins as well as their interactions with protein partners with the final aim of reconstructing at the molecular level the Fe/S assembly machinery. So far all Fe/S proteins involved in the Fe/S cluster biogenesis have been found in the mitochondrial matrix and in the cytosol. Our analysis identified an Fe/S protein, Ciapin1, as a substrate of the Mia40-based import mechanism responsible for localizing proteins into the mitochondrial intermembrane space. This finding opens a new view on the involvement of this compartment in the cluster biogenesis and in the connection of the mitochondrial and cytosolic cluster assembly machineries.



8 Highlights of the Conference

Advances in Bioimaging

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Advances in Methods and Spectroscopy

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Advances in BioNMR

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These presentations will eventually be published in the *Journal of Magnetic Resonance

9 Late Abstracts

Posters

P725
NMR Studies of Viral Envelope Proteins

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Envelope proteins play critical roles in viral entry by mediating the attachment of the virus to target cells and subsequently the fusion of the viral and cellular membranes. However, to date, structural information about viral envelope is often limited to studies of isolated domains, which do not adequately represent the structure of the larger complex bound to the viral membrane. We are currently using Saturation Transfer Difference (STD) NMR to characterize small molecule binding to the viral envelope proteins from Influenza and HIV in the preattachment and attachment conformations. The interactions under study include those of Influenza HA-sialyllactose receptor analogs and HIV gp120-inhibitor peptides. In the long term, the method could be exploited to lend insight into the viral entry mechanism, identify and improve small molecule or peptide therapeutics, and identify immunological "hotspots" for the development of vaccines.

P726
Structural Analysis of the Smad2-MAN1 Interaction that Regulates Transforming Growth Factor-ß Signaling at the Inner Nuclear Membrane

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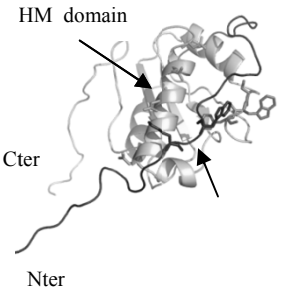

MAN1, an integral protein of the inner nuclear membrane, influences transforming growth factor-ß (TGF-ß) signaling by directly interacting with R-Smads[1]. Heterozygous loss of function mutations in the gene encoding MAN1 cause sclerosing bone dysplasias and increased TGF-ß signaling in cells. As a first step to elucidate the mechanism of MAN1, an integral protein of the inner nuclear membrane, influences transforming growth factor-ß (TGF-ß) signaling by directly interacting with R-Smads[1]. Heterozygous loss of function mutations in the gene encoding MAN1 cause sclerosing bone dysplasias and increased TGF-ß signaling in cells. As a first step to elucidate the mechanism of TGF-β pathway regulation by MAN1, we characterized the structure of the complex between the MAN1 C-terminal region and Smad2. Using NMR, we observed that this region is comprised of a winged helix domain[2], a structurally heterogeneous linker, a U2AF Homology Motif (UHM) domain and a disordered C-terminus. From NMR and SAXS data, we reconstituted the fluctuating 3D structure of the Smad2 binding region of MAN1. Our data indicate that the linker plays the role of an intramolecular UHM Ligand Motif (ULM) interacting with the UHM domain. We mapped the Smad2 binding site onto the MAN1 structure by combining GST-pulldown, fluorescence and yeast 2-hybrid approaches. The intramolecular interaction between the linker and the UHM domain is critical for Smad2 binding. On the basis of the structural heterogeneity and binding properties of the linker, we suggest that it can interact with other UHM domains, thus regulating the MAN1/Smad2 interaction.

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P727
^{31}P NMR studies of aluminophosphate species in the methanol-water mixture

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Aluminophosphate (AlPOs) system is one of the useful molecular sieves, and is widely utilized as catalysts and molecular sieves in the industrial processes.[1] Soluble aluminophosphate species, such as $[Al(H_2O)_4,(OH)(H_2PO_4)]^+$, have been suggested as the nutrients for the growth of aluminophosphate molecular sieves.[2] It is interesting to understand the effect of P and A1 concentration on the formation of soluble aluminophosphate species. Mortlock *et al.*[3] specified the presence of some complex such as $[Al(H_2O)_5(H_3PO_4)]^{3+}$, $[Al(H_2O)_5(H_2PO_4)]^{2+}$ and $[A1(H_2O)_4(H_2PO_4)_2]^+$ cations under acidic conditions. ^{31}P NMR and ^{27}Al NMR spectroscopy have been used to characterize the distribution of soluble aluminophosphate species in aqueous media[4]. Soluble aluminophosphate cations form from reactions of $[A1(H_2O)_6]^{3+}$ with phosphate ligands (i.e., H_3PO_4, $H_2PO_4^-$, and acid dimers $H_6P_2O_8$ and $H_5P_2O_7^-$).

In this work, Phosphorus-31 nuclear magnetic resonance techniques were used to characterize the distribution of soluble aluminophosphate species in methanol-water mixture. Working solutions were prepared in 5.0 ml volumetric flasks by addition of appropriate amounts of stock aluminate solution, H_3PO_4 (85%) and diluted by methanol-water mixture to the mark. Results indicated that new peaks are appeared in the ^{31}P NMR spectra by variation of methanol-water volume ratio. By considering of the ^{31}P NMR spectra it can be deduced that different species can be existence in the solution which attributed to the formation of complexes through interation of hexa-coordinated aluminum, methanol and phosphoric acid. These results help to better understanding of the synthesis of the $AlPO_4$ molecular sieves in non-aqueous media.

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P728
^{129}Xe NMR spectroscopic study of meso- and microporosity in soil components

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Pore environments of a series of samples representing porous soil constituents have been studied using conventional, i.e. thermally polarised (TP) and hyperpolarized (HP) ^{129}Xe NMR spectroscopy. Xenon gas behaved as an efficient probe for interrogating their pore structures through: i) higher sensitivity for probing micropores within polymeric organic structures as compared to common adsorption methods; ii) possibility to use elevated pressures of the adsorbate for increasing the pore accessibility; iii) evaluating not only the pore size range but also adsorption enthalpies that reflect the nature of Xe - pore surface interactions. A combination of the HP- and TP ^{129}Xe NMR was shown to be helpful for assessing the extent of pore attainability, since the latter affects relaxation phenomena which, in turn, determine appearance of the ^{129}Xe NMR spectra. First, the model samples representing soil porous components e.g.soil organic material (SOM), were tested for evaluating the electronic factors responsible for the ^{129}Xe resonance shifts detected in natural soils. By mixing model compounds in varying proportions with the following incubation experiments we tried to understand the mechanisms of interactions between the mineral and organic porous constituents. The SOM sorption within the model mineral (hydr)oxides was shown to be inhomogeneous, and the determined Xe adsorption enthalpies corresponded to the co-existing “empty” pores and those coated with organic species. At the next step, we used a combination of the TP- and HP ^{129}Xe NMR to investigate the structure of natural organic materials, such as charcoals. N_2 adsorption method has only limited application for these materials due to the restricted diffusion of N_2. In contrast, an increased sensitivity of the HP ^{129}Xe NMR allowed us detecting micropores in the studied samples. The observed differences between the HP- and TP ^{129}Xe patterns were explained by the slow xenon diffusion through the highly constricted pore system. The estimated width of those constricted pore openings was of the order of one or two diameters of the Xe atom. Similar “bottle neck” effects may also exist in the natural soil particle size fractions, as it was inferred from the increased pore access for Xe adsorption performed at elevated pressures (2-4 bar). Finally, we investigated particle size fractions derived from various soil types differing in their textural and chemical properties. Fine porosity could only be observed after the removal of SOM. Iron-induced broadening of the ^{129}Xe resonances was not directly proportional to the Fe oxide concentration. Large microporosity observed in some soils (e.g. Andosols) was attributed to the “multi-domain” structure of soil particles.

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P729

Initial mechanistic studies of small synthetic anti-microbial peptides by NMR and molecular modelling

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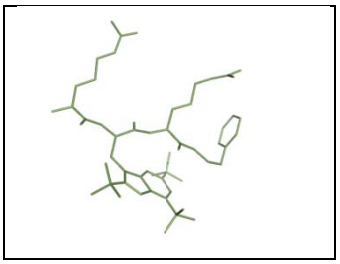
Synthetic antimicrobial peptides (SAMPs) with increased *in vitro* stability against proteolytic degradation and good selectivity for methicillin-resistant staphylococci over human erythrocytes have been developed. The exact mechanism of the antimicrobial effect is still unknown, though several models have been proposed, including pore formation, carpet models and intracellular targets.



Mechanistic studies have been initiated in an attempt to learn more about the mechanism behind the microbe killing and to allow for further optimization of the SAMPs through rational design. A number of SAMPs have been synthesised and studied in small unilamellar vesicle systems (SUVs) by liquid NMR and computer aided modelling. We here present some preliminary studies of peptide:liposome interactions.

P730

Nuclear Magnetic Resonance Studies on Amorphous and Crystalline Lanthanum-Aluminogalloborates

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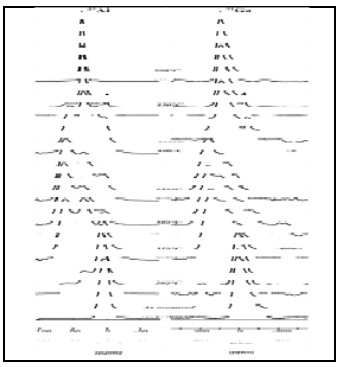
Up to now only one stable crystalline phase is known in the ternary lanthanum-aluminium-boron oxide system.[1] The composition of this crystalline phase is $LaAl_2$. $B_4O_{10.5}$ (124) and in its structure the La, Al and B atoms are surrounded by O atoms in trigonal prismatic, pyramidal and tetrahedral arrangements, respectively. It is an interesting oxide compound in which all cations exhibit less usually coordinations: lanthanum is hexacoordinated, aluminium pentacoordinated and boron tetracoordinated. This have being proved for aluminium and boron by MASNMR.[2] By substituting the aluminium with gallium it is expecting that also this atom will have similar coordination like aluminium. In order to check this hypothesis we synthesised the 124 phase where half of the aluminium have been substituted with gallium. As method of synthesis we choose the sol gel method as being much proper for following the local structural changes during the transformation from disordered system to the well-defined crystalline phase. The NMR results obtained by using an AVANCE Bruker 600 MHz spectrometer and a probe head with high MAS show that indeed the gallium took the pentacoordinated sites in 124 phase (Fig.1). By analysing the evolution of MASNMR spectra for ^{11}B, ^{27}Al and ^{69}Ga nuclei function on heat treatment temperature of the samples it was propose a model for local structure change that took place during the formation of crystalline 124 phase from its amorphous precursor.



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P731

Towards high-resolution RNA structures by solid-state NMR spectroscopy

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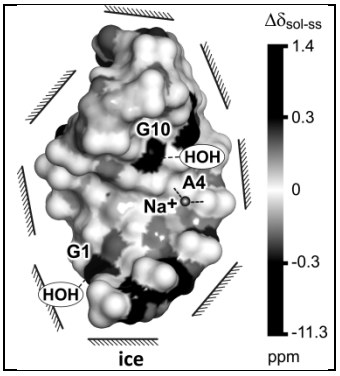
The nuclear magnetic resonance (NMR) assignment and conformational analysis of a uniformly labeled ribonucleic acid oligonucleotide (RNA) has been performed by high-resolution solid-state MAS NMR spectroscopy. A 14-mer RNA hairpin containing the very stable cUUCGg tetraloop was studied in frozen aqueous solution at 258 K. All ribose and most of the nucleobase carbon resonances, in total 88%, could be assigned in ^{13}C 2D dipolar recoupling experiments. 93% of the solid-state chemical shifts were found identical to those in solution within an average line width of 0.3 ppm. Analysis of ribose ^{13}C chemical shifts using an improved canonical equation model showed that sugar puckering modes and the exocyclic torsion angle conformers of the hairpin in ice are highly similar to that in solution. Minor modulation of the structure is attributed to a partial dehydration of RNA, binding of Na^+ ions and hydrogen bonding to water molecules at the ice interface. The results show that biologically-relevant RNAs can undergo the water/ice phase transition without significant structural changes and critical loss of NMR resolution and sensitivity. Use of uniformly labeled RNA is feasible because correlation experiments reveal remarkably sharp signals and sufficient chemical shift dispersion. Our findings pioneer the freeze-trapping studies of RNA structure-function in folding, ligand recognition and catalysis, form the basis and open new exciting possibilities for molecular analysis of RNAs and their complexes, advocating solid-state NMR to a broad RNA community.



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