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ABSTRACT BOOK

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PA-001 **Scalable biosynthesis of quantum dots: evolution of size selectivity, solubility and extracellular production**

Bryan Berger[1], Zhou Yang[2], Leah Spangler[1], Victoria Berard[1], Qian He[2], Li Lu[2], Robert Dunleavy[1], Christopher Kiely[2], Steven McIntosh[1]

1.-Department of Chemical and Biomolecular Engineering, Lehigh University, 2.-Department of Materials Science and Engineering, Lehigh University

Biological systems have evolved several unique mechanisms to produce inorganic nanomaterials of commercial interest. Furthermore, bio-based methods for nanomaterial synthesis are inherently "green", enabling low-cost and scalable production of nanomaterials under benign conditions in aqueous solutions. However, achieving regulated control of the biological processes necessary for reproducible, scalable biosynthesis of nanomaterials remains a central challenge. This is especially true of quantum dots (QDs), which are nanocrystals made from seminconducting metals whose diameter is smaller than the size of its exciton Bohr radius, leading to size-dependent changes in their optical properties. Several studies have described production of QDs from biological systems, but without control over particle size or composition. In this work, we describe the isolation, selection and characterization of a bacterial system capable of regulated, extracellular biosynthesis of metal sulfide QDs with extrinsic control over nanocrystal size. Using directed evolution, we isolated and engineered a bacterial strain (SMCD1) to (1) exhibit enhanced tolerance against aqueous cadmium acetate (2) produce soluble, extracellular nanocrystals and (3) regulate nanocrystal size by varying growth conditions. We estimate yields on the order of grams per liter from batch cultures under optimized conditions, and are able to reproduce the entire size range of CdS QDs described in literature. Furthermore, we are able to generalize this approach to not only cadmium, but PbS QDs as well. Investigation of purified QDs using ESI-MS reveals several putative proteins that may be involved in biosynthesis, and current work is aimed at improving photoluminescent properties as well as long-term aqueous stability. Nonetheless, our approach clearly demonstrates the ability of biological systems to produce advanced, functional nanomaterials, and provides a template for engineering biological systems to high-value materials such as QDs at cost and scale. This work was supported by the National Science Foundation (EFRI-1332349).

PA-002 **Protein and Cellular Engineering Platform for Selective and Inducible Apoptotic Proteolysis**

Charlie Morgan[1,2,3], Juan Diaz[3], Jim Wells[3]

1.-Chemistry and Chemical Biology Graduate Program, UCSF, 2.-Pharmaceutical Chemistry Department, UCSF, 3.-Molecular and Cellular Pharmacology, UCSF

Proteolysis is a fundamental process in biology; it plays a crucial role across development of multicellular organisms, aids in maintaining tissue homeostasis, and is integral in cell signaling. Intracellular proteolysis frequently focuses on proteasome mediated protein degradation, however the tightly regulated and selective proteolysis mediated by the cysteine-aspartyl specific proteases, caspases, leave their substrates intact. The growing list of caspase substrates now tops 1500 proteins; a key unmet question is to differentiate how individual substrate cleavages directly lead to the profound morphological transformations that are the hallmark of apoptotic cells. We employ an optimized site-specific and inducible split-protein protease to examine the role of a classic apoptotic node, the Caspase Activated DNase (CAD). We describe our engineering platform of post-transcriptional gene replacement (PTGR), where-by endogenous bi-allelic ICAD is knocked down and simultaneously replaced with an engineered allele that is susceptible to cleavage by our engineered TEV protease. Remarkably, selective activation of CAD alone does not induce cell death, although hallmarks of DNA damage are detected in human cancer cell lines. Additionally, we show the utility of our technology in deciphering synthetic lethality resulting from coordinated proteolysis of caspase substrates that control the apoptotic hallmark of chromatin fragmentation.

PA-003 **Improving microbial medium-chain fatty acid production using GPCR-based chemical sensors**

Stephen Sarria[1], Souryadeep Bhattacharyya[2], Pamela Peralta-Yahya[1]
1.- School of Chemistry and Biochemistry, Georgia Institute of Technology
2.- School of Chemical and Biomolecular Engineering, Georgia Institute of Technology

Increasing energy needs have accelerated the demand for renewable alternatives to petroleum-based fuels; engineered microbes for the production of biofuels have the potential to fulfill these energy needs. Fatty acids are the immediate precursors to the advanced biofuels fatty acid methyl esters (FAMEs), which can serve as a "drop in" replacement for D2 diesel. FAMEs derived from medium-chain fatty acids (C8-C12) have been shown to have better cold properties than traditional FAMEs (C16-C22). Here, we engineer a yeast strain for the production of medium chain fatty acids by screening different thioesterases. Our next goal is to couple a medium-chain fatty acid producing yeast strain to our previously developed medium-chain fatty acid GPCR-based sensor, in order to engineer a yeast strain with improved medium-chain fatty acid production via directed evolution.

PB-001 **Applications of 19F-NMR to study protein-ligand interactions and protein conformational changes in solution**

Martine I. Abboud[1], Jurgen Brem[1], Rasheduzzaman Chowdhury[1], Ivanhoe K. H. Leung[2], Timothy D. W. Claridge[1], Christopher J. Schofield[1]

1.-University of Oxford, Department of Chemistry, 2.-University of Auckland, School of Chemical Sciences

Nuclear magnetic resonance (NMR) is a powerful biophysical method for studying protein-ligand interactions in solution and elucidating the mechanism of action of potential inhibitors. However, protein NMR can be complicated by the overlap of 1H and other resonances, hence the resolution needed to assign spectra precisely can be hard to achieve[1]. 19F-NMR is increasingly being used to study conformational changes and protein-ligand interactions in solution because 19F is (i) a spin ½ nucleus, (ii) 100% naturally abundant, (iii) 83 % as sensitive to NMR detection as 1H, (iv) not present in most biological systems, and (v) its chemical shift is particularly sensitive to changes in local environment[2]. Recent advancements in NMR instrument and probe design have made 19F-NMR more sensitive and more widely available; consequently, 19F-NMR is finding growing application in research. Here, we report the use of 19F-NMR to study two biomedically important protein systems. Proteins can be fluorine-labelled either by biological incorporation of fluorinated amino acids or by site-specific chemical ligation[3]. 3-bromo-1,1,1-trifluoroacetone (BTFA) has been developed as a useful reagent for importing fluorine into proteins via nucleophilic substitution such as with a cysteinyl-thiol[4]. The São Paulo metallo-β-lactamase-1 (SPM-1), a B1 sub-family metallo-β-lactamase, containing only one cysteine (Cys221) coordinating the second Zn(II) cation in its active site, was 19F-labelled using BTFA. The interactions of SPM-1 with various potential inhibitors were reported by 19F-NMR, which enabled monitoring SPM-1 conformational changes on ligand binding and informed on binding strength by enabling KD measurements. In a second study, prolyl hydroxylase 2 (PHD2), an enzyme involved in human oxygen sensing, was 19F-labelled and its interactions with its co-substrate, 2-oxoglutarate (2OG), and peptide substrate (CODD) were monitored by 19F-NMR. Conformational changes of PHD2 on 2OG and CODD binding were consistent with crystallographic analyses. Finally, 19F-NMR was used to study solvent exposure of the complex and its dynamics in solution through relaxation dispersion of the 19F-nucleus at different temperatures. Overall, the results illustrate the power of 19F-NMR for monitoring ligand binding and conformational changes. [1] Wemmer, B. D. E., & Williams, P. G. (1994). Use of nuclear magnetic resonance in probing ligand-macromolecule. Methods in Enzymology. [2] Marsh, E. N. G., & Suzuki, Y. (2014). Using 19F NMR to Probe Biological Interactions of Proteins and Peptides. ACS Chemical Biology. [3] Chen, H., Viel, S., Ziarelli, F., & Peng, L. (2013). 19F NMR: a valuable tool for studying biological events. Chemical Society Reviews. [4] Rydzik, A. M., Brem, J., van Berkel, S. S., Pfeffer, I., Makena, A., Claridge, T. D. W., & Schofield, C. J. (2014). Monitoring conformational changes in the NDM-1 metallo-β-lactamase by 19F-NMR spectroscopy. Angewandte Chemie.

PB-002 **NMR solution structure of lacticin Q, a broad spectrum leaderless antimicrobial protein from Lactococcus lactis QU 5**

Jeella Acedo[1], Marco van Belkum[1], John Vederas[1]
1.-Department of Chemistry, University of Alberta

Bacteriocins are ribosomally synthesized antimicrobial peptides and proteins that can be potentially used as food preservatives and are promising alternatives to traditional antibiotics. Lacticin Q from Lactococcus lactis QU 5 belongs to an unusual class of bacteriocins classified by the absence of an N-terminal leader sequence. It is composed of 53 amino acids and has previously been reported to be active against a broad spectrum of Gram-positive bacteria in the nanomolar range. In this study, lacticin Q was expressed as a recombinant protein fused to SUMO (small ubiquitin-related modifier) protein. After cleavage of the SUMO-tag and subsequent purification, mass spectrometry was used to confirm the identity of the recombinant lacticin Q. Its three-dimensional nuclear magnetic resonance (NMR) solution structure was then elucidated. Both circular dichroism and NMR spectroscopy results reveal that lacticin Q is highly α-helical. It has a compact, globular overall fold and has a cationic surface and a hydrophobic core. These structural data support previously established mechanism of action, whereby antimicrobial activity is attributed to the binding of lacticin Q to anionic bacterial cell membrane and subsequent formation of pores that result in the leakage of cell contents. The elucidated structure of lacticin Q resembles the two-component leaderless bacteriocins enterocins 7A and 7B, which are 9- and 10- amino acids shorter than lacticin Q. This suggests that the observed overall fold might be conserved among this class of bacteriocins.

PB-003 **Sizing and interactions of proteins under native conditions from microfluidic diffusion measurements: application to molecular chaperones and single-step immunoassay**

Paolo Arosio[1], Thomas Müller[1], Luke Rajah[1], Francesco Aprile[1], Tom Scheidt[1], Jackie Carrozza[1], Maya Wright[1], Michele Vendruscolo[1], Christopher Dobson[1], Tuomas Knowles[1]
1.-Department of Chemistry, University of Cambridge

Characterizing the sizes and shapes of proteins and their interactions is of fundamental importance for understanding the behavior of a large variety of systems in the biological and biotechnological sciences. Defining these properties under native conditions, directly in solution and on a second timescale, remains, however, challenging. To address this problem, we have developed a method based on monitoring micron-scale diffusion in both space and time by acquiring, in a microfluidic format, diffusion profiles at different diffusion times under steady-state flow conditions. We show that the global analysis of this combined space-time acquisition enables the average sizes of the components of monodisperse and polydisperse solutions, as well as the sizes of individual species within binary mixtures, to be determined directly. We show that the ability to perform rapid and non-invasive sizing enables this technique to be used to quantify the thermodynamics and the kinetics of specific interactions between molecular chaperones and protein aggregates in complex polydisperse solutions, as well as to identify the oligomerization state of dynamic protein systems. We demonstrate further a quantitative immunoassay that enables specific interactions between biomolecules as well as the conformations of target protein species to be determined directly in solution even in heterogeneous mixtures.

PB-004 **Using α-chymotrypsin and elastase enzymatic degradation to control peptide self-assembly**

Valeria Castelletto[1], Ian Hamley[1]
1.-School of Chemistry, University of Reading

A micellar nanocontainer delivery and release system is designed on the basis of a peptide-polymer conjugate. 1 The hybrid molecules self-assemble into micelles comprising a modified amyloid peptide core surrounded by a PEG corona. The modified amyloid peptide previously studied in our group forms helical ribbons based on a β-sheet motif and contains β-amino acids that are excluded from the β-sheet structure, thus being potentially useful as fibrillization inhibitors. In the model peptide-PEG hybrid system studied, enzymatic degradation using R-chymotrypsin leads to selective cleavage close to the PEG-peptide linkage, break up of the micelles, and release of peptides in unassociated form. The release of monomeric peptide is useful because aggregation of the released peptide into β-sheet amyloid fibrils is not observed. This concept has considerable potential in the targeted delivery of peptides for therapeutic applications. In a separate work, the self-assembly of the alanine-rich amphiphilic peptides Lys(Ala)6Lys (KA6K) and Lys(Ala)6Glu (KA6E) with homotelechelic or heterotelechelic charged termini respectively has been investigated in aqueous solution.2 These peptides contain hexa-alanine sequences designed to serve as substrates for the enzyme elastase. Electrostatic repulsion of the lysine termini in KA6K prevents self-assembly, whereas in contrast KA6E is observed, through electron microscopy, to form tape-like fibrils, which based on X-ray scattering contain layers of thickness equal to the molecular length. The alanine residues enable efficient packing of the side-chains in a β-sheet structure, as revealed by circular dichroism, FTIR and X-ray diffraction experiments. In buffer, KA6E is able to form hydrogels at sufficiently high concentration. These were used as substrates for elastase, and enzyme-induced de-gelation was observed due to the disruption of the β-sheet fibrillar network. We propose that hydrogels of the simple designed amphiphilic peptide KA6E may serve as model substrates for elastase and this could ultimately lead to applications in biomedicine and regenerative medicine.

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PB-005 **Fluorescence-based techniques for the investigation of localization and functions of proteins**

Yuen-Yan Chang[1], Yau-Tsz Lai[1], Ligang Hu[1], Ya Yang[1], Ailun Chao[1], Hongzhe Sun[1]
1.-Department of Chemistry, The University of Hong Kong

Two fluorescence-based techniques are applied to label intracellular tagged proteins and monitor the interaction between a metalloprotein and Bi-based metallodrugs. First, we have synthesized, to our knowledge, the first membrane permeable fluorescent probe Ni-NTA-AC that enters cells and monitors intracellular His-tagged proteins in minutes, without functionally perturbing the target proteins (Figure 1) [1]. Arylazide photoactivation to covalently linked the His-tagged proteins by Ni-NTA-AC resulted in significant fluorescence enhancement (~13-fold). Ni-NTA-AC successfully traced the subcellular localization of His-tagged proteins with negligible toxicity in different biological systems, including bacterial and mammalian cells and even the plant tissues. We are currently developing fluorescent probes with different fluorophores using similar strategy to enable the simultaneous examination of tagged target proteins in cells. Besides, we have constructed two fluorescent sensors CYHpnl and CYHpnl_1-48 (with C-terminus glutamine-rich sequence deleted) to elucidate the role of metalloprotein Hpn-like by Fluorescence Resonance Energy Transfer (FRET) (Figure 2) [2]. We found the selective coordination of Ni(II) and Zn(II) to the purified sensors and in E. coli cells. Surprisingly, specific interaction between the FRET sensors and Bi(III) was observed. Our FRET analysis confirmed the role of Hpnl for Ni(II) storage and revealed the potential association of Hpnl with Bi-based antiulcer drugs in cells.

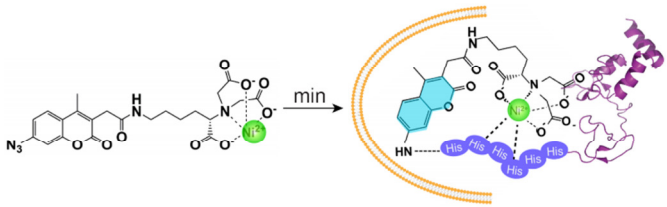

Figure 1

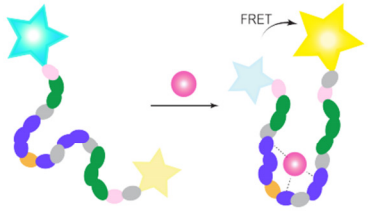

Figure 2

This work was supported by the Research Grants Council of Hong Kong (704909 and N_HKU75209, 704612P, 703913P), Livzon Pharmaceutical Group and the University of Hong Kong (for the emerging Strategic Research Theme – Integrative Biology).

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PB-006 **RNA Fate is Controlled by Highly-Regulated RNA Binding Proteins**

Irene Díaz-Moreno[1], Isabel Cruz-Gallardo[1], Sofía M. García-Mauriño[1], Rebecca Del Conte[2], B. Göran Karlsson[3], Andres Ramos[4], María L. Martínez-Chantar[5], Francisco J. Blanco[5], Myriam Gorospe[6], Jacqueline A. Wilce[7]

1.-IBVF - cicCartuja, University of Seville - CSIC, 2.-CERM, Department of Chemistry, University of Florence, 3.-Swedish NMR Centre, University of Gothenburg, 4.-Molecular Structure Division, MRC National Institute for Medical Research, 5.-CIC bioGUNE, 6.-Laboratory of Genetics, National Institute on Aging-Intramural Research Program, 7.-Department of Biochemistry and Molecular Biology, Monash University

RNA biology is tightly orchestrated by the interplay of RNAs with RNA-Binding Proteins (RBPs), which can be regulated by post-translational modifications, pH-dependence and oligomerization states. Phosphorylation of the RBP K-Homology Splicing Regulatory Protein (KSRP) induces protein unfolding and impairs the ability of KSRP to promote the degradation of its RNA targets [1,2]. This finding reveals the molecular mechanism that links the mRNA-degradation pathway with extracellular signaling networks through the reversible unfolding of a RNA binding domain (RBD). RNA binding is also controlled by pH conditions. This finding becomes relevant for RBPs such as T-cell Intracellular Antigen 1 (TIA-1), which shuttles between two cellular compartments (nucleus and cytoplasm) with slightly different pH values. In fact, RNA binding by TIA-1 is modulated by slight environmental pH changes due to the protonation/deprotonation of TIA-1 histidine residues [3,4]. The pH dependence of the TIA-1/RNA interaction provides a new insight into the function of TIA-1 in recognizing new RNA targets [5], like the 5' Terminal Oligopyrimidine Tracts (5´TOPs) of translationally-repressed mRNAs. Along with TIA-1, the RBP Hu antigen R (HuR) is involved in the assembly/disassembly of cytoplasmic Stress Granules (SG), which arise as a protective mechanism by preventing mRNA decay under stress situations. Despite wide acceptance that RBPs harboring aggregation-promoting Prion Related Domains (PRDs), such as TIA-1, stimulate rapid self-association and formation of SGs, we propose that scaffolding SGs may be driven by RBDs, since PRD-lacking RBPs, like HuR, often form oligomers [6,7,8] and are included in SGs. Under continuous stress, the transition from the physiological to pathological aggregation of RBPs in SGs may depend on post-translational modifications of RBDs. RNA-binding proteinopathies, characterized by the nucleation of irreversible SGs, are often found in neurodegenerative diseases. Altogether, resulting insights into RNA biology suggest that highly-regulated RBPs determine mRNA fate from synthesis to decay.

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PB-007 **Understanding promiscuous and selective ligand binding by liver FABP**

Mariapina D'Onofrio[1], Filippo Favretto[1], Serena Zanzoni[1], Silvia Perez Santero[1], Michael Assfalg[1], Henriette Molinari[2], Carlo Santambrogio[2], Rita Grandor[2]

1.-Department of Biotechnology, University of Verona, Strada Le Grazie 15, 2.-Laboratorio NMR, ISMAC-CNR

Fatty acid binding proteins (FABPs) act as intracellular carriers of lipid molecules, and play a role in global metabolism regulation. Liver FABP (L-FABP) is characterized by high versatility in terms of ligand binding capabilities.[1] Indeed, both long chain fatty acids as well as bulkier ligands can be accommodated into the protein's large internal cavity. The involvement of L-FABP in the transport of bile salts has been postulated but scarcely investigated.[2] This hypothesis is further supported by the realization that L-BABP (a type 2 intracellular lipid binding protein bile-salt carrier) is absent in mammals. We have used a variety of NMR experiments, as well as steady-state fluorescence spectroscopy, and mass spectrometry to gain insight, at molecular and atomic level, into the interactions established by human L-FABP with a pool of bile acids [3, 4], contributing to improve our understanding of the binding specificity for this important class of cholesterol-derived metabolites. An extensive comparison among L-FABP alone, in complex with bile acids, and in complex with oleate, has been performed in order to investigate the distinctive features of L-FABP binding promiscuity.[3] NMR relaxation experiments on different timescales suggest that human L-FABP is poorly selective in terms of ligand binding and a functional role is played by its internal dynamics. Taken together our findings expand the current knowledge about ligand recognition by L-FABP with implications in the intracellular transport of bile acids in physiological and pathological states. [1] Zimmerman AW, Veerkamp JH (2002) Cell Mol Life Sci. 59, 1096–1116. [2] Guariento M, Raimondo D, Assfalg M, Zanzoni S, Pesente P, Ragona L, Tramontano A & Molinari H (2008) Proteins 70, 462–472. [3] Favretto F, Assfalg M, Gallo M, Cicero DO, D'Onofrio M & Molinari H (2013) ChemBioChem 14, 1807–1819. [4] Favretto F, Santambrogio C, D'Onofrio M, Molinari H, Grandori R & Assfalg M (2015) FEBS J. 282,1271-88.

PB-008 **Antimalarial Agents With a Novel Mode of Action: Dual Inhibition of P. falciparum M1 and M17 Metalloaminopeptidases**

Nyssa Drinkwater[1], Shailesh Mistry[2], Komagal Kannan Sivaraman[1], Alessandro Paiardini[3], Vicky Avery[4], Peter Scammells[2], Sheena McGowan[1]

1.-Department of Biochemistry & Molecular Biology, Monash University, 2.-Monash Institute of Pharmaceutical Sciences, Monash University, 3.-Dipartmento di Scienze Biochimiche, Sapienza Universita di Roma, 4.-Eskitis Institute for Drug Discovery, Griffith University

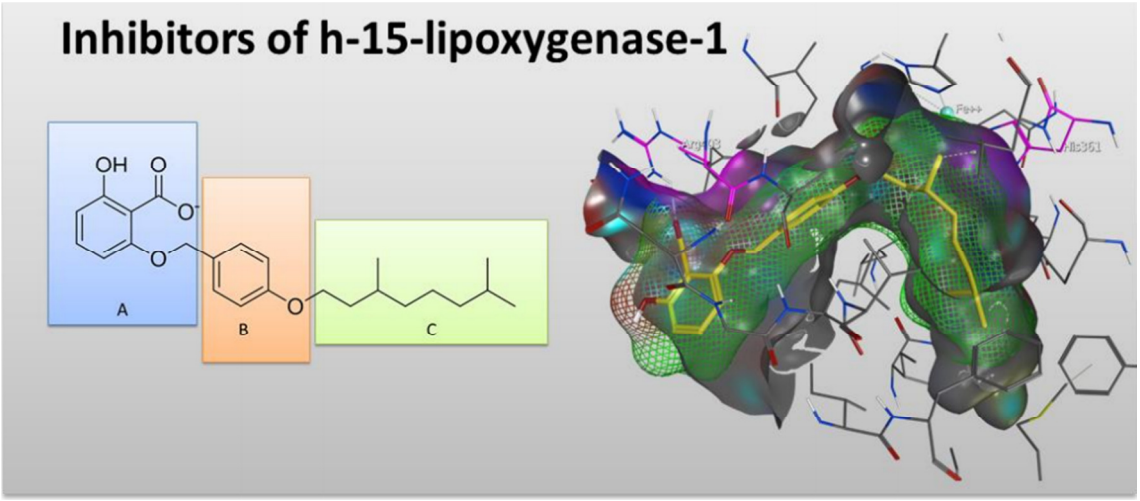
Malaria is caused by parasites of the genus Plasmodium, with P. falciparum (Pf) causing the most fatalities. The prevention and treatment of Pf malaria is becoming increasingly difficult due to the spread of drug resistant parasites. New therapeutics with a novel mode of action are desperately required. Two P. falciparum aminopeptidases, PfA-M1 and PfA-M17, play crucial roles in the erythrocytic stage of infection, and have been validated as potential antimalarial targets. Using compound-bound crystal structures of both enzymes, we were able to identify key similarities and differences in the mechanism of inhibitor binding by PfA-M1 versus PfA-M17, which we exploited to design inhibitors capable of potently inhibiting both enzymes. The resultant hydroxamic acid-based inhibitors represent the first compounds capable of potent dual inhibition of both PfA-M1 and PfA-M17. The compounds additionally possess nanomolar activity against 3D7 malaria parasites and no observable cytotoxicity, and are therefore extremely attractive lead molecules for further development into antimalarial therapeutics with a novel mode of action.

PB-009 **Different classes of inhibitors for human 15-lipoxygenase-1**

Nikolaos Eleftheriadis[1], Stephanie Thee[1], Johan te Biesebeek[1], Petra van der Wouden[1], Bert-Jan Baas[1], Frank J. Dekker[1]

1.-Groningen Research Institute of Pharmacy

Lipoxygenases metabolize polyunsaturated fatty acids into signalling molecules such as leukotrienes and lipoxins. 15-lipoxygenase (15-LOX) is an important mammalian lipoxygenase and plays a crucial regulatory role in several respiratory diseases such as asthma, COPD and chronic bronchitis. Novel potent and selective inhibitors of 15-LOX-1 are required to explore the role of this enzyme in drug discovery. In this study we present different classes of inhibitors for human 15-LOX-1. Kinetic analysis suggests competitive inhibition and the binding model of these compounds can be rationalized using molecular modelling studies. The new inhibitors show Ki values from 0.040 µM to 1.7 µM. These structure-activity relationships provide a basis to design improved inhibitors and explore 15-LOX-1 as a drug target.



PB-010 **Identification of Novel Inhibitors of 6-Phosphogluconate Dehydrogenase (6PGDH) in Trypanosoma brucei Through Virtual Drug Screening**

Victoria Gomez[1], Kavya Kolavasi[1], Josh Beckham[1], Jon Robertus[1]
1.-The University of Texas at Austin College of Natural Science

A threat to 70 million people in underdeveloped nations around the world, African trypanosomiasis (sleeping sickness) is a neglected tropical disease (NTD) caused by the protozoan parasite Trypanosoma brucei (T. brucei). T. brucei is transmitted to humans via the tsetse fly, and replicates in the blood before crossing into the brain, causing death for the infected individual. Current treatments that are available for African sleeping sickness are highly toxic and usually difficult to administer past the blood-brain barrier. It is our belief that coupling less toxic compounds with efficient drug delivery systems will contribute to the development of the most effective drug against African sleeping sickness. Our goal was to determine a novel and effective chemical inhibitor with the potential to prevent the replication of T. brucei in the human body. The enzyme target for inhibition studied in this research was 6-phosphogluconate dehydrogenase (6PGDH), a cytosolic enzyme in the pentose phosphate pathway (PPP) of T. brucei. 6PGDH is essential in the PPP due to its ability to oxidize 6-phosphogluconate into ribulose-5-phosphate, which is essential for the formation of nucleotides. Primer overlap extension Polymerase Chain Reaction (PCR) was used to synthesize the coding DNA sequence of the 6PGDH gene, which was then cloned into a pNIC-Bsa4 inducible expression plasmid with an N-terminal 6 Histidine tag, by way of ligation independent cloning. The protein was then expressed in BL21 (DE3) Escherichia coli (E. coli) cells and purified via nickel column affinity and size exclusion fast protein liquid chromatography (FPLC) to perform inhibition assays. Through virtual screening, various ligands obtained from the Chembridge Library and NIH Clinical Collection) were docked into the active site of the crystal structure of Tb6pgdh (Pubchem identification 1PGJ) using GOLD molecular docking software. The top scoring compounds were selected by utilizing parameters such as hydrophobic interactions, hydrogen bonds, and Van der Waals forces. The compounds with the best scores that also satisfied Lipinski's Rule of 5 criteria for druggability were then tested in spectrophotometric enzyme inhibition assays monitoring the absorbance of NADPH at 340 nm. Compounds that show inhibitory activity in the assays will be taken to higher levels of testing to determine their effect on T. brucei in other organisms.

PB-011 **NMR studies of the structural influence of phosphopantetheinylation in nonribosomal peptide synthetase carrier proteins and impact on binding affinities**

Andrew Goodrich[1], Dominique Frueh[1]
1.-Johns Hopkins University School of Medicine

Nonribosomal peptide synthetases (NRPSs) are modular enzymatic systems responsible for the production of complex secondary metabolites in bacteria and fungi. Each module is comprised of (at least) three core domains whose combined action leads to the selection, activation, and incorporation of a single small molecule into a growing peptide. Central to each module is the carrier protein (CP), which is first primed via attachment of a 4'-phosphopantetheine moiety (ppant arm) to a conserved serine to generate the active holo form. An adenylation (A) domain then covalently attaches an amino or aryl acid onto the ppant arm via formation of a thioester. The CP then shuttles activated monomers and growing peptides between the active sites of catalytic domains in both the same and adjacent modules. During CP priming and peptide elongation, a CP thus exists in multiple different post-translational states and interacts with numerous catalytic domains. Understanding how NRPSs are able to efficiently orchestrate this series of sequential protein-protein interactions between a CP and its partner catalytic domains is key to unraveling the molecular mechanism of NRP synthesis. Using a combination of isothermal titration calorimetry and nuclear magnetic resonance (NMR) titrations, we found that converting a CP from the apo to holo form alters its affinity for its partner A domain. This change in binding suggests a means by which directionality in protein-protein interactions is achieved in NRPSs. However, we also found that A domain binding affects the same subset of residues in both the apo and holo forms. In order to identify the molecular features underpinning this difference in affinity, we solved the NMR solution structures of the apo and holo forms of the CP. Here, we present the solution structures of an apo and holo CP and discuss them in light of their differential binding to an A domain.

PB-012 **Functional analysis of of conditional analog-sensitive alleles of essential protein kinases in the fission yeast Schizosaccharomyces pombe.**

Juraj Gregan[1, 2]
1.-Mfpl/imp, 2.-Comenius University

The genome of the fission yeast Schizosaccharomyces pombe encodes for 17 protein kinases that are essential for viability. Studies of the essential kinases often require the use of mutant strains carrying conditional alleles. To inactivate these kinases conditionally, we applied a recently developed chemical genetic strategy. The mutation of a single residue in the ATP-binding pocket confers sensitivity to small-molecule inhibitors, allowing for specific inactivation of the modified kinase. Using this approach, we constructed conditional analog-sensitive alleles of 13 essential protein kinases in the fission yeast S. pombe. I will present the functional analysis of these mutants during meiosis.

PB-013 **Peptide conjugates: From self-assembly towards applications in biomedicine**

Ian Hamley[1]
1.-University Of Reading, Dept of Chemistry

Self-assembling peptides and their conjugates offer exceptional potential in nanomedicine. I will present some of our recent work on nanoscale assembled peptides and their conjugates, focussing on lipopeptides [1, 2] and PEG-peptide conjugates [3]. PEGylation is an important technique in the development of conjugates for applications in therapeutics. It is found to greatly influence self-assembly of peptides and proteins - one example from our own work is a peptide which itself forms twisted fibrils but when PEG is attached, self-assembly of the conjugate leads to spherical micelles[4]. The conjugate can be enzymatically degraded using alpha-chymotrypsin, releasing the peptide. This nanocontainer delivery and release system could be useful in therapeutic applications. Thermoresponsive telechelic PEG/peptides with hydrophobic dipeptide end groups (di-tyrosine or di-phenylalanine) were developed, one of which shows a de-gelation transition near body temperature and which may be useful in bioresponsive delivery systems [5]. Examples from our recent work on self-assembling lipopeptides will also be outlined. Our focus is to investigate potential relationships between self-assembly and bioactivity, in particular in the fields of regenerative medicine [6-10], antimicrobial systems [11, 12] and immune therapies [13].

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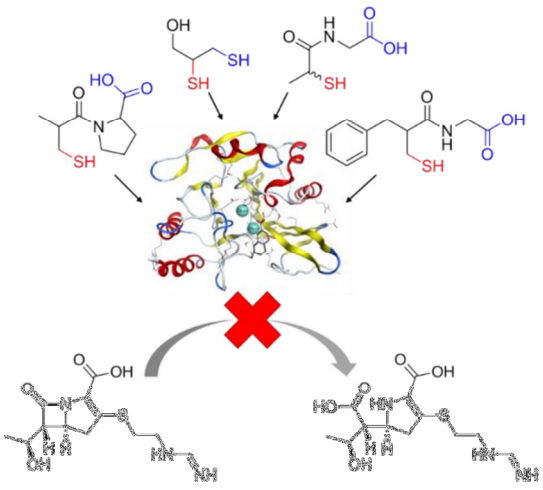
PB-014 **Approved Drugs containing Thiols as Inhibitors of Metallo-ß-Lactamases: A Strategy to Combat Multidrug-Resistant Bacteria**

Franca-M. Klingler[1], Ewgenij Proschak[1]
1.-Goethe University, Institute of Pharmaceutical Chemistry

Antibiotic resistance in bacterial pathogens is one of the major threats regarding human health. An alarming trend is the spread of metallo-β-lactamases (MBLs) among gram-negative pathogens that transfer resistance against almost all β-lactams including carbapenems.[1] The development of new anti-infective agents remains one of the most significant demands in modern medicine.[2] The aim of this work is to find an already approved drug which restores the activity of β-lactam antibiotic by protecting it from hydrolysis through the MBL. Thiol groups are known zinc chelators and therefore inhibit MBLs.[3] We established a novel sensitive fluorescence-based assay platform for studying inhibition of β-lactamases using the commercially available substrate Fluorocillin.[4] The reliability of the system was evaluated on three different class B MBLs: New-Delhi-Metallo- β-Lactamase-1 (NDM-1), Verona-Integron-Encoded-Metallo-β-Lactamase 1 (VIM-1) and Impenemase-7 (IMP-7). Remarkably, not all compounds inhibited MBLs, although every compound carried a thiol group. In order to discriminate between zinc-withdrawing and direct binding to the enzyme, thermal shift assay was conducted. This assay combination provides a method to find inhibitors which inhibit MBLs via direct binding to the active site.[5] Most promising compounds were passed to antimicrobial susceptibility testing using laboratory strains and patient isolates. Results showed that some of our compounds partially restored the efficacy of Imipenem against pathogenic bacteria. Overall, we found four approved drugs, which inhibit three clinically important MBLs, namely Captopril, Thiorphan, Dimercaprol and Tiopronin. This result yields a good starting point for the development of potent MBL inhibitors, with the primary optimization goal being the uptake and activity in pathogens.



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PB-015 **Protein Carbamylation at the Chemistry-Biology interface**

Victoria Linthwaite[1], Joana Janus[1], David R.W. Hodgson[2], Martin J. Cann[1]
1.-School of Biological and Biomedical Sciences, Durham University, 2.-Department of Chemistry, Durham University

Carbon dioxide (CO2) is a crucial regulator for all three domains of life (1), known for its role during respiration and photosynthesis. Despite this there is very little known about its molecular interactions with cellular components. CO2 combines rapidly but reversibly with amines at physiological temperatures and pressures to form carbamates (2). This modification is present in key proteins, such as RuBisCO and haemoglobin but remains unexplored in other systems. Carbamylation is caused by the nucleophilic attack of an uncharged amine (for example on an arginine or lysine side chain or an N-terminal group) on CO2 (2). This research aims to investigate this understudied modification.

We are resolving these limitations to investigate carbamate formation in cellular systems by trapping carbamates chemically thereby removing their labile nature3. We are developing chemical and analytical tools to meet this challenge. Main results: We have successfully proven the ability to trap carbamates on acetyl-lysine, Lys-Gly and Phe-Gly dipeptides, a tetra-peptide and haemoglobin. These results have been confirmed using ESI-MS combined with 12C and ^{13}C isotope incorporation. Further work using radioactive ^{14}C has been carried out on whole organism samples.

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PB-016 **A β-carboline substituted derivative displays selective anti-cancer activity through inhibition of translation**

Annelise de Carvalho[1], Jennifer Chu[2], Céline Meinguet[3], Robert Kiss[1], Guy Vandenbussche[4], Bernard Masereel[3], Yohan Wouters[3], Jerry Pelletier[2], Véronique Mathieu[1]

1.-Laboratoire de Cancérologie et Toxicologie Expérimentale, Faculté de Pharmacie, 2.-Biochemistry Department, 3.-Namur Medicine and Drug Innovation Center (NAMEDIC-NARILIS), 4.-Laboratory for the Structure and Function of Biological Membranes

Background : Reprogrammed cellular metabolism is one of the ten recognized hallmarks of cancer cells (Hanahan and Weinberg, 2011). More particularly, increased cell proliferation, migration, angiogenic induction and modifications to the tumor environment require elevated protein synthesis and turnover. Surprisingly, the only FDA-approved anticancer drugs that targets protein metabolism in cancer cells are recently launched proteasomal inhibitors and omacetaxine mepesuccinate, a translation elongation inhibitor. Regulation of protein synthesis in tumor cells differs significantly from their non-transformed counterparts due to alterations in MAPK and Akt/mTOR signaling pathways, two major signal transduction pathways that control initiation of translation. There is thus significant interest in the development of novel inhibitors of translation as potential anti-neoplastic agents. Purpose and results: In a previous study, we reported novel substituted β-carbolines as protein synthesis inhibitors (Frederick and Bruyere et al, 2012). CM16, the lead compound optimized in terms of pharmacological properties (Meinguet et al, 2015), was used to start to decipher their mode of action. CM16 exerts cytostatic anti-cancer effects in vitro without significant modification of the cell cycle profile. The National Cancer Institute COMPARE algorithm enabled us to compare the global growth inhibition profile of CM16 on 60 cancer cell lines (mean GI50 of 0.2 μM) to the >765,000 compounds of their database and revealed good correlation coefficients with protein synthesis inhibitors. Effects of CM16 on transcription could appear secondary since we didn't observed any effects before prolonged compound incubation on cells - at least 24h exposure to 5 μM CM16. In contrast, translation was quite sensitive to CM16, as assessed by metabolic labeling assays. Ribosomal subunit assembly and polysome profiling of cells exposed to CM16 were evaluated by sucrose gradient analysis. Exposure of cells to CM16 for only 3h was sufficient to disrupt polysomes and cause an increase in 80S complexes. Ongoing investigations of the expression and phosphorylation status of different initiation and elongation factors identified eIF2α; as a potential target of CM16. This effect unlikely results from a direct activity of CM16 on PERK, one of four eIF2α; kinases. CM16 penetrates into the cancer cells after few minutes of treatment and its distribution parallels an endoplasmic reticulum fluorescent probe. Interestingly, non-transformed cells are ~ 10 times less sensitive to CM16. Conclusions and Perspectives: CM16 is a synthetic harmine derivative that displays anti-neoplastic activity in vitro at concentrations of 0.1 to 0.5 μM. It appears to inhibit protein synthesis at the level of the initiation phase within in the first 3 hours following exposure to cells. Differences in the dependencies on deregulated protein synthesis in normal versus transformed cells appear to be exploitable for the development of new anti-cancer agents. Additional proteomic studies will be conducted in the near future to further our understanding of this concept and the effects of CM16.

PB-017 **Semi-chemical synthesis and characterization of a small heat shock protein bearing a nonenzymatic posttranslational modification found in vivo**

Maria Matveenko[1], Christian Becker[1]
1.-Institute of Biological Chemistry, Department of Chemistry, University of Vienna

Up to 50% of all human proteins are believed to be modified following their biosynthesis through posttranslational modifications (PTMs) [1]. PTMs can result from enzymatic and nonenzymatic reactions, and both types of modifications play an important role in a plethora of physiological and pathological processes [2,3]. Nonenzymatic modifications (NEMs) are increasingly recognized to affect cellular processes, with an involvement in age-related, metabolic and neurodegenerative diseases [4,5]. Human heat shock protein (Hsp27) has been shown to become derivatized with argpyrimidine, a prominent NEM that occurs on arginine residues [6], in certain human cancer tissues and cell lines [7,8]. This NEM was linked to the elevated antiapoptotic activity of the protein [7,8], whereby modification of Arg-188 appeared to be of particular significance [7]. In this work, Hsp27 homogeneously modified with argpyrimidine at position 188 is generated for the first time. Using expressed protein ligation [9], the first semisynthesis of the unmodified protein is achieved as well. Our approach, which combines organic chemistry, peptide synthesis and protein synthesis, enables complete control over protein composition and thus can provide previously unattainable insight into the properties of this vital chaperone following nonenzymatic modification. The synthesis of argpyrimidine-modified Hsp27 and the progress towards structural and functional characterization of the protein will be presented herein. [1] Khoury, G.A., Baliban, R.C., Floudas, C.A. Sci Rep 2011, 1(139). [2] Walsh, C.T., Garneau-Tsodikova, S., Gatto, G.J., Jr. Angew Chem Int Ed 2005, 44(45), 7342. [3] Vistoli G, De Maddis D, Cipak A, Zarkovic N, Carini M, Aldini G. Free Radic Res 2013, 47(Suppl. 1), 3. [4] Jaisson, S., Gillery, P. Clin Chem 2010, 56(9), 1401. [5] Rabbani, N., Thornalley, P.J. Amino Acids 2012, 42(4), 1133. [6] Shipanova, I.N., Glomb, M.A., Nagaraj, R.H. Arch Biochem Biophys 1997, 344(1), 29. [7] Sakamoto, H., Mashima, T., Yamamoto, K., Tsuruo, T. J Biol Chem 2002, 277(48), 45770. [8] Van Heijst, J.W., Niessen, H.W., Musters, R.J., van Hinsbergh, V.W., Hoekman, K., Schalkwijk, C.G. Cancer Lett 2006, 241(2), 309. [9] Muir, T.W., Sondhi, D., Cole, P.A. PNAS 1998, 95(12), 6705.

PB-018 **A new scaffold for inhibition of cysteine proteases: Structural and functional characterization of Kunitz inhibitors from potato**

Manasi Mishra[1], Jiri Brynda[1], Michael Mares[1]
1.-Institute of Organic Chemistry and Biochemistry, AS CR

Kunitz-type protease inhibitors belong to a widespread protein family present in many plant species and play an important role in plant defense against insect pests and pathogens. Members of this family are typically inhibitors of proteases of serine class. Interestingly, a few members were identified as inhibitors of proteases of cysteine class, however, they have not been functionally and structurally characterized. Our study is focused on Kunitz-type inhibitors of cysteine proteases (PCPIs) from potato (Solanum tuberosum). A series of 20 kDa PCPIs was purified using a multi-step chromatographical protocol, and two most abundant and effective isoinhibitors named PCI 1-5 and PCI 3 were characterized in detail. They were screened against a broad panel of model cysteine proteases and digestive cysteine proteases from herbivorous insects. PCI 1-5 and PCI 3 exhibit different inhibitory specificity pattern and potency up to the nanomolar range. Both isoinhibitors were crystallized and their spatial structures were solved and refined at 1.5 Å (PCI 1-5) and 1.7 Å (PCI 3) resolutions. A position of reactive sites against cysteine proteases on the conserved β-trefoil fold scaffold was proposed. The work provides the first analysis of PCPIs with respect to the structure-function relationships and evolution within the Kunitz-type inhibitor family.

PB-019 **Role of the ABCC2 transporter in the mode of action of the Bacillus thuringiensis Cry1Ac toxin in the Diamond Back Moth Plutella xylostella**

Josué Ocelotl[1], Jorge Sánchez[2], Raquel Arroyo[1], Isabel Gómez[1], Gopalan Unnithan[2], Bruce Tabashnik[2], Alejandra Bravo[1], Mario Soberón[1]

1.-Instituto de Biotecnología, Universidad Nacional Autónoma de México, 2.-Department of Entomology, University of Arizona

Due to being environmentally friendly and highly specific to their target insect, Bacillus thuringiensis three domain-Cry toxins (Bt toxins) are widely used as an alternative to chemical insecticides in formulations or in transgenic expression. To exert their toxic effect the protein crystals that contain this pore forming toxins must be ingested by susceptible insect larvae. Once in the midgut, the crystal is solubilized and activated by gut proteases. The protease resistant fragment, composed of three domains is able to interact with different insect proteins located in the apical membrane of the gut epithelium, putative Cry-toxins binding proteins include cadherin (CADR), aminopeptidase-N (APN), and alkaline phosphatase (ALP). Current model for the mode of action involves a low affinity interaction of Cry1A toxins with the highly abundant GPI-anchored-receptors, ALP and APN which concentrates the toxin close to the microvilli membrane, here the toxin binds in a high affinity interaction to the cadherin receptor, this event promotes the proteolytic cleavage of the N-terminal end including helix α-1 triggering the formation of an oligomer structure. The binding of oligomeric Cry1A structure to ALP and APN facilitates insertion of this pre-pore into the membrane causing pore-formation and cell lysis. It is known that mutations resulting in diminished or lack of expression of Cry-toxin binding proteins result in high levels of resistance of the insect pests. In recent years, a novel resistance mechanism involving an ABC transporter (ABCC2) has been reported in four lepidopteran insects, Helothis virescens YEE and YHD3 strains, Plutella xylostella NO-QA strain, Bombyx mori C2 strain and Trichoplusia ni GlenBtR strain. Although some studies have suggested that the ABCC2 transporter could be a receptor for Bt toxins in this insect pests, the precise role of this membrane protein in the mode of action of Cry toxins remains unclear. Interestingly insects with mutations in the ABCC2 transporter or cadherin show high resistance levels to Cry1Ab or Cry1Ac toxins but are susceptible to mutant toxins named Cry1AbMod or Cry1AC Mod, in which the N-terminal end of the toxin including helix-α1was deleted, these proteins do not require the presence of cadherin to form oligomeric structures. We analyzed and compared the binding interactions of Cry1Ac and Cry1AcMod toxins with brush border membranes from susceptible and resistant populations of P. xylostella and P. gossypiella, which display different Cry-toxin resistance mechanisms, ABCC2 or cadherin. Our data shows that ABCC2 and cadherin are involved in Cry1Ac toxin oligomerization in P. xylostella and P. gossypiella respectively showing that these receptor molecules fulfill the same role in the mode of action of Cry1Ac in different insect species.

PB-020 **Metabolic alkene labeling and in vitro detection of histone acylation via the aqueous oxidative Heck reaction**

Maria-Eleni Ourailidou[1], Paul Dockerty[1], Martin Witte[1], Gerrit J. Poelarends[1], Frank J. Dekker[1], 1.-University of Groningen

The detection of protein lysine acylations remains a challenge due to lack of specific antibodies for acylations with various chain lengths. This problem can be addressed by metabolic labeling techniques using carboxylates with reactive functionalities. Subsequent chemoselective reactions with a complementary moiety connected to a detection tag enable the visualization and quantification of the protein lysine acylome. In this study, we present EDTA-Pd(II) as a novel catalyst for the oxidative Heck reaction on protein-bound alkenes, which allows employment of fully aqueous reaction conditions. We used this reaction to monitor histone lysine acylation in vitro after metabolic incorporation of olefinic carboxylates as chemical reporters.

PB-021 **"Study of Bacillus thuringiensis Cry1Ab and Cry1Ac protoxins interaction with cadherin-like receptor from Manduca sexta"**

Arlen Peña-Cardeña[1], Alejandra Bravo[1], Mario Soberón[1], Isabel Gómez[1],
1.-Instituto de Biotecnología, Universidad Nacional Autónoma de México

The Gram-positive bacterium Bacillus thuringiensis (Bt) produces insecticidal crystal proteins (Cry toxins) to control insect pests. Cry toxins are recognized as pore forming toxins that kill larval epithelium midgut cells by causing an osmotic shock leading to cell lysis. To induce the pore formation of Cry toxins, the parasporal crystals have to be ingested by susceptible larva, solubilized by the pH conditions of the insect gut, and activated by midgut proteases to yield the resistant core of the activated toxin. In the case of Cry toxins that are active against lepidopteran insects, it has been shown that Cry1A toxins undergo a sequential binding mechanism with glycosyl-phosphatidyl-inositol anchored proteins such as alkaline phosphatase (ALP) or aminopeptidase-N (APN) and cadherin-like protein resulting in the formation of a pre-pore oligomeric structure that is proficient in membrane insertion and pore formation. Receptor recognition by Cry toxins has been recognized as a key step of Cry toxicity that is fundamental for insect specificity. Previously we reported that Cry1Ab protoxin or activated toxin bind cadherin-like receptor with similar affinities and two different pre-pores are produced depending on which of these molecules interacts with cadherin in the presence of insect midgut proteases. Here we test the interaction with a second protoxin the Cry1Ac that share 85% of identity with Cry1Ab and both have similar toxicity against Manduca sexta larva. However, we observe that Cry1Ac protoxin has no interaction with cadherin-like receptor as Cry1Ab, our results suggest that another protein may act as receptor for Cry1Ac protoxin.

PB-022 **Proton solvation in protic and aprotic solvents**

Emanuele Rossini[1], Ernst-Walter Knapp[1]
1.-Institute of Chemistry and Biochemistry, Freie Universität Berlin

Protonation pattern influence actively properties of molecules and play an essential role in biochemical mechanisms. For an accurate determination of the protonation equilibria, the absolute proton solvation free energy needs to be known. The determination of this energy represents one of the most challenging problems in physical chemistry. This is particularly difficult for protons solvated in water, where the solvation is dynamically performed by different water clusters and the proton is not attached to a single solvent molecule. The proton solvation is notably important in order to quantify mechanisms of proton transfer and such processes have been investigated for a long time based on different approaches, often leading to contradictory conclusions. A rigorous and accurate protocol for computing proton solvation in solvents of different nature is of prime importance for applied (pharmaceutical and material science) and fundamental sciences. In this study, proton affinities, electrostatic energies of solvation and pKa values of a reference set of organic molecules are computed in protic and aprotic solvents. Proportional to the free energy of proton dissociation, the pKa value calculation is therefore strongly dependent on the free energy of proton solvation. Such energy is then determined in acetonitrile (ACN), methanol (MET), water and dimethyl sulfoxide (DMSO) in order to obtain the best possible match between measured and computed pKa values. The computation of these values is based on a combination of quantum chemical (QC) and electrostatic approaches by using a thermodynamic cycle connecting gas-phase and solvent-phase of proton dissociation. The computed proton solvation energies in ACN, MET, water and DMSO of the present study are very precise (RMSD much lower than 1 pH value). They will be a basis for better understanding of proton solvation and help to predict pKa values of organic compounds in different solvents more precise.

PB-023 **Biochemical characterization of two evolutionary distant ten-eleven translocation enzymes and their utility in 5-methylcytosine sequencing in the genomes at single-base resolution**

Lana Saleh[1], Esta Tamanaha, June Pais[1], Romualdas Vaisvila[1], Nan Dai[1], Shengxi Guan[1], Ivan Correa[1], Christopher Noren[1], Richard Roberts[1], Yu Zheng[1]
1.-New England biolabs

The ten-eleven translocation (TET) enzymes iteratively oxidize 5-methylcytosine (5mC) on DNA to 5-hydroxymethylcytosine, 5-formylcytosine, and 5-carboxycytosine. Here, we examine the in vitro biochemical activity of two evolutionary distant TETs, mTET1 from mouse and NgTET1 from the single-celled protist Naegleria gruberi. We show that both of these enzymes are 5-methylpyrimidine oxygenases with activity on both 5mC (major activity) and thymidine (T) (minor activity) and preference to 5mCpG and TpG dinucleotide sites. Intriguingly, NgTET1 displays higher T oxidation activity in vitro than mTET1 supporting a closer evolutionary relationship between NgTET1 and the base J binding proteins from trypanosomes. In fact, unprecedented evidence for the formation of two new bases, 5-formyluridine and 5-carboxyuridine, is shown for NgTET1 activity in vitro. Mutagenesis studies performed in NgTET1 reveal a delicate balance between choice of 5mC or T as the preferred substrate. Steady-state kinetic analysis show that both mTET1 and NgTET1 are distributive in their oxidative chemistry with each oxidized species released from the enzyme upon formation. Furthermore, both enzymes are physically distributive in the recognition of their substrates on template DNA with no apparent preference to a specific 5mC oxidation intermediate. These data indicate a role for the TET enzymes in the maintenance of the three oxidized forms of 5mC and suggest that these bases are not simply intermediates in a methylation cycle but represent additional epigenetic states in genomic DNA with regulatory functions to be explored. Finally, we demonstrate the utility of both enzymes in 5mC sequencing technologies and present examples for the mapping of 5mC in different genomes at base resolution.

PB-024 **Contribution of Connexin37 Gene Polymorphism (C1019T) in the Incidence of Acute Myocardial Infarction in the Egyptians**

Fadwa El Tahry[1], Ingy Hashad[1], Mohamed Farouk[1], Mohamed Gad[1]
1.-German University In Cairo (GUC)

Contribution of Connexin37 Gene Polymorphism (C1019T) in the Incidence of Acute Myocardial Infarction in the Egyptians Fadwa A. El Tahry1, Ingy M. Hashad1, Mohamed F. Abdel Rahman1, Mohamed Z. Gad1 Clinical Biochemistry unit1 Faculty of pharmacy and Biotechnology, German University in Cairo, Egypt Background: Cardiovascular diseases are the leading cause of death worldwide and in Egypt. Acute myocardial infarction (AMI), the main outcome of ischemia, is caused mainly by atherosclerosis. Connexin 37 (Cx37) protein plays a protective role by decreasing the monocyte adhesion, therefore delaying the initiation of atherosclerosis and as a result the incidence of AMI. Gap junctions are protein structures present between cells that help in the electrical and metabolic coupling of cells. Their building subunits are connexins. To date 21 connexins (Cxs) have been identified in humans. CX37 is one of the Cxs forming the gap junction protein family. It is expressed mainly on vascular endothelial cells and monocytes. Several studies reported a single nucleotide polymorphism (SNP) C1019T in CX37 gene as a prognostic marker for atherosclerosis. Aim of work: To investigate the contribution of C1019T gene polymorphism of CX37 in the predisposition of AMI in the Egyptians. Subjects and Methods: The study consisted of 114 AMI patients and 100 controls. Blood was taken after taking a written consent from all subjects and the approval of the ethical committees of EL Demrdash hospital and the German university in Cairo. DNA was extracted from the whole blood using Thermo-scientific DNA extraction kit. The frequency of the genotypes (CC, TT and CT) was determined by (PCR-RFLP) using Drd I restriction enzyme. Results: The genotype distribution of CX37 gene in AMI patients was (CC 15.78%, TT 11.40%, CT 72.80 %) while in controls (CC 27%, TT 18%, CT 55%) (p =0.516). On the other hand, the alleles frequencies of AMI patients were (C allele=52.19%, T allele= 47.80%) and in control subjects (C allele= 54.5%, T allele=45.5%(p=0.697). Conclusion: The CX 37 gene polymorphism (C1019T) is not associated with the incidence of AMI in the Egyptians.

PB-025 **Delineating toxin:lipid:ion channel interactions for rationally sodium channel inhibitors design**

Christina Schroeder[1], Sónia Henriques[2], Mehdi Mobli[2], Stephanie Chaousis[1], Phillip Walsh[1], Panumart Thongyoo[1], David Craik[1]

1.-Institute for Molecular Bioscience, The University of Queensland, 2.-Centre for Advanced Imaging, The University of Queensland

In recent years, certain voltage-gated sodium channels (NaV) subtypes have emerged as validated chronic pain targets with loss-of-function and gain-of-function mutations in both NaV1.7 and NaV1.8 subtypes leading to an inability to perceive pain and painful neuropathies, respectively. However, as NaV ion channels are intimately involved in almost all aspects of physiology, only the most selective inhibitors would be suitable as drug leads. Disulfide-rich venom derived mini-proteins from cone snails and spiders are being actively pursued as novel therapeutics for pain, because of their high selectivity and potency at human ion channels, including sodium channels (NaV). Two main strategies of inhibition have been identified; blocking the pore and interacting with the voltage-sensor domains (VSD) surrounding the pore. The ion-conducting pore is highly conserved between all sodium channel subtypes whereas the voltage-sensor domain binding sites are less conserved. Therefore, inhibition of a specific NaV isoform is more achievable using inhibitors that modulate VSDs than with pore blockers. Gating modifier toxins from spider and cone snail venom inhibit NaV1.7 and NaV1.8 by interacting with the VSD. They appear to reach their target by partitioning into the lipid membrane surrounding the ion channel, thus enabling access to the VSD. Toxin pharmacology may therefore not only be driven by the peptide-ion channel interactions, but also including the lipids surrounding the channel protein, a feature that is very much under explored. It is therefore apparent that peptide-lipid interactions in combination with peptide-channel interactions need to be considered when designing potent inhibitors. Using a range of biophysical techniques, including surface plasmon resonance and nuclear magnetic resonance, we are studying the interactions underpinning the mechanism of action between toxins and membranes and toxins and ion channels. Initial results show that the lipid composition surrounding ion channels play a major role in terms of toxin:lipid interaction and that these interactions can be used in combination with traditional structure-activity relationship studies to design selective and potent NaV inhibitors, which will be discussed. We believe that our studies will ultimately delineate what drives toxin pharmacology and NaV subtype selectivity and will lead to improve rationally engineering of novel therapeutics for the treatment of pain.

PB-026 **Micelles promote Aß42 assembly into pore-forming oligomers**

Montserrat Serra-Batiste[1], Mariam Bayoumi[2], Margarida Gairí[3], Martí Ninot-Pedrosa[1], Giovanni Maglia[2], Natàlia Carulla[1]

1.-Institute for Research in Biomedicine (IRB Barcelona), 2.-Biochemistry, Molecular and Structural Biology Section, University of Leuven, 3.-NMR Facility, Scientific and Technological Centers, University of Barcelona

The formation of amyloid-β peptide (Aβ) oligomers at the cellular membrane is considered to be a crucial process underlying neurotoxicity in Alzheimer´s disease (AD).1-2 Therefore, it is important to understand how oligomers form within a membrane environment. Using solution nuclear magnetic resonance (NMR) spectroscopy, combined with size exclusion chromatography (SEC), we have studied the two major Aβ variants— Aβ40 and Aβ42, the latter having a more prominent role in AD than the former—under carefully selected micelle conditions intended to mimic a membrane environment. Our results indicate that after an incubation period, Aβ42, but not Aβ40, assembles into oligomers with specific structural properties, which we have named Stabilized Micelle Oligomers (SMOs). SMO complexes incorporate into lipid bilayers as well-defined pores, a feature linked to neurotoxicity. These results have important implications in the AD field as they provide a new perspective on how Aβ oligomers cause neurotoxicity. Indeed, our findings constitute a first step towards the establishment of a new therapeutic target for AD.3

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PB-027 **Molecular dynamics study on the key catalytic intermediates of threonine synthase**

Mitsuo Shoji[1], Yuzuru Ujiie[1], Ryuhei Harada[1], Megumi Kayanuma[1], Yasuteru Shigeta[1], Takeshi Murakawa[2], Hideyuki Hayashi[2]
1.-Univeristy of Tsukuba, 2.-Osaka Medical College

Threonine Synthase (ThrS) catalyzes a formation of L-threonine from O-phospho-L-homoserine. The series of reactions catalyzed by ThrS encompasses many regiospecific and stereospecific steps, which are controlled by the enzyme protein, However, the precise mechanism of the reaction control (product-assisted catalysis) is not fully elucidated. In this study, molecular dynamics (MD) simulations of ThrS were performed with the thermodynamics integration approach, and the accurate free energy differences between the key intermediates were evaluated by changing the phosphate ion to a sulfate ion. It is already known experimentally that the phosphate ion is one of the products of the enzyme reaction and plays an important role in the catalytic reaction. The free energy differences between the ions were well reproduced theoretically for the key intermediate states. By performing additional 100ns MDs, we analyzed the substrate conformations. We found that the substrate conformations are changed by the reaction-controlling ions. This finding suggests that the controlling of the substrate conformation will be an important molecular mechanism for the product-assisted catalysis.

PB-028 **Agrobacterium tumefaciens employs two distinct ClpS adaptors to modulate the N-end rule degradation pathway**

Benjamin J. Stein[1], Robert A. Grant[1], Robert T. Sauer[1], Tania A. Baker[1,2]

1.-Department of Biology, Massachusetts Institute of Technology, 2.-Howard Hughes Medical Institute, Massachusetts Institute of Technology

The N-end rule is a widely conserved proteolytic pathway, in which the N-terminal amino acid of a protein determines its in vivo stability. In E. coli and C. crescentus, the ClpS adaptor protein recognizes destabilizing N-termini and delivers them to the ClpAP AAA+ protease for degradation. Unlike the majority of proteobacteria, most α-proteobacteria contain two paralogs of ClpS, ClpS1 and ClpS2. Here, we investigate the binding specificity of the ClpS1 and ClpS2 proteins from A. tumefaciens. We demonstrate that both ClpS1 and ClpS2 deliver N-end rule substrates to ClpA, but ClpS2 has a narrower binding specificity than ClpS1. Importantly, crystal structures of ClpS2 reveal conformational changes in the substrate-binding pocket that are critical for N-end rule recognition. Moreover, we find evidence that ClpS1 and ClpS2 are differentially expressed in A. tumefaciens. We conclude that A. tumefaciens contains two ClpS proteins with differing N-terminal binding specificities, allowing fine-tuning of N-end rule recognition at the level of adaptor proteins.

PB-029 **Interactions between U24 from HHV-6A and 7 and Nedd4 or Smurf2 WW domains**

Yurou Sang[1], Rui Zhang[1], Walter R.P. Scott[1], A. Louise Creagh[2], Charles A. Haynes[2], Suzana K. Straus[1]

1.-Department of Chemistry, University of British Columbia, 2036 Main Mall, 2.-Michael Smith Labs, University of British Columbia

U24 is a protein found in both Human Herpes Virus type 6A (HHV-6A) and type 7 (HHV-7), with an N-terminus which is rich in prolines (PPxY motif in both HHV-6A and 7; PxxP motif in HHV-6A). Previous work tested the hypothesis that U24 may be implicated in multiple sclerosis (MS), because of a shared seven residue sequence identity between U24 and myelin basic protein (MBP), a key protein in the progression of MS. Our study showed however that the binding between U24 (via the PxxP motif) and its interaction partner Fyn-SH3 was weak [1]. The current study examines the interaction of U24 (via the PPxY motif) with WW domains, small protein-protein interaction domains of approximately 40 residues that derive their name from two highly conserved tryptophan residues, usually spaced 22-23 amino-acids apart. The interaction between U24 and WW domains is deemed to be important for endocytic recycling of T-cell receptors [2]. Binding affinities of a number of U24-WW pairs were determined in order to identify whether a specific binding partner exists. Data from ITC, NMR and molecular dynamics simulations will be presented and discussed in light of the function of U24 in disease, with a particular focus on MS. [1]- Y. Sang, A.R. Tait, W.R.P. Scott, A.L. Creagh, P. Kumar, C.A. Haynes, S.K. Straus, Biochemistry, 53(38):6092-102 (2014). [2]- B.M. Sullivan and L. Coscoy, J. Virol., 84(3):1265-75 (2010).

PB-030 **Ebola Virus Surface Glycoprotein GP2 Forms a Hydrophobic Fist to Enter Cells by Membrane Fusion**

Jinwoo Lee[1], Sonia Gregory[1], Lukas Tamm[1]
1.-University of Virginia

Ebola Virus (EboV) is a filamentous membrane-enveloped virus that enters cells by macropinocytosis and pH-triggered membrane fusion. The disulfide-stabilized fusion loop of Ebola GP2 is extended on the surface of circulating virus, but forms a compact hydrophobic "fist" in the Nieman-Pick late endosomal compartment. The pH-induced conformational change of the EboV fusion loop enables the virus to escape from the endosome into the cytoplasm where it replicates causing severe hemorrhagic fever in infected individuals. We determined the structure of the EboV fusion loop and several fusion-compromised mutants by NMR. The results show that the fist is stabilized by a hydrophobic triad consisting of a leucine, an isoleucine and a phenyalanine residue. The fist interacts with residues in the membrane-proximal and transmembrane domains of GP2, the structures of which we also determined by NMR, to catalyze membrane fusion and virus entry into the cytoplasm. These protein interactions that involve a dramatic refolding of the protein at the membrane surface provide potential targets for drug discovery and new strategies for vaccine development. Supported by NIH grant R01 AI30557.

PB-031 **Zero-Length Crosslinking of the ß Subunits of the Phosphorylase Kinase Complex by Periodate**

Jackie Thompson[1], Owen Nadeau[1], Gerald Carlson[1]
1.-Department of Biochemistry and Molecular Biology, University of Kansas Medical Center

Phosphorylase kinase (PhK) is a hexadecameric enzyme complex that regulates glycogen catabolism. The complex is composed of four copies of four subunits: α, β, γ and δ. The α, β and δ; subunits regulate the catalytic γ; subunit through quaternary constraints that inhibit its activity. Upon binding allosteric effectors or the covalent modification of the regulatory subunits, the γ subunit is activated. Despite nearly six decades of investigation, the subunit-interactions responsible for the inhibition and activation of the γ subunit are still largely unknown. Due to PhK's large size (1.3 megadaltons), studying its structure is challenging, but chemical crosslinking has proved a valuable tool to study interactions among its α, β, γ; and δ; subunits before and after activation by phosphorylation. Here we report selective, zero-length crosslinking of the regulatory β subunits of PhK by the general oxidizing agent periodate, even at concentrations as low as μM. SDS-PAGE, mass spectrometry, Western blot analysis, and size-exclusion chromatography were used to characterize crosslinked PhK and to identify the products formed. Oxidation of non-activated PhK at pH 6.8 with periodate produced a β-dimer, making periodate the most effective crosslinking agent to produce this homodimer. When periodate crosslinking of non-activated PhK was carried out in the presence of a synthetic peptide corresponding to the N-terminal 22 residues of β (Nβ peptide) known to compete with its counterpart region in the native enzyme and disrupt interactions between the β and γ subunits, there was an increased amount of β-dimer formation. It should be noted that this Nβ peptide contains the autophosphorylatable Ser-11 associated with PhK activation, and phosphorylated Nβ; peptide was considerably less effective in promoting β-dimer formation than non-phosphorylated peptide. These results suggest a role for Ser-11 autophosphorylation in mediating homodimeric β subunit interactions within the PhK complex, and augment previous studies on the activation of PhK by phosphorylation in which changes at the N-terminus of β are critical in the activation of the catalytic γ subunit. Summing these results leads to a new model of activation. In this model, in the inactive state, the nonphosphorylated N-terminus of β interacts directly or indirectly with the regulatory C-terminal domain of the γ subunit, inhibiting catalytic activity. Upon phosphorylation of the N-terminus of β, three important events occur: 1) the interaction between β and γ is disrupted, 2) the β subunits of the holoenzyme self-associate, and 3) the catalytic domain is activated. Thus, we envision that the N-terminus of β acts as an allosteric switch, with activation triggered by phosphorylation of this region, causing disruption of its previously inhibiting interactions with γ and promotion of β β dimerization to stabilize the activated conformation of γ .

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PB-032 **hSSB1 is involved in the cellular response to oxidative DNA damage**

Christine Touma[1], Nicolas Paquet[2], Derek J. Richard[2], Roland Gamsjaeger[1,3], Liza Cubeddu[1,3]
1.-School of Science and Health, University of Western Sydney, 2.-Queensland University of Te chnology, 3.-School of Molecular Bioscience, University of Sydney

Cellular DNA is subject to oxidative damage in the presence of reactive oxygen species. The 7,8-dihydro-8-oxoguanine (8-oxoG) adduct is the most common form of oxidative damage and results in G:C to T:A transversions; these lesions are normally processed by the Base Excision Repair (BER) pathway. Single-stranded binding (SSB) proteins of the oligonucleotide binding domain family are heavily involved in DNA repair processes, which involve the detection of DNA damage and recruitment of repair proteins to the site of damage. Using immunofluorescence we demonstrate that hSSB1 (a novel human SSB) levels increase in response to oxidative damage (H202). Cells depleted of hSSB1 are hypersensitive to oxidative damage and are also unable to efficiently remove 8-oxoG adducts. We show that hSSB1 forms dimers and tetramers under oxidative conditions and that this oligomerisation is likely mediated by inter-domain disulfide bond formation. Furthermore, using Surface Plasmon Resonance, we also show that oxidised hSSB1 binds to 8-oxo-G damaged ssDNA with higher affinity than non-damaged ssDNA, indicating a direct role for oxidised hSSB1 in the recognition of 8-oxo-G lesions. As oxidative stress is associated with aging, cancer and Alzheimer's disease, understanding the molecular mechanisms of how cells repair oxidative DNA damage will be crucial in the development of potential therapeutic treatments.

PB-033 **Virtual Screening for Novel Inhibitors of Acetoacetyl-CoA Reductase of Burkholderia pseudomallei**

Luis Valencia[1,2], Josh Beckham, Oscar Villarreal, Jon Robertus
1.-University of Texas at Austin, 2.-Freshman Research Initiative

Burkholderia pseudomallei is a gram-negative bacteria that causes the disease melioidosis, a potentially chronic and aggressive infection with a mortality rate of up to 90% and is listed as a category B critical biological agent by the National Institute of Allergy and Infectious Diseases (NIAID). The glyoxylate metabolism pathway of B. pseudomallei carries out the metabolism of fatty acids – a function responsible for the virulent ability of B. pseudomallei to survive after being engulfed by the host's macrophages. Virtual screening on the structure of acetoacetyl-CoA reductase, a protein suggested to be essential in the glyoxylate pathway of B. pseudomallei, was conducted using GOLD molecular docking program to identify potentially novel inhibitor ligands from large virtual chemical libraries. The gene sequence of the acetoacetyl-CoA was assembled through overlap PCR and inserted into the expression vector pNIC-Bsa4 using ligation independent cloning. The protein was then expressed using IPTG induction of the T7 polymerase Lac operon system in BL21(DE3) cells, purified using His-tag Ni-NTA affinity chromatography, and characterized using SDS-PAGE. Enzymatic activity was confirmed by using a spectrophotometric enzyme assay measuring the absorbance of NADPH at 340nm during the reduction of acetoacetyl-CoA into (R)-3-Hydroxy-butanoyl-CoA. From the virtual screening of 30,000 ligands of the Chembridge Diversity Library against the acetoacetyl-CoA reductase structure the highest scoring ligands were selected and ordered for inhibition assay experiments. Current research objectives involve identification of novel inhibitors from inhibition assays, determination of enzyme kinetics, and the development of an acetoacetyl-CoA reductase structure with ligands or inhibitors bound in the active site.

PB-034 **Use of Principal Component Analysis and Molecular Docking to Identify Novel Selective Plasmepsin II Non-Competitive Inhibitors with Antimalarial Activity**

Pedro Alberto Valiente Flores[1], Maarten G Wolf[2], Yasel Guerra[3], Isel Pascual[1], Isabelle Florent[4], Enrique Rudiño[3], Pedro Geraldo Pascutti[5], Tirso Pons[6], Gerrit Groenhof[2]

1.-Center of Protein Studies, Faculty of Biology, University of Havana., 2.-Max Planck Institute of Biophysical Chemistry, 3.-Biotechnology Institute. UNAM, 4.-CNRS-MNHN, 5.-Biophysics Institute. Federal University of Rio de Janeiro, 6.-Spanish National Cancer Research Centre

Plasmepsin II (PlmII) is an aspartic protease involved in the initial steps of the hemoglobin degradation pathway, a critical stage in the Plasmodium falciparum life cycle during human infection. However, most of the PlmII inhibitors obtained through structure-based ligand design have generally shown a low selectivity towards the human related protease Cathepsin D (hCatD), which is a notable drawback to their use as antimalarial drugs. Here, we presented a novel in silico approach based on the combined use of principal component analysis and molecular docking to identify non-competitive selective inhibitors of PlmII. We searched unique conformational states of PlmII that can not be adopted by the human aspartic proteases: Cathepsin D, Renin and Pepsin by comparing the conformational subspaces sampled by these proteins along molecular dynamic simulations of 1.2 μs. Specific conformations along the flap opening-siding mode of PlmII that can not be sampled by the human counterparts were identified. The specific conformations were used to perform virtual screening experiments and proposed putative PlmII selective-inhibitors. The hCatD was also targeted to exclude non-selective compounds. The first five ranked inhibitors, with inhibition constants (Ki) values in the μM-nM range, target a cryptic flap interior pocket formed by the residues M75, V82, V105, T108, and Y115 which is only exposed in the PlmII specific conformations. The inhibition assays showed that the inhibitors bind better PlmII than hCatD in a range from 70 to 100-fold of their Ki values. The kinetic characterization showed a non-competitive inhibition of PlmII for all the compounds. Molecular docking calculations suggest that these compounds probably target the other three Plms expressed in the digestive vacuole of Pf. Notably two of them (SPB07935 and HTS07519) inhibited red blood cell cultures infected with the Pf cloroquine resistant strain FcB1 with an IC50 value in the μM range.

PB-035 **Ain't gold all that glitters: missing gold atoms in the structure of lysozyme crystals used to co-crystallize gold nanoparticles**

Antonello Melrino[1], Irene Russo Krauss[2], Marco Caterino[1], Alessandro Vergara[1]
1.-Dept. Chemical Sciences, University of Naples Federico II, 2.-Institute of Biostructures and Bioimaging, CNR

Wei et al. (2011) reported the growth of gold nanoparticles within protein single crystals of hen egg white lysozyme (HEWL) [1]. We tried to reproduce the experiments performed by these authors obtaining red, well-diffracting crystals of HEWL after 1 month of soaking in the presence of the gold-nanoparticle precursor ClAuS(CH2CH2OH)2. However, when refining our crystal structures we found no gold atoms.[2] This finding prompted us to analyze the models by Wei et al. deposited in the Protein Data Bank (codes 3P4Z, 3P64, 3P65, 3P66, 3P68), where nine different gold atoms are present (four isolated gold atoms and a 5-atom cluster). For eight out of the nine gold atoms found in the structures deposited by Wei et al., we believe that the authors' interpretation is questionable. [2] Ultimately, three out of five crystal structures solved by Wei et al. likely correspond to HEWL with only one Au+ ion bound to His15, as previously reported in crystal structures of adduct between HEWL and gold-based drugs [3-4]. The last two crystal structures are gold-free. We consider the ability of gold nanoparticles to grow within protein single crystals a stimulating result that has interesting implications. However, the structural analysis by Wei et al cannot be used to unveil protein-gold nanoparticle interactions because no gold atom is unambiguously found in the reported HEWL structures, apart from one ion bound to His15 in the first three structures. This means that structural data on biomolecule-directed gold clusters is still lacking and that the molecular basis of protein-gold nanoparticle recognition requires further investigation. References 1. Wei, H. et al. Nature Nanotech. 6, 93–7 (2011). 2. Merlino, A. et al. Nature Nanotech. 10 (4), 285 (2015). 3. Messori, L. et al. Chem. Commun. 49 (86), 10100-2 (2013). 4. Russo Krauss et al. Dalton Trans 43 (46), 17483-8 (2014). Sponsored by the member Prof. Filomena Sica

PB-036 **Finding a novel treatment for the biological weapon threat of epidemic typhus by targeting ß-ketoacyl-ACP-reductase in Rickettsia prowazekii**

Oscar Villarreal[1], Josh Beckham[1], Jon Robertus[1]
1.-University of Texas at Austin, Department of Molecular Biosciences

Epidemic typhus, which is caused by the bacterial pathogen Rickettsia prowazekii, is a menacing disease world wide that the NIH lists as one of America's greatest biological weapons threats. This research seeks to find novel inhibitors of β-ketoacyl-ACP-reductase (FabG), an enzyme that catalyzes one of the reactions in the fatty acid synthesis type II system in bacteria. This pathway is essential for survival in bacteria. The FabG enzyme uses NADPH as a substrate, which facilitates the binding of the second substrate, acetoacetyl-ACP into the active site. The acetoacetyl-ACP is subsequently reduced into β -hydroxyacyl-ACP. The coding DNA sequence for the RpFabG protein was cloned into a pNIC vector and transformed into E.coli BL21(DE3), then the protein was expressed and purified using metal affinity and size exclusion chromatography methods. High throughput molecular docking software (GOLD) was used to screen a commercial library of ligands against the acetoacetyl-ACP region of the active site. The ligands with the best GOLD scores were selected to be tested in vitro. Spectrophotometric enzyme inhibition assays were performed to determine whether the drugs could inhibit RpFabG activity. Chlorogenic acid, a previously known inhibitor of homologous FabGs, was tested along with the other potential drugs, and was determined to have moderate inhibitory effects on RpFabG. Loop modeling using ICM software was performed in order to create a prediction of the complete RpFabG structure, including the disordered loops that are not a part of the 3F9I PDB structure. Co-crystallization of RpFabG with both substrates was carried out in order to obtain a structure, but only non-diffracting crystals resulted. Further inhibition assays and crystallography trials are being performed in order to continue the search for a novel inhibitor of RpFabG and ultimately a treatment for epidemic typhus.

PB-037 **Diazotransfer reagents to selectively functionalize a protein of interest with azido groups**

Martin Witte[1], Jonas Lohse[1], Remko Welker[1]
1.-Stratingh Institute for Chemistry, University of Groningen

The incorporation of a non-natural functional group onto proteins facilitates studying their role. It also led to the development of protein variants which can be activated (photo)chemically inside cells and it has become an important means to synthesize biopharmaceuticals. The functional group is generally incorporated using auxotrophic strains, amber suppression technology, enzymatic labelling or ligand-tethered labelling.1 These techniques, except for the latter, all require genetic modification and overexpression of the target protein, which can lead to artefacts. Our aim was to develop reagents that enable direct functionalisation of a target protein with a ligation handle in a complex mixture. As requirement, we set that the handle should not only facilitate further modification, but that it should also serve as a potential chemical turn-on/turn-off switch. Conversion of an amine into an azido group fulfils these requirements. It can be modified using bioorthogonal reactions, such as the copper-catalyzed click reaction and the Staudinger ligation. Moreover, modification of essential amine groups in the protein of interest may inhibit protein activity. Reduction of the azido group will give the unmodified target protein and restore the activity, and as such the azido group may also serve as a chemical turn-on/turn-off switch. Van Hest and coworkers revealed that azido groups can be introduced onto purified recombinant proteins using a diazotransfer reaction,2 but the described method cannot be used to selectively modify a proteins of interest within a complex mixture. We reasoned that this issue could be addressed by tethering the diazotransfer reagent to a ligand that binds to the protein of interest. As a proof of concept, we synthesized novel reagents to selectively functionalize the model proteins streptavidin and carbonic anhydrase II. The diazotransfer reagents react selectively with their corresponding target protein in mixture of proteins and when spiked in a cell lysate. Rapid labelling was observed in the presence of Cu(II), but the diazotransfer reaction also occurred in the absence of copper, albeit with reduced efficiency. To identify the site of modification, labelled proteins were analyzed with mass-spectrometry. A single lysine residue is modified in the case of streptavidin and tethering the reagents may thus enhance the site selectivity as well. However, multiple lysine residues were modified in carbonic anhydrase II indicating that careful design of the reagent is required to achieve site selective modification. The experiments with streptavidin and carbonic anhydrase II revealed that targeted diazotransfer reagents can be used to selectively modify a protein of interest, and we are currently extending our approach to other proteins, such as the proteasome.

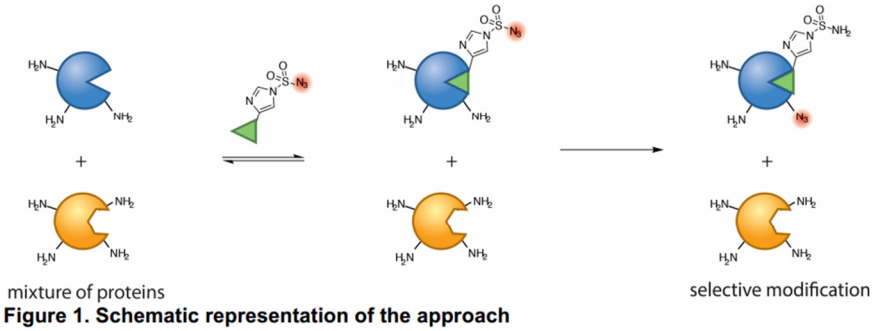

Figure 1. Schematic representation of the approach

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PB-038 **PENG: a neural gas-based approach for pharmacophore elucidation. method design, validation, and virtual screening for novel ligands of lta4h**

Sandra Kerstin Wittmann[1], Daniel Moser[1], Jan Sebastian Kramer[1], René Blöcher[1], Janosch Achenbach[3], Denys Pogoryelov[2], Eugen Proschak[1]

1.-Institute of Pharmaceutical Chemistry, LiFF/OSF/ZAFES, Goethe-University, 2.-Institute of Biochemistry, Goethe University, 3.-BASF SE

Leukotriene A4 hydrolase (LTA4H; EC 3.3.2.6) is a bifunctional zinc metalloprotease which displays both epoxide hydrolase and aminopeptidase activity [1]. With a high preference the leukotriene A4 hydrolase cleaves tripeptides at an arginyl bond on NH2 position [3]. To screen for new inhibitors of the enzyme we used a growing neural gas (GNG)-based approach for the extraction of the relevant features which we called PENG (pharmacophore elucidation by neural gas). The results of the prospective virtual screening have been validated using a fluorescence-based assay system. Therefore the non-fluorescent substrate L-arginine-7-amido-4-methylcoumarin hydrochloride is used. The LTA4H cleaves the arginyl bond what results in the fluorescent 7-amino-4-methylcoumarin. Additionally, we could show that the PENG approach is able to predict the binding mode of the ligand by X-ray crystallography. References [1] J.Z. Haeggström, F. Kull et all., Leukotriene A4 Hydrolase., Prostaglandins & other Lipid Mediators 68-69 (2002); 495-510. [2] Y. Michael Shim, Mikell Paige. Leukotriene A4 Hydrolase – An envolving target. Inflammatory Diseases - Immunopathology, Clinical and Pharmacological Bases, Dr Mahin Khatami (Ed.), ISBN: 978-953-307-9118-0. [3] Lars Orning, J.K. Gierse, and F. A. Fitzpatrick. The Bifunctional Enzyme Leukotriene-A, Hydrolase Is an Arginine Aminopeptidase of High Efficiency and Specificity. J. Biol. Chem. (1994); 269(15); 11269-73. [4] J. Z. Haeggström and C. D. Funk. Lipoxygenase and Leukotriene Pathways: Biochemistry, Biology, and Roles in Disease. Chem. Rev. (2011); 111; 5866 – 98. [5] Robert J. Snelgrove et al. A critical role for LTA4H in limiting chronic pulmonary. Science (2010) 330 (6000): 90-94

PB-039 **Stabilization of aspergillus parasiticus cytosine deaminase by immobilization on calcium alginate beads improved enzyme operational stability**

Hassan Zanna[1], Andrew Nok[2], Sani Ibrahim[2], Hauwa Inuwa[2]
1.-University of Maiduguri, 2.-Ahmadu Bello University

Abstract Cytosine deaminase (CD) from Aspergillus parasiticus, which has half-life of 1.10 hrs at 37ºC, was stabilized by immobilization on calcium alginate beads. The immobilized CD had pH and temperature optimum of 5 and 50ºC respectively. The immobilized enzyme also stoichiometrically deaminated Cytosine and 5-fluorocytosine (5-FC) with the apparent K'M values of 0.60 mM and 0.65 mM respectively, displaying activation energy of 10.72 KJ/mol. The immobilization of native CD on calcium alginate beads gave the highest yield of apparent enzymic activity of 51.60% of the original activity and the enzymic activity was lost exponentially at 37ºC over twelve (12) hours with half- life of 5.80 hrs. Hence, the operational stability of native CD can be improved by immobilization on calcium alginate beads. * Author for correspondence

PB-040 **Ubiquitin-nanoparticle interactions by solution NMR spectroscopy**

Serena Zanzoni[1], Michael Assfalg[1], Rajesh K Singh[2], Marco Pedroni[3], Adolfo Speghini[3], David Fushman[2], Mariapina D'Onofrio[1]

1.-NMR Laboratory, Department of Biotechnology, University of Verona, 2.-Center For Biomolecular Structure and Organization, Department of Chemistry and Biochemistry, University of Maryland, 3.-Solid State Chemistry Laboratory, Department of Biotechnology, University of Verona

The potential use of nanoparticles (NPs) in biomedical applications has attracted considerable interest in the last years. NPs introduced in a biological environment interact with a collection of biomolecules, including proteins. NPs associating with proteins may determine changes in protein conformation, interfere with protein-protein interactions, and affect signal communication pathways [1]. Therefore, the study of NP-induced functional perturbations of proteins implicated in the regulation of key biochemical pathways is particularly relevant. Ubiquitin (Ub) is a small cytosolic protein playing a central role in numerous biological processes including protein degradation, cell signaling, and DNA repair [2]. It can be predicted that its interaction with NPs may affect cellular pathways. In this respect, we characterized, at atomic level, the interactions of Ub with two different size and chemical composition NPs. The first NP that was tested was fullerenol, a polyhydroxylated [60]fullerene NP. These carbon based NPs have several potential biomedical applications, including their use as drug carriers, antiviral drugs, enzyme inhibitors, contrast agents, antioxidants, and photosensitizers [3]. To characterize the fullerenol-Ub interactions, site-resolved chemical shift and intensity perturbations of Ub's NMR signals, together with 15N spin relaxation rate changes, were used [4]. The obtained data were consistent with interactions involving fullerenol clusters adsorbing reversibly to monomeric Ub (and dimeric Ub), and targeting specific binding epitopes, coincident with functional recognition sites of Ub. Furthermore, we observed that fullerenol almost completely abolished the formation of di-Ub and longer chains in vitro, suggesting that fullerenol NPs may effectively interfere with protein-mediated functional communication, eliciting cytotoxic effects. The second kind of examined NPs were SrF2 NPs. Considerable importance in biomedical luminescence is the application of these NPs doped with rare lanthanide ions, due to their ability to produce up-conversion emission [5]. NMR spectroscopy, up-conversion luminescence measurements and isothermal titration calorimetry were used to probe the Ub-SrF2 NP interactions. As in the case of fullerenol NPs, the analysis of NMR data indicated the occurrence of a reversible equilibrium between free and NP-bound protein forms. The identification of similar interaction epitopes suggests a similar impact on functional biomolecular communication. Our findings support the view that NPs may affect fundamental interaction patterns of Ub, with possible nanotoxic consequences on cell homeostasis. On the other hand, the specific inhibition of critical Ub interactions through competitive binding of NPs to polyUb chains could represent a new potential opportunity for pharmacological intervention against cancer development. Reference 1. Saptarshi S.R. et al., J. Nanobiotechnology, 2013, 11, 26. 2. Aguilar R.C. et al., Curr. Opin. Cell Biol., 2003, 15, 184–190. 3. Bosi S. et al., Eur. J. Med. Chem., 2003, 38, 913–923. 4. Zanzoni S et al., Nanoscale, 2015, DOI: 10.1039/C5NR00539F 5. Dong N.N. et al., ACS Nano, 2011, 5, 8665–8671.

PB-041 **Chemical-Genetic Dissection of Protein Kinase Functions**

Chao Zhang[1], Ying-Chu Chen[1], Alvin Kung[1]
1.-Department of Chemistry, University of Southern California

Small molecules are extremely useful tools for elucidating protein functions in cells due to their acute and tunable effects. However, they often have difficulty distinguishing between target proteins that are highly homologous to each other. To address this deficiency and achieve high specificity, we are developing molecules that can covalently target a reactive feature in the target protein. The reactive feature, in the form of a cysteine residue near a ligand-binding pocket, can be naturally present or engineered via mutagenesis in the protein of interest. Using this chemical-genetic approach, we successfully identified specific inhibitors for a single isoform of Eph receptor tyrosine kinases. These isoform-selective Eph inhibitors allow us to evaluate the role of individual Eph kinases in cells. In addition, we applied the chemical-genetic approach to the study of the Raf serine/threonine kinases, a key player in the MAPK/ERK pathway (also known as the Ras-Raf-MEK-ERK pathway) that plays an essential role in the regulation of cell proliferation, differentiation and survival. Surprisingly, the selective inhibitors of oncogenic BRAF generated in this manner were unable to completely block the MAPK signaling in cells, which we attribute to the transactivation of endogenous wide-type RAF in cells. These results suggest that a pan-RAF inhibitor is required for suppression the MAPK signaling in cancer cells. These findings have direct implications for the drug resistance observed in the clinic and the development of second-generation melanoma therapies.

PB-042 **Selective modification of proteins and peptides by ruthenium porphyrin-catalyzed carbene transfer reaction**

Chi-Ming Ho[1], Jun-Long Zhang[1], Cong-Ying Zhou[1], On-Yee Chan[1], Jessie Jing Yan[1], Fu-Yi Zhang[1], Jie-Sheng Huang[1], Chi-Ming Che[1]
1.-The University of Hong Kong

Bioconjugation of proteins has emerged as a useful tool in the study of biological systems. There is an increasing need to develop new synthetic technologies for the bioconjugation reaction of proteins, and metal-catalyzed site-selective modification of proteins has attracted considerable interest in recent years. We have developed a ruthenium glycosylated porphyrin-catalyzed carbenoid transfer reaction for the site-selective modification of proteins. We firstly applied the catalysis to the selective modification of the N-terminus of peptides. By using ruthenium glycosylated porphyrin as catalyst, the N-terminus of a number of peptides can be modified through carbenoid N-H bond insertion in aqueous media with moderate to excellent conversion. The reaction is highly selective, for example, the reaction with YTSSSKNVVR, which contains various types of oxygen–hydrogen and nitrogen–hydrogen bonds possibly available for carbenoid insertion, catalyzed by the ruthenium glycosylated porphyrin gave the N-terminal-modified product with >99% conversion and without the formation of other modified peptides including doubly modified and oxygen–hydrogen bond insertion products. We next extended the N-terminal modification method to proteins. Eventually success was attained in the modification of RNase A and insulin. The reaction of RNase A with a diazoacetate mediated by ruthenium glycosylated porphyrin gave corresponding N-terminal-modified protein with 65% conversion. We also achieved a bioconjugation to ubiquitin via ruthenium glycosylated porphyrin-catalyzed alkene cyclopropanation in aqueous solution in two steps: (1) incorporation of an alkenic group by the reaction of N-hydroxysuccinimide ester with ubiquitin and (2) cyclopropanation of the alkene-tethered Lys6 ubiquitin with the fluorescent labeled diazoacetate in the presence of a catalytic amount of ruthenium glycosylated porphyrin. The corresponding cyclopropanation product was obtained with $\sim$ 55% conversion based on MALDI-TOF mass spectrometry. In conclusion, we developed a ruthenium porphyrin-catalyzed site-selective modification of peptides and proteins in aqueous media. The method provides an entry to new bioconjugation reactions for protein modifications using metalloporphyrins as catalysts.

PB-043 **Modulating the affinities of phophopeptides to human Pin1 WW domain using 4-substituted proline derivatives**

Jia-Cherng Horng[1], Kuei-Yen Huang[1]
1.-Department of Chemistry, National Tsing Hua University

Human Pin1 is involved in cancer developments and has been a pharmaceutical target. Thus, finding a high affinity inhibitor of Pin1 has become an attractive topic. The WW domain of human Pin1 can recognize the phosphoserine/phosphothreonine-proline (pS/pT-P) motifs, while its PPIase domain catalyzes the cis/trans isomerization of prolyl bonds to regulate the cell cycle. Here we incorporated a series of 4-substituted proline derivatives into the phosphopeptides and investigated their affinities to the WW domain of Pin1 to develop better inhibitors of Pin1. Based on the ligand Myt1-T412 [PPA(pT)PP], we synthesized several phosphopeptides in which proline residue in the pT-P motif was replaced with various 4-substituted proline derivatives. Isothermal titration calorimetry and fluorescence anisotropy analyses show that the replacement of proline by (2S,4R)-4-fluoroproline increases the binding affinity of the peptide. Circular dichroism measurements suggest that a more PPII-like structure of phosphopeptides make them bind to the WW domain more tightly. Chemical shift perturbation experiments also indicate that (2S,4R)-4-fluoroproline interacts with Trp34 of the WW domain in the binding site. Results of molecular modeling further suggested that a strong C-H... π interaction induced by (2S,4R)-4-fluoroproline is important in enhancing the affinity of the peptide to the WW domain. The results of our present study provide new valuable information for designing and developing effective inhibitors of human Pin1.

PB-044 **Applying an analytical ultracentrifuge equipped with fluorescence detection to the study of polyglutamine aggregation in Caenorhabditis elegans**

Bashkim Kokona[1], Carrie A. May[2], Nicole R. Cunningham[1], Franklin J. Garcia[1], Kathleen M. Ulrich[1], Christine M. Roberts[4], Christopher D. Link[4], Walter F. Stafford[3], Thomas M. Laue[2], Robert Fairman[1]

1.-Department of Biology, Haverford College, 2.-Department Of Molec., Cell., and Biomed. Sci., University of New Hampshire, 3.-Boston Biomedical Research Institute, 4.-Integrative Physiology, University of Colorado Boulder

This work aims to explore the heterogeneity of aggregation of polyglutamine fusion constructs in crude extracts of an animal model system, using transgenic Caenorhabditis elegans animals. The work takes advantage of the recent technical advances in fluorescence detection as coupled with analytical ultracentrifugation. Further, new methods of analysis of sedimentation velocity experiments, such as gravitational sweep experiments, are applied to improve the resolution of the measures of heterogeneity over a wide range of sizes. The focus here is to test the ability to measure sedimentation of polyglutamine aggregates in complex mixtures, as a prelude to future studies that will explore the effects of genetics and environment on aggregation and toxicity. Using sedimentation velocity methods, we can detect a wide range of aggregates, ranging from robust analysis of the monomer species, through an intermediate and quite heterogeneous population of oligomeric species, and all the way up to detecting species that likely represent intact inclusion bodies based on comparison to an analysis of fluorescent punctates in living worms by confocal microscopy. Our results support the hypothesis that misfolding of expanded polyglutamine tracts into insoluble aggregates involves transition through a number of stable intermediate structures, a model that accounts for how aggregation can be at the same time toxic and protective. An understanding of the details of small, intermediate, and large-scale aggregation for polyglutamine sequences, as found in neurodegenerative diseases such as Huntington's Disease, will help to more precisely identify which aggregated species may be involved in toxicity and disease.

PB-045 **Probing the selectivity of peptide carrier protein-tailoring enzyme interactions using analytical ultracentrifugation**

Robert Fairman[1], Bashkim Kokona[1], Emily S. Winesett[2], Alfred N. von Krusenstiern[2], Max J. Cryle[3], Louise K. Charkoudian[2]

1.-Department of Biology, Haverford College, 2.-Department of Chemistry, Haverford College, 3.-Max Planck Institute for Medical Research

Bacteria and fungi use non-ribosomal peptide synthetases (NRPSs) to produce peptides of broad structural diversity and biological activity. The impressive diversity of non-ribosomal peptides originates in part from the action of tailoring enzymes that modify single amino acids and/or the mature peptide. Studying the interplay between tailoring enzymes and the peptide carrier proteins (PCPs) that anchor and present the substrate is challenging because the transient nature of the protein-protein interactions. Using sedimentation velocity (SV) methods, we studied the collaboration between PCP and cytochrome P450 enzyme that is crucial for the installation of β-hydroxylated amino acid precursors in the biosynthesis of the depsipeptide skyllamycin. We show that sedimentation velocity (SV) is an ideally suited method for a quantitative exploration of PCP-enzyme equilibrium interactions. Our results suggest that the PCP itself and the presence of substrate covalently tethered to the PCP together facilitate productive PCP-P450 interactions, thereby revealing one of nature's intricate strategies for installing interesting functionalities using natural product synthases.

PB-046 **Uridine Monophosphate Synthase: Architecture Versatility in the Service of Late Blight Control**

Francisco Tenjo Castaño[1,2], Manuel Garavito[1,2], Leonor García[1,2], Silvia Restrepo[2], Barbara Zimmermann[1]

1.-Biochemistry and Molecular Biology Research Group, Universidad de los Andes., 2.-Mycology and Plan Pathology Laboratory, Universidad de los Andes

Uridine monophosphate synthase (UMPase), a bifunctional enzyme in the de novo pyrimidine biosynthetic pathway, is a protein comprised of orotate phosphoribosyl transferase (OPRTase) and orotidine monophosphate decarboxylase (ODCase). Different fusion orders of the two domains have been documented to exist in nature. In some organisms OPRTase and ODCase are monofunctional proteins, and act as a complex. Here, UMPase from Solanum tuberosum (potato) and from Phytophthora infestans (an oomycete) were examined. P. infestans causes late blight disease in S. tuberosum, destroying crops and increasing production costs. Since pyrimidines are fundamental cellular components, we have proposed that UMPase could serve as a target to control P. infestans infection. The enzymes from P. infestans and S. tuberosum differ in their fusion order of OPRT and ODC. The study of these two UMPase could facilitate the design of species-specific inhibitors, and might shed light on the effect of fusing UMPase domains in one order or the other. To this end we carried out bioinformatic and biochemical characterization of the enzymes. Sequence analyses showed 20 residue differences among the P. infestans UMPase sequences from three strains: 4084, 1306 and T30-4. Strain T30-4 was found to have a duplicated UMPase, but neither sequence corresponded to the ones predicted previously from the genome. A recombinant UMPase from 4084 strain was expressed in bacteria and purified but it showed low solubility and was inactive in vitro. The recombinant UMPase from the 1306 strain complemented both OPRTase and ODCase deficient E. coli strains. A soluble, active, recombinant protein was expressed and purified in the presence of high salt and the product UMP (specific activity ≈ 0.2 µmol min-1 mg-1). The sequence SKQ was found at the C-terminus of the P. infestans UMPase sequences and resembles a peroxisome signal peptide (SKL). The predicted hydrophobicity of this UMPase and its architecture (OPRT at the C-terminus and ODC at the N-terminus) resembles that of the UMPase from Leishmania donovani, which has been localized to the peroxisome. We suggest that P. infestans UMPS could also be located in this organelle. In contrast to the oomycete enzyme, S. tuberosum UMPase is highly soluble, and has a higher specific activity (Vmax= 8.8 µmol min-1 mg-1). We measured the kinetic parameters KM(orotate)= 16.2 µM, KM(PRPP)= 25.5 µM, and found that it exhibited product inhibition by pyrophosphate. In conclusion, the different architectures of the two UMPS might be related to distinct biochemical characteristics, further supporting this protein as a good candidate for P. infestans control.

PB-047 **Three Antimicrobial Peptides: MD Simulation Studies Supporting Experiment**

Walter Scott[1], Vivien Schubert[2], Andi Mainz[2], Suzana K. Straus[1], Roderich Suessmuth[2]
1.-Department of Chemistry, University of British Columbia, 2.-Institut fuer Chemie, Technische Universitaet Berlin

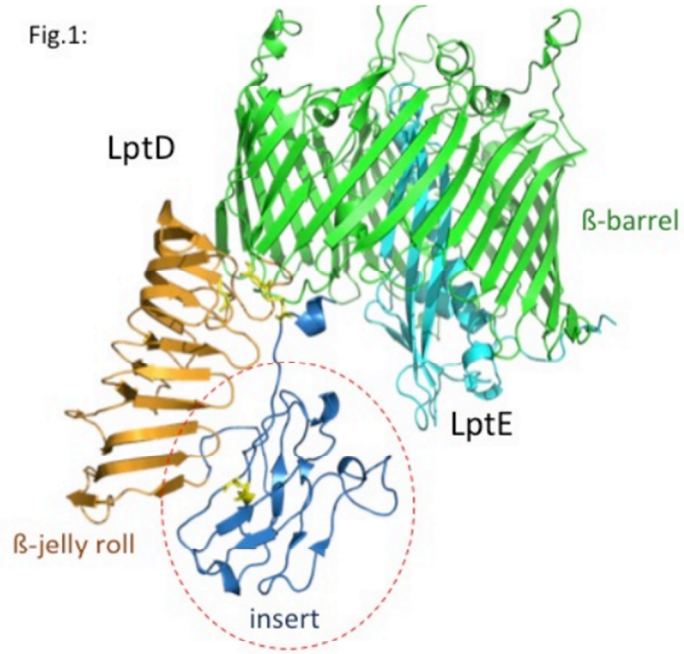
We present computer simulation studies of three different antimicrobial peptides we have been studying by MD computer simulation in collaboration with experimentalists. The first is Daptomycin, a potent lipopeptide currently licensed to treat infections caused by multi-drug-resistent bacteria. The mechanism of action of Daptomycin is currently not completely understood. We have solved the NMR structure of this molecule, and attempted to determine the size of its oligomer by small angle neutron scattering (SANS) supported by computer simulation. Feglymycin is a 13-amino-acid peptide with a high percentage of unusual amino acids such as 4-hydroxyphenylglycine and 3,5-dihydroxyphenylglycine. Feglymicin inhibits MurA and MurC enzymes which are involved in bacterial peptidoglycan synthesis, while also displaying anti-HIV activity by interaction with the viral envelope protein gp120. A previous X-ray structure shows the molecule forming a dimer. Here, the molecule was studied by NMR in water and DMSO. In water, the molecule is clearly at least a dimer, while in DMSO it is a monomer. We have performed NOE refinement simulations in order to elucidate a structure, however, due to a lack of long-range NOE contacts, a unique structure cannot be determined. Labyrinthopeptin A2 is a lantibiotic that contains labionin, a unique carbacyclic posttranslationally modified amino acid that links the protein backbone in three different locations. Labyrinthopeptin A2 has shown promising activity as a pain killer. Starting from the X-ray structure, we present results from the first MD simulation studies of this unique peptide. Because of the extensive cross-linking, this peptide is observed to be highly rigid in its native form. Simulation results of mutants are also presented.

PB-048 **Studying the Outer Membrane ß-barrel Protein LptD, the Target of a New Peptidomimetic Antibiotic**

Katja Zerbe[1], Gloria Andolina[1], Laszlo Bencze[1], Kerstin Moehle[1], John A. Robinson[1]
1.-Department of Chemistry, University Zurich

Antibiotics with new mechanism of action are urgently required to combat the growing health threat posed by resistant pathogenic microorganisms. Here we report the discovery of a new peptidomimetic antibiotic (L27-11), which is active with a minimum inhibitory concentration (MIC) in the low nanomolar range, only against Pseudomonas sp., and with a non-membrane-lytic mechanism of action. A drug target identified both in a forward genetic screen for resistance determinants and by photoaffinity labeling is the ß-barrel protein LptD, which plays an important role in LPS transport and the outer membrane biogenesis. The X-ray structure of LptD in complex with LptE from Shigella flexneri shows a 26 stranded β-barrel linked to a periplasmatic N-terminal jelly-roll domain. Interestingly the homology model structure for LptD from Pseudomonas shows a significant difference: an insertion of around 100 amino acids in the N-terminal domain. The results of our attempts to purify and characterize this large outer membrane protein and to determine the binding site of the peptidomimetic antibiotic will be shown.

Fig.1:



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PB-049 **Structure and catalytic properties of peptides based on sequences of P-loop from ATP binding domains**

Wioletta Zmudzinska[1], Marcel Thiel[1], Stanislaw Oldziej[1]
1.-IFB, University of Gdansk and Medical University of Gdansk

The theory of how life on Earth begun still remains unclear. Nevertheless, according to some theories, at the beginning level proteins did not emerge as a complex globular forms as know today. At the times, when solely RNA molecules stored both genetic information and catalyzed the chemical reactions in primitive cells, peptides acted as a proteins nowadays [1,2]. Literature postulate that the possible role of primordial short peptides was to catalyze reactions in RNA-world, as they possess an excellent ability to self-assemble into well-ordered nanostructures [3,4]. Elementary Functional Loops (EFLs) can be considered as a small structures (blocks) having specific signatures and providing functional residues important for binding/activation as well as principal chemical transformation steps of the enzymatic reaction [5]. P-Loop EFL is a widespread structure across vast majority of protein families such as motor domains, AAA+, RecA, PEPCK and many others. Sequential alignment of these protein families reveals existence of a conserved P-loop motif, that is able to bind ATP molecule. We investigated the structure and ATPase activity of peptides, which sequences possessed strongly conserved GXGK[T/S] motif from P-loop. The goal of our work was to check if peptides corresponding to the most conserved P-loop motif fragment are able to bind and hydrolyze ATP molecule. All peptides under study were chemically synthesized and their structures was investigated by NMR spectroscopy. The ability to bind ATP molecules was analyzed by using HPLC chromatography. Results of our study show, that peptides with conserved P-Loop motif have a suitable structures to promote binding of the molecules with phosphate group, but cannot accelerate pyrophosphate hydrolysis process. Conference participation for W. Ż. supported by the FP7 project MOBI4Health (grant agreement no 316094). Computational resources were provided by the Informatics Center of the Metropolitan Academic Network (IC MAN TASK) in Gdansk, Poland. 1] Carny O & Gazit E (2005) A model for the role of short self-assembled peptides in the very early stages of the origin of life. Faseb J 19(9):1051-1055
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PB-050 **The atp-binding site of CK2 carries two regions with antagonistic electrostatic potential that atracts charged ligands**

Maria Winiewska[1], Jarosław Poznański[1]
1.-Institute of Biochemistry and Biophysics Polish Academy of Sciences

CK2 is a ubiquitous serine/threonine protein kinase, being one of the most pleiotropic of all protein kinases1. CK2 plays a key role in cell growth, differentiation, cell death and survival, and become the therapeutic target in cancer treatment, since its level is significantly increased in cancer cells2. Halogenated ligands have been widely developed as potent inhibitors of protein kinases. Among them 4,5,6,7-tetrabromobenzoteriazole (TBBt) is one of the first potent and selective inhibitor of CK2α, directed towards the conserved ATP binding site3. To assess contribution of electrostatic interactions to the specificity and strength of binding of multi halogenated inhibitors by a protein kinase, we have studied interaction between CK2α and nine benzotriazole derivatives, representing all possible patterns of halogenation on the benzene ring. Herein, we present results that support existence of two alternative regions that are involved in ligand binding. Aspartic acid 175 is known for its function in coordination of a Mg2+ ion, which is required for ATP binding 4. Asp175 has been identified in crystal structure of CK2:TBBt complex (PDB1j91, Fig. 1) as the charged residue closest to TBBt. There is also Lys68 proximal to TBBt, interaction with which may favor anionic form of ligands5 (pK for TBBt <5), however it is involved in the intramolecular salt bridge, and thus its mutation may significantly change stability of the protein.

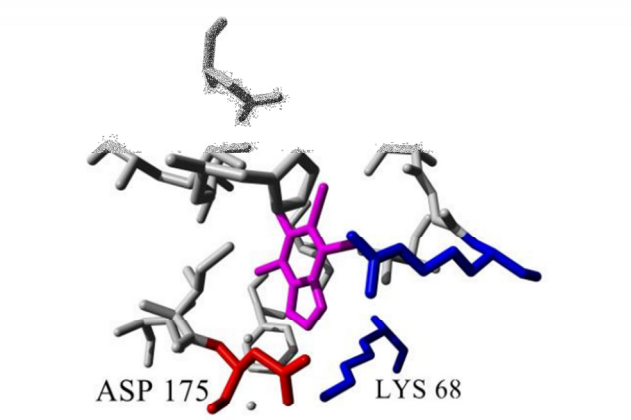

Fig. 1. Crystal structure of TBBt complexed with CK2 (PDB:1j91). Residues with a distance to TBBt (Magenta) shorter than 5Å are shown. Red residue is negatively charged, blue ones are protonated.

Comparison of Kdiss values determined for ligands at pH 8 and at pH 7 shows that strength of the complex significantly varies upon deprotonation of the triazole ring. This confirms former hypothesis that a negatively charged ligands cluster at the ATP binding site region proximal to Lys685, which is beneficial both to the specificity and to strength of the binding. We have also observed for the tested ligands variations in their binding to either wild type protein and its D175N mutant (with less negative charge distributed over ATP binding site). All ligands displaying higher pKa for dissociation of the triazole proton bind to the mutant visibly weaker than to the wild-type protein. Altogether reveals the predominance electrostatic intermolecular interactions. Although, negatively charged ligands most probably cluster at the ATP-binding site proximal to Lys68, beneficial for the strength of binding, the less dissociated forms are favored due to unfavorable interactions of the anionic form of ligands with Asp175.

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PB-052 **NMR Solution Structure Elucidation of Phenol Soluble Modulins; Virulence Factors in Staphylococcus aureus**

Kaitlyn M. Towle[1], Christopher T. Lohans[2], Marco J. van Belkum[1], Mark Miskolzie[1], John C. Vederas[1]

1.-University of Alberta, 2.-University of Oxford

Phenol soluble modulins (PSMs) are a class of toxins isolated from the Staphylococcus sp..1 Of particular interest are the PSMs, produced by the infectious Methicillin Resistant Staphylococcus aureus (MRSA). MRSA infections have been on the rise through both community associated MRSA (CA-MRSA) and hospital associated MRSA (HA-MRSA) strains.1 There are many virulence factors produced by these strains, many of which are encoded on mobile genetic elements.2 PSMs are of specific interest because these virulence factors are encoded on the core genome of the bacteria and therefore all strains of staphylococci bacteria produce some variation of PSMs with a variety of biological functions.2 The specific mechanism by which PSMs act as virulence factors has been poorly understood until recently. Biological functions of PSMs include cell lysis, biofilm formation and the ability to kill neutrophils after phagocystosis.1 These toxins are of special interest to our research group due to their genetic similarities to certain bacteriocins, namely leaderless bacteriocins.3 Both groups of peptides are ribosomally synthesized with a N-terminal formyl methionine and secreted from the bacteria by ATP-binding cassette (ABC) transporters without any leader sequence or signal peptide. ABC transporters may also play a role in immunity towards PSMs and leaderless bacteriocins. These similarities led our group to investigate the solution structure of these peptides through nuclear magnetic resonance (NMR). Isolating PSMs from the producer organisim, S. aureus, typically involves lengthy extractions and low yields.4 For these reasons, we opted to chemically synthesize the desired peptides using solid phase peptide synthesis (SPPS). Utilizing a variety of SPPS techniques, PSM α1 and PSM α3 were successfully synthesized, however, due to the hydrophobic nature of PSM β2, an alternate genetic approach was devised to isolate PSM β2. Formation of a fusion protein between PSM β2 and the small ubiquitin like modifier (SUMO) protein allowed for heterologous expression. Upon cleavage of the fusion protein with SUMO protease, and subsequent purification and isolation of the cut peptide, PSM β2 was obtained. As previously reported, the PSMs were found to be alpha-helical in structure inducing solvents.5 A series of 2 dimensional (2D) NMR experiments were ran to determine chemical shift assignments and to obtain NOE data. Importing the chemical shift assignments and NOE data into the structure calculating software, CYANA, we were able to elucidate the solution structure of PSM α1 and PSM α3 and we are currently working towards the elucidation of PSM β2. The synthesis, isolation, characterization and solution structures of the aforementioned PSMs will be discussed here. 1. Peschel, A.; Otto, M. Nature Reviews Microbiology, 2013, 11, 667. 2. Cheung, G.Y.C.; et al. FEMS Microbiol Rev. 2014, 38, 698. 3. Cintas, L. M.; et al. J. Bacteriol. 1988, 180. 4. Wang, R.; et al. Nature Medicine 2007, 13, 1510. 5. Laabei, M.; et al. Biochimica et Biophysica Acta 2014, 1838, 3153.

PB-053 **Mitochondrial iron as a potential therapeutic target in friedreich's ataxia neurodegeneration: desferioxamine-peptide conjugate**

Roxana Yesenia Pastrana Alta[1], Maria Teresa Machini[2], Breno Pannia Espósito[1]1.-Laboratory for Bioinorganic Chemistry and Metallodrugs, Instituto de Química, Un, 2.-Laboratory of Peptide Chemistry, Instituto de Química, Universidade de São Paulo

Transition metals are critical for enzyme function and protein folding, but their excess can mediate neurotoxic oxidative processes [1]. As, energy production involves oxidative phosphorylation, a process requiring a continuous flow of electrons, mitochondria are particularly vulnerable to oxidative damage [2]. As such, mitochondria are the major sites of Reactive Oxygen Species (ROS) generation, which are produced as byproducts of the electron transport chain. Since free iron and certain ROS can engage into potentially deleterious processes such as Fenton reaction, mitochondrial iron homeostasis must be tightly controlled, and dysregulation of iron metabolism in this organelle has been associated with various diseases, including Friedich´s ataxia (FA), Alzheimer's, and other neurodegenerative disorders [3]. Engineering an efficient mitochondria-targeting, cell-permeable vector is a challenge due to the fact that mitochondrion is impermeable to a wide range of molecules. The development of delivery vectors has been made possible by a greater understanding of mitochondrial structure and chemical features of molecules that selectively localize within this organelle. From these findings, two generalized requirements for mitochondrial localization are delocalized positive charge and lipophilicity [4, 5]. Targeting iron in this organelle is proposed as a means to ameliorate FA symptoms. Desferrioxamine (DFO) is a bacterial siderophore with high affinity for iron, but low cell penetration. We prepared conjugates of DFO with Mitochondria Penetrating Peptides and studied their iron-binding characteristics in vitro. The lipophilic and charged peptides TAT49-57 (H-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg-OH)[6], 1A (H-Cha-Arg-Cha-Lys-Cha-Arg-Cha-Lys-NH2)[6], SS-02 (H-Dmt-Arg-Phe-Lys-NH2)[7] and SS-20 (H-Phe-Arg-Phe-Lys-NH2) [7], are known to permeate cytosolic and mitochondrial membranes. They were prepared and conjugated to DFO in solid-phase [8], an alternative synthetic route. Once detached from the resin, fully deprotected, purified and characterized by means of LC/MS and aminoacid analysis, it was observed that the DFO-conjugated peptides displayed iron-binding abilities identical to the free chelator DFO. DFO-conjugated peptides were also able to quench the iron-catalysed oxidation of ascorbate (a model of oxidative stress in plasma of iron-overloaded patients), as probed by a high throughput fluorimetric method [9,10]. These results indicate that our synthesis and conjugation strategy were successful in preserving the iron-binding moiety and the antioxidant ability of the free chelator DFO.

PB-054 **The proteolytic activity and oligomerization status of the human HtrA3 protease functioning as a tumor suppressor**

Przemyslaw Glaza[1], Tomasz Wenta[1], Jerzy Osipiuk[3], Agnieszka Kowalska[1], Ewa Gebal[1], Dorota Zurawa-Janicka[1], Adam Lesner[4], Barbara Lipinska[1]

1.-Department of Biochemistry, Faculty of Biology, University of Gdansk, 2.-Midwest Center for Structural Genomics, Argonne National Laboratory, 3.-Structural Biology Center, Biosciences Division, Argonne National Laboratory, 4.-Department of Biochemistry, Faculty of Chemistry, University of Gdansk

HtrA3 protease belongs to the high-temperature requirement A (HtrA) family of serine proteases which take part in cellular stress response including heat shock, inflammation and cancer. HtrA3 is composed of an N-terminal domain not required for proteolytic activity, a central serine protease domain and a C-terminal PDZ domain. The latter serves as a substrate or regulator binding domain and may participate in oligomerization. HtrA3S, its short natural isoform, lacks the PDZ domain which is substituted by a stretch of 7 C-terminal amino acid residues, unique for this isoform. Down-regulation of HtrA3 in tumors, shown by other groups and us, suggests HtrA3s involvement in oncogenesis [1]. HtrA3 acts as a proapoptotic protein and is suggested to function as a tumor suppressor. It promotes cytotoxicity of etoposide and cisplatin in lung cancer cell lines [2,3]. To date, HtrA3 has been poorly characterized from the biochemical point of view, mainly due to the fact that it is difficult to purify recombinant HtrA3. We were able to express in bacterial system and purify HtrA3 in quantities sufficient to perform structural studies. The aim of this study was to characterize and compare the proteolytic properties and quaternary structure of the HtrA3 isoforms. Both studied isoforms lacked the N-terminal domain. HtrA3 with the PDZ domain removed (HtrA3-ΔPDZ) and HtrA3S (HtrA3S) were fully active at a wide range of temperatures and their substrate affinity was not impaired. This indicates that the PDZ domain is dispensable for HtrA3 activity. As determined by size exclusion chromatography, HtrA3 formed stable trimers while both HtrA3-ΔPDZ and HtrA3S were monomeric. This suggests that the presence of the PDZ domain, unlike in other human HtrAs (HtrA1 and HtrA2), influences HtrA3 trimer formation. The unique C-terminal sequence of ΔN-HtrA3S appeared to have little effect on activity and oligomerization [4].

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PB-055

Cyclodextrins moderately affects binding of halogenated benzotriazoles by protein kinase CK2

Katarzyna Kuciñska[1], Maria Winiewska[1] Jarosław Poznański[1]
1.-Institute of Biochemistry and Biophysics Polish Academy of Sciences

Cyclodextrins (CDs) are cyclic oligosaccharides that have been recognized as useful pharmaceutical excipients. In aqueous solution CDs are capable to form complexes with various ligands, hosting inside their cavity either a whole molecule, or part of a ligand. Inclusion complexes with CDs offers a variety of physicochemical advantages over the biologically active ligands, including the improved aqueous solubility, solution stability or an increase of bioavailability. CK2 is an ubiquitous, highly pleiotropic and constitutively active Ser/Thr protein kinase. Halogenated benzotriazoles have been developed as potent and selective inhibitors of this enzyme. The interaction of the catalytic domain of human protein kinase CK2 with a series of brominated ligands, which represent all possible patterns of halogen substitutions to the benzene ring of benzotriazole, was previously studied by microscale thermophoresis (MST) [1]. This method alloweddetermination of binding affinities for seven ligands, all of which were found consistent with the values determined independently by isothermal titration calorimetry (ITC). However, a very limited aqueous solubility of some brominated benzotriazoles may decrease their bioavability, thus affectingtheir apparent activity[2]. To overcome this limitation, the aqueous solubility of halogenated benzotriazoles in the presence of cyclodextrins has been tested. The formation of inclusion complexes with β-cyclodextrin (β-CD), hydroxypropyl-β-cyclodextrin (HP-β-CD) and γ-cyclodextrin (γ-CD) in aqueous solutions, followed by UV–Vis spectroscopy, substantially improved the solubility of TBBt and its derivatives. The interaction between protein kinase CK2 and cyclodextrins, and also with their inclusion complexes with halogenated benzotriazoles , was followed with the aid of the microscale thermophoresis. The results obtained clearly demonstrate that the binding of halogenated benzotriazoles by CK2 is only moderately affected by cyclodextrins.

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PB-056 **Antibody Activation using DNA-Based Logic Gates**

Maarten Merkx[1], Brian Janssen[1], Martijn van Rosmalen[1], Lotte van Beek[1]1.-Laboratory of Chemical Bology, Eindhoven University of Technology

Oligonucleotide-based molecular circuits offer the exciting possibility to introduce autonomous signal processing in biomedicine, synthetic biology, and molecular diagnostics. Here we introduce bivalent peptide–DNA conjugates as generic, noncovalent, and easily applicable molecular locks that allow the control of antibody activity using toeholdmediated strand displacement reactions. Employing yeast as a cellular model system, reversible control of antibody targeting is demonstrated with low nm concentrations of peptide–DNA locks and oligonucleotide displacer strands. Introduction of two different toehold strands on the peptide–DNA lock allowed signal integration of two different inputs, yielding logic OR- and AND-gates. The range of molecular inputs could be further extended to protein-based triggers by using proteinbinding aptamers.

PB-057 **Insights of a novel kind of cell wall binding domain that cleaves the peptidoglycan muropeptide: the CW_7 motif**

Noemí Bustamante[1,3], Manuel Iglesias, Noella Silva-Martín, Isabel Uson, Pedro García, Juan Hermoso, Marta Bruix, Margarita Menéndez

1.-Institute of Physical-Chemistry 'Rocasolano', CSIC, 2.-Institute of Physical-Chemistry 'Rocasolano', CSIC, 3.-Ciber of Respiratory Diseases (CIBERES), 4.-Center of Biological Research (CIB), CSIC, 5.-Institucio Catalana de Recerca i Estudis Avançats, CSIC-IBMB

Enzybiotics constitute a hopeful alternative to current treatments to fight against bacterial infections. Phage endolysins are consider as enzybiotics due to their capacity to cleave the peptidoglycan (PG) of Gram-positive bacteria in a generally species-specific manner and kill bacteria when exogenously added (1,2). The Cpl-7 endolysin, a lysozyme encoded by the pneumococcal Cp-7 bacteriophage, is a remarkable exception among all the PG hydrolases produced by Streptococcus pneumoniae and its bacteriophages due to its capacity of degrading pneumococcal cell walls containing either choline or ethanolamine (3, 4). This fact confers to Cpl-7 the advantage of displaying a broader microbicide spectrum comparing to choline binding proteins (5). This behavior results from the acquisition of a cell wall binding module (CWBM) made of three identical repeats of 48 amino acids each (CW_7 motifs), with unknown specificity and totally unrelated with the choline-binding motives present in pneumococcal hydrolases. Interestingly, CW_7 repeats have been identified in many putative proteins potentially involved in cell wall metabolism (Pfam entry: PF08230) from different species of Gram positive and Gram negative bacteria, and some bacteriophages (6). Preliminary studies of thermal stability in presence of a small cell wall structural-analogue (GlcNAc-MurNAc-L-Ala-D-IsoGln) point to the muropeptide as the cell wall target recognized by CW_7 motifs (7). In this communication we have gone in depth in the characterization of CW_7 repeats. We present the first crystal structure of the CW_7 motif, which reveals a three-helical bundle folding. Using STD_NMR spectroscopy the epitope of binding of the disacharide dipeptide to this repeats has been identified. Interestingly, the β anomer of the MurNAc moiety, the form present in the peptidoglycan, seems to be preferentially recognized with respect to the α anomer. Finally, a docking model of the complex CW_7/GMDP compatible with STD results was built allowing to identify the major contacts between the protein and the muropeptide and to propose the relevant role of a conserved Arginine residue in this interaction.

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PB-058 **Engagement of the ClpS NTE by the ClpAP machinery inhibits substrate recognition and processing**

Amaris Torres-Delgado[1], Robert T. Sauer[1], Tania A. Baker[1,2]

1.-Department of Biology, Massachusetts Institute of Technology, 2.-Howard Hughes Medical Institute

Energy-dependent AAA+ proteases carry out regulated proteolysis to ensure protein quality control and post-translational regulation of many cellular processes. Control of proteolysis occurs primarily at the level of substrate recognition, which can be modulated by adaptor proteins. The ClpS adaptor protein enhances and inhibits degradation of different classes of substrates, and thus triggers a specificity switch in ClpA. Whereas the mechanism for substrate delivery by ClpS has been described in detail, the inhibition mechanism is poorly understood. We show that ClpS inhibits ssrA substrate recognition and processing, instead of simply preventing substrate binding. We demonstrate that ClpA engagement of the ClpS N-terminal extension (NTE) is necessary, and may even be sufficient, for inhibition. In addition, we find that inhibition of substrate processing requires a longer NTE, as compared to inhibition of substrate recognition. Interestingly, the NTE length required for inhibiting substrate processing is also necessary for suppression of the ClpA ATPase rate. Furthermore, preliminary data suggests that ClpS slows down substrate translocation. These results support a model where there is an ssrA•ClpA•ClpS inhibitory complex in which the ClpA pore engages the ClpS NTE. This engagement of the NTE causes suppression of ATPase activity, and therefore slower substrate translocation and processing. This model illustrates how an adaptor protein can inhibit recognition of one type of substrate while efficiently promoting degradation of a different substrate.

PB-059 **Single-molecule assay development for studying Human RNA Polymerase II Promoter-Proximal Pausing**

Yazan Alhadid[1], Benjamin Allen[2], Sangyoon Chung[1], Dylan Taatjes[2], Shimon Weiss[1]
1.-University of California, Los Angeles, 2.-Univeristy of Colorado Boulder

Single-molecule assay development for studying Human RNA Polymerase II Promoter-Proximal Pausing Yazan Alhadid, Benjamin Allen, Sangyoon Chung, Dylan Taatjes, Shimon Weiss Abstract: Promoter-proximal RNA Polymerase II (PolII) pausing has been shown to play a significant role in transcription regulation of elongating PolII complexes in a large number of metazoan and mammalian genes(1). The traditional understanding of transcription regulation in mammals involved controlling PolII recruitment to promoters and controlling initial steps at the promoter, including pre-initiation complex formation and promoter escape. Most works investigating promoter-proximal PolII pausing have employed chromatin immunoprecipitation followed by sequencing to determine PolII localization or in vitro transcriptional assays using nuclear extracts analyzed with radio-active gel electrophoresis. In order to gain greater mechanistic insight into the regulation of promoter-proximal PolII pausing, we have been developing a diffusion-based single-molecule method using alternating laser excitation on the micro-second timescale (µsALEX). The method detects RNA transcripts generated by a reconstituted human PolII system in vitro using complementary doubly dye-labeled single-stranded DNA (ssDNA) probes. The human gene HSPA1B for heat shock protein 70 (Hsp70) is used as a model system due to its extensive characterization in drosophila. The method would provide a rapid, sensitive and robust avenue to screen for protein factors regulating promoter-proximal PolII pausing. Controlling of the PIC composition using the reconstituted system allows for dissection of the functional roles of different PIC components in facilitating regulation of PolII pausing. We have demonstrated the hybridization of double dye-labeled ssDNA probe to complementary ssDNA mimicking RNA transcripts and to transcripts generated with bacterial RNA polymerase. Also, a functional reconstituted human PolII system has been verified using radioactive polyacrylamide gel electrophoresis of transcripts from in vitro transcription assays. 1. H. Kwak, J. T. Lis, Control of transcriptional elongation. Annu. Rev. Genet. 47, 483–508 (2013).

PB-060 **Structural characterization of Plasmodium falciparum CCT and fragment-based drug design approach for targeting phospholipid biosynthesis pathway**

Ewelina Guca[1], François Hoh[2], Jean-François Guichou[2], Henri Vial[1], Rachel Cerdan[1],
1.-DIMNP, UMR 5235, University of Montpellier, 2.-Centre de Biochimie Structurale, INSERM

Malaria is a major global health problem. In 2013, there were an estimated 128 million case of malaria and 584 000 deaths, most of them children under 5 years old [1]. Among the 5 malaria species that affect humans, Plasmodium falciparum is the most deadly form. Since no efficient vaccine is available yet, the fight against malaria includes vector control, protection from mosquito bites and artemisinin combined therapy. However, resistances to all known treatments have been observed. Therefore, new antimalarial strategies involving novel targets and new mechanisms of action are needed. During its life cycle, in erythrocytic stage, which causes all the malaria symptoms, Plasmodium falciparum relies on phospholipids to build the membranes necessary for daughter cell development. Approximately 85% of parasite phospholipids consist of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) synthesized by the parasite through the de novo Kennedy pathways. In the pathway of phosphatidylcholine biosynthesis, the second step catalyzed by CTP:phosphocholine cytidylyltransferase [EC 2.7.7.15] is rate limiting and appears essential for the parasite survival at its blood stage [2-3]. We are focused on the structural characterization of this enzyme, the identification of effectors by fragment-based drug design approach (FBDD) and then their optimization to eventually design a lead. The first reported crystal structure of the catalytic domain of the enzyme target (PfCCT) has been solved at resolution 2.2 Å, 3 enzyme-substrates complexes (CMP-, phosphocholine- and choline-bound forms) at resolutions 1.9-2 Å and an enzyme-product (CDP-choline) complex structure at resolution 2.4 Å that give detailed images of binding pocket, demonstrate conformational changes between apo- and holo-protein forms and provide the information about the mechanism of the catalytic reaction at atomic level. The FBDD method uses a library of small molecules (fragments) with molecular weight that does not exceed 300 Da to explore target binding sites. Although fragments often have too low affinities to evoke a biological response, their probability of binding is high because they are small enough to prevent unfavorable interactions with target protein-binding sites. Moreover, they represent more attractive and synthetically tractable starting points for medicinal chemistry compared to more complex compounds. As the affinity is low, fragment screening usually depends on detecting binding rather than inhibition. Screenings of a fragment library (300 molecules) has been performed by fluorescence-based thermal shift assay and Nuclear Magnetic Resonance Saturation Transfer Difference (NMR STD) [4]. This combination of techniques identified so far 4 fragment hits that are currently evaluated for their binding modes and affinities. Co-crystallization of the protein-fragments complexes is carrying out to provide accurate information on the molecular interactions. Topology of interactions will be used to rationally monitor every iterative round of the optimization process allowing subsequent rational design. [1] World Health organization, World Malaria Report (WHO Press, Geneva, Switzerland), http://www.who.int/malaria/publications/world_malaria_report_2014/wmr-2014-no-profiles.pdf?ua=1 [2] Dechamps S, et al., Mol. Biochem. Parasitol., 173:69-80, 2010; [3] Alberge B, et al., Biochem. J., 425:149-158, 2009; [4] Mayer and Meyer, Angewand Chem. Int. Ed. 38:1784-8,1999

PB-061 **14-3-3 proteins as a scaffold for small-molecule controlled signaling platforms**

Anniek Den Hamer[1], Lenne Lemmens[1], Tom de Greef[1], Christian Ottmann[1], Maarten Merkx[1], Luc Brunsveld[1]

1.-Eindhoven University of Technology

Protein scaffolds play a crucial role in signaling pathways by generating signal specificity and increasing signal efficiency and amplitude. Engineered protein scaffolds can be used as key regulators for signal transduction in artificial signal transduction cascades where they can regulate in- and output of the network. In this research a 14-3-3 protein scaffold is developed which induces dimerization of proteins mediated by the small molecule stabilizer fusicoccin. As proof of principle caspase 9 is used to constitute proximity induced dimerization. Dimerization of caspase 9 leads to its activation and consecutively initiates the caspase cascade involved in the programmed cell death pathway. Caspase 9 does not naturally bind to 14-3-3 proteins, therefore the caspase 9 monomer is conjugated to a 14-3-3 binding motif which is known to bind into the binding grooves of a 14-3-3 dimer. This interaction can be stabilized by the small molecule fusicoccin. We showed that upon addition of small molecule fusiccocin caspase dimerization is induced, resulting in caspase activity which is measured using a synthetic caspase substrate. Moreover the biphasic effect of the 14-3-3 scaffold could be proven. Additionally, the activated caspase 9 is also able to cleave its natural substrate caspase 3, downstream in the caspase cascade. These results indicate that the 14-3-3 platform is a versatile small molecule induced dimerization platform which can be used as tool for engineering of a synthetic signaling network.

PB-062 **The G308E variant of the apoptosis inducing factor, responsible of a rare encephalopathy, is hampered in NAD+/H binding**

Luca Sorrentino[1], Laura Rigamonti[1], Mirvan Krasniqi[1], Alessandra Calogero[1], Vittorio Pandini[1], Maria Antonietta Vanoni[1], Alessandro Aliverti[1]

1.-Department of Biosciences, Università degli Studi di Milano

The apoptosis inducing factor (AIF) is a highly conserved mitochondrial flavoprotein known to play two opposite roles in eukaryotic cells: in mitochondria it is required for efficient oxidative phosphorylation (OXPHOS), while, when released into the cytoplasm, it triggers caspase-independent apoptosis (1). The mechanism of AIF-induced apoptosis was extensively investigated, whereas its mitochondrial role is poorly understood. There are many evidences of AIF importance for mitochondrial correct morphology and functions and recently the discovery of its direct interaction with CHCHD4, a key regulator of respiratory complexes subunits import and folding in mitochondria, was reported (2). A unique feature of AIF, probably pivotal for its vital function, is the ability to form a tight, air-stable charge-transfer (CT) complex with NAD+ and undergo dimerization. Although some aspects of AIF interaction with NAD+/H have been analyzed, its precise mechanism is not fully understood. We investigated the effect of the pathogenic G308E replacement, associated with OXPHOS defect and neurodegeneration (3), to understand how it could alter AIF properties at the molecular level. To do so, we analysed how the wild type and the G307E forms of murine AIF interact with NAD+/H and nicotinamide mononucleotide (NMN+/H), finding that the pathogenic replacement resulted in a dramatic and specific decrease of the rate for CT complex formation and consequent protein dimerization only in the case of the physiological ligand. Our results demonstrate that the adenylate moiety of NAD+/H is crucial for the ligand binding process and that the G307E replacement causes an alteration of the adenylate-binding site of AIF that drastically decreases the affinity for and the association rate of the ligand. In addition, we shed new light on the mechanism of the dimerization process, demonstrating that FAD reduction rather than NAD+/H binding initiates the conformational rearrangement of AIF that leads to quaternary structure transitions. Taken together, our results contribute to define how AIF works at the molecular level in binding NAD+/H and undergoing dimerization and also point out that the G308E replacement, responsible of a rare neurodegenerative disease, has the selective effect of slowing down the formation of AIF dimeric CT complex.

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PB-063 **Understanding the mechanism of action of human MICAL1, a multidomain flavoenzyme controlling cytoskeleton dynamics**

Teresa Vitali[1], Gabriella Tedeschi[2], Simona Nonnis[2], Maria Antonietta Vanoni[1]
1.-Dipartimento di Bioscienze, Università degli Studi di Milano, 2.-Dipartimento di Scienze Veterinarie e Sanità Pubblica, Università degli Studi di

MICAL, from the Molecule Interacting with CasL, indicates a family of conserved cytoplasmic multidomain proteins that catalyze a NADPH-dependent F-actin depolymerization activity through their essential N-terminal FAD-containing monooxygenase-like domain (MO) in response to semaphorin signaling [1]. This domain is followed by calponin homology (CH) and LIM domains, proline- and glutamate-rich regions and a C-terminal coiled-coil motif that mediate the interaction with various proteins (e.g: CRMP, CasL, Plexin, G proteins, NDR) [1]. To contribute to establish the catalytic properties of MICAL MO and their modulation by the additional domains and by the interacting proteins, we have produced and are characterizing the human MICAL1 (MICAL-FL) and forms containing the MO [2], MO-CH and MO-CH-LIM domains. All MICAL forms contain stoichiometric amounts of FAD in the MO domain and 2 Zn++ ions in the LIM domain. MICAL-MO catalyzes a NADPH oxidase (H_2O_2-producing) activity. The CH, LIM and C-terminal domains lower its catalytic efficiency (kcat/Km, NADPH) mainly due to an increase of Km for NADPH. The kcat is similar for all forms excepted for MICAL-FL where a 7-fold drop is observed, in agreement with the proposed autoinhibitory function of the C-terminal domain [3]. The pH dependence of the kinetic parameters of MO, MOCH and MOCHLIM is complex suggesting that it does not reflect the ionization state of individual groups, but rather the overall protein charge. MICAL-MO, -MOCH and -MOCHLIM catalyze a NADPH-dependent F-actin depolymerization with a similar apparent Km for actin. F-actin (but not G-actin) stimulates the rate of NADPH oxidation by increasing kcat and lowering KNADPH. The extent of NADPH oxidation exceeds total F-actin which is in contrast with the proposal of specific modification of actin Met44 or Met46 reported for Drosophila and mouse MOCH [4-5], but it suggests that F-actin stimulates the NADPH oxidase activity or a case of substrate recycling. Accordingly, with hMICAL MO and MOCH several actin residues are oxidized beside Met44 and Met46. Thus, the CH and LIM domains do not seem to be important for the MICAL-actin interaction and actin modification may be mediated by in situ H_2O_2 production. In HEK293T and COS-7 cells mouse collapsin response mediator protein-1 (mCRMP1) interacts with MICAL1 inhibiting H_2O_2 production [3], suggesting that CRMP1 could be a hydroxylatable substrate of MICAL-MO. We have produced the same mCRMP1 form (8-525 aa) and we have shown that under conditions that limit non specific interactions a mild stimulation (up to 20%) of NADPH oxidation is observed. F-actin reversed the effect of mCRMP1 suggesting their competition for MICAL. These results suggest that CRMP1, a major microtubules regulator, is not the substrate of the MO domain, but actin and microtubules cytoskeleton components may be linked through the formation of CRMP-MICAL complex in response to semaphorin-plexin signaling. Experiments are in progress to complete the characterization of MOCHLIM and full length MICAL forms.

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PB-064 **Protective function of enhanced green fluorescent protein against reactive oxygen species photo-sensitized by N-doped nanoTiO2**

Beata Wielgus-Kutrowska[1], Joanna Krasowska[1], Agnieszka Bzowska[1], László Forró[2], Andrzej Sienkiewicz[3]

1.-Department of Biophysics, Institute of Experimental Physics, Warsaw University, 2.-Laboratory of Physics of Complex Matter (LPMC), 3.-ADSresonances

Green fluorescent protein (GFP), owing to its genetically encoded strong fluorescence, has become one of the most important tools in modern biology [1]. Enhanced GFP (EGFP, F64L/S65T-GFP), frequently used variants of this protein, is thermodynamically more stable and ~35-times brighter than GFP [2]. Due to the improved fluorescent properties, EGFP is commonly used as a fluorescent intracellular marker in bio-imaging in vitro and in vivo. Despite sustained interest of the scientific community and numerous practical applications, the actual biological role of GFP remains elusive. Recent reports put forward a hypothesis of antioxidant and photo-protective functions of GFP [3]. In this study, we focused on the photo-protective role of EGFP against reactive oxygen species (ROS) photo-generated by visible light in water suspensions of nano-particular nitrogen-doped titanium oxide (N-doped nano-TiO2), that is in the system: 'N-doped nano-TiO2)/visible light'. N-doped nano-TiO2 (Sumitomo TP-S201) was chosen as a photo-catalyst, since it is widely accepted that nitrogen doping enhances visible light photoactivity of TiO2. 4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl (TEMPOL), a paramagnetic water-soluble compound, belonging to the nitroxide class o superoxide dismutase (SOD) mimetics, was used as a target for photo-generated ROS. A solar simulator, with the flux output intensity of ~1 kW/m2, was used as a visible light source. Electron spin resonance (ESR) was employed to monitor the changes in the paramagnetic signal of TEMPOL exposed to the action of ROS in the absence and presence of EGFP. In the absence of EGFP and after 50 min of illumination, due to a combined action of superoxide (O2•-) and hydroxyl (OH•) radicals generated by the system 'N-doped nano-TiO2)/visible light, the ESR signal of 100 uM TEMPOL decayed by ~20%. Moreover, the growth of a new signal, interpreted as 4-oxo-2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPONE), resulting from the attack of OH•radicals on TEMPOL, was also observed. In contrast, in the presence of EGFP (7.5 uM), the ROS-induced decay of the ESR signal of TEMPOL was markedly smaller, not exceeding 5%. Concomitantly, the growth of the ESR signal of TEMPONE was also partially inhibited (~30% smaller amplitude), as compared to the process performed in the absence of EGFP. In summary, our results point to a significant inhibition of the photo-decomposition of TEMPOL in the presence of EGFP and support the hypothesis of the protective role of this fluorescent protein against ROS generated by the system 'N-doped nano-TiO2)/visible light'.

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PB-065 **Selective Recognition and Assembly in Protein-Small molecule Interactions**

Aishling M. Doolan[1], Maike C. Jürgens[1], Amir R. Khan[2], Peter B. Crowley[1]
1.-School of Chemistry, National University of Ireland Galway, 2.-School of Biochemistry and Immunology, Trinity College Dublin

By studying a variety of anionic ligands and their interactions with cationic cytochrome c, we are building knowledge of protein recognition geared towards regulating activity. In previous work it was shown that p-sulfonatocalix[4]arene selectively binds to, and encapsulates, three Lysine side chains on cytochrome c 1. Here, the binding of two small molecule ligands to cytochrome c was investigated. NMR spectroscopy was used and in one case, a crystal structure of the complex was obtained (Fig 1). The calixarene bound to cytochrome c, reveals a crystal packing assembly that suggests it is a key mediator of crystal formation. NMR data analysis indicates the calixarene's binding site on cytochrome c. The pillararene, a relatively new class of compound, is a symmetrical arrangement with a π-rich cavity 2, related structurally to calixarenes. This suggests good host-guest complexation properties. Previously, the carboxylatopillararene showed selective binding to Arginine, Lysine and Histidine 2. With this ligand, an interaction with cytochrome c was observed and a complex formed. Additionally, biphasic binding behaviour was observed through analysis of the chemical shift perturbations. This may indicate more than one binding event taking place. The data from these studies indicate that recognition is occurring and again that Lysine side chains play an essential role.

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PB-066 PB-066 **Macromolecular crowding modulates enzyme catalysis**

Annelise Gorensek[1], Luis Acosta[1], Gary Pielak[1,2,3]

1.-Department of Chemistry, University of North Carolina, 2.-Department of Biochemistry and Biophysics, University of North Carolina, 3.-Lineberger Comprehensive Cancer Center, University of North Carolina

The enzyme dihydrofolate reductase (DHFR) is necessary for the growth and development of all organisms.1 The structure and function of Escherichia coli DHFR have been characterized in buffer. However, DHFR exists in living cells, where the protein concentration can exceed 300 g/L.2 We know that weak, non-specific chemical interactions with cytosolic proteins alter protein conformation and dynamics,3,4 both of which are expected to influence DHFR catalysis. Investigators have examined steady-state enzyme kinetics under crowded conditions, but conclusions can be conflicting.5,6 Here, the effects of crowding on E. coli DHFR catalysis are assessed through specific activity measurements in solutions of synthetic polymers. These kinetics studies are complemented by in-cell and in vitro 19F NMR data from fluorinated tryptophan residues. Preliminary results suggest that the effects of polymeric crowders on DHFR activity are non-monotonic, which may arise from the polymer's transition from the dilute to semi-dilute regime. The data suggest that synthetic polymers are not a valid representation of the cellular interior.

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PB-067 **Biophysical and biochemical characterization of Arabidopsis thaliana Calmodulin-like protein CML14**

Rosario Vallone[1], Valentina La Verde[1], Mariapina D'Onofrio[1], Alessandra Astegno[1], Paola Dominici[1]

1.-Biotechnology Department, University of Verona

Calcium (Ca2+) is one of the most important second messengers in eukaryotes. Ca2+ binding proteins can be subdivided into two categories: “Ca2+ buffers” that modulate Ca2+ ion concentrations in cells, and “Ca2+ sensors” that decode Ca2+ signals in a wide array of physiological processes in response to external stimuli. Calmodulin (CaM) is the prototypical example of Ca2+ sensor proteins in both animals and plants. In addition to conserved CaM, plants possess a unique family of 50 CaM-like proteins (CMLs). Many of these CMLs still remain uncharacterized and the investigation of their biochemical and biophysical properties will provide insight into Ca2+ signalling in plants. Herein, a detailed characterization of Arabidopsis thaliana CML14 is reported. CML14 is a protein of 148 amino acids with a theoretical molecular weight of 16,579 Da and 50% amino acid sequence identity with AtCaM2. CML14 is predicted to have one functional Ca2+ binding site despite the presence of three EF-hand motifs (Prosite). We overexpressed CML14 in E. coli and analyzed its biochemical and biophysical characteristics, i.e. calcium affinity and stoichiometry and eventual changes in conformation, thermal stability and proteolytic susceptibility upon Ca2+ binding. Isothermal titration calorimetry (ITC) and nuclear magnetic resonance (NMR) spectroscopy identified one Ca2+ binding site in CML14 and showed that Ca2+ and Mg2+ compete for the same binding site. The Kd values determined by ITC established that CML14 has higher affinity for Ca2+ than for Mg2+. Our data were consistent with the sequence based prediction of one functional calcium binding site. Differential scanning calorimetry (DSC) showed that Ca2+ and Mg2+ have the same stabilizing effects on protein folding. Apo-CML14 undergoes two thermal unfolding transitions, but in the presence of Ca2+ or Mg2+ only one unfolding event at an intermediate temperature occurs. Limited proteolysis experiments showed that Ca2+ binding affords protection against CML14 digestion by trypsin. Surprisingly, CML14 exhibits very few conformational changes upon calcium binding, which were evaluated by ANS fluorescence and Stokes radius measurements in the apo- and Ca2+ bound-forms. These results suggest that CML14 does not show the characteristics of a classical Ca2+ sensor protein. To better understand the physiological role of CML14 in plants, in vivo analysis will be performed.

PB-068 **FBP17 controls the hepatocyte morphology through Rho signaling**

Jun Zhang[1], Mingming Ling[1], Qianying Zhang[1], Yunhong Wang[1], Deqiang Wang[2]
1.-The Department of Cell Biology and Genetics, 2.-Key Laboratory of Molecular Biology on Infectious Disease

The formin-binding protein 17 (FBP17) widely expressed in eukaryotic cells was previously identified to play a role in morphological maintenance in hepatocyte, but the molecular mechanism keeps still unclear so far. In the present investigation, it was found that Rho family proteins CDC42/RAC1 signaling was involved in the morphological regulation controlled by FBP17. Knockdown of endogenous FBP17 expression with RNAi technique or dominant negative mutant of FBP17 could trigger the cell morphological remodeling from the epithelioid to fibroid following the significant down-regulation of CDC42/RAC1 activities and dephosphorylation of paxillin. While the Rho protein specific activator could restore the CDC42/RAC1 activities, and in turn abrogated the silence effect. Overexpression of wild type FBP17 could not result in any of the morphological transition. Furthermore, withdrawal of the silence could induce morphological recovery when the FBP17 expression, CDC42/RAC1 activities and paxillin phosphorylation were restored to the normal level. The experimental evidences strongly indicated that FBP17 was implicated in morphological control probably via Rho signaling pathway in hepatocyte. Key words: FBP17; Rho signaling; paxillin; morphological control; hepatocyte This work was supported by a grant from National Natural Science Foundation of China (NSFC, no. 20803098)

PB-069 **Energetics of proton transport in Cytochrome c oxidase: Investigation of proton entry in the K-channel of Paracoccus denitrificans**

Jovan Dragelj[1], Anna-Lena Woelke[1], Ulrike Alexiev[2], Ernst-Walter Knapp[1]
1.-Fachbereich Biologie, Chemie, Pharmazie/Institute of Chemistry and Biochemistry, 2.-Fachbereich Physik/Department of Physics

Cytochrome c oxidase (CcO) is the final enzyme in the respiratory chain of mitochondria but also an integral part of the metabolism of many types of bacteria. In a complex, stepwise redox-reaction, CcO catalyzes the reduction of molecular oxygen to water and utilizes the resulting free energy to pump protons across the membrane thereby creating an electrochemical gradient [1,2]. To investigate proton pumping spectroscopically it is possible to label the entrance of the proton entrance channel with fluorescein, a pH sensitive dye, which allows determining time resolved local changes in proton concentration at the cytoplasmic CcO surface and related properties. It has already been shown that the redox state of copper and heme centers affects such properties at the cytoplasmic surface. [3] This study is a theoretical approach to investigate changes of pKA values of the fluorescein label at the entrance of the K-channel for different protonation pattern in both oxidized and reduced CcO by performing molecular dynamics (MD) simulations. Further work is based on calculations of pKA values of the fluorescein using software Karlsberg+[4,5].

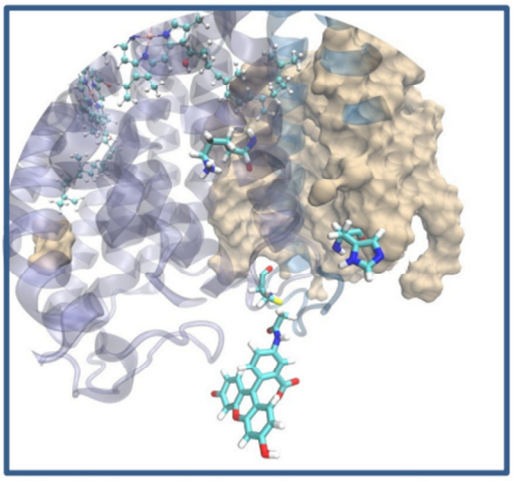

Fig 1. *Entrance of the K-channel of cytochrome c oxidase with attached fluorescein.*

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PB-070 **Efficient Methods in the Production of Unnatural Amino Acid Containing Proteins**

Christopher Walters[1], Solongo Batjargal[1], Anne Wagner[1], E. James Petersson[1]
1.-University of Pennsylvania

Methods for genetically and synthetically manipulating protein structure enable a greater flexibility in the study of protein function. We have shown that using inteins as traceless, cleavable purification tags enables the separation of full length unnatural amino acid (Uaa) containing proteins from their corresponding truncation products. This method has been used to incorporate Uaas in previously unattainable positions in a variety of proteins using a myriad of Uaas, inteins, and purification tags. In other applications, we have used E. coli aminoacyl transferase (AaT) to selectively modify the N-termini of proteins with Uaas in denaturing conditions and conditions that maintain folding. Applications of particular interest include overcoming the need for an N-terminal Cys residue in expressed protein ligation, transfer of reactive handles for "click" chemistry labeling of proteins, and transfer of fluorogenic molecules for photophysical experiments. We have found that AaT can transfer protected cysteine, homocysteine, and selenocysteine to expressed proteins. After ligation, these residues can be converted to Met or Ala, making the ligation traceless. We continue to develop variants of AaT to broaden the substrate scope of both its transferred substrate and N-terminal recognition element. In addition, expressed protein ligation is being used to incorporate backbone modifications, such as the thioamide, into various positions in the protein calmodulin to determine how these modifications can impact the structure and function of an ordered protein. In general, by working at the interface of several protein modification technologies, we have made beneficial discoveries that might be missed by more focused approaches.

PB-071 **Function and modularity of CW_7 motives in the C-terminal region of the endolysin Cpl-7 encoded by the Cp7 pneumococcal bacteriophage**

Manuel Iglesias-Bexiga[1,2], Noelia Bernardo-García[3], Rubén Martínez-Buey[4], Noemí Bustamante[1,2], Guadalupe García[1,2], Marta Bruix[1], Juan Hermoso[3], Margarita Menéndez[1,2]
1.-Dept. of Biological Physical-Chemistry, IQFR-CSIC, 2.-Ciber of Respiratory Diseases (CIBERES), 3.-Department of Crystallography and Structural Biology, IQFR-CSIC, 4.-University of Salamanca

Bacteriophage lytic murein-hydrolases have been proposed as enzybiotics, an efficient way to fight bacterial infections. However, the use of these enzymes is normally restricted to Gram-positive bacteria since the outer membrane of the Gram-negative bacteria hampers the access of the hydrolases to the peptidoglycan substrates. All the murein hydrolases reported in the pneumococcal system, both from host or phage origin, depend on the aminoalcohol choline to be fully active. There is only a unique exception to this rule, the Cpl-7 lysozyme. This hydrolase is encoded by the lytic pneumococcal phage Cp-7 and, instead of the common cell wall binding module (CWBM) that recognizes choline, Cpl-7 harbors a completely different cell wall binding structure. Recent studies have revealed that reducing the net charge of the CWBM, from -14.9 to +3.0, leads to an improvement in the antibacterial activity of Cpl-7 (1). The CWBM of Cpl-7 is composed by three identical repeats of 48 amino acids, the CW_7 motives, and it folds both in the presence and in the absence of the N-terminal catalytic module (2). This module shows the capacity of recognize the GlcNAc-MurNAc-L-Ala-D-isoGln muropeptide (GMDP), structurally related with the peptidoglycan basic unit (3). Here, we report the high resolution structure of the cell wall binding module of the Cpl-7 endolysin. Each CW_7 repeat is composed of a bundle of three α-helices with a highly negative electrostatic charge at the surface. The strong inter-repeat interactions and the high ionic strength used in the crystallization conditions allow them overcoming the electrostatic repulsions inducing a closed-packed structure with a three-fold symmetry. The module dimensions (49 x 38 x 34 Å) and the repeat arrangement in the crystal structure are inconsistent with the GMDP binding characterization, the activity displayed by Cpl-7 truncated variants with one or two CW_7 repeats, or the experimental determined hydrodynamic properties. Using the small angle X-ray scattering (SAXS) technique and the ATSAS computational platform (4), a different arrangement of the CW_7 repeats is envisaged in solution (Fig. 1), whose rather opened structure (70 x 44 x 46 Å) is consistent with the experimental data. Additionally, employing the SAXS-based structure and the honeycomb structure proposed for the peptidoglycan, a model, where each CW_7 repeat of the cell wall binding module fit in adjacent glycan chains, has been derived.

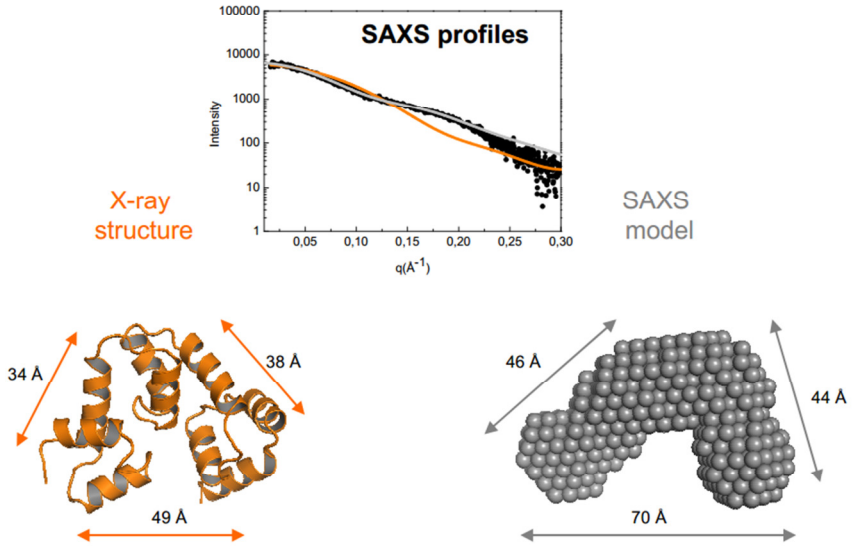

Fig. 1. Experimental SAXS data of C-terminal domain of Cpl-7 (black dots) and the theoretical scattering profiles calculated by CRYSOL (4) from the x-ray structure (orange line and cartoon) and from the ab initio SAXS model (grey line and beads model).

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PB-072 **Utilizing computational and experimental chemistry to characterize the functions of Structural Genomics proteins in the Crotonase Superfamily**

Caitlyn Mills[1], Pengcheng Yin[1], Penny Beuning[1], Mary Jo Ondrechen[1]
1.-Northeastern University

In 2000, the Protein Structure Initiative (PSI) was started as to determine three-dimensional structures of proteins within every family. Once solved, structures are deposited into the Protein Data Bank (PDB) and termed Structural Genomics (SG) proteins. As of June 2015, there are over 13,300 SG proteins deposited in the PDB and most of them are of unknown or uncertain biochemical function. In addition, many of these SG proteins have a putative functional assignment based on their sequence and structural similarities with proteins of known function; such comparisons can be made against large databases using programs such as BLAST or Dali. However, these putative functional assignments are often incorrect. This project analyzes members of the Crotonase Superfamily (CS). The CS consists of five diverse functional subgroups that are well characterized structurally and functionally, representing different types of reactivity, including hydrolase, isomerase, hydratase, and dehalogenase activities. This superfamily also contains at least 70 SG proteins, so it is ideal to test predictions of protein function. Our approach is based on local structure matching at the computationally predicted active site. First, Partial Order Optimum Likelihood (POOL) is used to predict the functionally important residues of each SG protein and of the proteins of known function in the superfamily. Next, Structurally Aligned Local Sites of Activity (SALSA) is used to align the predicted catalytic residues of the well-characterized members in the superfamily. From this analysis we generate chemical signatures for each functional subgroup and compare them to the sets of catalytic residues predicted for the SG proteins. We demonstrate based on these computational methods that the majority of the putative annotations in the CS superfamily are likely incorrect. Currently, biochemical assays are being used to test these predictions. Preliminary biochemical results show that one SG protein, Thermus thermophilus Q5SLS5_THET8, classified as a probable enoyl-CoA hydratase, possesses hydrolase activity as predicted by our methods. The outcomes of this project will be to successfully classify the biochemical functions of SG proteins based on their local structure at the predicted active sites and to provide a conceptual framework for the functional classification of the remaining SG proteins within the PDB. This work is supported by NSF-CHE-1305655.

PB-073 **Directly observing the synergistic dynamics in F-actin and microtubule assembly**

Jun Zhang[1], Deqiang Wang[2]

1.-The Department of Cell Biology and Genetics, 2.-Key Laboratory of Molecular Biology on Infectious Disease

Although important in cellular activities, little attention was paid to the synergistic effects of actin and microtubule cytoskeleton assembly. With the time-lapse atomic force microscope (TL-AFM), we directly observed the large-scale dynamic structure of actin filaments formed in the presence or absence of microtubulin in solution. In absence of microtubulin, the G-actin could be polymerized into ordered filamentous structures with different diameter from the slimmest filament of single F-actin to giant filament in tree-like branched aggregates. The polymerized actin filaments, to which our most intense attention was attracted, was discretely arranged and showed obvious polymorphism in structures completely distinct from those in the presence of microtubulin. The supra-molecular complex structures of the latter were mainly composed of single F-actin and/or multifilaments clearly consisting of several single F-actin and regularly cross-linked with the assembled microtubular bundles. The experimental results demonstrated that the F-actin dynamics could be coordinated by microtubule assembly. Further analyses implied that the interactions between F-actin and microtubule could prevent the emergence of structural polymorphism of F-actin alone, and give rise to organization of specific complex structures instead. It was suggested that dynamic synergy between the F-actin and microtubule would be implicated in living cells Key words: TL-AFM; actin; microtubule; assembly dynamics; synergy

PB-074 **Bivalent phosphonate inhibitors for extracellular 14-3-3 protein targets**

Jeroen Briels[1], Maria Bartel[1], Elvan Yilmaz[2], Philipp Thiel[3], Markus Kaiser[2], Christian Ottmann[1]
1.-Laboratory of Chemical Biology, Eindhoven University of Technology, 2.-Centre for Medical Biotechnology, University of Duisburg-Essen, 3.-Department of Computer Science, University of Tübingen

The adaptor protein 14-3-3 is found in a diverse range of pathologically relevant protein-protein interactions (PPIs). As 14-3-3 is a hub protein with very diverse interactions, it is able to influence the intracellular localization of their binding partners and they are key regulators of signal transduction processes as well as regulators of cell cycle functions.Nevertheless, there are only few examples of 14-3-3 acting extracellularly. One of the extracellular targets for 14-3-3 is Aminopeptidase N (APN). APN is an extracellular trans-membrane enzyme that acts as a receptor for 14-3-3. Binding to APN, 14-3-3 excreted by keratinocytes can upregulate the excretion of matrix metalloproteinase-1 (MMP1) in fibroblasts. MMP1, by breaking down collagens, is key in the remodeling of the extracellular matrix. Modulation of the 14-3-3/APN interaction thereby may play a crucial role in the fundamental understanding and ultimately treatment of wound healing, respiratory diseases and tumor growth.

In the eukaryotic cell, the 14-3-3 dimer operates as an adapter platform for binding partners. A wide range of classes of (small) molecules, natural products and peptides has been used to modulate the PPIs, providing either stabilization or inhibition of the interactions of 14-3-3 with its binding partner. Binding partner fragments or peptides are known to bind to the 14-3-3 binding groove via arecognition motif containing a phosphorylated serine or threonine.

Making use of the dimeric structure of 14-3-3, novel small-molecule inhibitors may be tethered to exploit the bivalent effect. From a large virtual screening and experimental validation, a scaffold containing a phenyl phosphonic moiety was identified, showing inhibitory properties for 14-3-3 PPIs. Potent derivatives of this scaffold were bridged by polyethylene glycol (PEG) linkers of varying lengths, thereby facilitating the compound to reach both binding sites of the 14-3-3 dimer and concurrently increasing the compound's solubility in aqueous solution. Similar bivalent inhibitors have been proven to synergistically increase their efficacy.

Biophysical evaluation by means of fluorescence polarization (FP) inhibition competition assays, revealed an increase of the half maximal inhibitory concentration (IC50) from approximately 81 µM for the monomeric phenyl phosphonate to approximately 1.8 µM for the bivalent inhibitor with a 60Å linker. This demonstrates a 45-fold increase of inhibitory effect towards 14-3-3 and its binding partner peptide mimic. Extensive thermodynamic, kinetic and structural analysis of the interaction is in progress.Phosphonic moieties have been shown to pass the cell membrane poorly, due to their highly charged character. By being able to specifically inhibit the extracellular interaction between 14-3-3 and APN, these inhibitors are prevented from interfering with the extensive intracellular 14-3-3 interactome. Hence, these bivalent phenyl phosphonate inhibitors provide a promising strategy towards extracellular application.

PB-075 **Probing the extremely high metal-to-protein affinity of interprotein zinc hook domain of Rad50 protein from P. furiosus**

Tomasz Kochanczyk[1], Michal Nowakowski[2], Dominika Wojewska[1], Artur Krezel[1]
1.-Laboratory of Chemical Biology, Faculty of Biotechnology, University of Wroclaw, 2.-Laboratory of NMR Spectroscopy, Center of New Technology, University of Warsaw

The Mre11 complex is an oligomeric assembly comprising of dimmers of Mre11 and Rad50 proteins in Archea and additionally Nbs1 subunit present in Eukaryote. It is the central player in the DNA damage response - a functional network comprising DNA damage sensing, signal transduction, cell cycle regulation and DNA double strand breaks (DSBs) repair [1]. Recent structural studies revealed that Rad50 hinge domain is rather a short kink in the coiled-coil region and adopts unusual dimerization mode by intermolecular coordination of Zn(II) and formation of so-called zinc hook domain [2]. To date, very limited structural data on the zinc hook domain have been reported, the only known structure was resolved for Rad50 homologue from hyperthermophilic archaeon – P. furiosus. Unusual Zn(II) coordination mode in zinc hook domain raises question of how zinc hook domain assembles to form interprotein zinc binding site with sufficient stability to function at low intracellular free Zn(II) concentrations [3]. Our study on minimal zinc hook domain fragment (14 aa) indicated low femtomolar affinity towards Zn(II) [4]. Extended zinc hook domain fragment (45 aa) reveals even zeptomolar affinity. Therefore, our main goal was to probe the thermodynamic and structural effects that are hidden in the small interprotein interface and are responsible for the dimerization of the large and critical protein machinery. Probing of those effects was achieved by detailed biophysical characterizations (including potentiometry, NMR, HDX MS and CD spectroscopy) of 18 protein fragments of zinc hook domains with a number of point mutations. We showed that extremely high stability of zinc hook domain from P. furiosus is achieved by the formation of hydrogen bond network in β-hairpins and interprotein hydrophobic core.

This work was supported by the National Science Centre, grant: 2014/13/B/NZ1/00935 and Polish Foundation for Science (F1/2010/P/2013).

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PB-076 **DNA-directed control of enzyme-inhibitor complex formation: A modular approach to reversibly switch enzyme activity**

Wouter Engelen[1], Brian Janssen[1], Maarten Merkx[1]
1.-Eindhoven University of Technology

DNA-based molecular circuits have become a very attractive tool in molecular imaging, synthetic biology, molecular diagnostics and biomolecular computing. The highly modular and predictable nature of Watson-Crick base pairing allows the construction of complex circuits using a limited set of logic gates and building blocks. However, the lack of generic approaches to interface DNA-based molecular circuits with protein activity limits their application in biomedicine and molecular diagnostics. Here we present a new, highly modular approach to control the activity of a reporter enzyme based on the DNA-directed assembly and disassembly of a complex between TEM1-β-lactamase and its inhibitor protein BLIP. Both proteins are conjugated to a unique oligonucleotide, allowing the assembly of the enzyme-inhibitor pair and inhibition of enzyme activity by the addition of a complementary template strand. Addition of an oligonucleotide that is complementary to a loop sequence in the template results in the formation of a rigid dsDNA spacer that disrupts the enzyme-inhibitor complex, restoring enzyme activity. Using this noncovalent approach allowed easy tuning of the template and target sequences with only a single set of oligonucleotide-functionalized enzyme and inhibitor. To show the modularity of the system, a panel of 8 different template sequences were selected. Only in the presence of their complementary viral DNA sequences restoration of enzyme activity was observed. In addition to this excellent specificity the system showed to by higly sensitive towards its target, since the presence of as little as 2 fmol of target resulted in an observable increase in enzyme activity. The use of a stable and well-characterized enzyme-inhibitor pair, complemented by the modular design of our reversible DNA-directed protein switch make it an attractive system to implement in DNA-based molecular circuits.

PB-077 **Carboxylic Acids: a versatile classe of carbonic anhydrase inhibitors**

Giuseppina De Simone[1], Simone Carradori[2], Emma Langella[1], Simona Maria Monti[1], Claudiu T. Supuran[3], Katia D'Ambrosio[1]

1.-Istituto di Biostrutture e Bioimmagini-CNR, 2.-Department of Pharmacy, 'G. D'Annunzio' University of Chieti-Pescara, 3.-Università Degli Studi Di Firenze, NEUROFARBA Department

Several studies demonstrated important roles of human Carbonic anhydrases (hCAs) in a variety of physiological and pathological processes. Consequently, in recent years the 12 catalytically active hCA isoforms have become an interesting target for the design of inhibitors with biomedical applications [1]. Derivatized sulfonamides of type R-SO2NH2 represent the class of CA inhibitors (CAIs) mostly used and best characterized. The large number of crystallographic studies so far available on these molecules clarified the main factors responsible for the binding of the sulfonamide moiety to the CA active site.1 In particular, it has been highlighted that even though these molecules generally behave as very potent CAIs, they do not show selectivity for the different isoforms. Indeed, the sulfonamide moiety plays a predominant role in the interaction with the enzyme, while any change in the nature of the R substituent has generally a rather marginal effect on the enzyme-inhibitor affinity. These characteristics make difficult the design of sulfonamide derivatives selective for the different CA isoforms. Consequently, much efforts were dedicated in last years to the development of new inhibitors that, although presenting lower affinity for the CA active site, would be able to be more selective toward the different isoforms. Carboxylic acids have been recently investigated as CAIs, showing that these molecules can adopt different binding modes to the enzyme active site. In particular, they can coordinate directly to the zinc ion or be anchored to the zinc-bound water molecule. However, the structural reasons responsible of this peculiar behavior have not been clarified yet. In a general research project aimed at providing insights into the binding mode of these molecules to CAs, we have undertaken the characterization of two carboxylic acids, namely an ortho-substituted benzoic acid [2] and a saccharine derivative, by means of kinetic, crystallographic and theoretical studies.

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PB-078 **Exploring the mechanism of fibril formation using fluorescently labelled human lysozyme variants**

Ana Bernardo Gancedo[1]
1.-University of Cambridge

'Exploring the mechanism of fibril formation using fluorescently labelled human lysozyme variants' Human lysozyme is a widely characterised protein whose mutational variants misfold into fibrils that are associated with systemic amyloidosis (1). Although the process of aggregation for human lysozyme has been well studied, the details of early events within this process are not fully characterised. Single molecule fluorescence microscopy has been used to determine the oligomeric distributions present in the aggregation process of a number of disease-related intrinsic disordered proteins (IDPs) (2). Recent advances in site-specific labelling of human lysozyme (3) have made this protein amenable to these single molecule fluorescence studies. We have introduced Alexa-fluorophores into the I59T variant of human lysozyme and have demonstrated that the process of in vitro fibril formation is not significantly altered. Using these fluorophore-labelled proteins we can apply single molecule fluorescence to study the early aggregation events within this system, allowing us to compare protein aggregation in a globular protein and with the aggregation process of IDP's.

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PB-079 **A new lead compound for the development of Carbonic Anhydrase inhibitors**

Anna Di Fiore[1], Giuseppina De Simone[1], Alessandro Vergara[1,2], Marco Caterino[2], Vincenzo Alterio[1], Simona M. Monti[1], Joanna Ombouma[3], Pascal Dumy[3], Claudiu T. Supuran[4], Jean-Yves Winum[3]

1.-Istituto di Biostrutture e Bioimmagini-CNR, via Mezzocannone 16 - 80134 Naples., 2.-University of Naples Federico II, Via Cinthia - 80126, Naples., 3.-Institut des Biomolécules Max Mousseron-CNRS, Université de Montpellier., 4.-Università Degli Studi Di Firenze, NEUROFARBA Department

Carbonic anhydrases (CAs) are ubiquitous metalloenzymes, which catalyze the reversible hydration of carbon dioxide to bicarbonate ion and proton. These proteins are present in prokaryotes and eukaryotes, and are encoded by six evolutionarily unrelated gene families.[1,2] Human CAs (hCAs) are widely distributed in many tissues and organs. Since at these sites CAs play a crucial role in various physiological processes, they have recently become interesting targets for pharmaceutical research. Indeed, several CA inhibitors (CAIs) incorporating a sulfonamide/sulfamate/sulfamide moieties are currently clinically used for the treatment or prevention of a multitude of diseases such as glaucoma, solid tumors, etc.[1] However, these compounds are generally poorly selective towards the different CA isoforms. For this reason, despite many encouraging results, new zinc binding groups (ZBGs) are continuously tested in order to advance upon the identification of isoform selective CAIs.[1] Here, we report the synthesis, inhibition and structural studies of hydroxylamine-O-sulfonamide, a molecule containing two ZBGs, namely the sulfonamide and hydroxylamine moieties.[3] The inhibitor action of this molecule was tested against all catalytically active hCA isoforms, revealing that it possesses a rather variable behavior against the different human isozymes. To elucidate the binding mode of hydroxylamine-O-sulfonamide to CA active site, an original crystallography-assisted Raman spectroscopy approach was utilized. In particular, the X-ray structures of hydroxylamine-O-sulfonamide in complex with hCA II, the best characterized hCA isoform, showed that hydroxylamine-O-sulfonamide is in part coordinated in the classical manner, as all sulfonamides/sulfamates, binding the catalytic zinc ion through the sulfonamide nitrogen and in part through hydroxylamine moiety in a side-on fashion, which is an unusual inhibition pattern for this family of enzymes. This surprising observation was further proved by Raman microspectroscopy experiments. Altogether our data indicate that the hydroxylamine-O-sulfonamide versatility can be exploited for drug design purposes for obtaining new effective and selective CAIs.

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PB-080 **Secondary transporter structure and function in synthetic lipid bilayer systems**

Heather Findlay[1], Sowmya Purushothaman[2], Oscar Ces[2], Paula Booth[1]
1.-Kings College London, 2.-Imperial College London

Biological membranes are complex environments, where membrane proteins are surrounded by a bilayer composed of many different types of lipid. The physical properties of the bilayer influence protein structure, folding and function, while specific interactions with lipid molecules can also contribute towards the biological activity of some membrane proteins. Improving understanding of the interactions has resulted in the development of artificial lipid systems that allow the bilayer properties to be rationally manipulated in vitro to control protein behaviour. The bacterial transporter LacY is a well known integral membrane protein from the Major Facilitor Superfamily, responsible for the proton-driven uptake of D-lactose in E. coli. With a high resolution structure available and considerable understanding of mechanistic detail, and with observed changes to both structure and function in different bilayer environments, LacY is a good model system for examining the behaviour of a major class of membrane proteins in these lipid systems. Purified LacY has been reconstituted into liposomes and droplet interface bilayer systems of varying lipid composition and the effect on protein function and bilayer properties examined.

PB-081 **Targeting Abeta oligomers by Trehalose-conjugated peptides: a molecular dynamics study**

Emma Langella[1], Ida Autiero[1], Michele Saviano[2]
1.-National Research Council, Institute of Biostructures and Bioimaging, 2.-National Research Council, Institute of Crystallography

Targeting Abeta oligomers by Trehalose-conjugated peptides: a molecular dynamics study Emma Langellaa, Ida Autieroa and Michele Savianob a National Research Council, Institute of Biostructures and Bioimaging, 80138 Naples, Italy b National Research Council, Institute of Crystallography, 70126 Bari, Italy Alzheimer's disease (AD) is currently one of the most common and devastating forms of dementia correlated with beta-amyloid peptide (Abeta) accumulation in human brain tissue [1,2]. Inhibiting Abeta self-oligomerization in brain tissue remains one of the main strategies to prevent or treat this disorder. As a consequence, in recent years much efforts have been spent in the understanding of the amyloid fibril growth process and its modulation by putative drug molecules. An interesting class of compounds able to prevent Abeta fibrillogenesis, is represented by beta-sheet-breaker (BSB) peptides [3]. Although these molecules are thought to recognize in a self-complementary manner the Abeta hydrophobic core region, however their precise mechanism of interaction is still unclear. In this context, we have studied the structural basis underlying the inhibitory effect of Abeta(1-42) fibrillogenesis explicated by two promising trehalose-conjugated BSB peptides (Ac-LPFFD-Th (ThCT) and Th-Succinyl-LPFFD-NH2 (ThNT))[4] using an all-atom molecular dynamics (MD) approach [5,6]. The pentameric NMR structure [7] of Abeta(1-42) has been used to model amyloid protofibril, and the two protofibril ends have been investigated as putative binding sites. Our simulations suggest that the interaction with the two protofibril ends occurs through different binding modes. In particular, binding on the odd edge (chain A) is guided by a well defined hydrophobic cleft, which is common to both ligands (ThCT and ThNT). Moreover, targeting chain A entails a significant structure destabilization leading to a partial loss of β structure and is an energetically favoured process, as assessed by MM/PBSA calculations. A significant contribution of the trehalose moiety to complexes stabilities emerged from our results. The energetically favoured hydrophobic cleft detected on chain A could represent a good starting point for the design of new molecules with improved anti-aggregating features. [1] Hardy J. and Dennis J.S., Science, 2002, 297, 353-356 [2] Adessi C. and Soto C., Drug Dev Res. 2002, 56, 184-193 [3] Bieler S., Soto C., Curr Drug Target, 2004, 5(6):553-8. [4] De Bona P., Giuffrida M.L., Caraci F., Copani A., Pignataro B., Attanasio F., Cataldo S., Pappalardo G. and Rizzarelli E., J. Pept Scie., 2009, 15, 220-228. [5] Autiero I, Saviano M, and Langella E. Mol Biosyst. 2013, 8, 2118-24. [6] Autiero I., Langella E. and Saviano M. Mol. BioSyst., 2013, 9, 2835-41. [7] Luhrs T., Ritter C., Adrian M., Riek-Loher D., Bohrmann B., Doeli H., Schubert D. and Riek R., Proc.Natl. Acad. Sci. U. S. A., 2005, 102, 17342–17347.

PB-082 **Establishing a tool box for generating designer nucleosomes**

Diego Aparicio Pelaz[1], Henriette Mahler, Dirk Schwarzer, Wolfgang Fischle
1.- University of Tuebingen

The basic structural unit of chromatin is the nucleosome, which is composed of histone proteins forming a scaffold with about 150 base pairs of DNA wrapped around. Chromatin compacts eukaryotic genomes and regulates gene activity, which is mediated in part by posttranslational modifications (PTMs) on the N-terminal tails of the histones. Uncovering the detailed relationship between histone tail modifications and gene activity is a major topic of biomedical sciences and general techniques for generating nucleosomes with defined modification patterns in large numbers would greatly facilitate such investigations. To this end we are establishing a chemical toolbox for designer chromatin with defined histone PTM patterns. A protein semysinthesis approach is used that bases on “ligation-ready nucleosomes” with truncated histone H3 that can be ligated with the corresponding synthetic histone tail. We resorted to sortase-mediated ligation as chemoselective ligation method. Here we report our recent developments in establishing the envisioned chemical toolbox for designer chromatin.

PB-083 **Evaluating cation-pi and pi-pi interaction in proteins using various biophysical methods**

Jinfeng Shao[1], Andy-Mark W.H. Thunnissen[1], Jaap Broos[1]
1.-Laboratory of Biophysical Chemistry, University of Groningen Nijenborgh

In proteins the aromatic residues phenylalanine (Phe), tyrosine (Tyr), and tryptophan (Trp) can be involved in aromatic interactions known as cation-pi and pi-pi interactions (Dougherty 2000). Compared to other non covalent interactions in proteins, like H-bonds, dipole-dipole, or van der Waals interactions, relatively little is known about the pi-pi and the cation-pi interactions. The strength of both aromatic interactions is dependent on the pi-electron density in the aromatic residues. A lowering of electron density can be created by introducing strong electron-withdrawing substituents like fluorine atoms in the aromatic ring (Dougherty 2000). In this way a nearly isosteric change in the aromatic system results in a marked change in electron density. Substitution with methyl groups is known to slightly increase the electron density. The published protein labeling method only yields ng quantities of labeled proteins, limiting the scope to channel proteins and their characterization via patch-clamp. The protein LmrR is a transcription factor in Lactococcus lactis that regulates the drug-induced expression of the ABC type multidrug efflux transporter LmrCD (Madoori et al. 2009). LmrR was crystallized in its drug-free state and in complex with the aromatic substrates Hoechst 33342 (H33342) and daunomycin (Madoori et al. 2009). Each substrate is sandwiched between two Trp residues, suggesting pi-pi interactions are operative. To study this interaction in more detail LmrR was biosynthetically labeled with Trp analogs which differ in electron density in the indole moiety. This was achieved by co-expressing LmrR and the Trp-tRNAsynthetase in a Lactococcus lactis Trp auxotroph (Petrovic et al. 2013), yielding mg quantities of labeled proteins. 5-Fluoro-Trp, 5,6-difluoro-Trp, and 5-methyl-Trp were incorporated into LmrR successfully with very high efficiencies. Various biophysical methods including steady state and stopped-flow fluorescence spectroscopy, surface plasmon resonance, and X-ray crystallography are used to evaluate the binding interaction. Our results indicate pi-pi interactions play a role in binding of drugs to LmrR. Recently, we also demonstrated the efficient incorporation of β-(1-azulenyl)-L-alanine in LmrR (Shao et al. 2015). Give its spectroscopic properties (UV-Vis, fluorescence, infrared), this blue colored isostere of tryptophan offers great potential as protein spectroscopic probe under in vitro and in vivo conditions. Dougherty,D.A. 2000. Unnatural amino acids as probes of protein structure and function. Current Opinion in Chemical Biology 4:645-652. Madoori,P.K., Agustiandari,H., Driessen,A.J.M., and Thunnissen,A.M.W.H. 2009. Structure of the transcriptional regulator LmrR and its mechanism of multidrug recognition. EMBO J. 28:156-166. Petrovic,D.M., Leenhouts,K., van Roosmalen,M.L., and Broos,J. 2013. An expression system for the efficient incorporation of an expanded set of tryptophan analogues. Amino Acids 44:1329-1336. Shao,J., Korendovych,I.V., and Broos,J. 2015. Biosynthetic incorporation of the azulene moiety in proteins with high efficiency. Amino Acids 47:213-216.

PB-084 **Synthesis and application of chemical probes for histone deacetylases**

Julia Sindlinger[1], Alexander Dose[1], Jan Bierlmeier[1], Frank Essmann[1], Markus Hartl[2], Iris Finkemeier[3], Dirk Schwarzer[1]

1.-Interfaculty Institute of Biochemistry, University of Tuebingen, 2.-Max Planck Institute of Biochemistry, 3.-Max Planck Institute for Plant Breeding Research

Histone Deacetylaces (HDACs) and Histone Acetylaces (HATs) are responsible for maintaining the global acetylation-level of proteins, including histones. Lysine-acetylation influences gene expression by varying the accessibility of DNA for transcription factors and other chromatin binding proteins [1]. Alterations in the HAT and HDAC activities and the resulting changes in gene expression are therefore considered as key factors in the pathogenesis of cancer and other diseases. Consequential HDACs represent promising drug targets for cancer therapy. Several potent HDAC inhibitors have been reported to date and some of them have been approved as drugs [2]. However, the poor accessibility of recombinant HDACs hampers biochemical research on this important class of enzymes. We have developed peptide-based probes that allow investigations on endogenous HDACs in cellular lysates. These probes were used in pull-down experiments to isolate HDAC complexes and study their substrate selectivity. [1] Strahl, B.D., T. and Allis, C. D., Nature 403, 41-46 (2000) [2] Minucci. S. and Pelicci, P. G., Nat. Rev. Cancer 6, 38-51 (2006)

PB-085 **Exploring the Substrate Selectivity of Oxygen Sensing Prolyl Hydroxylases**

Kerstin Lippl[1], Martine Abboud[1], Christoph Loenarz[2], Christopher Schofield[1]
1.-Department of Chemistry, University of Oxford, United Kingdom, 2.-Department of Chemistry, University of Nottingham, United Kingdom

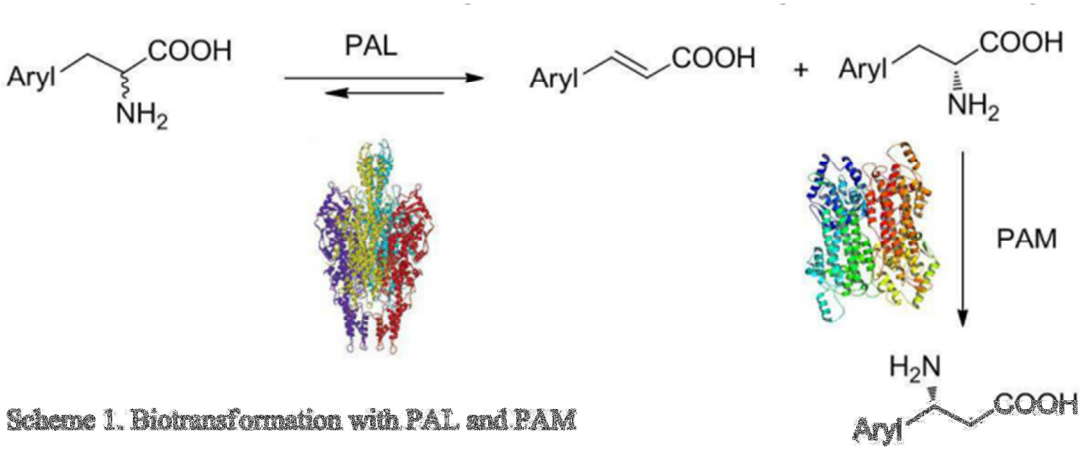
The response to low cellular oxygen levels in humans and other animals is induced by the hypoxia inducible transcription factors (HIFs). These transcription factors are regulated by hypoxia inducible factor prolyl hydroxylases (PHDs), which act as 'oxygen sensors' by hydroxylating HIFs, thus leading to the proteomic degradation of the transcription factors. Over the last years, there have been multiple reports that describe additional PHD substrates other than HIFs. Among them are the large subunit of RNA Pol II, several transcription factors, and components of signalling pathways. Validating these reports is of major medicinal relevance given that PHD inhibitors are now in the late stage Phase 3 clinical trials. In order to investigate the selectivity of PHDs, the reported proteins have been tested as substrates for hydroxylation by Mass Spectrometry, and as binders or competitors of the PHDs. Initial work on peptides that contain the putative hydroxylation sites has indicated that the PHDs are much more selective for their well-established substrate HIF. However, in ongoing work these initial results are going to be validated on protein level by co-expressing PHDs with the reported substrates. Additionally, peptides of reported substrates were screened for their ability to alter the kinetics of HIF-hydroxylation by PHD2. An inhibitory effect of at least two different peptides on PHD2 was observed, suggesting that there is an interaction between the prolyl hydroxylase and these peptides. In order to investigate the mode of binding and inhibition, NMR studies have been carried out and binding of the two inhibitory peptides on PHD2 has been shown. Altogether, these results indicate that, although PHDs might be more selective for HIF as a substrate as it was initially thought, the enzymatic activity of the prolyl hydroxylases is possibly influenced by a number of other proteins that can directly bind to PHDs.

PB-086 **Non-natural aminoacids via the MIO–enzyme toolkit**

Alina Filip[1], Judith H Bartha-Vári[1], Gergely Bánóczy[2], László Poppe[2], Csaba Paizs[1], Florin-Dan Irimie[1]

1.-Biocatalysis and Biotransformation Research Group, Department of Chemistry, UBB, 2.-Department of Organic Chemistry and Technology

An attractive enzymatic route to enantiomerically pure to the highly valuable α- or β-aromatic amino acids involves the use of aromatic ammonia lyases (ALs) and aminomutases (AMs). All these enzymes have in common an auto-catalically formed 5-methylene-3,5-dihydroimidazole-4-one (MIO) electrophilic prosthetic group, and show high structural and sequence similarities. The recent advances in improving the functional properties of these enzymes increased both their biocatalytic and therapeutic applications.



Scheme 1. Biotransformation with PAL and PAM

We aimed to create a library of recombinant MIO-enzymes consisting of the PALs and PAMs with large substrate promiscuity in order to provide access to various non-natural aminoacids through enzymatic ammonia addition and/or ammonia elimination reactions of the substrate library already available in our researchgroup. The developed complementary substrate and enzyme library would provide the MIO-enzyme toolkit useful for the synthesis of nonnatural aminoacids. The synthetic gene of the enzymes (PcPAL, RtPAL, AvPAL, PaPAM) were cloned into pET19b_J906 expression vector using XhoI and Bpu1102I cloning sites. The plasmid DNA was transformed to several E.coli host strains (Rosetta, BL21, Origami 2) in order to optimize the expression yields. The enzymes containing an N-terminal His10-tag were purified with affinity chromatography, followed by ion-exchange or/and size-exclusion chromatography, obtaining pure and homogenous proteins, in their tetrameric, presumably native fold. The enzyme activity and the kinetic parameters of the purified enzymes was determined towards the natural substrate L-phenylalanine, as well as towards novel bulkier aromatic substrates (heteroaryl alanines, styryl alanines, biphenylalanines). Furthermore to enhance their biocatalytic applicability we covalently immobilized the enzymes to carboxylated single-walled carbon nanotubes (SwCNT COOH) using linkers with different lengths, and tested the activity and recycling of the immobilized enzyme. Acknowledgements: AF thanks for the financial support of the Sectorial Operational Program for Human Resources Development 2007-2013, co-financed by the European Social Fund, under the project number POSDRU/159/1.5/S/132400 and of the Romanian National Authority for Scientific Research, CNCS-UEFISCDI,project number PN-II-ID-PCE-2011-3-0775. LP thanks for financial support from Hungarian OTKA Foundation (NN-103242) and from the New Hungary Development Plan (TÁMOP-4.2.1/B-09/1/KMR-

2010-0002: Development of quality-oriented and harmonized R+D+I strategy and functional model at BME). The authors also thank the support from COST Action CM1303 (SysBiocat) and Jody L. McGinness

PB-087 **High affinity synthetic antibodies as biological tools**

Mateusz Lugowski[1], Malgorzata Nocula-Lugowska[1], Somnath Mukherjee[1], Anthony Kossiakoff[1]
1.-Department of Biochemistry and Molecular Biology, The University of Chicago

Antibodies that bind protein antigens are indispensable tools in biochemical research and modern medicine. Utilizing a phage display selection strategy, we have obtained synthetic antigen binders (sABs), based on a Fab fragment of IgG, to a wide array of proteins as distinct as membrane proteins, structural proteins, scaffold proteins and nuclear targets. Here we demonstrate the applicability of the sABs towards the native, full-length proteins in cells. We show that the generated sABs are able to pull-down endogenous proteins from mammalian cell extracts along with their natural binding partners. We developed a method of utilizing our high affinity and specificity binders as fluorescently labeled tools to visualize target proteins in their native environment in the cells without the need of secondary antibodies or blocking reagents. Our system also includes a method of efficient delivery of generated antibodies to living cells, where they can perform their function. The sABs have been successfully used for altering biological processes in a controllable manner.

PB-088 **In vitro evolution from pluripotent peptide libraries with natural neurotoxin scaffolds to target receptors, proteases and trophic factors**

Tai Kubo[1], Mohammed Naimuddin[1], Seigo Ono[1]
1.-National Institute of Advanced Industrial Science and Technology (AIST)

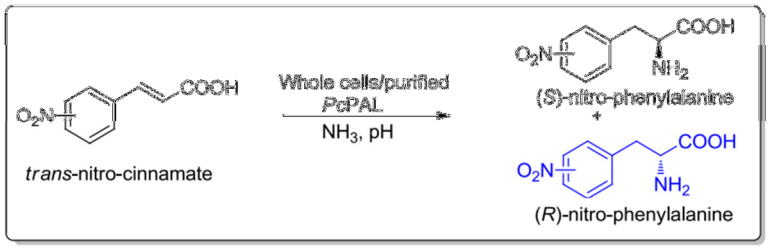
In vitro evolution from pluripotent peptide libraries with natural neurotoxin scaffolds to target receptors, proteases and trophic factors Small molecule natural products are precious resources for drug discovery. During millions of years of evolution, natural products must have been exposed to various selection pressures and have been refined in structure and function to obtain the present features. In some peptide neurotoxins, however, the basic molecular scaffold mainly configured by disulfide (S-S) bridges and/or alpha/beta structures, is strictly conserved within each family even under the evolution pressure. On the other hand the loop regions, which are not heavily involved in scaffold formation, are highly diverged. This mode of molecular evolution named 'accelerated evolution', is reasonable to quickly adapt to the vigorous change of the environment. The evolutionally selected scaffold is compact harboring both rigidity and flexibility in nature, and it may support a topology appropriate for target recognition and selective interaction. Inspired by the system, we designed random peptide libraries from the peptide neurotoxins of the accelerated evolution. A three-finger (3F) shaped snake neurotoxin consists of huge family evolved by accelerated gene evolution. We prepared a 3F-peptide library by introducing random sequences in each fingertip. Another random peptide library with an ICK (inhibitor cystine knot) motif was prepared based on a neurotoxin GTx1-15 from spider; originally identified as a T-type Ca2+ channel modulator. Each library was subjected to in-vitro evolution directed to specific target molecules. For the 3F-peptide library cDNA display method was applied to select binders. When interleukin-6 (IL-6) receptors were targeted, the selected 3F peptides showed binding affinities (Kd ~100 nM) comparable to the native ligand IL-6. When trypsin was targeted, peptides with serine protease inhibitor activities similar to STI and BPTI (Ki ~30 nM) were isolated. Specific binders to a trophic factor VEGF were also generated from the 3F library. To target membrane proteins, we developed a unique in-vitro evolution system, and named it as the PERISS (intra periplasm secretion and selection) method. In the system, target membrane proteins are expressed in inner membrane of E. coli and peptides are secreted to the periplasmic space, in between the inner and outer membranes; and the space is served for interaction and selection. The PERISS method enabled us to identify a peptide specific to muscarinic receptor m2 subtype from the ICK peptide library. In conclusion, it was proved that the library designed from the scaffold of peptide toxin, which evolved in the mode of accelerated gene evolution, has pluripotency in target recognition, interaction and even bioactivity. The library, when combined with in-vitro evolution technologies, may open a new platform to develop antibody alternatives.

PB-089 **Stereoselectivity of PAL under non-optimal conditions**

Andrea Varga[1], Botond Nagy[1], Melinda Miklós[1], Florin-Dan Irimie[1], László Poppe[1], Csaba Paizs[1]
1.-Biocatalysis and Biotransformation Research Group, Department of Chimie, UBB

Phenylalanine ammonia lyase from Petroselinum cripsum (PcPAL) belongs to the class of enzymes containing 4-methylideneimidazole-5-one (MIO) as a prostetic group and it is responsible for the conversion of L-phenylalanine into trans-cinnamic acid. This reaction is reversibile under high ammonia concentration.1

We analyzed several factors that can influence the enantioselective synthesis of nitrophenylalanine mediated by whole cells as well as purified MIO-containing and MIO-less PcPALs. First we investigated the behaviour of the enzymes depending on the ammonia concentration. We also inspected the influence of the pH on the PcPAL catalyzed biotransformations. Based on our results, we concluded that variation of ammonia concentration and the pH leads to decrease of enantioselectivity, suggesting that PcPAL is able to catalyze the formation of both L- and D- enantiomers of electron-deficient structures.



Scheme 1. *Pc*PAL mediated amination of *trans*-nitro-cinnamate in non-stereoselective manner

Acknowledgements: AV thanks for the financial support of the Sectoral Operational Programme for Human
Resources Development 2007-2013, co-financed by the European Social Fund, under the project
POSDRU/159/1.5/S/137750 and of the Romanian National Authority for Scientific Research, CNCSUEFISCDI, project number PN-II-ID-PCE- 2011-3-0799. The authors also thank the support to Jody L.

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PC-001 **Biochemical Characterization and Amino Acid Sequence Analysis of Thermostable Endo-ß-1, 4-glucanase from Trichoderma viride**

Nidhee Chaudhary[1], Monendra Grover[2]

1.-Amity Institute of Biotechnology, Amity University Uttar Pradesh, Sector-125, 2.-1Centre for Agricultural Bioinformatics, IASRI

Abstract In the present investigation, Endo-β-1,4-glucanase has been purified to homogeneity from Trichoderma viride using (NH4)2SO4 fractionation, DEAE-cellulose chromatography and CM-cellulose chromatography. The purified enzyme had a molecular mass of 44.67 kDa with pH and temperature optima 4.8 and 500C, respectively, and thermostability upto 500C for 48h. The purified enzyme showed a novel N-terminal 15 amino acid sequence 'SYPNKQPYGPSGFWM'. However, T. viride endo- β-1,4-glucanase, like all microbial cellulase appears to have a conserved 'SG' amino acid sequence at an identical position in the N-terminal domain. The properties of the N-terminal 15 amino acid sequence were also predicted computationally. This analysis showed that N-terminal sequence of the enzyme is unstable. The N-terminal sequence also showed potential cleavage sites by different proteases which may contribute to its instability. The secondary structure analysis showed that the N-terminal sequence has 40% of the 15 a.a. sequence in extended strand and 60% in random coil conformation. The N-terminal sequence was also analyzed for potential phosphorylation sites. While no potential serine and threonine sites were predicted, two tyrosine phosphorylation sites were predicted in the N-terminal sequence. The N-terminal sequence was also examined for the presence of kinase specific phosphorylation sites. The results showed the presence of one potential site which may be phosphorylated by PKC at position 1 of the N-terminal sequence. The analysis for the prediction of the presence of OGlcNAc sites revealed that two such sites may potentially be present in the sequence. We have also predicted the ligand binding site in the N-terminal sequence of the protein.

Keywords: Endo-β-1,4-glucanase, Trichoderma viride, N-terminal amino acid sequence, phosphorylation, secondary structure

PC-002 **Modulation of the enzymatic activity of protein arginine methyltransferase 1 by small molecules**

Wey-Jing Lin[1]
1.-National Yang-Ming University

Protein arginine methylation catalyzed by protein arginine methyltransferases (PRMTs), is a pivotal protein post-translational modification involved in a growing number of physiological and pathological processes including signal transduction, proliferation, differentiation and malignancy. PRMT1 accounts for the majority of protein arginine methyltransferase activity in mammalian cells and, in consistence, a large amount of cellular substrates have been identified. Several studies have reported that the activity of PRMT1 changes upon stimulation in various cellular processes. In mammalian cells, PRMT1 exists in a high molecular weight complex. The interacting partners of PRMT1, such as antiproliferative proteins BTG1 and BTG2, protein phosphatase 2A, the orphan receptor TR3, and CCR4-associated factor 1(hCAF1) are shown to play a role in modulating the methyltransferase activity and the substrate selectivity of PRMT1. Due to the pivotal roles of PRMT1 in physiological and pathological conditions, intensive efforts have been put on the search of small synthetic chemical molecules which can efficiently modulate the activity of PRMT1 for the potential development of therapeutics. In light of this, the intracellular small molecules that either transmit extracellular stimulation or act as cofactor to dictate the activity of PRMTs in cells are still poorly understood. Our study focused on examining how cellular ions might affect the activity of PRMT1 and found that divalent and monovalent ions differentially modulated the catalytic activity of PRMT1 toward different substrates.

PC-003 **Oligomerisation properties of light-dependent protochlorophyllide oxidoreductase**

Michal Gabruk[1], Anna Piszczek[1], Bozena Skupien-Rabian[1], Sylwia Kedracka-Krok[1,2], Jerzy Kruk[1], Beata Mysliwa-Kurdziel[1]

1.-Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, 2.-Malopolska Centre of Biotechnology, Jagiellonian University

Light dependent protochlorophyllide oxidoreductase (POR, E.C. 1.3.1.33) is a key enzyme on chlorophyll biosynthesis pathway. It catalyses the conversion of protochlorophyllide (Pchlide) to chlorophyllide using NADPH in a light-dependent manner. This special property makes the enzyme unique and interesting subject of study. In our research, we have obtained recombinant PORA from A. thaliana and a fusion protein of PORA-GFP. We have confirmed that the enzyme works as an oligomer using different methodological approaches. Using native gel electrophoresis, we have shown that the enzyme simultaneously forms monomers, dimers and much larger oligomers without substrates in the lipid-free environment. However, addition of Pchlide, but not NADPH, promotes formation of the oligomers. In order to get insights into the structure of Pchlide:POR:NADPH oligomer, we have applied cross-linking assay followed by mass-spectrometry analysis to identify which domains of the enzyme interact with each other. We have analysed our results with respect to the homology model of POR since there is no crystal structure of the enzyme known. Taking advantage of the broad spectrum of applied techniques, we have been able to propose the model of POR oligomerization. ACKNOWLEDGEMENT This work was supported by the grant Preludium 5 NCN2013/09/N/NZ1/00200 obtained form the National Science Centre.

PC-004 **Preparation of insect prothoracicotropic hormone with complicated disulfide-bond structure, by the heterologous expression in Brevibacillus choshinensis**

Kazuki Saito[1], Tadafumi Konogami[1], Yiwen Yang[1], Yusuke Yamashita[1], Masatoshi Iga[1], Tamari Hoshikawa[1], Hiroshi Kataoka[1]

1.-Dept. of Integrated Biosciences, Grad. Sch. of Frontier Sciences, Univ. of Tokyo

Prothoracicotropic hormone (PTTH) is one of the most important neuropeptide regulators for insect molting and metamorphosis. However, preparation of its recombinant protein has hardly been successful, because it is a homodimer protein with very complicated disulfide-bond structure. For example, silkworm PTTH has three intramolecular disulfide bonds in its 109-residue polypeptide chain, and the two chains are further linked by an additional intermolecular disulfide bond to form the homomeric dimer. Although the recombinant silkworm PTTH was previously expressed in Escherichia coli, the product was obtained only in precipitation fractions, and refolding of the precipitated protein provided the active dimer PTTH in very poor yield. Under such reductive conditions as in cytosol of the E. coli cells, formation of the correct disulfide-bond arrangement must be difficult. Alternatively, for the heterologous expression of the silkworm PTTH, we employed Brevibacillus choshinensis (formally referred to as Bacillus brevis), which has achieved good results in expression of various disulfide-bond-containing proteins. In this study, the silkworm PTTH was expressed in the Brevibacillus cells with an additional His6-tag sequence at the C-terminus, for easier detection and purification. First of all, since the Brevibacillus bacteria are equipped with a secretory system of the expressed proteins, a secretory signal sequence to be attached before the silkworm PTTH was carefully selected. Among four candidates in a commercially-available kit, a signal sequence derived from an intrinsic cell-wall protein MWP gave better results in expression levels of the protein. Second, incubation time of the cells was optimized, because an oligomerization state of the secreted PTTH in the cell culture medium changed with the time. In the medium, various PTTH oligomers including a monomer and a dimer were initially observed, but higher oligomers became a major portion of the secreted product after longer incubation than 48 h. Incubation for 24–36 h may be suitable for obtaining the native dimer form of the silkworm PTTH. To remove the undesired monomer and higher oligomers, which mostly retained free sulfhydryl groups, the secreted proteins were treated with maleimide-PEG2-biotin. In the purification using a Ni+-NTA column, the dimer of the His6-tagged silkworm PTTH was eluted with an imidazole gradient, separately ahead of other biotinylated proteins, probably due to interaction of the PEG2 spacer with the Ni+-NTA groups of the resin. After the reversed-phase HPLC purification, the final product showed a single band on the non-reductive SDS-PAGE, and it had adequate ecdysone-releasing activity from isolated silkworm prothoracic glands. The Brevibacillus bacteria are most promising host cells for the heterologous production of the insect PTTH.

PC-005 **Role of the disulfide bridges in the transmembrane region of the insect prothoracicotropic-hormone receptor, Torso**

Tadafumi Konogami[1], Yiwen Yang[1], Mari H. Ogihara[1], Juri Hikiba[1], Hiroshi Kataoka[1], Kazuki Saito[1]

1.-Dept. of Integrated Biosciences, Grad. Sch. of Frontier Sciences, Univ. of Tokyo

Torso is an insect cellular-membrane protein, which was recently identified as a receptor for prothoracicotropic hormone (PTTH). Although PTTH is one of the important regulatory molecules in insect molting and metamorphosis, activation mechanism of Torso by the ligand has not been elucidated yet. In this study, an oligomerization manner of the silkworm Torso was examined, using heterologous expression in Drosophila S2 cultured cells, because Torso is a single-polypeptide receptor tyrosine kinase (RTK), and activation of such RTKs is often triggered by the ligand-induced receptor dimerization on the cellular membrane. When activated with silkworm PTTH, dimerization of the silkworm Torso in the S2 cells was observed, using a cross-linking reagent BS3, and the subsequent receptor autophosphorylation and downstream ERK phosphorylation were also detected. Surprisingly, however, the Torso dimerization was revealed to occur even without the ligand stimulation, while the autophosphorylation and the ERK phosphorylation were held in response to the stimulation. When fractionated by non-reductive SDS-PAGE, the silkworm Torso showed an obvious dimer band, in addition to a faint monomer band, both with and without the PTTH simulation, even though the receptor was not treated with the cross-linking reagent. This indicates that the Torso protein is expressed originally as a disulfide-bond-linked dimer. In addition, by examining oligomerization states of several truncation and substitution mutants, cysteine residues in the transmembrane region were found to participate in the intermolecular disulfide bridges, linking the two receptor molecules in the dimer. When all of the three cysteines in the transmembrane region were replaced by phenylalanines, the disulfide-bond-linked Torso dimerization was not observed, but spontaneous, ligand-independent association of the Torso molecules was detected using the cross-linker BS3. This spontaneous dimerization caused the apparent Torso autophosphorylation, but it could not induce the downstream ERK phosphorylation. Consequently, without the intermolecular disulfide bridges, Torso loses its responsiveness to the PTTH stimulation. In conclusion, the disulfide bridges in the transmembrane region may play a role to preserve suitable relative position between the two Torso molecules, which could induce ligand-dependent autophosphorylation leading to activation of the downstream signaling pathways in the cells.

PC-006 **Structural study of the yeast enzyme neutral trehalase Nth1 and pNth1:Bmh1 protein complex**

Miroslava Kopecka[1, 2], Zdenek Kukacka[3], Petr Man[3], Tomas Obsil[2], Veronika Obsilova[2]
1.-2nd Faculty of Medicine, Charles University in Prague, 2.-Institute of Physiology of the Czech Academy of Sciences, 3.-Institute of Microbiology of the Czech Academy of Sciences

The yeast enzyme neutral trehalase (Nth1, EC 3.2.1.28) from Saccharomyces cerevisiae hydrolyses the non-reducing disaccharide trehalose which serves as an energy source and a universal stress protectant in many different organisms. Enzymatic activity of Nth1 is enhanced by the yeast 14-3-3 protein (Bmh1 and Bmh2) binding in a phosphorylation-dependent manner. Nth1 activity is also regulated by Ca2+ binding to the EF-hand-like motif containing domain of Nth1 [1]. The native TBE PAGE and analytical ultracentrifugation show that Nth1 forms very stable complexes with Bmh1 and Bmh2 [1]. To study the structure of Nth1 alone and its complex with the 14-3-3 protein we used circular dichroism, H/D exchange coupled to mass spectrometry, chemical cross-linking [2] and small angle X-ray scattering (SAXS) [3]. At the same time protein crystallography of Nth1 alone and its complex with Bmh1 is performed.

The low resolution structure of pNth1:Bmh1 protein complex revealed that binding of Bmh1 induces a rearrangement of the whole Nth1 molecule and that the region containing the EF-hand motif forms a separate domain which interacts with both Bmh1 and catalytic domain of Nth1. We proved that integrity of the EF-hand motif is crucial for the Bmh1 mediated activation of Nth1 and Ca2+ binding. Our data suggest that the EF hand-like motif functions as the intermediary through which Bmh1 modulates the function of the catalytic domain of Nth1. These structural changes probably enable the substrate entry into the enzyme active site [3]. Our study of 14-3-3 protein complex with the fully active enzyme Nth1 offers a unique structural view of Nth1 activation enabling us to better understand the role of the 14-3-3 proteins in regulation of other enzymes. This work was supported by the Czech Science Foundation (Project P207/11/0455) and by Grant Agency of Charles University (Grant 644313).

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PC-007 **Development and use of a molecular purge valve to maintain reduction/oxidation balance in synthetic biochemistry systems**

Tyler Korman[1], Paul Opgenorth[1], James Bowie[1]
1.-Department of Chemistry and Biochemistry, University of California Los Angeles

The assembly of self-regulating synthetic biochemical pathways in vitro has great potential as alternative catalysts for the high-yield production of low value/high volume commodity chemicals from biomass. High yields of low-value/high volume compounds that are required for economic viability is particularly difficult via traditional in vivo metabolic engineering of microbes due to competing biochemical pathways and toxicity. We have developed an alternative approach, called synthetic biochemistry, where the glycolysis pathway of central metabolism is reconstituted in vitro with an anabolic pathway that can produce useful compounds at high yield. In the specific synthetic biochemistry system described, reducing equivalents, ATP, and carbon from glycolysis are funneled through the anabolic mevalonate pathway to produce the monoterpene limonene from glucose. The successful implementation of the in vitro pathway required development of a molecular purge-valve consisting of an NAD+ and NADP+ specific reductase (ie wild-type and mutant pyruvate dehydrogenase), and NADH oxidase, NoxE, to maintain proper NADP+/NADPH cofactor balance while allowing continuous carbon flux. We find that the purge-valve concept is readily transportable to other NAD(P)H generating steps in central metabolism and can be used to convert glucose to limonene at high yield.

PC-008 **Evolution of Structure and Mechanistic Divergence in Di-Domain Methyltransferases from Nematode Phosphocholine Biosynthesis**

Soon Goo Lee[1], Joseph Jez[1],
1.-Washington University in St. Louis

The phosphobase methylation pathway is the major route for supplying phosphocholine to phospholipid biosynthesis in plants, nematodes, and Plasmodium. In this pathway, phosphoethanolamine N-methyltransferases (PMT) catalyzes the sequential methylation of phosphoethanolamine to phosphocholine. In the PMT, one domain (MT1) catalyzes methylation of phosphoethanolamine to phosphomonomethylethanolamine and a second domain (MT2) completes the synthesis of phosphocholine. The x-ray crystal structures of the di-domain PMT from the parasitic nematode Haemonchus contortus (HcPMT1 and HcPMT2) reveal that the catalytic domains of these proteins are structurally distinct and allow for selective methylation of phosphobase substrates using different active site architectures. These structures also reveal changes leading to loss of function in the vestigial domains of the nematode PMT. Divergence of function in the two nematode PMT provides two distinct anti-parasitic inhibitor targets within the same essential metabolic pathway. The PMT from nematodes, plants, and Plasmodium also highlight adaptable metabolic modularity in evolutionarily diverse organisms.

PC-009 **Glycoside hydrolase family18 chitinase from the stomach of fish: characteristics of isozymes**

Masahiro Matsumiya[1], Hiromi Kakizaki[1], Mana Ikeda[1]
1.-College of Bioresource Sciences, Nihon University

Chitinases (EC 3.2.1.14) are enzymes that randomly hydrolyze β-1,4 glycosidic bonds of chitin and produce N-acetylchitooligosaccharide ((GlcNAc)n) that has various physiological functions such as immunostimulatory activity. Most of fish takes crustacean such as shrimp and crab as food. Therefore, the fish has chitinase in the stomach to chemically disrupt the chitinous envelope of crustacean. Four chitinase isozymes (42-60 kDa), PaChiA[1] and PaChiB[2], and PtChiA and PtChiB, [3] were purified from the stomach of silver croaker Pennahia argentatus and threeline grunt Parapristipoma trilineatum, by ammonium sulfate fractionation and column chromatographies, respectively. All the chitinases were stable and showed activity in the acidic pH range (pH3-5). PaChiA and PtChiA preferentially degraded the second glycosidic bond from the non-reducing end of (GlcNAc)n and PaChiB and PtChiB had a preference for the third glycosidic bond of those. All the chitinases showed different substrate specificity toward insoluble long substrates. Moreover, chitinase cDNAs (PaChi-1 and PaChi-2) encoding PaChiA and PaChiB, and cDNAs (PtChi-1 and PtChi-2) encoding PtChiA and PtChiB were obtained by cDNA cloning using the RT-PCR and RACE method. The deduced amino acid sequences of all the chitinase cDNAs contained N-terminal signal peptide, GH family 18 catalytic domain, linker region, and chitin-binding domain. Phylogenetic tree analysis of vertebrate chitinase revealed that fish stomach chitinases form unique chitinase isozyme groups, acidic fish chitinase-1 (AFCase-1) including PaChiA and PtChiA, and acidic fish chitinase-2 (AFCase-2) including PaChiB and PtChiB , which was different from an acidic mammalian chitinase (AMCase) group.[3,4] The previously reported purified fish stomach chitinases[5] can also be classified into two chitinase isozyme groups, AFCase-1 and AFCase-2, by the N-terminal amino acid sequence. This study suggested that fish have excellent chitin degrading enzymatic system in which two different chitinases isozyme groups, AFCase-1 and AFCase-2, with different degradation patterns are expressed in the stomach. [1] M. Ikeda, K. Miyauchi, A. Mochizuki, and M. Matsumiya, Purification and characterization of chitinase from the stomach of silver croaker Pennahia argentatus. Protein Expr. Purif. 65, 214–222 (2009). [2] M. Ikeda, K. Miyauchi, and M. Matsumiya, Purification and characterization of a 56 kDa chitinase isozyme (PaChiB) from the stomach of silver croaker Pennahia argentatus. Biosci. Biotechnol. Biochem. 76, 971–979 (2012). [3] M. Ikeda, Y. Kondo, and M. Matsumiya, Purification, characterization, and molecular cloning of chitinases from the stomach of the threeline grunt Parapristipoma trilineatum. Process Biochem. 48, 1324–1334, (2013). [4] M. Ikeda, D. Shirase, T. Sato, M. Ueda, S. Hirabayashi, and M. Matsumiya, Primary structure and enzymatic properties of chitinase isozymes purified from the stomach of the marbled rockfish Sebastiscus marmoratus. J. Chitin Chitosan Sci.2, 106-116 (2014). [5] M. Matsumiya, Y. Arakane, A. Haga, S. Muthukrishnan, and K. J. Kramer, Substrate specificity of chitinases from two species of fish, greenling, Hexagrammos otakii, and common mackerel, Scomber japonicus, and the insect, tobacco hornworm, Manduca sexta, Biosci. Biotechnol. Biochem., 70, 971-979 (2006).

PC-010 **A bifunctional cold active lipase with protease activity isolated from an Antarctic yeast, Glaciozyma antarctica PI12**

Mohd Shukuri Mohamad Ali[1,2], Ira Maya Haris[1,2], Raja Noor Zaliha Raja Abd Rahman[1,2], Mahiran Basri[3], Abu Bakar Salleh[1,2]

1.-Enzyme and Microbial Technology Research Center, 2.-Faculty of Biotechnology and Biomolecular Sciences, 3.-Faculty of Science

Recently, the enzymes produced by psychrophilic organisms have gained huge interest especially in the studies of temperature adaptation of the protein. Previously, a cold-adapted yeast, Glaciozyma antarctica PI12 was isolated from a marine environment in Antarctica and the yeast was known to produce lipolytic and proteolytic enzymes. A gene encoding a unique recombinant bifunctional enzyme (LipPI12) with cold active lipase with protease activity was successfully expressed, purified and characterized. Temperature profile of the bifunctional LipPI12 enzyme showed that the lipase functions optimally at 20°C whereas the protease was more active at 40°C. pH profile showed that both LipPI12 lipase and protease were active at near neutral condition. Activity of LipPI12 lipase and protease were also activated in the presence of CaCl2 but its protease counterpart seemed to be more active in the presence of ZnCl2. Effect of surfactants showed LipPI12 lipase was activated by Tween 80 and SLS and in contrast, LipPI12 protease was almost deactivated in all surfactants tested. The presence of organic solvents did not affect both the lipase and protease activities. The lipase was more stable at solvents with higher log P value whereas the protease was slightly activated at low log P value particularly with dimethylsulfonyl. Inhibitor studies revealed that LipPI12 lipase was partially inhibited with EDTA and PMSF whereby the LipPI12 protease was inhibited by pepstatin, EDTA and PMSF. LipPI12 enzyme was successfully crystallized via vapour diffusion method. Crystal of LipPI12 enzyme was diffracted via synchrotron radiation. The three-dimensional structure of cold-adapted PI12 provided insight into cold adaptation and better understanding of the structural properties of LipPI12 enzyme. The bifunctional properties of the enzyme could be potential candidate for low temperature industrial application.

PC-011 **Conformation-specific antibodies as enhancers and inhibitors of phosphatase activity of DEP 1**

Malgorzata Nocula-Lugowska[1], Mateusz Lugowski[1], Anthony A. Kossiakoff[1]
1.-The University of Chicago

DEP-1 (CD148/PTP-η) is a transmembrane receptor-like protein tyrosine phosphatase (PTP) that has been implicated in the density-dependent regulation of cell growth, differentiation and transformation. It counteracts protein kinases by dephosphorylating a number of their substrates as well as the kinases themselves, thus potentially controlling the specificity of signals. For example EGFR, VEGFR 2, Met, PDGF β receptor have been shown to be dephosphorylated by this phosphatase. DEP-1 has been shown to act as a tumor suppressor and it has been proposed as a molecular target in anti-angiogenesis therapy. As a result, both enhancers and inhibitors of DEP-1 activity have the potential of elucidating pathways responsible for abnormal cell behavior. We generated synthetic antibodies against intracellular catalytic domain of DEP-1 that act as modulators of the enzyme's phosphatase activity. By applying a combination of selection pressures an array of antibodies has been raised from phage display libraries of Fab fragments which are capable of either enhancing or inhibiting DEP-1 activity. In phosphatase assays with catalytic domain of DEP-1 the antibodies demonstrate non-competitive or mixed kinetics. The crystal structure of DEP-1-inhibitor complex shows that this antibody binds to the part of the protein that is distant from the active site and acts by locking the enzyme in the non-natural catalytically inactive state by hindering the closure of the WPD loop which is crucial for the reaction to occur. By contrast, as judged from the crystal structure of a complex of DEP-1 with the antibody that enhances its phosphatase activity, this antibody seems to act by stabilizing the naturally found active state of DEP-1 with WPD loop in the closed conformation. The antibodies are also able to recognize DEP-1 in cells, as they stain DEP-1 in immunofluorescence experiments. To test the applicability of raised antibodies in cells the activator was additionally used to pull down full-length endogenous DEP-1 after being delivered to live cells. Inhibition and enhancement of DEP-1 activity by locking the enzyme in conformations which are either natural or imposed by allosteric binding of antibodies seems to be a mechanism that can be utilized to modulate activity of other tyrosine phosphatases.

PC-012 **Investigating Acinetobacter baumannii pathogenesis: crystal structure of WbjB epimerase from a polysaccharide biosynthesis cluster**

Bhumika S. Shah[1], Karl A. Hassan A. Hassan[1], Heather E. Clift[1], Stephen J. Harrop[2], Ian T. Paulsen[1], Bridget C. Mabbutt[1]

1.-Department of Chemistry and Biomolecular Sciences, Macquarie University, 2.-School of Physics, University of New South Wales

Acinetobacter baumannii is a multi-drug resistant opportunistic pathogen emerging as a major health threat in clinical and community settings worldwide. Recent comparative genome analysis across Acinetobacter spp. highlights that large portions of its genetic material are not fixed, but rather held within a mobile pangenome. Acquired through lateral transfer, these mobilized genes cluster within genomic islands (GIs), encoding highly adaptive traits such as virulence, pathogenicity and drug resistance. I have analyzed three new A. baumannii genome sequences derived from Australian hospitals and indigenous sources, so complementing previously studied genomes from USA and Europe. My work identified GIs resident within these strains, clusters of novelty account for 9-16% of each genome. One GI of interest, found in an A. baumannii strain from a community-acquired infection in an indigenous settlement in Northern Australia, clearly encodes discrete steps of lipopolysaccharide (LPS) biosynthesis, so likely influencing virulence factors for its host pathogen. I have focused on the encoded epimerase WbjB, a member of the extended shortchain dehydrogenase/reductase family (SDR). The crystal structure solved to 2.65 Å reveals an unusual hexameric form of SDR, with cofactor NADP bound. Assay of WbjB indicates responsiveness to UDP sugar molecules such as UDP-GlcNAc and UDP-Glc. I describe screening and first knock out strains created in A. baumannii. These aim to assess the role of the GI cluster in carbon metabolism, as well as its impact on biofilm formation. Phenotypic differences of wild type and knock out strains will subsequently be investigated to identify adaptive traits attributable to this LPS cluster.

PC-013 **Role of the Hydrogen Bonding Interactions in the O2 Sensitivity of HIF-Prolyl Hydroxylase (PHD2)**

Serap Pektas[1, 2], Michael Knapp[1]

1.-University of Massachusetts Amherst, 2.-Recep Tayyip Erdogan University

Oxygen homeostasis is regulated by hypoxia inducible factor, a transcription factor. When the oxygen level becomes too low (hypoxia), hypoxia-inducible-factor 1 (HIF-1α) activates the expression of over a hundred genes, associated with angiogenesis, erythropoiesis, VEGF (vascular endothelial growth factor), cell migration, and energy metabolism etc. HIF-1α cellular level is highly dependent on oxygen concentration and regulated by oxygen sensor enzyme, HIF prolyl hydroxylase (PHD2). PHD2 is a 2-oxoglutarate, non-heme Fe2+ dependent dioxygenase, which require O2 for catalytic activity. PHD2 regulates the HIF-1α cellular level by hydroxylating two proline residues in ODD domain of HIF-1α, which targets HIF-1α for proteasomal degradation. In order to understand how O2 activation of PHD2 enzyme works, we investigated the effect of residues, which have hydrogen bonding interactions with O2 ligand of PHD2. Point mutations and steady state kinetic assays were performed and compared with wild type PHD2. Eliminating certain hydrogen bonding interactions of O2 ligand led increased turnover rate at low O2 concentration. Our results revealed that hydrogen bonding interactions with O2 ligand determines the O2 sensitivity of PHD2.

PC-014 **New pharmacological therapies against congenital erythropoietic porphyria (CEP)**

Pedro David Urquiza[1], Ana Laín[1], Arantza Sanz[1], Juan Manuel Falcón[1, 2], Oscar Millet[1]
1.-CIC bioGUNE, 2.-Ikerbasque

Congenital erythropoietic porphyria (CEP) is produced by deleterious mutations in uroS gene. Among the most aggressive mutations, C73R drastically reduces the activity and stability of uroporphyrinogen III synthase enzyme (UROIIIS), present in almost one of third of all the reported CEP cases. Previous studies in our laboratory demonstrated that the catalytic activity UROIIIS was fully restored, by incorporating residues prone to interact with 73R to stabilize the hinge region, as well as a modulated increase in the kinetic stability of the enzyme. These results provide an unprecedented rationale for a destabilizing missense mutation. At the present time we are screening different molecular chaperones (library of 2500 compounds) as a therapy against CEP which should be able to upregulate the protein's homeostasis by binding to the enzyme in order to stabilize the folded conformation. At the moment, some chaperones stabilize the hotspot C73R in vitro, the results were monitored by circular dichroism (CD) and nuclear magnetic resonance (NMR). On the other hand, we are studying the effects of the compounds in UROIIIS-GFP mutant cells. The results obtained at HC automated fluorescent microscope suggest that the compounds enter into the cells and interact with the protein UROIIIS-C73R-GFP. This could indicate that they could be acting as chaperones.

PC-015 **Delicate Balance of Noncovalent Forces Control the Electron Transfer Complex between Ferredoxin and Sulfite Reductase to Optimize Enzymatic Activity**

Juyaen Kim[1], Misaki Kinoshita[1], Takahisa Ikegami[1,2], Genji Kurisu[1], Yuji Goto[1], Toshiharu Hase[1], Young-Ho Lee[1]

1.-Institute for Protein Research, Osaka University, 2.-Yokohama City University

Plant sulphite reductase (SiR) forms an electron transfer complex with ferredoxin (Fd) for the reductive conversion of sulphite to sulphide. Although previous studies have highlighted electrostatic interactions between oppositely-charged residues of the two proteins, detailed thermoenergetics of the intermolecular interaction for the complexation remains unknown. We herein carried out isothermal calorimetry of Fd:SiR complex formation at various NaCl concentrations. Driving force plot constructed from calorimetry showed that the complex was thermodynamically stabilized by both enthalpy and entropy through favourable electrostatic and non-electrostatic interactions. Increasing NaCl concentrations weakened interprotein affinity and contribution of the negative enthalpy changes became decreased, while no such significant decrease was found in the contribution of positive entropy changes. Furthermore, a negative heat capacity change obtained from the enthalpy changes at distinct temperature indicated a contribution of hydrophobic interactions. These findings suggested that both electrostatic and non-electrostatic interprotein interactions were energetically important for the complex formation. Fd-dependent SiR activity assay revealed a bell shaped activity curve with a maximum under a certain NaCl concentration, while the methyl viologen-dependent assay of SiR exhibited a profile of saturating curve, suggesting that an optimized interprotein interaction is a crucial factor in control of Fd-dependent-SiR activity. A residue-based NMR measurement of 15N-labeled Fd upon complex formation with SiR revealed that charged and non-charged residues were differentially contributed in the complex formation depending on NaCl concentrations. We proposed that non-electrostatic forces were also critical for forming the Fd:SiR complex, and an optimized complex conformation for maximum enzymatic activity was achievable by a delicate balance among non-covalent intermolecular forces. These results may be extended for understanding of complexation between redox proteins containing biased charge clusters.

PC-016 **Ornithine transcarbamylase has a spatially extended active site as computationally predicted**

Lisa Ngu[1], Kevin Ramos[1], Nicholas DeLateur[1], Penny Beuning[1], Mary Jo Ondrechen[1]
1.-Department of Chemistry & Chemical Biology, Northeastern University

Understanding how an enzyme catalyzes a reaction is a fundamental problem in protein science. Biochemical experimentation has revealed catalytic mechanisms of many enzymes; however these studies have focused almost exclusively on amino acid residues in direct contact with the reacting substrate molecule(s). Here we report on the computational prediction and experimental verification of the importance of distal residues in enzyme catalysis, using E. coli ornithine transcarbamylase as an example. Partial Order Optimum Likelihood (POOL), developed at Northeastern University, is a machine learning technique that only requires the tertiary structure of a protein to predict important catalytic residues, based on computed, residue-specific electrostatic and chemical properties. POOL has been shown to predict accurately the catalytic residues and to discern between compact and spatially extended active sites. Dynamic conformational changes during catalysis and strong electrostatic interactions give rise to significant coupling between remote residues and the canonical active site residues of an enzyme. This suggests that at least some enzyme active sites are spatially extended, with second- and third- shell residues playing significant roles in catalysis. In this project, we focus on ornithine transcarbamylase (OTC), for which dynamic processes are believed to play a role in its catalytic mechanism. OTC is reported to undergo induced-fit conformational changes upon binding carbamoyl phosphate, which affects the subsequent binding of ornithine. Residues predicted by POOL to be catalytically important include five in direct contact with the substrate, R106, H133, D231, C273 and R319. POOL also predicted remote residues to form a spatially extended, triple-layer active site. Guided by computational predictions and using site-directed mutagenesis and kinetics assays of Asp140, His272, Glu299 and Arg57 variants, we show that these POOL-predicted remote residues, located in the second and third layers, are important for catalysis. Kinetics assays of wild-type OTC resulted in catalytic efficiencies of 170 ± 52 x 105 M-1s-1 for ornithine and 590 ± 86 x 105 M-1s-1 for carbamoyl phosphate, consistent with previous studies. OTC variants R57A, D140N, Y160S, H272L and E299Q show significant loss of catalytic efficiency. The results indicate that the charge on Glu299 and polarity of His272 play roles in catalysis and demonstrate the importance of remote residues, up to 10 Å away from canonical active site residues, for OTC activity. In addition, we verify the power of POOL to predict important catalytic residues accurately. This example, along with others, illustrates the importance of distal residues in natural enzymes and also in protein engineering. Understanding how distal residues contribute to catalysis has important implication for protein design for applications as diverse as therapeutics, renewable energy systems, and green industrial chemical processes. Supported by NSF MCB-1158176.

PC-018 **Identification, Characterization, and Modification of Fatty Acid Alkyl Esterases Found in Staphylococcus aureus**

Benjamin Saylor[1]
1.-San Diego State University

Alternative energy is a major focus of current research efforts. Biodiesel, a mixture of fatty acid alkyl esters, is one of the most versatile alternative fuels currently in use. This is due to the fact that it is similar to gasoline and compatible with diesel engines found throughout the existing global infrastructure. Biodiesel precursor lipids are abundant in cultivated feedstock organisms such as algae and bacteria. However, the standard process for converting oil to biodiesel is heat-intensive and requires complete removal of water, reducing the overall net energy gained in its production. Our work constitutes an attempt to explore enzymatic synthesis of biodiesel from lipids such as those derived from emerging fuel crops. Previous literature describes fatty acid alkyl ester formation in human patients with MRSA Staphylococcus aureus wound lesions. These esters are formed by partially characterized esterase activity from an unidentified source. We have identified two MRSA enzymes responsible for this activity by using a combination of size exclusion chromatography, gas chromatography-mass spectrometry, and mass spectrometric protein sequencing. These two highly similar enzymes in the glycerol ester hydrolase (geh) family of proteins catalyze the synthesis of fatty acid alkyl esters in aqueous conditions at or near room temperature. We have demonstrated that other non-Staphylococcal lipases do not exhibit this behavior. We have expressed these Staphylococcal esterases in E. coli, and shown via gas chromatography that the expressed proteins catalyze the formation of fatty acid alkyl esters. Based on sequence similarity to homologous proteins that have already been crystallized, we have predicted a structure for these enzymes and have engineered mutant fusions with higher rates of catalysis. Our design hypothesis is that increased avidity for substrate molecules will yield a higher substrate concentration in the vicinity to the enzyme. To increase substrate concentration we have designed and expressed one of the enzymes as a chimeric fusion with the Drosophila melanogaster alcohol-binding protein LUSH. GC-MS determination of biodiesel production rate indicates that the chimeric fusion has a lower-order rate constant with respect to ethanol. In other words, the fusion enzyme is less dependent on substrate concentration and is a superior catalyst at low ethanol concentrations. This result indicates that the rationally designed modification of binding avidity constitutes a potential avenue for improving the ability of enzymes to catalyze reactions with low-concentration or low-solubility substrates.

PC-019 **Functional elements of a human antizyme essential for binding and inhibiting human ornithine decarboxylase**

Ju-Yi Hsieh[1], Yi-Liang Liu[1], Guang-Yaw Liu[2], Hui-Chih Hung[1]

1.-Department of Life Sciences and Institute of Bioinformatics, National Chung Hsin, 2.-Institute of Microbiology & Immunology, Chung Shan Medical University, and Divis

Ornithine decarboxylase (ODC) plays an essential role in various biological functions, including cell proliferation, differentiation and cell death. In this study, we revealed that overexpression of ODC in HeLa and MCF-7 cells decreased cellular ROS (Reactive oxygen species) after low dose of ultraviolet B radiation (UVB), leading autophagy inhibited, and it was restored by knocking down ODC (shODC) in ODC overexpressing HeLa and MCF-7 cells. Furthermore, the results demonstrated that AMPK was increased after high dose of UVB radiation in ODC ovexpressing HeLa and MCF-7 cells, leading autophagy induced and apoptosis inhibited. We demonstrated that knocked down autophagy by shRNA (shAtg5, shBECN1, and shAtg12) and chloroquine (CQ) could enhance high dose of UVB induced cell death in ODC overexpressing HeLa and MCF-7 cells. Here, we also observed that knocked down ODC in ODC overexpressing HeLa and MCF-7 cells inhibited autophagy and enhanced high dose of UVB radiation. Because of Atg12 can regulate cell apoptosis and autophagy. Site directed mutagenesis was used to mutant the amino acid which can regulate cell apoptosis and autophagy on Atg12, respectively in these two ODC overexpressing cells. According to the results, mutated the amino acid which can regulate apoptosis on Atg12 leading the cells more survival. Relatively, mutated the amino acid which can regulate autophagy on Atg12 leading the cells died. Therefore, inhibition of ODC and autophagy could be a promising strategy for adjuvant chemotherapy in human breast and cervical cancers.

PC-020 **Structure-Function Relationships of human Aldo-Keto Reductase 1B15, AN enzyme active with 9-cis-Retinaldehyde**

Joan Giménez Dejoz[1], Michal H. H. Kolář[2,3], Francesc Xavier Ruiz[4], Isidro Crespo[1], Alexandra Cousido-Siah[4], Alberto Podjarny[4], Jindřich Fanfrlík[2], Xavier Parés[1], Jaume Farrés[1], Sergio Porté[1]
1.-Universitat Autònoma de Barcelona, 2.-Institute of Organic Chemistry and Biochemistry, 3.-Institute of Neuroscience and Medicine and Institute for Advanced Simulation, 4.-Institut de Génétique et de Biologie Moléculaire et Cellulaire

Human aldo-keto reductase 1B15 (AKR1B15) is a recently identified member of the human AKR family (Weber et al. J. Biol. Chem. 290, 6531-45, 2015). The enzyme displays 92% sequence identity with AKR1B10, which efficiently catalyzes the reduction of a wide variety of endogenous and exogenous carbonyls, such all-trans-retinaldehyde, and it is linked to the development of several cancer types. In contrast, the enzymatic activity and physiological role of AKR1B15 are still poorly understood. In this work, we have improved the expression and purification of AKR1B15 using a detergent-free system. AKR1B15 enzymatic activity has been characterized together with inhibitor screening and structural modeling analyses. The results show that AKR1B15 is active towards a variety of carbonyl substrates, including ketones and dicarbonyl compounds, with lower Km and kcat values than those of AKR1B10. Moreover, AKR1B15 exhibits superior catalytic efficiency towards 9-cis-retinaldehyde, the best substrate found for this enzyme. Several typical AKR inhibitors do not significantly affect AKR1B15 activity. Structural modeling reveals that AKR1B15 active site is smaller and more rigid than the AKR1B10 pocket, mainly due to hydrophobic residue substitutions clustered in loops A and C.

PC-021 **Significance of protein substrate structure and dynamics in proteolysis: insights from Kunitz-BPTI family canonical serine protease inhibitors**

Olumide Kayode[1, 2], Thomas R. Caulfield[3], Ruiying Wang[2], Devon Pendlebury[2], Alexei Soares[4], Evette S. Radisky[2]

1.-Mayo Graduate School, 2.-Department of Cancer Biology, Mayo Clinic Cancer Center, 3.-Department of Neuroscience, Mayo Clinic College of Medicine, 4.-Biology Department, Brookhaven National Laboratory

Proteases are ubiquitous enzymes that catalyze the hydrolysis of peptide bonds within protein substrates; they have served as key model enzymes for studying the molecular basis for catalytic power and specificity. Protease substrate specificity is most often defined in terms of linear sequence motifs that flank the cleavage site; however, the natural substrates of proteases are proteins with 3-dimensional shapes and complex conformational dynamics that are not well represented by 1-dimensional sequence alone. These structural and dynamical properties can impact recognition and binding of substrates by proteases, as well as the efficiency of catalysis itself. In this study, we explore the importance of substrate structure and dynamics for proteolysis using as our model the cleavage of the Kunitz-BPTI family of canonical serine protease inhibitors by mesotrypsin. Bovine pancreatic trypsin inhibitor (BPTI), an archetypal serine protease inhibitor of the Kunitz family, has a high affinity interaction with trypsin, yet its peptide bond hydrolysis is many orders of magnitude slower than other peptide substrates. Mesotrypsin, a trypsin variant, has been shown to hydrolyze Kunitz family inhibitors at accelerated rates; this is especially true of human Kunitz domain inhibitors. Amyloid precursor protein inhibitor (APPI) and amyloid precursor like protein-2 (APLP2), two human Kunitz domain family members, are hydrolyzed by mesotrypsin several hundred times faster than BPTI. Here, we present a new, unpublished crystal structure of a cleavage intermediate APLP2 bound to mesotrypsin, refined to 1.4Å resolution, revealing a dramatic substrate conformational change we hypothesize to be required during cleavage of a Kunitz domain. Using this structure along with published structures of APPI and BPTI complexes, we have modeled acyl-enzyme intermediates of mesotrypsin, and we have carried out molecular dynamic simulations that explore the transition of the initially formed native-like acyl-enzyme through the conformational transformation that allows the progression of the hydrolysis reaction. We further identify a specific hydrogen bond, present in BPTI but not APPI, which forms a stabilizing feature of the BPTI scaffold. Using site directed mutagenesis, we probe the contribution of this bond to the proteolytic stability of BPTI. Collectively our data for these highly structured substrates show that proteolysis rates are limited by a necessary conformational change in the substrate as the reaction progresses. Rigid substrates possessing stabilizing features that render them highly resistant to this conformational change are proteolyzed more slowly than more flexible substrates of similar structure.

PC-022 **Determinants for regioselectivity in Lytic Polysaccharide MonoOxygenases (LPMOs)**

Barbara Danneels[1], Magali Tanghe[1], Henk-Jan Joosten[2], Tom Desmet[1]
1.-Centre for Industrial Biotechnology and Biocatalysis, University of Ghent, 2.-Bioprodict

INTRODUCTION With the discovery of the lytic polysaccharide monooxygenases (LPMOs), new light has been shed on the degradation of lignocellulosic biomass. LPMOs are copper metalloenzymes that carry out the oxidative cleavage of the β-1,4-glycosidic bond, generating new chain ends that can subsequently be processed by cellulases, boosting the cellulose degradation. LPMOs have a β-sandwich conformation with a flat binding surface, allowing for the enzyme to bind to crystalline cellulose. The Cu2+ ion, required for activity, is located in a so-called "histidine brace", in which the N-terminal histidine is highly conserved. REGIOSELECTIVITY According to the carbon atom being oxidized, 3 LPMO types are identified: type 1 and type 2 oxidizing at the C1 and the C4 respectively, type 3 LPMOs oxidizing both the C1 and the C4 adjacent to the glycosidic linkage. We were able to express a type-1 LPMO (Phanerochaete chrysosporium GH61D) and a type-3 LPMO (Trichoderma reesei Cel61A) in P. pastoris. This has proven to be very challenging, as LPMO activity requires a perfect cleavage of the signal sequence. After activity assays on PASC, characteristic HPAEC-PAD traces were obtained which will serve as a reference for engineering experiments. ENZYME ENGINEERING Using the 3DM database, a structure based multiple sequence alignment tool, it is possible to identify residues specifically conserved in subsets of protein sequences. By defining a subset for each LPMO type, we were able to identify residues contributing to regioselectivity. These positions are now being rationally engineered in subsequent rounds of mutagenesis, using TrCel61A as a template. The effect of the mutations will be determined by analyzing the HPAEC-PAD trace released from PASC. The main goal is to investigate the possibility of deleting the C4 specificity in a type 3 LPMO.

PC-023 **Folding topology determines substrate binding order in the ribokinase superfamily**

Alejandra Herrera-Morandé[1], Victor Castro-Fernández[1], Felipe Merino[1], César Ramírez-Sarmiento[1], Francisco Fernández[2], Cristina Vega[2], Victoria Guixé[1]

1.-Departamento de Biología, Facultad de Ciencias, Universidad de Chile., 2.-Centro de Investigaciones Biológicas (CIB-CSIC)

Folding topology determines substrate binding order in the ribokinase superfamily 1Alejandra Herrera-Morandé, 1Victor Castro-Fernández, 1Felipe Merino, 1César. A. Ramírez-Sarmiento, 2Francisco Fernández, 2Cristina Vega and 1Victoria Guixé. 1Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile. 2Centro de Investigaciones Biológicas (CIB-CSIC), Madrid, España Ribokinase superfamily comprises three enzyme families: the ADP-dependent sugar kinases family, the ATP-dependent coenzyme kinases family and the ATP-dependent sugar kinases family. In all these families there is a large domain composed by a Rossmann motif but only the ATP-dependent enzymes have a β-meander motif in the C-terminal end. Interestingly, these enzymes display an ordered kinetic mechanism where the substrate that will be phosphorylated binds first to the enzyme. The ADP-dependent enzymes present a topological re-ordering of the secondary structural elements which produces an equivalent tertiary structure, which can be thought as a non-circular permutation (NCP) of the β-meander region. These enzymes also display an ordered kinetic mechanism but with an inversed order being the nucleotide the first substrate to bind to the enzyme. As this β-meander region of the proteins constitutes almost entirely the nucleotide binding site, and given that the permutation is the major structural difference between ADP and ATP-dependent kinases, it could the responsible for the nucleotide specificity. To test this hypothesis we introduce, by permutation, an ATP-dependent topology in the homologous ADP-dependent glucokinase from T. litoralis (perGK). Size exclusion chromatography and circular dichroism spectra show that both the wild type and the permutated enzyme eluted as monomers with similar hydrodynamic behavior, and have the same secondary structure content. Kinetic assays employing ATP or ADP as substrate demonstrate that even in the presence of 10 mM ATP, the perGK enzyme is not able to carry out the phosphoryl transfer. To test if the NCP has an impact in the kinetic constants and substrate binding order we determine the kinetic mechanism through classical protocols, involving initial velocity studies, product inhibition and dead end inhibitors. The results demonstrate that the perGK enzyme presents an altered substrate binding order compared to the wild type enzyme, where glucose was the first substrate to bind to the enzyme and glucose-6-P the last product to be released. Also, ligand-induced conformational changes were determined in the crystal structures. The apo, the enzyme-glucose and enzyme-glucose-ADPβS structures were determined at 2.14 Å, 1.95 Å and 2.44 Å resolutions, respectively. Structure analysis reveals that glucose binding provokes major conformational changes in the perGK enzyme, whereas ADP binding does not cause further changes in the conformation of the protein. The results show that although the permutation has no effect on the nucleotide preference it provokes a change in the substrate binding order that correlates well with that those observed in the crystal structures. Also, they demonstrate that during the evolutionary history of the ribokinase superfamily folding topology dictates the substrate binding order

(Fondecyt 1150460).

PC-024 **Thrombin Proteolytically Hinders the Antioxidant Activity of Human Ceruloplasmin: Implications in the Pathogenesis of Rheumatoid Arthritis**

Laura Acquasaliente[1], Giulia Pontarollo[1], Alexiej V. Sokolov[2], Simone Tescari[1], Vadim B. Vasilyev[2], Vincenzo De Filippis[1]

1.-Department of Pharmaceutical and Pharmacological Sciences, University of Padua, 2.-State University of Saint-Petersburg

Background: Human ceruloplasmin (CP) is a circulating copper-containing glycoprotein produced in the liver and first described as a component of alpha2-globulin fraction of human plasma. CP belongs to the multicopper oxidase family and it is nowadays regarded as a "moonlighting" protein, because it changes its function according to substrate, localization and expression. CP plays a key role in copper transport and iron metabolism and it is also a potent inhibitor of leukocyte myeloperoxidase (MPO) (Kd=130nM), a major source of oxidants in vivo. The protein is extremely susceptible to proteolysis. In fact, CP is a structural homolog of coagulation factors V and VIII, that are physiological substrates of thrombin (FIIa). Interestingly, thrombin participates in both haemostatic and inflammatory responses: in some focus of inflammation, such as rheumatoid arthritis (RA), the high activity of FIIa has been documented. It was demonstrated that FIIa can promote the chemotaxis of neutrophils and monocytes and their adhesion to endothelial cells, to increase vascular permeability. All these effect are mediated by PAR-1 interaction, that are abundantly expressed in inflamed rheumatoid synovial tissues. Aims: In this study the interaction of CP with thrombin was investigated to confirm the participation of FIIa in "spontaneous" proteolytic degradation of CP. In fact, in vivo the integrity of CP is essential for its role in the transport or metabolism of copper. Results: Our results indicated that thrombin cleaves CP in vitro at 481Arg-Ser482 and 887Lys-Val888 bonds, generating a nicked species that retains the native-like fold and the ferroxidase activity of the intact protein, whereas the MPO inhibitory function of CP is abrogated. Analysis of the synovial fluid of 24 RA patients reveals that CP is proteolytically degraded to a variable extent, with a fragmentation pattern similar to that observed with FIIa in vitro, and that proteolysis is blocked by hirudin, a highly potent and specific thrombin inhibitor. We demonstrate that FIIa has intrinsic affinity for CP (Kd = 60-270 nM), independently of proteolysis, and inhibits CP ferroxidase activity (KI = 220±20 nM). Mapping of thrombin binding sites with specific exosite-directed ligands (i.e. hirugen, fibrinogen gamma-peptide) and thrombin analogues having the exosites variably compromised (i.e. prothrombin, prethrombin-2, alpha-thrombin), reveals that the positively charged exosite-II of thrombin binds to the negative upper region of CP, while the protease active site and exosite-I remain accessible. These results suggest that thrombin can exacerbate inflammation in RA by impairing via proteolysis the MPO inhibitory function of CP and by competitively inhibiting CP ferroxidase activity.

PC-025 **An artificial pathway for isobutene production by direct fermentation : combining metabolic engineering and protein engineering**

Benoit Villiers[1], François Stricher[1]
1.-Global Bioenergies

The purpose of Global Bioenergies is to develop innovative metabolic pathways for the production of light olefins from renewable resources, by direct fermentation. Light olefins (ethylene, propylene, linear butylene, isobutene and butadiene) are the core of the petrochemical industry. However, microorganisms do not naturally produce light olefins and no bioprocess to convert renewable resources to these molecules has been industrialized so far. Global Bioenergies has developed an artificial metabolic pathway including all the necessary enzymatic reactions from feedstock to isobutene. The metabolic route leading to isobutene can be divided in three parts, the first one being the use of natural reactions occurring in the host microorganism. Second, heterologous natural reactions were introduced into the same host microorganism. Finally, in contrast with most former approaches, non-naturally occurring reactions as enzymatic key steps were used, for example the decarboxylation of hydroxyisovaleric acid into isobutene. Such non-natural critical steps were made possible by taking advantages of the natural catalytic and substrate promiscuity of exogenous enzymes. Candidate enzymes are then evolved using systematic, random and semi-rational approaches in successive rounds in order to reach the desired catalytic efficiency. Since all these reactions are enzymatic, isobutene can be obtained by direct fermentation, e.g. a process wherein all the chemical transformations are carried on by the host microorganism. The scale-up of this process began in November 2014 in a pilot plant installed in Pomacle-Bazancourt, France, with an annual capacity of 10 tons of oxidation-grade isobutene. Importantly, production of a volatile compound such as isobutene (and other light olefins) by direct fermentation presents two major advantages: first, the product is spontaneously removed from the culture broth, which alleviates the limitations linked with titer issues. Second, the purification process is considerably easier and cheaper since no energy consuming methods such as distillation or phase separation are necessary to purify the end product. For the first time, batches of industrially produced isobutene from renewable resources have been obtained in the first half of 2015. This isobutene has been in turn converted into isooctane, an additive currently used to improve gasoline quality, which could also be used as a standalone fuel. A demonstration plant is planned in Leuna, Germany, with an annual capacity of 100 tons of polymer-grade isobutene and IBN-One, a joint venture with Cristal Union (4th European beet processor), has been formed to build and operate the first plant in France converting renewable resources into isobutene. Finally, while the isobutene process is progressing towards industrial scale, Global Bioenergies is also developing new artificial metabolic pathways enabling direct bio-production of Butadiene and Propylene.

PC-026 **The development of a coupled enzyme assay to detect isochorismate pyruvate lyase activity**

Linda Jäger[1], Christian Jäckel[1], Peter Kast[1], Donald Hilvert[1]
1.-LOC, ETH Zürich

Most bacteria and their pathogens exhibit a sensitive iron balance and can acquire extracellular iron via the secretion of siderophores. In Escherichia coli, iron starvation leads to the production of isochorismate from chorismate by isochorismate synthase such as, for example EntC. However, under normal growth conditions the synthesis of isochorismate is suppressed. This complicates the in vivo analysis of enzymes downstream of isochorismate, such as the isochorismate pyruvate lyase from Pseudomonas aeruginosa. Thus far, only a few isochorismate pyruvate lyases have been biochemically and biophysically characterized. To further the understanding of this group of enzymes a sensitive enzymatic activity screening assay was lacking. Here we describe the implementation and optimization of a high throughput screening assay that measures the siderophore salicylate, a fluorescence product of the isochorismate pyruvate lyase reaction. The assay employs a sequential enzyme cascade involving the isochorismate synthase EntC, and PchB variants with varying isochorismate pyruvate lyase activities. Upon salicylate production, a feedback loop involving an engineered version of the salicylate mediated transcription activator NahR further enhances the isochorismate pyruvate lyase production and hence salicylate production. The assay afforded a 500-fold dynamic range between EcCM, a chorismate mutase of the same AroQ fold lacking isochorismate activity, and PchB with a kcat/Km of 1.13 x 106 M-1s-1. The assay will greatly facilitate current efforts to identify novel enzymes with promiscuity for the isochorismate pyruvate lyase activity.

PD-001 **3-D interaction homology. Do hydropathic microenvironments dictate amino acid sidechain conformations?**

Mostafa Ahmed[1,2], Martin Safo[1,2], J. Neel Scarsdale[1,3], Glen Kellogg[1,2]

1.-Institute For Structural Biology and Drug Discovery, Virginia Commonwealth University, 2.-Department of Medicinal Chemistry, Virginia Commonwealth University, 3.-Center For The Study of Biological Complexity, Virginia Commonwealth University

Protein folding is typically defined in terms of the spatial arrangement of structural elements, i.e. helices, sheets and loops. We have, however, been developing an alternative and complementary paradigm based on conserved hydropathic interaction networks within proteins. These networks can be viewed as environments comprised of a mixture of polar and hydrophobic interaction fields, and may be the most important factor driving protein folding. This concept applies even to the lowest structural level within a protein: the sidechain conformations (or rotamers). Exhaustive statistical analysis of existing crystallographic structures of proteins showed rotameric preferences and led to the creation of rotamer libraries frequently used in multiple aspects of structural biology, e.g., crystallography of relatively low-resolution structures, homology modeling and biomolecular NMR. However, little is actually known about the forces and factors driving the preference or suitability of one rotamer over another. In our study, tyrosine was analyzed since its sidechain has a comprehensive set of hydropathic properties that made it ideal as a proof of concept residue. Construction of 3D hydropathic interaction maps of tyrosine residues in our dataset, reveals the environment around each, in terms of hydrophobic (π-π stacking, etc.) and polar (hydrogen bonding, etc.) interactions. After partitioning the tyrosines into backbone-dependent bins, a map similarity metric based on the correlation coefficient was applied to each map-map pair to build matrices suitable for clustering. Notably, the first bin representing 631 tyrosines, reduced to 14 unique hydropathic environments with most diversity arising from favorable hydrophobic interactions with many different residue partner types. Polar interactions for tyrosine include ubiquitous hydrogen bonding with the phenolic OH and somewhat surprisingly a handful of unique environments for the tyrosine backbone. All but one of the 14 environments are dominated by a single rotamer, the exception being an environment defined by a paucity of interactions with the tyrosine ring and as a consequence its rotamer is indeterminate. This is consistent with it being composed of mostly surface residues. Each tyrosine residue attempts to fulfill its hydropathic valences and thus, structural water molecules are seen in a variety of roles throughout these environments. Alanine was analyzed using the same protocol as well. Having the smallest sidechain (and small hydropathic interaction maps), alanine allowed us to investigate a significantly larger database, permitting us to examine the correlation between hydropathic maps and various structural features. In conclusion, the analysis of hydropathic environments strongly suggests that the orientation of a residue in a three-dimensional structure is a direct consequence of its hydropathic environment, which leads us to propose a new paradigm, interaction homology, as a key factor in protein structure. It is not the surrounding residues that direct sidechain conformations, but rather the hydropathic “field” of the surrounding atoms.

PD-002 **Folding studies of independent domains of Lysine, Arginine, Ornithine binding protein (LAO)**

Tania Raquel Berrocal Gama[1], Jesús Renan Vergara Gutiérrez[1], Andrés Escandón Flores[1], Alejandro Sosa Peinado[1]

1.-National Autonomus University of Mexico, Faculty of Medicine

Protein folding problem has been addressed from the past 50 years until nowadays, however, we still can not explain how proteins acquire their native structure from their amino acid sequence. Different approaches has been taken in order to study protein folding, for example, the comparative study of folding mechanism between homologues proteins with high identity of sequence and structure, and the study of independent regions within a single protein. Previously in our laboratory, thermodynamic and kinetic folding properties of lysine, ornithine, arginine binding protein (LAO), a 238 amino acid periplasmic binding protein (PBP), composed by two Rossmann fold domains (one continuous and the other discontinuous) attached by a hinge region, has been studied. Even there is a functional research about binding characteristics of histidine binding protein´s (His J) domains of when expressed independently (Chu, B. 2013); there are no folding studies in these conditions for this or another PBP´s. It should be noted that His J shares 70% of sequence identity and tertiary structure (RMSD≈ 1Å) with LAO. In order to know the folding effect of encoding different domains in the same poly peptidic chain, as well as its influence in function, we are studying the thermodynamic and kinetic characteristics of folding of independently expressed lobes of LAO, and comparing with those of native protein. By now, we expressed and purified the discontinuous domain. Circular dichroism (CD) and fluorescence intensity spectra show that this independent domain has primary and tertiary structure. Thermal denaturation has a single cooperative transition, which indicates this domain is folded. Thermodynamic analysis of temperature and urea-induced experiments suggest that LAO´s folding characteristics are not just the addition of those from independent domains. Furthermore, folding and refolding kinetics suggest the presence of a burst phase intermediate.

PD-003 **A hypothesis to reconcile the physical and chemical unfolding of proteins**

Guilherme de Oliveira[1], Jerson Silva[1]
1.-Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro

A comprehensive view of protein folding is crucial for understanding how misfolding can cause neurodegenerative diseases and cancer. When using physical or chemical perturbations, NMR spectroscopy is a powerful tool to reveal a shift in the native conformation toward local intermediates that act as seeds for misfolding. High pressure (HP) or urea is commonly used to disturb folding species. Pressure favors the reversible unfolding of proteins by causing changes in the volumetric properties of the protein–solvent system. However, no mechanistic model has fully elucidated the effects of urea on structure unfolding, even though protein– urea interactions are considered to be crucial. Here, we provide NMR spectroscopy and 3D reconstructions from X-ray scattering to develop the “push-and-pull” hypothesis, which helps to explain the initial mechanism of chemical unfolding in light of the physical events triggered by HP. In studying MpNep2 from Moniliophthora perniciosa, we tracked two cooperative units using HP-NMR as MpNep2 moved uphill in the energy landscape; this process contrasts with the overall structural unfolding that occurs upon reaching a threshold concentration of urea. At subdenaturing concentrations of urea, we were able to trap a state in which urea is preferentially bound to the protein (as determined by NMR intensities and chemical shifts); this state is still folded and not additionally exposed to solvent [fluorescence and small-angle X-ray scattering (SAXS)]. This state has a higher susceptibility to pressure denaturation (lower p1/2 and larger ΔVu); thus, urea and HP share concomitant effects of urea binding and pulling and water-inducing pushing, respectively. These observations explain the differences between the molecular mechanisms that control the physical and chemical unfolding of proteins, thus opening up new possibilities for the study of protein folding and providing an interpretation of the nature of cooperativity in the folding and unfolding processes.

PD-004 **Zinc: A Promoter or Inhibitor for IAPP aggregation?**

Feng Ding[1], Praveen Nedumpully-Govindan[1]
1.-Clemson Unversity

Zinc ions have been found to play an important and yet complex role in human islet amyloid polypeptide (hIAPP) aggregation, which is associated with β-cell death in type-II diabetes (T2D). Both concentration-dependent promotion and inhibition of IAPP aggregation by zinc ions have been observed in vitro. Similarly, at the population level, both positive and negative correlations were reported between the activity of a β-cell specific zinc transporter and T2D risk. Zinc ions are able to bind a single histidine in hIAPP and coordinate the formation of zinc-bound hIAPP oligomers. We hypothesize that the relative zinc/hIAPP concentration determines the population of zinc-bound hIAPP oligomers with different molecular weights. We have applied molecular dynamics (MD) simulations to systematically study the structure and dynamics of a range of zinc-coordinated hIAPP oligomers, including monomers, dimers, trimers, tetramers, and hexamers. Our computational results suggest that different zinc-bound oligomers have distinct aggregation propensities. High-molecular weight oligomers (≥2 peptides) have higher aggregation propensity than zinc-free and zinc-bound hIAPP monomers at ~2 mM concentration in silico. Therefore, our results provide a molecular insight into the complex role of direct zinc binding on hIAPP aggregation. At low zinc/hIAPP stoichiometry, zinc binding promotes aggregation. As the stoichiometry increases and zinc ions bind to single hIAPP peptides, the aggregation of hIAPP is inhibited due to electrostatic repulsion between the charged zinc ions. Our computational study sheds light on the complex role of zinc on hIAPP aggregation and T2D development.

PD-005 **Macromolecular Crowding: From the test tube to the cell**

David Gnutt[1], Michael Senske[1], Simon Ebbinghaus[1]
1.-Department of Physical Chemistry II, Ruhr-University Bochum

Biomolecules function in the densely crowded and highly heterogeneous cell, which is filled up to a volume of 40 % with macromolecules [1]. Often, artificial macromolecular crowding agents are used to mimic these conditions in vitro and the excluded volume theory is applied to explain the observed effects [2]. However, recent studies emphasize the role of further contributions aside from a pure volume effect including enthalpic and solvent effects [3, 4]. We study cosolute effects at high molecular and macromolecular concentrations via a thermodynamic analysis of the thermal unfolding of ubiquitin in the presence of different concentrations of cosolutes (glucose, dextran, polyethylene glycol, potassium chloride) [5]. In contrast to the excluded volume theory, we observed enthalpic stabilization and entropic destabilization forces for all tested cosolutes. The enthalpic stabilization mechanism of ubiquitin in macromolecular polysaccharide solutions of dextran was thereby similar to the effects observed in monomeric glucose. Further, it remains unclear how such cosolutes reflect the physicochemical properties of the complex cell environment as a characterization of the in-cell crowding effect is lacking. Thus, we developed a FRET-based macromolecular crowding sensor to study the crowding effect in living cells [6]. The averaged conformation of the sensor is similar to dilute aqueous buffer and cell lysate. We find that the in-cell crowding effect is distributed heterogeneously and can change significantly upon osmotic stress. The presented method allows to systematically study in-cell crowding effects and understand them as a modulator of biomolecular function. References [1] S. Zimmerman, S. Trach, J. Mol. Biol. 1991, 222, 599-620. [2] H.-X. Zhou, G. Rivas, A. Minton, Annu. Rev. Biophys. 2008, 37, 375-397. [3] Y. Wang et al., The Journal of the American Chemical Society, 2012, 134, 16614-16618 [4] R. Gilman-Politi and D. Harries, Journal of Chemical Theory and Computation, 2011, 7, 3816-3828 [5] M. Senske, L. Tork, B. Born, M. Havenith, C. Herrmann, S. Ebbinghaus, J. Am. Chem. Soc. 2014, 136, 9036-9041. [6] D. Gnutt, M. Gao, O. Brylski, M. Heyden, S. Ebbinghaus, Angew. Chem Int. Ed. 2014, DOI: 10.1002/anie.201409847

PD-006 **Breaking the deleterious effect of urea-unfolded state: consequences for the reversibility of intermediate species**

Angelo Figueiredo[1], Sivanandam Veeramuthu[2], Oscar Millet[2], Eurico Cabrita[1]
1.-Faculdade De Ciências E Tecnologia, Universidade Nova De Lisboa, 2.-CIC BioGUNE, Structural Biology Unit and Metabolomics Unit

The stability of biomolecules under co-solvent conditions is dependent on the nature of the co-solvent [1]. This can alter a protein's properties and structural features through biomolecular interactions between its functional groups and the co-solvent molecules. Ionic liquids (ILs) represent a rather diverse class of co-solvents. The design flexibility of these molten salts is an attractive feature, allowing the properties of the IL to be tuned to meet the requirements of different applications [2]. Particularly, the modulation of reaction pathways between folding states, offering possibilities to control irreversibility in non-native protein aggregation [2]. This has led us to investigate the impact of ILs as co-solvents with the well-known protein denaturant urea. Urea is considered to be a non-ionic chaotrope disturbing considerable the grid of hydrogen bonds with the protein backbone. Urea interacts preferentially with the protein surface, mainly apolar residues and that dispersion, rather than electrostatic interactions, is the main energetic contribution to explain the stabilization of the unfolded state of the protein and the irreversibility of the unfolding process in the presence of urea [3]. Remarkably, upon addition of choline chloride (ChlCl), 6 M urea-unfolded Im7 refolds and the 1H-15N HSQC NMR spectrum is very similar to the folded state in 50 mM phosphate buffer, suggesting that Im7 is in a compact, folded native state in this IL solution. The IL strongly attenuates the denaturation action of urea on Im7. To better understand how ILs particularly choline chloride can attenuate the deleterious action of urea we performed 15N chemical shift perturbation, NMR relaxation dispersion and ZZ-exchange experiments in order to gain access to the physicochemical mechanism regarding the thermodynamic stability of the species and kinetic barriers of the folding reaction, particularly on the intermediate species. Our results show significant changes on conformational dynamics showing that the native state of Im7 is in equilibrium with an intermediate state that is considerable populated at equilibrium. Comparison of kinetic and thermodynamic parameters describing the equilibrium intermediate state (EIS) obtained by NMR with previously reported parameters describing the kinetic intermediate state (KIS) obtained from stopped-flow fluorescence studies shows that the KIS and EIS are the same species. Thus the Urea:ChlCl mixture induce significant accumulation of folded intermediates with a high degree of secondary structure content, containing three of the four helices (I, II and IV) that are docked around a specific hydrophobic core, whilst non-native docking of these elements create long-lived intermediate states. The possibility to use ILs for stabilising intermediates open scenarios for mechanistic studies of protein unfolding/refolding. It is hoped that the results obtained from the above study will be useful in recommending tailor-made ILs for various applications in biological systems.

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PD-007 **Highly concerted domain folding and subunit association of a multidomain multimeric L asparaginase from hyperthermophile : A mechanistic underpinning of complex protein folding in extreme environement**

Dushyant K. Garg[1], Bishwajit Kundu[1]
1.-Indian Institute of Technology Delhi

A large body of multidomain protein folding work has been devoted to study monomeric proteins. How do multidomain multimeric protein fold, avoiding accumulation of stable intermediate is yet to be studied in detail. Our present study is focussed on understanding the folding and assembly of the domains of a homodimeric L-aspraginase from a hyperthermophile Pyrococcus furiosus (PfA). Each monomer of PfA consists of distinct N-and C-terminal domains (NPfA and CPfA, respectively), connected by a linker. The folding mechanism of each domain with respect to full length protein was studied by mutating one out of two tryptophans, one in each domain. Domains were purified and studied individually to obtain parallel account of the folding of each domain in isolation. Subunit assembly was studied by analytical size exclusion chromatography (SEC), Multiangle light scattering and functional activity. Through far UV CD, intrinsic Trp fluorescence and SEC, we demonstrated that domain folding and subunit association were intimately linked in full length PfA. Interestingly, en route to its folding there was complete absence of hydrophobic intermediates as probed by ANS fluorescence. Folding of NPfA was highly cooperative and, it provides interacting surfaces for CPfA to fold and also facilitates subunit assembly. The folding cooperativity of isolated domains was very less compared to the folding cooperativity of their full length counterparts, as indicated by equilibrium m values. To our surprise, during pH induced denaturation, at pH 2 and 13, the dimer dissociates into highly hydrophobic folded monomers which readily underwent amyloidogenesis. We showed that at such extreme conditions, co-operativity in folding process in multidomain multimeric protein is not solely governed by the folding of individual domains, rather by concomitant folding and association of domains directly into a quaternary structure. In other case, where subunit folding occurred prior to association, protein readily underwent extensive aggregation.

PD-008 **GroEL assisted folding of multiple recombinant proteins simultaneously over-expressed in E.coli**

Megha Goyal[1], Tapan Kumar Chaudhuri[1]
1.-Indian Institute of Technology Delhi

Aggregation prone recombinant proteins very often form inclusion bodies and also exhibits poor yield of functional protein during in vitro refolding process from chemically denatured form. Bacterial chaperonin GroEL provides folding assistance to several proteins, when over-expressed with one of the recombinant proteins. There are instances that GroEL in presence of few other co-expressed chaperones like DnaJ, DnaK etc provides better yield of folded protein during homologous and heterologous expression. Considering the ongoing events in the cells, it is known that molecular chaperone GroEL assists in the folding of various proteins in the cytoplasm. Hence attempt to fold multiple recombinant proteins over-expressing simultaneously with the co-expression of chaperones can be worth trying. This approach may cut down various complexities in the functional recombinant protein preparation, including time and effective cost. Keeping this view in mind, folding of two simultaneously expressed aggregation prone proteins, 69 kDa E.coli maltodextrin glucosidase (MalZ) and 82 kDa yeast mitochondrial aconitase have been investigated with the co-expression of GroEL and GroES in E.coli cytosol. It has been previously reported that both the chosen proteins undergo co-expressed GroEL-GroES assisted folding in E.coli cytosol, when they over-express alone. In this study we have optimized the over-expression of MalZ and aconitase simultaneously in E.coli. Further optimisation was carried out to co-express GroEL along with MalZ and aconitase. Based on the basic philosophy that soluble protein mainly contains folded fraction, the event of GroEL/ES assisted folding of simultaneously over-expressed proteins, MalZ and aconitase was monitored through the attainment of soluble proteins under various sets of conditions such as temperature. The major outcome of the present study is that, with the GroEL-GroES assistance, the yield of soluble proteins (MalZ and aconitase) together constitutes higher percentage of folded protein in contrast to the percent yield when a single protein was over-expressed. Significance of this type of study relies on the fact that the cells can over-produce higher amount of recombinant proteins, when multiple over-expression takes place. Not only pushing up cell's capability of over-expression, co-expression of GroEL and GroES efficiently assists in the folding of multiple proteins simultaneously over-expressed in E.coli. References:

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PD-009 **Complexity of the Post-transition State Folding of Rd-apocytochrome b562**

Shuanghong Huo[1], Mojie Duan[1,2], Hanzhong Liu[1], Minghai Li[1]

1.-Clark University, 2.-Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences

Long time-scale computer simulations powered by Anton, a specialized supercomputer, and the recent advance in force field development open up a new era in the study of protein folding, allowing us to investigate the folding of medium-sized proteins with an explicit solvent model. The folding intermediates that exist after the rate-limiting step are called hidden intermediates. Structures of the mimics of hidden intermediates of Rd-apocytochrome b562 are resolved by NMR. Based upon the structural features of the mimics of hidden intermediates, the folding of Rd-apocytochrome b562 after the rate-limiting step was proposed to follow a specific pathway. We performed molecular dynamics simulations starting from a hidden intermediate and the native state of Rd-apocytochrome b562 in explicit solvent, for a total of 37.18 μs mainly with Anton. Markov state model was used to analyze the simulation results. Besides the experimentally identified hidden intermediates, we have found other partially unfolded states and misfolded states and verified that these states occur after the rate-limiting step. Transition-path theory was employed to calculate the folding flux. To better describe the complexity of folding, we propose a folding flux network model to replace the specific pathway model. The experimentally putative hidden intermediates are the constituent nodes of the network, but the conformational transitions from them to the final state are not in a sequential manner. Our folding flux network gives a more detailed and complete description of the complex folding behavior than the simplistic pathway model. Our observation of the misfolded states occurring near the native state may have implications in the study of misfolding diseases.

PD-010 **Establishment of thermodynamics of protein aggregation using isothermal titration calorimetry**

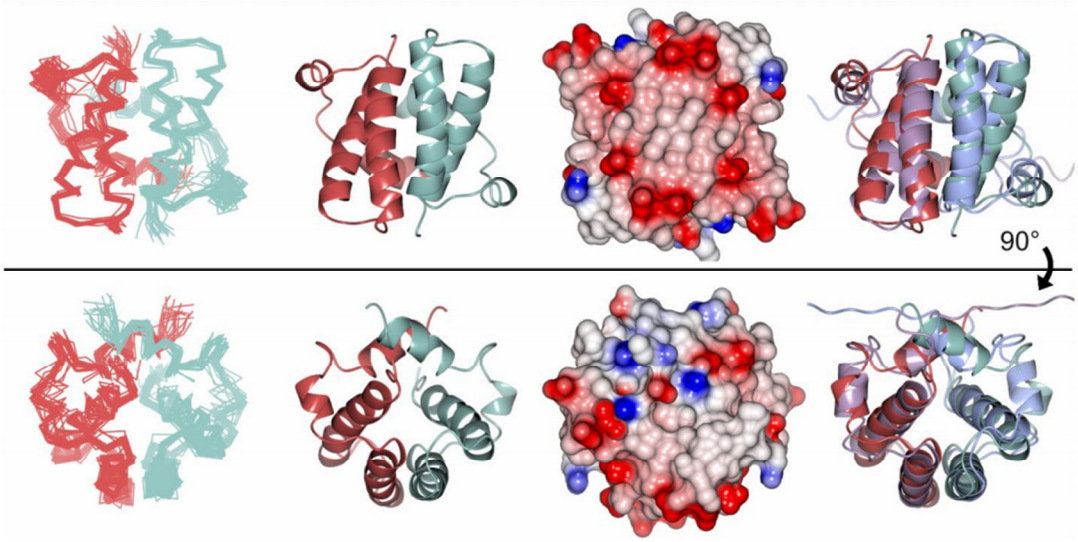
Tatsuya Ikenoue[1], Lee Young-Ho[1], Tetsuhei Uenoyama[1], Daniel Otzen[2], Yuji Goto[1]
1.-Institute for Protein Research, Osaka University, 2.-Interdisciplinary Nanoscience Center (iNANO), Aarhus University

Amyloid fibrils associated with serious diseases including Alzheimer's, Parkinson's, and prion diseases promoted the challenge of studying protein misfolding, leading to the development of amyloid structural biology. Amyloid fibrils form in supersaturated solutions via a nucleation and growth mechanism. Although the structural features of amyloid fibrils have become increasingly clearer, knowledge on the thermodynamics of fibrillation is limited. Furthermore, protein aggregation is not a target of calorimetry, one of the most powerful approaches used to study proteins. Here, with β2-microglobulin, a protein responsible for dialysis-related amyloidosis, we show direct heat measurements of the formation of amyloid fibrils using isothermal titration calorimetry (ITC). The spontaneous fibrillation after a lag phase was accompanied by exothermic heat. The thermodynamic parameters of fibrillation obtained under various protein concentrations and temperatures were consistent with the main-chain dominated structural model of fibrils, in which overall packing was less than that of the native structures. We also characterized the thermodynamics of amorphous aggregation, enabling the comparison of protein folding, amyloid fibrillation, and amorphous aggregation. In order to obtain general thermodynamic properties of protein aggregations, we further investigated aggregation of glucagon and insulin, two of the most famous amyloidogenic peptide hormones, using ITC. We also observed characteristic heat of spontaneous amyloid fibrillation of both proteins after a lag time. Taken all together, we showed that thermodynamic studies on amyloid fibrillation and amorphous aggregation were indeed possible by means of ITC-based qualitative and quantitative calorimetric analyses. ITC will become a promising approach for clarifying the thermodynamic properties of protein aggregates. The more case studies are required toward the establishment of thermodynamics of protein misfolding and aggregation

PD-011 **Molecular Mechanisms of Cytoplasmic Protein Quality Control**

Rivka Isaacson[1], Ewelina Krysztofinska[1], Santiago Martínez-Lumbreras[1], Arjun Thapaliya[1]
1.-Chemistry Department, King's College London

Molecular Mechanisms of Cytoplasmic Protein Quality Control In the crowded environment of the cell, quality control mechanisms are vital. Proteins that are obsolete or have strayed from their operative environments must be recycled or rehoused. When hydrophobic proteins are, for any reason, exposed to the cytosol they are rapidly captured by protective complexes which shield them from the aqueous surroundings and decide their fate (by either targeting them to their correct membrane homes or marking them for degradation by the ubiquitin/proteasome system). The BAG6 holdase is a heterotrimeric protein complex, comprising BAG6, UBL4a and TRC35, which works closely with the cochaperone SGTA to triage hydrophobic proteins and pass them along the appropriate pathway. SGTA also interacts with viral proteins and hormone receptors and is upregulated in numerous cancer types. These functions require further investigation to determine the scope of SGTA as a therapeutic target. Our lab has solved the solution structure of the N-terminal dimerization domain of SGTA and characterised its interaction with two different ubiquitin-like (UBL) domains in the BAG6 holdase (one from UBL4A and the other from BAG6 itself) using NMR chemical shift perturbation data and other biophysical techniques including isothermal titration calorimetry and microscale thermophoresis. At this meeting I will report on the progress we have made in structurally characterising further key players that participate in this quality control, with the aim of clarifying the intricate network of molecular interactions that governs these processes in health and disease.



Ensemble, ribbon and electrostatics spacefill views of the SGTA dimerization domain structure. The final panel shows the structure overlaid with its yeast homologue.

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PD-012 **Interaction of curcumin analogs with α-Synuclein: Modulation of Aggregation and Toxicity**

Narendra Jha[1], A. Anoop[1], Narasimham Ayyagari[1], Pradeep Singh[1], I.N.N. Namboothiri[1], Samir Maji[1]

1.-Indian Institute of Technology Bombay

Alpha synuclein is a small protein (~14 kDa) expressed at high levels in dopaminergic neurons. Fibrillar aggregates of α-synuclein inside the dopaminergic neuron are the major components of Lewy bodies and Lewy neuritis inclusion, which are considered as potential hallmark of Parkinson's disease (PD). Both in vitro as well as in vivo studies suggest that the soluble, oligomeric forms of α-Syn are the more potent neurotoxic species, responsible for neuronal injury and death in PD. Therefore, molecules that inhibit the toxicity of oligomers either by reducing their formation or by converting their more toxic oligomeric state to less-toxic fibrillar state would be effective agents for the drug development against PD. Curcumin is one of the Asian food ingredients which has shown a potential role as therapeutic agent against many neurological disorders including PD. However, the instability and low solubility makes it less attractive for use as potential therapeutic agent. The present work focuses on screening of the compounds similar to curcumin but having better effects on the morphology and toxicity of oligomeric and fibrillar assemblies of α-Syn, which could be used as therapeutic agent preferentially over the naturally occurring curcumin. We synthesized and analyzed the effects of nine compounds, which are structurally similar to curcumin, on different stages of α-Syn amyloid aggregation. Here, we showed that curcumin and its analogs accelerate α-Syn aggregation to produce morphologically different amyloid fibrils in vitro. However, there is no significant effect of curcumin and its analogs on the secondary structure of preformed α-Syn fibrils. Furthermore, these curcumin analogs showed differential binding affinities with the preformed α-Syn aggregates, possibly due to difference in their chemical structures. The present data suggest the promising role of curcumin analogs in the treatment of α-synucleinopathy disorders.

PD-013 **In vitro folding mechanisms determine the forces applied during co-translational folding**

Adrian Nickson[1], Jeff Hollins[1], Ola Nilsson[2], Gunnar von Heijne[2], Jane Clarke[1]
1.-Department of Chemistry, University of Cambridge, 2.-Department of Biochemistry and Biophysics, Stockholm University

There is currently much debate as to whether experiments conducted in vitro describe the folding of proteins in vivo. In particular, it is often suggested that the co-translational folding of nascent protein chains is dominated by the presence of the ribosome and associated chaperones, and that folding mechanisms will be affected by the vectorial nature of translation. Here we use an arrest peptide assay to investigate the co-translational folding of a number of all-α spectrin domains that exhibit a range of thermodynamic stabilities and in vitro folding rates. Our unexpected finding is that that the force exerted on the ribosome by these domains is not related to either the thermodynamic stability of the domain, or to the folding (loading) rate, but rather to the in vitro folding mechanism. We infer that the in vitro folding mechanisms of these domains are unaffected by the presence of the ribosome – even when part of the nascent chain is retained within the ribosome exit tunnel. There has been much work to date investigating the intermediates present in stalled translation complexes – but now, for the first time, we can begin to directly explore the rate limiting transition state in the co-translational folding of homologous proteins.

PD-014 **Can the structure of a protein (H3.1) depend on the treatment of a solvent medium (explicit vs effective) in a coarse-grained computer simulation?**

Ras Pandey[1], Barry Farmer[2]
1.-University of Southern Mississippi, 2.-Air Force Research Laboratory

Solvent medium plays a critical role in orchestrating the structure and dynamics of a protein. In computer simulation modeling of protein structure in a solvent medium, explicit, implicit, effective-medium, approaches are often adopted to incorporate the effects of solvation. Because of the complexity in incorporating all atomic and molecular details, the multiple components, reaching the large-scale, etc. implicit solvent or effective medium approach is generally more viable than the explicit solvent methods. Some of the pertinent characteristics such as excluded volume of the solvent constituents, its concentration, and the underlying fluctuations which may be important in probing some issues are generally ignored in effective medium or implicit solvent approaches. Using a coarse-grained approach, we investigate the structure and dynamics of a protein (a histone, H3.1) in the presence of both effective as well as explicit solvent media over a range of temperatures with the Monte Carlo simulations. The protein is represented by a coarse-grained chain of residues whose interactions are described by knowledge-based residue-residue and hydropathy-index-based residue-solvent interactions. In effective medium approach, each empty lattice site around the protein structure acts as a solvent. Only a fraction of lattice sites are occupied by mobile solvent constituents along with the protein chain in explicit solvent medium. Large scale simulations are performed to analyze the structure of the protein for a range of residue-solvent interactions and temperature in both explicit and effective solvent media. We study a number of local (e.g. solvation and mobility profiles) and global (radius of gyration and structure factor) physical quantities as a function of temperature. We find that the response of the radius of gyration of the protein in explicit solvent is different from that in effective medium solvent. Thus, the presence of fluctuations in explicit solvent approach have considerable effects on the structure and dynamics of protein H3.1. Differences due to type of solvent on the response of some of these quantities as a function of temperature as well as general similarities will be presented.

PD-015 **Single-molecule vectorial folding and unfolding through membrane pores**

David Rodriguez-Larrea[1], Hagan Bayley[2],
1.-University of the Basque Country, Dept. Biochemistry and Molecular Biology, 2.-Univerisity of Oxford, Dept. of Chemistry

Protein folding and unfolding in vivo is frequently vectorial. For example, proteins are synthesized at the ribosome and emerge N-terminal first. As the polypeptide chain emerges from a 2 nm wide pore is free to fold, interact with partners or misfold1. In another example, proteins are unfolded at the proteasome by pulling from either the N or C terminus against a 1-2 nm wide pore, applying a tension on the residues surrounding the terminus of the protein2. Under this conditions, proteins may behave differently than when unfolded/refolded with temperature or urea. This may have important implications, as protein folding and unfolding in vivo is related to both function and disease. We noticed that vectorial folding is inherently linked to nanometer size pores. Making use of nanopore technology we developed a method to monitor protein unfolding during membrane translocation at the single-molecule level3. Briefly, an oligonucleotide attached at either end of a protein threads a single protein nanopore inserted in a lipid membrane. In response to an applied membrane potential, the oligonucleotide pulls the protein through the pore and as it is forced to translocate it unfolds. Analysing the ionic current we obtain the unfolding pathway and information on the polypeptide sequence. This methodology has shown that proteins unfold with different kinetics when pulled from one terminus or the other4. Remarkably, it is also possible to say whether the protein has been phosphorylated or not, and where5. We have recently advanced our model system to study protein folding after translocation at the single-molecule level6. A single-protein molecule was translocated through a pore and forced to translocate back at predetermined times. We measured the stability of the refolded state at different times and we obtained the vectorial folding pathway of the protein. Further, we observed that the protein was capable of co-translocational folding and that this premature folding contributed to the complete translocation of the protein. Our results show that nanopore technology applied to proteins can be used to describe the vectorial folding and unfolding of proteins, providing insight to how these processes may work in vivo. Further, single-molecule protein sequencing is a possibility that could revolutionise our knowledge on biological processes.

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PD-016 **Reversibility and two state behavior in the thermal unfolding of oligomeric TIM barrel proteins from three bacterial phyla**

Sergio Romero Romero[1], Miguel Costas[2], Adela Rodríguez-Romero[3], D. Alejandro Fernández-Velasco[1]

1.-Facultad de Medicina, Universidad Nacional Autónoma de México., 2.-Facultad de Química, Universidad Nacional Autónoma de México., 3.-Instituto de Química, Universidad Nacional Autónoma de México

Thermodynamics studies of oligomeric proteins, which are the dominant protein natural form, have been often hampered because irreversible aggregation and/or slow reactions are common. There is not a single report on the reversible equilibrium thermal unfolding of proteins composed by (β/α)8 barrel subunits, albeit this "TIM barrel" topology is one of the most abundant and versatile in nature. The eponymous TIM barrel, Triosephosphate isomerase (TIM) is a ubiquitous glycolytic enzyme that catalyzes the isomerization of glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. The unfolding of several TIMs, mainly of eukaryotic organisms, has been extensively studied. Regarding thermal unfolding, eighteen TIMs, mainly from eukaryotes, as diverse as Amoebozoa, Euglenozoa, Ascomycota and Chordata, have been studied. Even though a full thermodynamic characterization has been hampered by irreversible aggregation and/or the presence of hysteresis in all of them, the activation parameters that describe the kinetic control of five eukaryotic TIMs have been reported. We characterized the structure, catalytic properties, association state and temperature-induced unfolding of the eponymous TIM barrel, Triosephosphate Isomerase (TIM), belonging to five species representative of different bacterial taxa: Deinococcus radiodurans (DrTIM), Nostoc punctiforme (NpTIM), Gemmata obscuriglobus (GoTIM), Clostridium perfringens (CpTIM) and Streptomyces coelicolor (ScTIM). Irreversibility and kinetic control were observed in the thermal unfolding of NpTIM and GoTIM, while for DrTIM, ScTIM and CpTIM, the thermal unfolding was found to follow a two-state equilibrium reversible process, a behavior not observed previously for others TIMs. Shifts in the global stability curves of these three proteins are related to organismal temperature range of optimal growth and modulated by variations in maximum stability temperature and in the enthalpy change at that temperature. Reversibility appears to correlate with low isoelectric point, the absence of residual structure in the unfolded state, small cavity volume in the native state structure, low conformational stability and a low melting temperature. Furthermore, the strong coupling between dimer dissociation and monomer unfolding may reduce the possibility of aggregation and favor reversibility. It appears that there is a delicate balance between several contributions whose concerted interplay is necessary to achieve thermal reversibility in oligomeric enzymes. Furthermore, the finding that the three reversible proteins come from organisms from different phyla suggests that unfolding reversibility may be more common than what is currently known Supported by CONACyT 99857, PAPIIT IN-219913, Facultad de Medicina-UNAM, and Posgrado en Ciencias Bioquímicas-UNAM.

PD-017 **Structural insights into HIV-1 Gag binding to the plasma membrane during virus assembly**

Jamil Saad[1], Jiri Vlach[1], Ruba Ghanam[1]
1.-Department of Microbiology, University of Alabama at Birmingham

A critical step in the late phase of human immunodeficiency virus type 1 (HIV-1) infection is targeting of the virally encoded Gag proteins to the plasma membrane (PM) for assembly. Prior to assembly, the HIV-1 Gag polyprotein adopts a compact "folded over" conformation and exists in the monomeric or low-order oligomeric states. Whereas it is established that the nucleocapsid domain of Gag specifically recognizes motifs in the viral RNA genome for packaging, there is compelling evidence that the myristoylated matrix (MA) domain also binds to cellular RNA to prevent premature Gag targeting to intracellular membranes. Upon transport of Gag to the PM, the interaction of MA with RNA is exchanged for an interaction of MA with PM components. This molecular switch induces an extended conformation of Gag, leading to formation of high-order Gag oligomers on the PM. Because Gag is anchored and therefore captured by its interaction with the available phospholipids, the intracellular targeting of Gag is likely to be determined by the relative strength of its interaction with the dominant lipids composing each membrane subcompartment. The key to understanding this essential molecular switch is elucidating at the molecular level the interaction of MA with specific PM components. For over two decades, biochemical, in vivo, in vitro and genetic studies have focused on factors that modulate binding of retroviral Gag proteins to membranes but only recently the structural and molecular determinants of Gag assembly have begun to emerge. In addition to the electrostatic interactions between a highly conserved basic region of MA and acidic phospholipids, it is now believed that the hydrophobicity of the membrane interior represented by the acyl chains and cholesterol also play important roles. We employ NMR methods to elucidate the molecular determinants of Gag binding to the membrane. Our structural studies revealed that phosphatidylinositol-4,5-bisphosphate (PI(4,5)P2), phosphatidylserine (PS), phosphatidylethanolamine (PE), and phosphatidylcholine (PC) all interact directly with the MA domain of Gag. Striking, our data revealed a novel binding mode by which the 2'-acyl chains of PI(4,5)P2, PS, PE, and PC lipids are buried in hydrophobic pockets in MA, while the 1'-acyl chains are exposed. These findings led us to propose a trio engagement model by which HIV-1 Gag is anchored to the PM via the 1'-acyl chains of PI(4,5)P2 and PS/PE/PC, and the myr group, which collectively bracket a basic patch projecting towards the polar leaflet of the membrane. In-depth understanding of the precise role of lipids in virus assembly, and elucidation of the molecular requirements of Gag-membrane interaction may aid in the development of new antiviral therapeutic strategies.

PD-018 **The structure and function of the Surrogate Light Chain**

Natalia Catalina Sarmiento Alam[1], Johannes Buchner[1]
1.-Department Chemie, Technishe Universität München

The production of functionally antibodies depends on the transition of immature B cells to mature plasma cells and is tightly linked to several “quality control” check points. During B cell development, the pre-B Cell Receptor (pre-BCR) is the first checkpoint which determines the viability and proliferation of the pre-B cell. The pre-BCR is composed of an immunoglobulin (Ig) heavy chain molecule associated with an Ig light chain-like molecule called the Surrogate Light Chain (SLC). The SLC is composed by two proteins λ5 and VpreB which possess a unique region at the N- or C-terminus, respectively. VpreB lacks a β-strand which is provided by the λ5 protein allowing the non-covalent interaction essential for formation of the SLC heterodimer. Our understandings of the molecular mechanism of SLC function and assembly are still at an early stage. In particular, we do not know how the SLC associates and forms the pre-BCR for the selection of all heavy chains (HCs). Our study focuses on dissecting the “Fab fragment” of the pre-BCR to study the effect of the unexpected structural features of the SLC to gain insight in HC selection. The analysis of the assembly of the SLC revealed a significant difference between the single domains and the complexes in terms of stability and assembly. The folding behavior of the CH1 domain in the presence of the SLC is key for the first quality control mechanism in the endoplasmic reticulum (ER) prior to surface expression. Our results show that the SLC interacts with CH1 domain in a similar manner to the CL domain. Thus, the folding of the naturally disordered CH1 domain upon interaction with the SLC releases the HC retention in the ER by BiP. Taken together, our study provides new insights into the folding and assembly of the “Fab fragment” of the pre-BCR and paves the way for a detailed mechanistic understanding of HCs selection by the unique SLC.

PD-019 **2D IR spectroscopy reveals a ß-sheet intermediate that dictates the fiber formation of hIAPP**

Arnaldo Serrano[1], Ling-Hsien Tu[2], Daniel Raleigh[2], Martin Zanni[1]

1.-Department of Chemistry, University of Wisconsin-Madison, 2.-Department of Chemistry, Stony Brook University

Though the 22-29 (SFGAILSS) region of human Islet Amyloid Polypeptide (hIAPP) has long been known to be crucial for amyloid fiber formation, lack of β-ordering of this region in structures of the final fiber as determined by both NMA and X-ray has been puzzling. New evidence now suggests that the FGAIL region forms ordered β structures only in early intermediates. We present new 2DIR studies on the FGAIL region of hIAPP, with uniformly 13C 18O labeled amides, along with spectral and kinetic modelling. Evolution of the peak frequency and 2D lineshape of the labeled region clearly present a transition from random coil to a stable β sheet, a conclusion which is substantiated by simulation of the 2D IR spectra. As determined from kinetic modeling, the FGAIL β-sheet creates a free energy barrier that is the cause of the lag phase during aggregation. These findings help to rationalize a broad range of previous fragment and mutation studies as well as provide a mechanism for fiber formation that has self-consistent kinetics and structures.

PD-020 **The temperature dependence of protein stability in living cells**

Austin E. Smith[1], Larry Z. Zhou[1], Annelise H. Gorensek[1], Michael Senske[2], Gary J. Pielak[1,3,4]
1.-Department of Chemistry, 2.-Department of Physical Chemistry II, 3.-Department of Biochemistry and Biophysics, 4.-Lineberger Comprehensive Cancer Center

Theory predicts that the cytoplasm, where the concentration of macromolecules can exceed 300 g/L, alters protein-folding thermodynamics. Specifically, the intracellular environment is hypothesized to increase the free energy of protein unfolding (i.e., increase stability) by reducing the available space, which is a purely entropic effect. However, in vitro studies under crowded conditions show that enthalpy plays an underappreciated role. Here, we present the first insight into the enthalpic destabilization of globular proteins in living cells. Using 19F NMR and the 7-kDa N-terminal SH3 domain of the Drosophila signal transduction protein drk, we show that the intracellular environment destabilizes the protein via attractive electrostatic interactions between the cytoplasmic components and protein's unfolded ensemble. Our results contradict classic crowding theory but provide a more complete picture of physiologically relevant protein thermodynamics.

PD-021 **Molecular crowding effects on the native and equilibrium intermediate state of a 29 kDa TIM Barrel protein**

Ramakrishna Vadrevu[1], Jagadeesh Gullipalli[1], Rajashekar Kadumuri[1], Srividya Subramanian[1], Koushik Chandra[2], Hanudutta Atreya[3]

1.-Dept. of Biological Sciences, Birla Institute of Technology & Science, 2.-NMR Research Centre, Indian Institute of Science, 3.-Solid State and Structural Chemistry Unit, Indian Institute of Science

Studies addressing the consequence of crowding that exist in the interior of cells have reached an interesting stage. Experimental data so far, predominantly from, small to medium sized proteins are indicating that, in general, natively folded proteins including, intrinsically disordered, gain structure and stability under conditions mimicking cell interior. However, on the other hand, a few studies on small proteins indicate destabilization of the native state. In very few instances, crowding resulted in compaction and aggregation of the unfolded and partially folded states. Experimental data on the consequences of cell-like crowding situation on relatively large proteins with complex folding free energy landscape are absent. alpha subunit of tryptophan synthase, a 29 kDa TIM barrel protein, provides a unique opportunity to address the consequence of crowding on the structure and stability of the native state and also on a partially folded state stable equilibrium intermediate populated in its (un)folding reactions. In the presence of increasing amounts the most commonly used crowding agent, Ficoll-70, a non-monotonous increase in the far UV-CD is observed for the native state. A steady increase up to 250mg/ml Ficoll followed by a decrease in far-UV CD region is observed, indicating loss of structure at increased concentrations of the crowding agent. 1H-15N HSQC NMR and fluorescence (FL) spectra confirm the of loss of structure at higher concentrations of Ficoll-70. Loss of native base line in the urea induced unfolding reaction monitored by CD and FL clearly confirms the destabilization of the native state. Similar to the structural changes observed for the native state, for the equilibrium intermediate state maximally populated at 3 M urea also, non-monotonous changes in the far UV CD and fluorescence spectra are observed. The highly populated equilibrium intermediate shows an initial steady increase in the far UV CD signal followed by a sudden decrease. Our results suggest that the structure of both native and partially folded states may be affected under crowding conditions.

PD-022 **Co-translational protein folding studies of alpha-1 antitrypsin**

Conny Wing-Heng Yu[1,2], Lien Chu[1,2], Xiaolin Wang[1,2], Christopher A. Waudby[1,2], John Christodoulou[1,2], Lisa D. Cabrita[1,2]

1.-Institute of Structural and Molecular Biology, University College London, 2.-Institute of Structural and Molecular Biology, Birkbeck College London

Alpha-1 antitrypsin (AAT) is a 44-kDa serine protein inhibitor (serpin), which acts as an inhibitor of neutrophil elastase within the lungs. During inhibition, the protein undergoes a dramatic conformational change in which its exposed reactive centre loop (RCL) is cleaved and inserts into the central A-sheet as an extra beta-strand. This highly dynamic protein is also susceptible to mutations, resulting in misfolding and the accumulation of ordered polymers as intracellular inclusions within the endoplasmic reticulum of hepatocytes, where AAT is synthesized. Despite much knowledge of the folding and misfolding properties of AAT as an isolated protein, very little is understood of how AAT acquires its structure during biosynthesis. Like all proteins, the biosynthesis of AAT takes place on the ribosome, and protein folding occurs in a co-translational manner as the nascent polypeptide chain emerges from the ribosome's exit tunnel. This study aims to develop the biochemical and NMR structural strategies to characterize the co-translational folding characteristics of AAT as it is being synthesized on the ribosome. For these studies, we have designed a series of SecM-stalled ribosome nascent chain complexes (RNC) of AAT of different lengths, which mimics the "snapshots" of the protein synthesis, capturing the folding process of the nascent chain during its emergence from the ribosome. Using this library, we have recently developed a strategy to produce large quantities of the RNCs both in vitro and in vivo within E. coli, a prerequisite for detailed biochemical and structural studies. Using the AAT-RNCs, we are developing a suite of biochemical strategies to probe the capacity for AAT nascent chains to adopt native structure on the ribosome. We have combined protease inhibition assays, Western blot and native-PAGE analysis to demonstrate that AAT can fold while bound to the ribosome. In addition, we have employed a cysteine-based modification "PEGylation" assay to probe low-resolution structural information of AAT-RNC and this will guide our structural studies by NMR spectroscopy to provide a detailed understanding of AAT folding on the ribosome at high resolution.

PD-023 **PH and Temperature dependent Folding-Unfolding transition of BBL protein under various Urea concentration**

Sangyeol Kim[1,2,4], Wookyung Yu[3], Bora Kwon[3], Seongjun Park[2], Iksoo Chang[1,2,3]

1.-Research Institute, Daegu Gyeongbuk Institute of Science and Technology, 2.-Center for Proteome Biophysics,Daegu Gyeongbuk Institute of Science and Technolo, 3.-Department of Brain Science, Daegu Gyeongbuk Institute of Science and Technology, 4.-Department of Physics, Pusan National University

Thermodynamic properties of proteins vary with the environmental solvent condition (temperature, ions, pH, denaturants, etc.). Although the effect of each environmental factor on proteins has been well studied, the complex effect of more than two environmental factors was not studied thoroughly. In this study, we investigate the simultaneous effect of urea denaturation (disruption of non-covalent bonds in proteins) and acid denaturation (titration of protein residues) on the nature of the folding transition for 2CYU protein. We performed the molecular dynamics simulations of BBL (PDB code: 2CYU) protein in various urea concentration at 300K. We calculated pH-dependent free energy landscape using the extended Munoz-Eaton model and described the phase diagram for the folding transition of BBL at various pH value and urea concentration. We mapped out the phase diagram of the folding transition of 2CYU, which clarifies the condition with which it undergoes the cooperative folding transition or the barrierless folding transition.

PD-024 **Biophysical analysis of partially folded states of myoglobin in presence of 2,2,2-trifluoroethanol**

Paurnima Talele[1], Nand Kishore[1]
1.-Indian Institute of Technology Bombay

The protein folding process involves one or more distinct populated intermediates. One such partially folded structure of particular importance observed during protein folding pathway is molten globule state. The properties of a molten globule state are intermediate between those of native and unfolded protein molecules. The importance of studying equilibrium molten globule is in its greater stability and flexible structure which has been shown to bind a variety of substrates and play a definite role in certain human diseases via aggregation, misfolding or some other mechanism. A protein must assume a stable and precisely ordered conformation to perform its biological function properly. The stability of a protein under specific conditions depends on its interactions with the solvent environment. Therefore it is essential to understand protein folding intermediates, protein solvent interactions and protein stabilization. We have made attempts to thoroughly investigate the formation of stable molten globule state of the protein induced by alcohol using combination of calorimetric and spectroscopic techniques. The presentation will cover the topic on biophysical studies on partially folded states of myoglobin in presence of 2,2,2-trifluoroethanol. The thermal denaturation of myoglobin was studied in the presence of 2,2,2-trifluoroethanol (TFE) at various pH values using differential scanning calorimetry and UV-visible spectroscopy. The most obvious effect of TFE was lowering of the transition temperature with increasing concentration of TFE up to 1.5 mol•dm-3, beyond which no thermal transitions were observed. The conformation of the protein was analyzed by a combination of fluorescence and circular dichroism measurements. At pH 5.0 and 11.0, partially folded states of myoglobin were confirmed by CD spectroscopy. Quantitative binding of ANS to the TFE induced molten globule state of myoglobin was studied by using isothermal titration calorimetry (ITC). The results enable quantitative estimation of the binding strength of ANS with the molten globule state of myoglobin along with the enthalpic and entropic contributions to the binding process. The results also suggest occurrence of common structural features of the molten globule states of proteins offering two types of binding sites to ANS molecules which has been widely used as a fluorescence probe to characterize partially folded states of proteins.

PD-025 **Structural duality in peptides derived from choline binding repeats**

Hector Zamora-Carreras[1], Roberto Silva-Rojas[1], Beatriz Maestro[2], Erik Strandberg[3], Anne Ulrich[3, 4], Jesús M Sanz[2], Marta Bruix[1], M Angeles Jimenez[1]

1.-Instituto de Química Fisica Rocasolano (IQFR-CSIC), 2.-Instituto de Biología Molecular y Celular, Universidad Miguel Hernández, 3.-Institute of Biological Interfaces, Karlsruhe Institute of Technology (KIT), 4.-dInstitute of Organic Chemistry, Karlsruhe Institute of Technology (KIT)

The C-terminal domain of the pneumococcal choline-binding protein LytA (CLytA) consists of six choline binding repeats (CBRs) organized as a ββ-solenoid structure, which is characteristic of choline binding modules. Each CBR comprises a β-hairpin core followed by a short linker sequence. Choline molecules are bound between two consecutive repeats through hydrophobic and cation-π interactions with aromatic side chains. Apart from its biotechnological applications as an affinity tag for protein immobilization and purification, CLytA is useful as a model for understanding the folding and stability of repeat proteins. In this sense, we proposed to get minimal peptides encompassing the sequence of a single CBR or even only its β-hairpin core able to maintain the native fold and the ability to bind choline. To that end, we first proceeded to analyze the peptide comprising the third β-hairpin core, denoted as CLyt3. Based on CD and NMR data we demonstrate that the peptide CLyt3 conserves its native β-hairpin structure in aqueous solution, but forms a stable, amphipathic α-helix in detergent micelles and as well as in small lipid vesicles [1]. Considering the great differences in the distribution of hydrophobic and polar side chains shown by CLyt3 β-hairpin and α-helix, we propose that amphipathic structures are stabilized in micelles or lipid vesicles. This “dual” behavior is the only up-to-now reported case of a micelle-induced conformational transition between two ordered peptide structures. To check whether other CBR repeats also undertake β-hairpin to α-helix transition in the presence of micelles, so that it represents a general tendency ascribed to all pneumococcal choline-binding modules, we will show new experimental evidences based on CD and NMR structural studies on peptides derived from the β-hairpin cores of other CLytA repeats, as well as in modified CLyt3 peptides.

1. Zamora-Carreras et al. (2015). Chemistry-Eur. J. doi:10.1002/chem.201500447 Financial support from the Spanish MINECO projects no CTQ2011-22514 and BIO2013-47684-R is acknowledged

PD-026 **Conformational analysis of fragments of the human Pin1 protein: the influence of charged amino-acid residues on the ß-hairpin structure**

Joanna Makowska[1], Dorota Uber[1], Wioletta Żmudzińska[2], Caterina Tiberi[3], Lech Chmurzyński[1], Anna Maria Papini[3]

1.-Faculty of Chemistry, University of Gdansk, 2.-Laboratory of Biopolymer Structure, Intercollegiate Faculty of Biotechnology, Medical University of Gdansk, 3.-Dipartimento di Chimica Organica 'Ugo Schiff ', Universit`a di Firenze

Continuing our studies of the effect of like-charged residues on protein-folding mechanisms, in this work, we investigated, by means of NMR spectroscopy and molecular-dynamics simulations, two short fragments of the human Pin1 WW domain [hPin1(14-24); hPin1(15-23)] and one single point mutation system derived from hPin1(14-24) in which the original charged residues were replaced with non-polar alanine residues. Results, for both original peptide fragments of hPin1 demonstrate the presence of ensembles of structures with a tendency to form a β-chain reversal.

PD-027 **Understanding the biology of Huntington's disease via the pathogenic huntingtin monomer**

Estella Newcombe[1], Yasmin Ramdzan[1], Ashish Sethi[1], Michael Lee[2], Dorothy Loo[3], Bim Graham[2], James Swarbrick[2], Anthony Purcell[3], Paul Gooley[1], Danny Hatters[1]

1.-Department of Biochemistry and Molecular Biology, University of Melbourne, 2.-Monash Institute of Pharmaceutical Science, Monash University, 3.-Department of Biochemistry and Molecular Biology, Monash University

Huntington's disease (HD) is caused by an abnormal extension of the polyglutamine (polyQ) region within exon 1 of the protein huntingtin from typically 25 glutamines to over 36. Disease onset correlates with the huntingtin misfolding and causing the formation of aggregates, however recent studies have postulated that pathogenic huntingtin monomer may form compact structures that are responsible for neuronal toxicity in HD. We sought to examine the conformation of huntingtin monomers, how polyQ sequence length affects monomer structure and which protein-binding partners in the cell may exert a gain-of-toxic mechanism in pathology. Hydrogen-deuterium exchange mass spectrometry was used to measure the degree of structure in both non-pathogenic (25Q) and pathogenic (46Q) huntingtin, with results showing that both forms exchanged 79% of potential NH hydrogen bond donors within 30 seconds (n=3), with little to no further exchange over the following ten minutes. This result suggested that the pathogenic conformations are not stabilized by slow exchanging hydrogen bonds. Binding partners to the monomer were assessed in Neuro2a cell culture by immunoprecipitation and quantitative MS/MS proteomics approaches after depletion of aggregates by pelleting. Proteins that more prevalently co-precipitated with pathogenic huntingtin included Fused in sarcoma (Fus), glycine-tRNA ligase (Gars), peroxiredoxin 6 (Prdx6), phosphatidylethanolamine-binding protein 1 (Pebp1/Rkip), and histone subunit Hist1H4a, all of which were significantly enriched by two-fold or greater. RNA-seq analysis indicated that none of these proteins had altered expression levels, suggesting that the binding interactions are not due to changes in background abundance. Overall we found that the conformational differences are subtle, yet are sufficient to generate several specific proteome interactions that offer clues to a toxic gain-of-function mechanism in pathology. Work is ongoing to probe the more subtle changes in conformation and the importance of these interactors to mediating mechanisms of dysfunction.

PD-028 **Molecular basis of tyrosinemia and identification of possible pharmacological chaperones targets**

Iratxe Macias[1], Arantza Sanz[1], Ana Laín[1], Oscar Millet[1]
1.-CIC bioGUNE

Hereditary tyrosinemia type I is an autosomal recesive disorder caused by deficiency of fumarylacetoacetate hydrolase (FAH) enzyme. Deficiency of FAH leads to cellular accumulation of toxic metabolites which include mainly, succinylacetone (SA), maleylacetoacetate (MAA) and fumarylacetoacetate (FAA) in many body tissues. FAH is mainly expressed in hepatocytes and renal proximal tubular epithelium. Therefore, liver and kidney are the two primary organs affected by this disorder, and development of hepatocellular carcinoma is the major symptom. Missense mutations leads to a loss of enzymatic efficiency which, in a high number of mutations, correlates with loss of kinetic and thermodynamic stability of the enzyme. In our ongoing project, we are trying to elucidate the molecular basis of tyrosinemia by means of biophisical and structural characterization of FAH wild type along with its mutations. This knowledge should help us design new therapies based on the identification of pharmacological chaperones that could restore the altered enzymatic stability of the enzyme. Human FAH wild type and 19 selected mutants were synthesized and inserted in an expression vector for E. coli. The proteins were purified in a FPLC and, their thermodynamic and kinetic stability investigated using circular dichroism. Our preliminary results confirm the loss of termodinamic stability of different mutants and its variability compared to wild type protein.

PD-029 **Repulsion between net charges of subunits during ferritin assembly**

Daisuke Sato[1], Hideaki Ohtomo[1], Atsushi Kurobe[1], Satsuki Takebe[1], Yoshiteru Yamada[2], Kazuo Fujiwara[1], Masamichi Ikeguchi[1]

1.-Department of Bioinformatics, Graduate School of Engineering, Soka University, 2.-JASRI/SPring-8

The organisms have a lot of spherical shell-shaped supermolecules consisting of identical or distinct subunits (e.g., ferritin, virus capsid, lumazine synthase and encapsulin). Such multimeric proteins spontaneously assemble into their native structures from the subunits to acquire the specific functions. However, the assembly mechanism of such supermolecules has not been understood in detail. Hence, to investigate the assembly mechanism is biologically important. Escherichia coli non-heme ferritin (Ftn) consists of 24 identical subunits, which are assembled into a spherical shell-shape with 4/3/2 symmetry. Ftn is able to store iron inside cavity. The subunit includes A-D helices forming 4-helix bundle, a long BC-loop between B and C-helices and a short E-helix at the C-terminal. Ftn dissociates into dimers at acidic pH. The dimer was shown to maintain the native-like secondary and tertiary structures by circular dichroism spectra and small angle X-ray scattering (SAXS). The acid-dissociated Ftn is able to reassemble into the native structure when pH increases. To clarify Ftn assembly mechanism, we performed the stopped-flow time-resolved SAXS (TR-SAXS) experiments. The SAXS profiles could be acquired every 15 ms after the initiation of reassembly. The initial velocity calculated from the forward scattering intensity increment was proportional to the square of the protein concentration, implying that the reaction is second-order. We propose the sequential bimolecular reaction, in which two dimers bind to form tetramer, then another dimer attaches to the tetramer to form a hexamer, and so on. The assembly rate depended on pH and ion strength, indicating that the electrostatic interaction plays an important role in the assembly reaction. The assembly rate decreased with increasing pH in the range from 6.0 to 8.0 and increased with increasing NaCl concentration. This indicates that there are repulsive electrostatic interactions between assembly units and that they increases with increasing pH from 6.0 to 8.0. A possible interaction is the repulsion between net charges of dimers since pI of Ftn is expected to be 4.6. To test this possibility, we made several mutants with different net charges. As mutational sites, we selected charged residues that are far from the subunit interface. Selected sites were Glu5, Glu8, Glu12, Glu85 and Glu89. We constructed the mutants with one, two, three or four Glu -> Gln substitutions of selected sites. The structures of those mutants were similar to that of wild-type Ftn. If aforementioned hypothesis is correct, the assembly rate is expected to increase with increasing the number of substitution. The result agreed well with this expectation and strongly suggested that the electrostatic repulsion between dimers is an important factor determining the assembly rate of Ftn.

PD-030 **Improved modeling of protein unfolding rates and pathways through solvation and modeling of beta-barrels**

Benjamin Walcott[1,2], Luís Garreta[3], Christopher Bystroff[1,2,4]

1.-Department of Biology, Rensselaer Polytechnic Institute, 2.-Center for Biotechnology and Interdisciplinary Studies, 3.-Department of Computer Science, Universdad del Valle, 4.-Department of Computer Science, Rensselaer Polytechnic Intitute

An understanding of the folding and unfolding pathways of proteins is integral to improving our ability to associate the structural impact of point mutations and disease etiology. Information gained here can also be used for protein structure prediction and design. To model unfolding pathways in proteins we utilize a computational method called GeoFold. This approach uses recursive hierarchical partitioning of protein structure and finite elements simulation. GeoFold considers three types of partitioning operations: translational motion (break), single point revolute joints (pivot), and rotation around two points (hinge). From these operations, a directed acyclic graph (DAG) is constructed where nodes correspond to the substructures created by these operations and the edges represent the operations. For each operation in the DAG, its dissociation and reassociation rates are determined as a function of solvent-accessible surface area, hydrogen bonds, voids, and conformational entropy. Finite element simulations are carried out to simulate the kinetics of unfolding. This model accurately predicts changes in unfolding pathways due to disulfides in a four-protein case-study , but it fails to produce a realistic pathway for β-barrel proteins such as green fluorescent protein (GFP). To better model these barrel proteins, a new partitioning operation is introduced involving the breaking of all contacts between an adjacent set of β-strands, called a seam. In addition, to improve the accuracy of kinetic modeling, several updates have been made to the energy function, including an improved solvation model and a contact-order-based estimation of the reassociation rates. The predicted unfolding rates and pathways using this improved GeoFold are compared with experimentally measured values in KineticDB for proteins with multi-state unfolding kinetics, point mutations, circular permutations, and engineered disulfides.

PD-031 **In the Multi-domain Protein Adenylate Kinase, Domain Insertion Facilitates Cooperative Folding while Accommodating Function at Domain Interfaces**

V. V. Hemanth Giri Rao[1], Shachi Gosavi[1]

1.-National Centre for Biological Sciences, Tata Institute of Fundamental Research

The presence of multiple domains in a protein can result in the formation of partially folded intermediates, leading to increased aggregation propensity. This can be reduced by cooperative, all-or- nothing folding of the multi-domain protein. In good agreement with ensemble folding experiments, a coarse-grained structure-based model of E. coli Adenylate kinase (AKE) folds cooperatively. AKE has three domains, NMP, LID and CORE. We examine the role of the interfaces between these domains in facilitating folding cooperativity in AKE. Mutants in which these interfaces are deleted exhibit similar folding cooperativities as wild-type AKE. On closer inspection, we observe that unlike a typical multi- domain protein in which one domain is singly-linked to its adjacent domain, NMP and LID are inserted into CORE, i.e. they are both connected to CORE by two linkers each. We create circular permutants of AKE in which the inserted domains are converted to singly-linked domains, and find that they fold less cooperatively than wild-type AKE. Domain insertion in wild-type AKE facilitates folding cooperativity even when the inserted domains have lower stabilities. The N- and C-termini of NMP and LID are constrained upon the folding of CORE and this facilitates their folding. Thus, NMP and LID which undergo large conformational changes during catalysis can be smaller with fewer stabilizing interactions. In addition, inter-domain interactions need not be optimized for folding, and can be tuned for substrate binding, conformational transition and catalysis. Analysis of protein domains using structural bioinformatics suggests several examples of multi-domain proteins in which domain insertion is likely to facilitate folding cooperativity.

PD-032 **Tuning cooperativity on the free energy landscape of protein folding**

Pooja Malhotra[1], Jayant Udgaonkar[1]
1.-National Centre for Biological Sciences, Tata Institute of Fundamental Research

The mechanism by which a protein explores the free energy landscape during a folding or unfolding reaction is poorly understood. Determining whether these reactions are slowed down by a continuum of small (~ kBT) free energy barriers or by a few large (> 3 kBT) free energy barriers is a major challenge. In this study the free energy landscape accessible to a small protein monellin is characterized under native-like conditions using hydrogen exchange in conjunction with mass spectrometry. Cooperative and non-cooperative opening processes could be directly distinguished from the mass distributions obtained in the EX1 limit. Under native conditions, where the native state is maximally stable, the unfolded state is transiently sampled in an entirely non-cooperative and gradual manner. Under conditions which stabilize the unfolded state or destabilize the native state of the protein, the slowest structure opening event becomes cooperative. The present study provides an understanding of the relationship between stability and folding cooperativity. It suggests that the cooperative transitions observed in unfolding reactions maybe a consequence of the changes in the stabilities of the unfolded state and the transition state. It also provides rare experimental evidence for a gradual unfolding transition on a very slow timescale.

PD-033 **Role of electrostatic repulsion between unique arginine residues on the assembly of a trimeric autotransporter translocator domain**

Eriko Aoki[1], Kazuo Fujiwara[1], Masamichi Ikeguchi[1]
1.-Department of Bioinformatics, Graduate School of Engineering, Soka University

Haemophilus influenzae adhesin (Hia) belongs to the trimeric autotransporter family. The autotransporter consists of an N–terminal signal peptide, an internal passenger domain and a C-terminal translocator domain. The signal peptide directs to export across the inner membrane via the Sec system and is cleaved, the passenger domain is a virulence factor, and the translocator domain (HiaT) is embedded in the outer membrane. The crystal structure of Hia translocator domain (HiaT) has shown that HiaT forms a transmembrane β-barrel of 12 β-strands, four of which are provided from each subunit. The β-barrel has a pore that is traversed by three α-helices, one of which is provided from each subunit. The protein has a unique arginine residue at 1077. Arg1077 side chains from three subunits protrude from the β-strand toward the center of the barrel and are close to each other. These residues seem to have an unfavorable electrostatic effect on the assembly and decrease the trimer stability. To investigate the role of this residue on the trimer assembly and stability of HiaT, we replaced this arginine with the neutral amino acid, methionine (R1077M) or the positively charged residue, lysine (R1077K), and properties of these mutants were investigated. HiaT and two mutants were dissociated by formic-acid treatment, and they were able to reassemble in the presence of the detergent. To measure the time course of trimer reassembly, amounts of reassembled trimer and monomer were quantified by SDS-PAGE at different assembly times. Although the neutralized mutation increased the rate of reassembly, the final amount of reassembled trimer decreased, especially at higher protein concentration. These suggest that the neutralized mutation cause the incorrect oligomer formation. The far-UV CD spectrum of reassembled WT HiaT was nearly identical with that of the native WT HiaT. However, the spectrum of the reassembled R1077M mutant was more intense that of the native R1077M mutant, although the proportion of trimer was much lower than that of the WT HiaT. This suggests that the incorrect oligomer has a secondary structure different from the WT HiaT. R1077K mutant showed assembly properties similar to those of the WT HiaT. Therefore, the repulsion between positively charged residues seems to be important for preventing HiaT from misassembly. Similar proximity of arginine residues is observed for HIV capsid protein, carboxysome shell protein, lumazine synthase and so on. The electrostatic repulsion between arginine residues may be a general mechanism for protein assembly.

PD-034 **Kurozu Increases HSPA1A Expression and Ameliorates Cognitive Dysfunction in Aged SAM P8 Mice**

Toshiaki Kakimoto[1], Hideya Nakano[1], Yuji Nakai[2], Kazuhiro Shiozaki[3], Kohei Akioka[4], Konosuke Otomaru[5], Mitsuharu Matsumoto[6], Masanobu Nagano[7], Yasushi Sugimoto[8], Hiroaki Kanouchi[1]

1.-Department of Veterinary Pathobiology, Kagoshima University, 2.-Institute for Food Sciences, Hirosaki University, 3.-Faculty of Fisheries, Kagoshima University, 4.-Department of Veterinary Histopathology, Kagoshima University, 5.-Veterinary Clinical Training Center, Kagoshima University, 6.-Department of Veterinary Anatomy, Kagoshima University, 7.-Sakamoto Kurozu Inc., 8.-The United Graduate School of Agricultural Sciences, Kagoshima University

Kurozu is a traditional Japanese rice vinegar. During fermentation and aging of the Kurozu liquid in an earthenware jar over 1 year, solid residue called Kurozu Moromi is produced. In the present study, we evaluated whether concentrated Kurozu or Kurozu Moromi could ameliorate cognitive dysfunction in the senescence accelerated P8 mouse. Senescence accelerated P8 mice were fed 0.25% (w/w) concentrated Kurozu or 0.5% (w/w) Kurozu Moromi for 4 or 25 weeks. Kurozu suppressed cognitive dysfunction and amyloid accumulation in the brain, while Kurozu Moromi showed a tendency to ameliorate cognitive dysfunction, but the effect was not significant. We hypothesize the effect is caused by the antioxidant effect of concentrated Kurozu, however, the level of lipid peroxidation in the brain did not differ in senescence accelerated P8 mice. DNA microarray analysis indicated that concentrated Kurozu increased HSPA1A mRNA expression, a protein that prevents protein misfolding and aggregation. The increase in HSPA1A expression by Kurozu was confirmed using quantitative real-time PCR and immunoblotting methods. Therefore, the suppression of amyloid accumulation by concentrated Kurozu may be associated with HSPA1A induction. However, concentrated Kurozu could not increase HSPA1A expression in mouse primary neurons, suggesting it may not directly affect neurons.

PD-035 **Cold Denaturation of Alpha-Synuclein Amyloid Fibrils**

Young-Ho Lee[1], Tatsuya Ikenoue[1], Yasushi Kawata[2], Yuji Goto[1]

1.-Laboratory of Protein Folding, Institute for Protein Research, Osaka University, 2.-Department of Chemistry and Biotechnology, Tottori University

Although amyloid fibrils are associated with a number of pathologies, their conformational stability remains largely unclear. We herein investigated the thermal stability of various amyloid fibrils. α-Synuclein fibrils, freshly prepared at 37 ºC at neutral pH, cold-denatured to monomers at 0-20 ºC and heat-denatured at 60-110 ºC. Meanwhile, the fibrils of β2-microglobulin, Alzheimer's Aβ1-40/Aβ1-42 peptides, and insulin exhibited only heat denaturation, although they showed a decrease in conformational stability at low temperature in the presence of chemical denaturants. A comparison of structural parameters with positive enthalpy and heat capacity changes which showed opposite signs to protein folding suggested that the burial of charged residues in the fibril cores contributed to the cold denaturation of α-synuclein fibrils. Reinforced electrostatic repulsion at low temperatures may promote cold denaturation, leading to a unique thermodynamic property of amyloid fibrils. We propose that although cold-denaturation is common to both native proteins and misfolded fibrillar states, the main-chain dominated amyloid structures may explain amyloid-specific cold denaturation due to the unfavorable burial of charged side-chains in fibril cores.

PD-036 **Key structural differences between TbTIM and TcTIM revealed by thermal unfolding molecular dynamics simulations**

Ángel Piñeiro[1], Miguel Costas[2], Andrea Gutiérrez-Quezada[2]

1.-Dept of Applied Physics, University of Santiago de Compostela, 2.-Lab. of Biophys. Chem., Dept of Physical Chemistry, Fac. of Chemistry, UNAM

The thermal unfolding pattern obtained by differential scanning calorimetry for Trypanosoma cruzi and Trypanosoma brucei triosephosphate isomerase (TcIM and TcTIM) are significantly different although the crystal structure of both proteins is almost indistinguishable and the sequences are highly homogolous. In order to explain these differences at molecular level a set of molecular dynamics simulations were performed at different temperatures between 400 and 700 K. The obtained trajectories were analyzed in detail and the residues that showed to be key in the unfolding pathway of each species were identified. A set of residues that behave significantly different between both proteins were selected and proposed for mutations. The general aim is to identify the minimum amount of residue mutations that allow providing TbTIM with the behaviour of TcTIM and vice versa. Experimental complementary work is also being performed on the same protein.

PD-037 **Repositioning SOM0226 as a potent inhibitor of transthyretin amyloidogenesis and its associated cellular toxicity**

Salvador Ventura[1], Ricardo Sant'Anna[1], Maria Rosário Almeida[2], Natàlia Reixach[3], Raul Insa[4], Adrian Velazquez-Campoy[5], David Reverter[1], Núria Reig[4]

1.-Universitat Autònoma de Barcelona, 2.-Instituto de Biologia Molecular e Celular, ICBAS, 3.-The Scripps Research Institute, 4.-SOM-Biotech, 5.-Universidad de Zaragoza

Transthyretin (TTR) is a plasma homotetrameric protein implicated in fatal amyloidosis. TTR tetramer dissociation precedes pathological TTR aggregation. Despite TTR stabilizers are promising drugs to treat TTR amyloidoses, none of them is approved by the Food and Drug Administration (FDA). Repositioning existing drugs for new indications is becoming increasingly important in drug development. Here, we repurposed SOM0226, an FDA-approved molecule for neurodegenerative diseases, as a very potent TTR aggregation inhibitor. SOM0226 binds specifically to TTR in human plasma, stabilizes the tetramer in vivo and inhibits TTR cytotoxicity. In contrast to most TTR stabilizers, it exhibits high affinity for both TTR thyroxine - binding sites. The crystal structure of SOM0226-bound TTR explains why this molecule is a better amyloid inhibitor than Tafamidis, so far the only drug in the market to treat the TTR amyloidoses. Overall, SOM0226, already in clinical trials, is a strong candidate for therapeutic intervention in these diseases.

PD-038 **Neurometals as modulators of protein aggregation in neurodegenerative diseases**

Sónia S. Leal[1], Joana S. Cristóvão[1], Cláudio M. Gomes[1]
1.-Faculdade de Ciências Universidade de Lisboa - BioISI, FCUL

Protein misfolding and aggregation is a hallmark across neurodegenerative diseases such as Alzheimer's disease and Amyotrophic lateral sclerosis (ALS). Since these diseases are mostly sporadic, the formation of protein amyloids in the nervous system depends of chemical and biological triggers within the neuronal environment, such as metal ions [1]. In this communication I will overview the metallobiology of neuronal calcium, zinc and copper, which are key players in brain function and have altered homeostasis in most neurodegenerative conditions. Our recent work will illustrate how this allows establishing molecular mechanisms in neurodegenerative diseases [2-6]. In the pursuit of this goal, in the last years we have been investigating superoxide dismutase 1 (SOD1), a Cu/Zn metalloenzyme that aggregates in the fatal neurodegenerative disorder ALS, as a model. In SOD1-ALS cases, this ubiquitous protein selectively aggregates in motor neurons, implicating a local biochemical factor in the process: interestingly, Zn2+ and Ca2+ levels are upregulated in the spinal and brain stem motor neurons of ALS patients, and increased Ca2+ triggers multiple pathophysiological processes which include direct effects on the SOD1 aggregation cascade [2,3]. Recently we established that calcium ions promote SOD1 aggregation into non-fibrillar amyloid, suggesting a link to toxic effects of calcium overload in ALS [4]. We showed that under physiological conditions, Ca2+ induces conformational changes on SOD1 that increase SOD1 β-sheet content and decrease SOD1 critical concentration and nucleation time during aggregation kinetics. We also observed that calcium diverts SOD1 aggregation from fibrils towards amorphous aggregates. Interestingly, the same heterogeneity of conformations is found in ALS-derived protein inclusions. We thus hypothesized that transient variations and dysregulation of cellular Ca2+ and Zn2+ levels contribute to the formation of SOD1 aggregates in ALS patients [4,5]. In a follow up study we combined experimental and computational approaches to show that the most frequent ligands for Ca2+ are negatively-charged gatekeeper residues located in boundary positions with respect to segments highly prone to edge-to-edge aggregation. Calcium interactions thus diminish gatekeeping roles by shielding repulsive interactions via stacking between aggregating β-sheets, partly blocking fibril formation and promoting amyloidogenic oligomers such as those found in ALS inclusions. Interestingly, many fALS mutations occur at these positions, disclosing how Ca2+ interactions recreate effects similar to those of genetic defects, a finding with relevance to understand sporadic ALS pathomechanisms [6]. FCT/MCTES is acknowledged for grants EBB-BIO/117793/2010 and PTDC/QUI-BIQ/117789/2010 (to CMG) and BPD (SFRH/BPD/47477/2008 to SSL.) 1. Leal et al (2012) Coord. Chem. Reviews 256(19-20):2253-227 2. Botelho et al. (2012) J Biol Chem 287(50):42233-42 3. Cristovão, et al (2013) Int. J. Mol. Sci. 14(9):19128-45 4. Leal et al (2013) J. Biol. Chem. 288, 25219-25228 5. Leal et al (2015) Metallomics 7(2):333-46 6.Estacio et al (2015) Biochim Biophys Acta 1854(2):118-26

PD-039 **Single-molecule FRET reveals proline dynamics in transmembrane helices**

Gustavo Fuertes[1,2,3], Ismael Mingarro[3], Edward A. Lemke[1]

1.-Structural and Computational Biology Unit, European Molecular Biology Laboratory, 2.-European Molecular Biology Laboratory, 3.-Department of Biochemistry and Molecular Biology, University of Valencia

The amino acid proline is well-known by its disorder promoting and helix breaking properties. Prolines can be accommodated within transmembrane (TM) alpha-helices and participate in important biological tasks like signal transduction, ligand binding and helix-helix packing. X-ray crystallography and NMR indicate that proline residues in membrane proteins induce distortions of the helix geometry to different extents ranging from small bends to severe kinks. However, such studies provide essentially a static snapshot of membrane-embedded helices. Therefore, the link between proline dynamics and function is not completely understood. In this work we have used single-molecule Förster resonance energy transfer (smFRET) and fluorescence correlation spectroscopy (FCS) to probe the structure and dynamics of the TM domain of human glycophorin A (GpA), a widely used model membrane protein for oligomerization studies. A fluorescent dye pair has been attached to both ends of the membrane-spanning region of GpA, which allowed monitoring the average distance and distance fluctuations between the attachment points. Site-specifically double-labeled GpA has been reconstituted into two membrane-mimetic systems: SDS micelles and phospholipid bilayers assembled into nanodiscs. Using proline-scanning mutagenesis we have systematically evaluated the impact of proline residues in different positions along the membrane normal on transmembrane helix length and lateral packing. Furthermore, we have investigated the distance distribution in TM helices containing native prolines, namely the insulin receptor and the nesprin protein. Our results shed light into the relation between proline dynamics and the folding and function of TM helices.

PD-040 **Thermodynamic contributions of specific mutations of L30e protein in the RNA : protein interface region measured by analytical ultracentrifugation and gel shift assay**

Bashkim Kokona[1,2], Sara Kim[1], Margaret Patchin[1], Britt Benner[1], Susan White[1]
1.-Department of Chemistry, Bryn Mawr College, 2.-Department of Biology, Haverford College

In Saccharomyces cerevisiae, ribosomal protein L30e acts as an autoregulator by inhibiting the splicing of its pre-mRNA and translation of its mRNA. The L30e protein-RNA binding site has been previously studied, revealing a RNA kink-turn motif, which is characterized by a sharp bend in the phosphodiester backbone due to unpaired nucleotides and internal tertiary interactions. L30e structural flexibility at the RNA-binding interface makes such interaction an excellent model to explore the energetics of RNA protein binding. We made L30e K28A, F85A, and F85W mutants to quantify the thermodynamic contributions of such interactions to the protein-RNA complex. We used analytical ultracentrifugation sedimentation equilibrium (SE) and sedimentation velocity (SV) to investigate conformational changes and protein-RNA binding free energy changes due to mutations. Our computed changes of binding free energy based on the sedimentation equilibrium experiments were consistent with the gel shift assay results. In addition, sedimentation velocity experiments on the L30e wild type indicate that protein-RNA interaction is highly dynamic and involves conformational changes of the kink-turn RNA induced by L30e protein. Our results provide new insights on understanding the binding between ribosomal proteins and their RNA molecules counterpart, which can be used to complement the x-ray structure.

PD-041 **Role of a non-native α-helix in the folding of equine β-lactoglobulin**

Takahiro Okabe[1], Toshiaki Miyajima[1], Kanako Nakagawa[1], Seiichi Tsukamoto[1], Kazuo Fujiwara[1], Masamichi Ikeguchi[1]

1.-Department of Bioinformatics, Soka University

Equine β-lactoglobulin is a small globular protein (162 residues). Although ELG adopts a predominantly β-sheet structure consisting of nine anti-parallel β-strands (A-I) and one major α-helix in the native state, it has been shown that a non-native α-helical intermediate accumulates during the burst-phase of folding reaction from the unfolded state in the concentrated denaturant. To ask whether the non-native helix formation is important for acquiring the native β-sheet structure, we determined first where the non-native α-helix is formed. A stable analogue of the burst-phase folding intermediate was observed at acid pH (A state). The amide hydrogen exchange experiment and proline-scanning mutagenesis experiment have shown that the non-native α-helix is formed at the region corresponding to the H strand in the A state. To investigate the role of this non-native α-helix on refolding reaction of ELG, we constructed several mutant proteins, which were designed to destabilize the non-native α-helix in the folding intermediate without perturbation on the native structure. A mutant, A123T, fulfilled this requirement, that is, A123T showed a native structure similar to that of the wild-type protein, and largely reduced CD intensity in the A state. Then, the refolding kinetics were investigated by the CD and fluorescence stopped-flow method. A123T mutation resulted in reduction of the burst-phase CD intensity, which confirmed that the non-native α-helix is formed around the H strand region. Subsequent to the burst-phase, four kinetic phases were observed for A123T and the wild-type protein. Importantly, the folding rate constants of the four kinetic phases were similar between both proteins. Furthermore, interrupted refolding experiments demonstrated that the native state was formed in the two parallel pathways in the two slower phases of the four kinetic phases. The relative amplitudes of the two pathways were similar between A123T and the wild-type protein. These results clearly showed that the formation of the non-native helix has little effect on the folding rates and pathways, and suggested that the non-native helix formation may not be a severe kinetic trap for protein folding reaction.

PD-042 **Impact of the chaperonin CCT in α-Synuclein(A53T) amyloid fibrils assembly**

Ahudrey Leal_Quintero[1], Javier Martinez-Sabando[1], Jose María Valpuesta[1], Begoña Sot[1]
1.-Centro Nacional de Biotecnología (CNB/CSIC)., 2.-Centro Nacional de Biotecnología (CNB/CSIC)., 3.-Centro Nacional de Biotecnología (CNB/CSIC)., 4.-Centro Nacional de Biotecnología (CNB/CSIC) and Fundación IMDEA-Nanociencia

CCT is a eukaryotic chaperonin that uses ATP hydrolysis to encapsulate and fold nascent protein chains. Moreover, it has recently been shown that CCT is able to inhibit amyloid fibers assembly and toxicity of the polyQ extended mutant of Huntingtin, the protein responsible of Huntington disease. Although this opens the possibility of CCT being also able to modulate other amyloidopathies, this has not addressed yet. The work presented here intends to determine the effect of CCT in the amyloid fibers assembly of α-Synuclein(A53T), one of the mutants responsible of Parkinson disease. It is demonstrated that CCT is able to inhibit α-Synuclein(A53T) fibrillation in a nucleotide independent way, suggesting that this effect is based on binding rather than on active folding. Furthermore, using deletion mutants and assaying the interaction of CCT with monomers, soluble oligomers and fibres, it has been possible to unravel the mechanism of this inhibition: CCT interferes with fibers assembly by interacting with α-Synuclein(A53T) NAC domain once soluble oligomers are formed, thus blocking the reaction before the fibers start to grow.

PD-043 **Amyloid-like aggregation of Nucleophosmin regions associated with acute myeloid leukemia mutations**

Daniela Marasco[1], Concetta Di Natale[1], Valentina Punzo[1], Domenico Riccardi[1], Pasqualina Scognamiglio[1], Roberta Cascella[2], Cristina Cecchi[2], Fabrizio Chiti[2], Marilisa Leone[3], Luigi Vitagliano[3]

1.-Department of Pharmacy, CIRPEB: Centro Interuniversitario di Ricerca sui Pepti, 2.-Section of Biochemistry, Department of Biomedical Experimental and Clinical Scie, 3.-Institute of Biostructures and Bioimaging

Nucleophosmin (NPM1) is a multifunctional protein involved in a variety of biological processes and implicated in the pathogenesis of several human malignancies. NPM1 has been identified as the most frequently mutated gene in acute myeloid leukemia (AML) patients, accounting for approximately 30% of cases (1). The most frequent human NPM1 mutations lead to variants with altered C-terminal sequences of the C-Terminal Domain (CTD) that, in its wild form, folds as a three helix bundle. AML modifications lead to (a) an unfolding of the CTD in the mutated protein and (b) its accumulation in the cytoplasm due to the loss of nuclear localization sequences with mutations of Trp290 (mut E) and also of Trp288 (mut A) (2). To gain insights into the role of isolated fragments in NPM1 activities we dissected the CTD in its helical fragments. Here we describe the unexpected structural behavior of the fragments corresponding to the helices H2 and H3 in both wild-type and AML-mutated variants. H2 region shows a remarkable tendency to form amyloid-like assemblies while only the MutA sequence of H3 region is endowed with and β-sheet structure, under physiological conditions, as shown by circular dichroism, Thioflavin T and dynamic light scattering. The aggregates of H2, are also toxic to neuroblastoma cells, as determined by using the MTT reduction and Ca^{2+} influx assays (3). Furthermore the effects of the local context on the different tendencies to aggregate of H2 and H3 were investigated and appeared to influence for the aggregation propensity of the entire CTD. Since in AML mutants the CTD is not properly folded, we hypothesize that the aggregation propensity of NPM1 regions may be implicated in AML etiology. These findings have implications to elucidate the pathogenesis of AML caused by NPM1 mutants and aggregation phenomena should be seriously considered in studies aimed at unveiling the molecular mechanisms of this pathology.

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PD-044 **Transition from α-helix to β-sheet structures occurs in myoglobin in deuterium oxide solution under exposure to microwaves**

Emanuele Calabrò[1], Salvatore Magazù[1]
1.-Department of Physics and Earth Sciences, University of Messina

We report a resume of our study regarding the effects of microwaves in the range 900-1800 MHz on a typical protein, Myglobin. Previous literature have concerned the effects on living and in vitro organic systems induced by high frequencies electromagnetic fields. We have focused our attention on a typical protein, Myoglobin, because proteins are the simplest organic systems that are fundamentals in organic functions of livings. Myoglobin is a protein found mainly in muscle tissue of vertebrates, consisting of a single protein chain with 153 amino acids and one heme group that stores oxygen in the muscle cells. The physiological importance of Myoglobin is mainly related to its ability to bind molecular oxygen. In particular, we focused our attention on the secondary structure of this protein in order to highlight whether exposure to microwaves unfold the protein producing transitions from α-helix component to β-sheet features. To this aim Fourier Transform Infrared (FTIR) spectroscopy have been used. The importance of this study is related to previous literature which indicated that transition from α-helix to β-sheet structure in a protein can be responsible for aggregation mechanisms that can lead to neurotoxicity and neurodegenerative disorders that can be considered as the first step to some pathologies [1-3]. The aggregates consist of fibers containing unfolded proteins with a prevalent β-sheet structure termed amyloid [4]. In our studies Myoglobin in deuterium oxide (D2O) solution was exposed for 3 h to mobile phone microwaves at 900 and 1800 MHz at a power density of 1 W/m2. FTIR spectra were recorded by a spectrometer Vertex 80v from Bruker Optics, following the protocol accurately described in [5-7]. FTIR spectroscopy analysis evidenced an increase in intensity of β-sheet structures and a significant shift to lower frequencies of about 2.5 cm-1 of the amide I vibration after exposure [8,9]. These results led to conclude that mobile phone microwaves induce proteins unfolding and formation of aggregates [10, 11].

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PD-045 **Investigating the insertion and folding of membrane proteins into lipid bilayers using a cell free expression system**

Nicola Harris[1], Kalypso Charalambous[1], Eamonn Reading[1], Paula Booth[1]
1.-Kings College London, Department of Chemistry

Membrane proteins play a vital role in many biological processes, and yet remain poorly understood as they are frequently unstable in vitro. The goal of this project is to investigate the insertion and folding of membrane proteins into lipid bilayers, using a cell free expression system. We have used both E.coli-based cell extracts (S30), and commercial translation systems (PURExpress) in combination with synthetic liposomes of defined lipid composition. These studies will aid understanding of cooperative folding, folding intermediates, and the effects of the lipid bilayer on folding and insertion. Model E.coli proteins have been investigated, as they can offer important insights into other proteins, and thus facilitate the further study of more biologically relevant proteins. It has been found that the rhomboid protease GlpG spontaneously inserts into liposomes without the aid of an insertase such as SecYEG. This spontaneously inserted GlpG is functional, and is able to cleave BODIPY-labeled casein, yielding a fluorescent product. The Major Facilitator Superfamily (MFS) transport proteins LacY, GalP and GlpT have also been found to insert spontaneously into liposomes. It has been shown that the lipid composition of the liposomes has an effect on the amount of protein inserted into the bilayer, with all proteins tested to date preferring liposomes containing at least 50 mol% DOPG. Ongoing and future work will involve the use of rare codons to alter the rate of translation, to investigate the effect this has on the final folded structure of the protein. Preliminary work is also currently being done into whether the two domains of the MFS family transporters fold cooperatively or independently, thus aiding understanding into the folding and stability of membrane transport proteins.

PD-046 **Study of rabies virus by Differential Scanning Calorimetry: Identification of Proteins Involved in Thermal Transitions**

Frederic Greco[1], Audrey Toinon[1], Nadege Moreno[1], Marie Claire Nicolaï[1], Catherine Manin[1], Francoise Guinet-Morlot[1], Frederic Ronzon[1]

1.-Sanofi Pasteur, Analytical Research and Development, Biophysical and Biochemical

Study of rabies virus by Differential Scanning Calorimetry: Identification of Proteins Involved in Thermal Transitions A.Toinon, F. Greco, N. Moreno, M.C. Nicolaï, C. Manin, F.Guinet-Morlot & F. Ronzon Sanofi Pasteur, Analytical Research and Development, Biophysical and Biochemical Characterization, 1541 Avenue Marcel Merieux, 69280 Marcy l'étoile, France Abstract Rabies virus (RABV) is the causative agent of rabies. Rabies remains an important worldwide health problem that causes a fatal encephalomyelitis [1]. Currently, rabies in humans is under control in Europe and North America following the use of efficient vaccines for dogs and wild animals. However, it still kills more than 55,000 people every year mainly in Africa and Asia [2]. Human vaccination prevents infection with very high efficacy. The vaccine contains an inactivated RABV produced on Vero cells. RABV is an enveloped, negative single stranded RNA virus which encodes five proteins, namely the nucleoprotein (N), the phosphoprotein (P), the matrix protein (M), the glycoprotein (G), and the viral RNA polymerase (L) [3]. The viral envelope is covered by trimer spikes of G-glycoprotein which is the most significant surface antigen for generating virus-neutralizing antibodies. Here we illustrate the use of DSC (Differential Scanning Calorimetry) to identify structural domains or proteins involved in thermal transitions. The DSC thermogram for intact Beta-propiolactone inactivated RABV samples in PBS buffer reveals two major thermal transitions with a Tm respectively at 61°C and 71°C. We have initially focused our investigations on one of the major proteins encode in RABV, Glycoprotein G [4]. Glycoprotein G contains disulfide bridges on the ectodomain [6], is sensitive to Bromelain cleavage [5] and shows reversible conformation changes at low pH [7]. Considering these characteristics, our results provide evidence on the identity of one thermal transition observed by DSC. Keywords: rabies virus, Differential Scanning Calorimetry, protein unfolding [1] - Hemachudha, T., Laothamatas, J., Rupprecht, C.E., 2002. Human rabies: a disease of complex neuropathogenetic mechanisms and diagnostic challenges. Lancet Neurology 1 [2] - WHO Fact Sheet n°99, updated July 2013 [3] - Finke, S., Conzelmann, K.K., 2005. Replication strategies of rabies virus. Virus Res. 111, 120–131. [4] - Coll, J.M., 1995. The glycoprotein G of Rhabdoviruses. Archives of Virology. 140:827-851. [5] - Gaudin, Y., Tuffereau, C., Segretain, D., Knossow, M., Flamand, A., 1991. Reversible Conformational Changes and Fusion Activity of Rabies Virus Glycoprotein. Journal of Virology. 4853-4859 [6] - Gaudin, Y., 1997. Folding of rabies Virus Glycoproteein: Epitope Acquisition and Interaction with Endoplasmic Reticulum Chaperones. Journal of Virology. 3742-3750. [7] - Gaudin, Y., Ruigrok, R., Knossow, M., Flamand, A., 1993. Low-pH Conformational Changes of Rabies Virus Glycoprotein and Their Role in Membrane Fusion. Journal of Virology. 1365-1372.

PD-047 **Domain swapping of the DNA-binding domain of human FoxP1 is facilitated by its low folding stability**

Exequiel Medina[1], Sandro L. Valenzuela[1], Cristóbal Córdova[1], César A. Ramírez-Sarmiento[1], Jorge Babul[1]

1.-Departamento de Biología, Facultad de Ciencias, Universidad de Chile

Domain swapping of the DNA-binding domain of human FoxP1 is facilitated by its low folding stability Exequiel Medina, Sandro L. Valenzuela, Cristóbal Córdova, César A. Ramírez-Sarmiento and Jorge Babul Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile Protein folding and dimerization (or oligomerization) are biologically relevant processes when reaching the quaternary structure is required for function. Proteins that form dimers by exchanging segments or domains of their tertiary structure with another subunit, the so-called domain swapping phenomenon, are examples where folding and dimerization are tightly concerted processes. Previous studies on domain swapping proteins, such as p13suc1 and diphtheria toxin, have shown that, in general, a high kinetic barrier separates monomers and domain swapped dimers, and that this barrier can be lowered by promoting protein unfolding and refolding at high protein concentrations, thus favoring the swapped oligomer. Recent crystal structures of the DNA-binding domain of several human forkhead box (Fox) proteins have shown that the P subfamily of these transcription factors (FoxP) can form swapped dimers. The human FoxP proteins are interesting models of domain swapping, because mutations of the DNA-binding domain of these proteins are linked to diverse inherited disorders in humans, such as IPEX and language deficits, and some of these mutations are located in the hinge region that connects the exchanged segment with the rest of the protein. Moreover, FoxP1 and FoxP2 have been described to reach monomer-dimer equilibrium in solution after hours of incubation, suggesting that a low kinetic barrier separates both species. Using FoxP1 as a model of domain swapping, we analyzed the temperature and protein concentration effects on the dimer dissociation, obtaining the free energy change and enthalpy of the process by van't Hoff analysis (ΔHo of 23.1 kcal•mol-1, ΔSo of 0.082 kcal•mol-1•K-1 and ΔGo at 25oC of -0.95 kcal•mol-1). These results indicate that the monomer-monomer association is an example of an enthalpy-driven process. To understand how FoxP1 domains swap without protein unfolding, we performed equilibrium unfolding experiments using GndHCl as denaturant, showing that the wild-type protein has a low stability (ΔGU = 6 kcal•mol-1, Cm =3.5 M at 25oC), in contrast to other domain swapping proteins with high kinetic barriers. We further explore the domain swapping mechanism of FoxP1 through biased targeted molecular dynamics simulations, showing that the exchange process can occur by specific local destabilization and unfolding of the hinge region and helix H3. To further corroborate that the low stability of wild-type FoxP1 facilitates its domain swapping, we engineered a monomeric version of FoxP1 through a single-point mutation in the hinge region, which has been previously described in the literature, and used this protein to visualize the effect of monomer stability in the dimer formation. Comparison of the folding stability of the monomeric mutant A39P and wild-type FoxP1 shows that ΔΔGU (mutant- wild-type) is ~2.5 kcal/mol, concluding that the ability of FoxP1 to domain swap rapidly can be explained through its low monomer stability and local unfolding of the exchange region.

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PD-048 **Determining the coupled interactions that stabilize the structural framework of the ß-propeller fold**

Loretta Au[1], David Green[2,3,4]

1.-Department of Statistics, The University of Chicago, 2.-Department of Applied Mathematics and Statistics, Stony Brook University, 3.-Graduate Program in Biochemistry and Structural Biology, Stony Brook University, 4.-Laufer Center of Physical and Quantitative Biology, Stony Brook University

β-propeller proteins are a highly evolved family of repeat proteins that are involved in several biological pathways, such as signal transduction, cell-cycle modulation and transcription regulation, through interactions with diverse binding partners, despite having a similar fold. As for all repeat protein families, there is a consistent pattern in secondary structure for each repetitive region, in addition to the entire family. Typically, four to ten propeller blades (each containing four anti-parallel β-sheets) are arranged in a toroidal shape, thus providing a large binding surface for ligands or other proteins. About 1% of known proteins adopt this distinctive fold, and although the requirements for tertiary structure and protein function are fundamentally encoded in primary structure, this relationship is not fully understood, and addressing it could provide insight on why the β-propeller fold is common. Many techniques in comparative sequence analysis can successfully identify amino-acid conservation between closely related proteins, but molecular interactions between amino acids are often neglected, and further experimentation is still needed to determine the reasons underlying conservation. To explore how primary structure can dictate fold and function, we devised a computational approach to perform large-scale mutagenesis, by adapting the dead-end elimination and A* search algorithms (DEE/A*), and also leveraged the structural conservation of each repeating region to understand how sequence variation influences protein fitness, defined here as a combination of stabilizing and binding interactions. DEE/A* can evaluate low-energy protein sequences and their corresponding three-dimensional structures, and we used the β-subunit of a G-protein heterotrimer (PDB: 1GP2, Giα1β1γ2) as a model system to demonstrate: (1) how the multiple roles of individual amino acids in protein fitness can be deconvolved, and (2) how epistatic interactions between them can contribute to structural stability. In doing so, we were able to identify important patterns in sequence complementarity between repeating regions that cannot be found using sequence-based methods alone. These results suggest that computational approaches can be used to determine important protein interactions, and help elucidate the prevalence of β-propeller proteins in biology.

PD-049 **Temperature induced conformational changes of the villin headpiece miniprotein**

Stanislaw Oldziej[1], Wioletta Żmudzińska[1], Anna Hałabis [1]
1.-Intercollegiate Faculty of Biotechnology, UG and MUG

The C-terminal subdomain of the actin-binding protein villin called HP35 (villin headpiece) has been used as a model protein in a number of studies of protein folding kinetics and protein folding mechanism [1,2]. The HP35 is a 35 residue miniprotein with an alpha-helix bundle three-dimensional fold. The goal of our work was to determine conformational ensemble of polypeptide chain of the investigated miniprotein at a wide range of temperatures to get detailed information about how protein structure is influenced by temperature. 2D NMR spectra of the title miniprotein were registered at 278, 293 and 313 K. The three-dimensional structure of the HP35 based on restraints derived from NMR spectra registered at 278 K is almost identical with structure deposited in the PDB database in the record 2F4K [2]. At higher temperatures (293 and 313 K) the general shape of the protein remains unchanged, with well packed hydrophobic core. However, with temperature increase alpha-helices start to melt. At 313 K structure of the protein remains compact and in general shape similar to structure observed at 278K, but none of the alpha-helices could be observed. Results obtained for HP35 protein are in agreement with previous observation for the Trp-cage miniprotein [3], that with temperature increase regular secondary structure elements melt first before the break-up of the hydrophobic core of the protein. Acknowledgements: Conference participation for S.O supported by the FP7 project Mobi4Health (grant agreement no 316094). Calculations were carried out with the use of the resources of the Informatics Center of the Metropolitan Academic Network (IC MAN – TASK) in Gdańsk, Poland.

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PD-050 **Comparative equilibrium folding of a membrane transporter within detergent and lipid environments**

Michael Sanders[1], Heather Findlay[1], Paula Booth[1]
1.-Department of Chemistry, King's college London

Biological membranes provide a selective and chemically sealed barrier for cells. Transport of ions and small molecules across the membrane is mediated by transporter proteins and the breakdown of a cell's ability to produce functionally folded membrane transport proteins can lead to dysfunction and has been implicated in many diseases1. However little is known about the processes that govern the misfolding of α-helical integral membrane proteins, taking into account that these proteins fold and maintain functional structures within membranes of various organelles. The neurotransmitter sodium symporter (NSS) protein family is an example of α-helical transporter proteins. The NSS family encompasses a wide range of prokaryotic and eukaryotic ion-coupled transporters that regulate the transport of neurotransmitter molecules whose dysfunction has been implicated in multiple diseases and disorders2. We have investigated the folding processes of prokaryotic homologue of the NSS family LeuT responsible for the transport of neurotransmitters and amino acids to the sodium electrochemical gradient. Previously folding processes of membrane transporters have mainly been characterised within detergent micelles. However, detergent micelles are not an accurate depiction of the environment of the membrane bilayer, with this in mind we have also attempted to investigate folding processes within a bilayer

PD-051 **NMR Investigation of pH-induced unfolding of B domain of an Escherichia Coli mannitol transporter II Mannitol in the bacterial phosphotransferase system**

Kim Gowoon[1], Yu Taekyung[1], Suh Jeongyong[1]
1.-Department of Agricultural Biotechnology, Seoul National University

The bacterial phosphotransferase system (PTS) mediates sugar phosphorylation and translocation across the cytoplasmic membrane. Cytoplasmic B domain (IIB Mtl) of the mannitol transporter enzyme II Mannitol , a PTS family protein, delivers a phosphoryl group from A domain to an incoming mannitol that is translocated across the membrane. IIB Mtl is comprised of a four-stranded ß-sheet and three helices, representing a characteristic Rossmann fold. We found that the IIB Mtl of Escherichia coli unfolded at a mildly acidic condition. We made IIB Mtl mutants to investigate the mechanism of the pH-induced unfolding using NMR spectroscopy. We monitored backbone amide groups and side chain imidazole groups of histidine residues using 2D HSQC NMR, and pointed out a potential histidine residue that might be responsible for the unfolding. Histidine residues may be generally important to the folding stability in response to environmental pH changes.

PD-052 **Can site-directed mutagenesis shed light on the refolding pattern of human glucose 6-phosphate dehydrogenase (G6PD)?**

Nurriza Ab Latif[1,2], Paul Engel[1]

1.-Conway Institute, Univerversity College Dublin, 2.-Faculty of Biosciences and Medical Engineering, Universiti Teknologi Malaysia

Human glucose 6-phosphate dehydrogenase (G6PD) is the first enzyme involved in the pentose phosphate pathway (PPP). This oligomeric enzyme catalyses the reaction of glucose 6-phosphate to form 6-phosphogluconolactone with concomitant reduction of NADP+ to NADPH. In erythrocytes NADPH is important mainly for protection against oxidative stress. In connection with its role as the sole source of NADPH, G6PD deficiency commonly causes haemolytic disease and is known as the most common human enzyme deficiency globally. Protein folding problems and instability are believed to be the major defects in the deficient enzymes. In this study, we employed site directed mutagenesis with hope to give more information on the role of –SH groups in the refolding of human G6PD. Two mutants were created: 1) one in which all 8 Cys residues were replaced by Ser and 2) one in which only C13 and C446 were retained. The refolding of recombinant human G6PD has been studied primarily by measuring the enzyme activity after refolding. We also used a combination of intrinsic protein fluorescence, ANS (8-anilino-1-naphthalenesulphonic acid) binding and limited proteolysis to look at the conformational change during the refolding. The results showed that GdnHCl-denatured recombinant human G6PD wild type could be refolded and reactivated by rapid dilution technique. Even though, as recombinants in E. coli, the mutants were well expressed and active, they remained inactive after attempts were made to refold them in vitro. The methods we applied may have provided some insights on the refolding pattern of this oligomeric protein, albeit qualitatively rather than quantitatively.

PD-054 **A single aromatic core mutation converts a designed 'primitive' protein from halophile to mesophile folding**

Connie Tenorio[1], Liam Longo[1], Ozan S. Kumru[2], C. Russell Middaugh[2], Michael Blaber[1]
1.-Department of Biomedical Sciences, Florida State University, 2.-Department of Pharmaceutical Chemistry, University of Kansas

Experiments in prebiotic protein design suggest that the origin of folded proteins may have favored halophile conditions. These results are consistent with salt induced peptide formation which shows that polymerization of amino acids is also promoted by high salt concentrations. As a result of various origin of life studies, a consensus on which amino acids likely populated early earth has emerged. These residues were synthesized by abiotic chemical and physical processes from molecules present in the surrounding environment. The properties of the consensus set of common prebiotic amino acids (A,D,E,G,I,L,P,S,T,V) are compatible with known features of halophile proteins, meaning these proteins are only stable in the presence of high salt concentrations. The halophile environment, thus, has a number of compelling aspects with regard to the origin of structured polypeptides. Consequently, a proposed key step in evolution was, movement out of the halophile regime into a mesophile one commensurate with biosynthesis of “phase 2” amino acids – including the aromatic and basic amino acids. We tested the effects of aromatic residue addition to the core of a “primitive” designed protein enriched for the prebiotic amino acids (A, D, E, G, I, L, P, S, T, V) that required halophilic conditions for folding. The subsequent results show that the inclusion of just a single aromatic residue was sufficient for movement to a mesophile folding environment. Thus, the inclusion of aromatic residues into the codon table could have conferred key stability to early proteins enabling adaptive radiation outside of a halophile environment.

PD-055 **From Sequence Data to Protein 3D Structure Using Evolutionary Couplings**

Robert Fieldhouse[1,2], Sikander Hayat[1,2], Robert Sheridan[1], Debora Marks[2], Chris Sander[1]
1.-Computational Biology Center, Memorial Sloan Kettering Cancer Center, 2.-Systems Biology, Harvard Medical School

Contact prediction methods that rely on sequence information alone, such as EVfold, can be used for de novo 3D structure prediction and identification of functionally important residues in proteins. Large multiple sequence alignments of protein families consisting of evolutionarily related and plausibly isostructural members reveal co-variation patterns that can be used to identify interactions between pairs of amino acids. We use a global probability model to disambiguate direct and indirect correlations. Specifically, we use a maximum entropy approach called pseudo-likelihood maximization (PLM) to distinguish causation (residue interactions) from correlation (correlated mutations) and compute evolutionary couplings (ECs). The inferred set of residue interactions can then be interpreted as physical contacts and used in de novo 3D structure prediction. Furthermore, the interactions that are inferred can help guide experiments that measure the phenotypic consequences of protein substitutions, making the method useful for functional studies. The present work can be divided into three areas: (i) methodological improvements related to alignment, folding procedure, structure refinement and ranking; (ii) folding of proteins of known structure for benchmarking and prediction of proteins of unknown structure; and (iii) focused exploration of specific cases of interest.

PD-056 **Developing SHuffle as a platform for expression and engineering of antibodies**

Na Ke[1], Alana Ali-Reynolds[1], Bryce Causey[1], Berkmen Berkmen[1]
1.-New England Biolabs

SHuffle is a genetically engineered E.coli strain that allows disulfide bond formationin its cytoplasm with high fidelity. Many proteins containing disulfide bonds have been successfully expressed in SHuffle. In this study, we expressed, purified and characterized full-length monoclonal antibody IgG in SHuffle. For the first time, a full-length IgG can be functionally expressed in the cytoplasm compartment of an E.coli strain. In order to improve the folding and assembly of IgG, we have investigated the expression of IgG in various formats and vectors; we have co-expressed chaperones and other helper proteins with IgG. Several-fold increase in the yield of full-length IgG was observed. We characterized the SHuffle produced IgG and found it comparable to hybridoma produced IgG. Optimization of fermentation conditions for a large-scale production is in progress. We aim to develop SHuffle as an easy, fast, robust platform for antibody engineering, screening and expression.

PE-001 **Experimental and computational studies of the effects of highly concentrated solutes on proteins: Insights into the causes and consequences of quinary protein structure and cytoplasmic organization**

Luciano Abriata[1], Matteo Dal Peraro[1]
1.-École Polytechnique Fédérale de Lausanne

Most studies of protein structure and function focus on pure, diluted samples; however, real-world biochemistry and typical biotechnological applications of proteins take place in complex media with very high concentrations of solutes (100-400 g/L) of varied size and chemical nature. On one side, this has recently fostered the study of proteins in vivo, in cell, or at least in media mimicking the native conditions. On the other hand, physical chemistry has for a long time studied the general effects of crowded and viscous conditions on proteins, looking mainly at coarse traits like diffusion and stability. But the general effects on traits relevant at atomic/residue resolutions have been less studied, and one fundamental issue remains unsolved: to what extent are proteins forced into interactions with highly concentrated solutes, and with what direct consequences? I will present here our ongoing efforts to dissect the fine effects of high solute concentrations and macromolecular crowding on proteins, based on NMR experiments and MD simulations, two complementary techniques of high spatial and temporal resolutions. Our results show that smaller solutes are prone to extensive interactions with proteins when at high concentrations while large solutes act chiefly through excluded-volume effects. Overall, we observe location-specific perturbations of a protein's surface, its internal dynamics and internal dielectrics, and its hydration, all very dependently on the solute's size and chemical nature. Our results support the growing notion that proteins should be studied in native-like media, adding that not only macromolecular crowders but also small molecules should be considered in these studies. Last, the fact that high-concentration conditions affect far more than a protein's diffusion rate and stability suggests critical consequences of quinary protein structure and cytoplasmic organization on the regulation of proteins within cellular biochemistry.

PE-002 **Protein-ligand interactions**

Aldona Jelińska[1], Anna Lewandrowska[1], Robert Hołyst[1]
1.-Institute of Physical Chemistry Polish Academy of Sciences

We developed an analytical technique for the study of interactions of ligands (e.g. cefaclor, etodolac, sulindac) with most abundant blood protein (e.g. bovine serum albumin) using the Flow Injection Method. The experiments were conducted at high flow rates (31 cm/s) in a long (>15m), thin (250μm) and coiled capillaries. The compound of interest (10 μl) was injected into carrier phase, which moved by the Poisseule laminar flow. At the detection point we measure the concentration distribution of the analyte. The width of the final profile of the analyte concentration is inversely proportional to the effective diffusion coefficient of the analyte. From the differences between the widths of the concentration distribution of free and bound ligand we can determine value of the association constant. Acknowledgement: We thank the National Science Center for funding the project from the funds granted on the basis of the decision number: UMO-2012/07/B/ST4/01400 (Opus 4).

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PE-003 **Carbohydrate Binding Modules. Structural and thermodynamic study**

Benjamin Garcia[1], Patricia Cano Sánchez[1], Siseth Martínez-Caballero[1], Romina Rodríguez-Sanoja[1], Adela Rodríguez-Romero[1]

1.-Instituto de Química, UNAM, 2.-Instituto de Química, UNAM, 3.-Instituto de Química, UNAM, 4.-Instituto de Investigaciones Biomédicas, UNAM, 5.-Instituto de Química, UNAM

Carbohydrate binding modules (CBMs), which are defined as contiguous amino acid sequences within a carbohydrate-active enzyme, have been found in both hydrolytic and non-hydrolytic proteins and are classified into 71 families, according to their primary structure similarity. The characterization of CBMs by different methods has shown that these modules concentrate enzymes on the surface of polysaccharide substrates. It is thought that maintaining the enzyme in proximity with the substrate leads to more rapid degradation of the polysaccharide. Therefore, the study of these kinds of modules or domains is relevant, since they are involved in multiple processes in organisms, like signaling, defense and metabolism; and some of them are involved in allergenic responses. In the present work we studied two different models: The first one is a CBM of the family 26 from Lactobacillus amylovorus (LaCBM26) that binds starch These domains are present in a α-amylase like a repetitive tandem of five modules that are consecutive and do not present connectors. By means of ITC and using a single recombinant LaCBM26 domain we determined a Ka = 2.31x104 M-1 for β- cyclodextrin and a Ka = 8.54x104 M-1 for α-cyclodextrin. When the number of consecutive recombinant modules increased to three or five tandem modules, the Ka values increased to 106 M-1; however, these constants did not show an additive or a synergic effect. For these experiments we fitted the isotherms to different models and used different algorithms. Additionally, we used circular dichroism in the UV-far region to determine if there existed conformational changes upon binding of the cyclodextrin molecules to the different tandem modules. We could only observe slight changes in a positive band centered around 220-240 nm, which has been explained in terms of π-π; interactions of the aromatic residues at the binding site. These CBMs have been used as carriers for in vivo vaccine delivery and affinity tags. The second model is a hevein-like CBM of the family 18 present in a chitinase-like protein from Hevea brasiliensis (HbCBM18). Hevein is a lectin from H. brasiliensis that shows a 63% identity with HbCBM18. These CMBs are connected to the catalytic domain, in proteins such as chitinases, by a linker of approximately 10 residues. In these experiments we used fluorescence techniques to determine the affinity constants for chitotriose. We previously reported a Ka = 2.08x106 M-1 when using a HbCBM18 that has a Met residue at the N-terminal region. Besides the aromatic residues at the binding site, the Met residue also interacts with the ligand, as determined using crystallographic and docking techniques. The mutant HbCBM18-R5W that does not have the Met residue showed a Ka of 2.8x104 M-1 with chitotriose, similar to the value reported for hevein using ITC (Ka 1.42x104 M-1). Interestingly, there exists an isoform of the HbCBM18 that has a connector between one CBM18 and a half CBM18 (1.5xHbCBM18). This protein has a Ka of 6.7x105 M-1 with the same ligand.

PE-004 **Initiating vesicle formation at the Golgi complex: auto-regulation and protein interactions govern the Arf-GEFs Gea1 and Gea2**

Margaret Gustafson[1], J. Chris Fromme[1]
1.-Cornell University

Molecular decision-makers play critical roles in the effort to maintain efficient and accurate cellular functions. In the case of vesicular traffic at the Golgi complex, the decision to initiate vesicle formation is made by a set of guanine nucleotide exchange factors (GEFs) that activate the small GTPase Arf1, which is the master controller for the recruitment of cargos and coat proteins. Saccharomyces cerevisiae possess three Golgi Arf-GEFs, Gea1, Gea2, and Sec7, which work at distinct sub-compartments of the Golgi to activate Arf1 only when and where appropriate. In the case of Sec7 at the trans-Golgi network (TGN), this requires a positive feedback loop in which active Arf1 relieves autoinhibition of Sec7, as well as recruitment to the Golgi membrane and catalytic stimulation by signaling Rab GTPases. We know far less about the decision-making process for Gea1 and Gea2, which are responsible for retrograde traffic within the Golgi and to the endoplasmic reticulum. I have found that both Gea1 and Gea2 can bind membranes weakly in vitro, an ability which is counteracted by their C-terminal HDS3 domains. In addition, I have discovered membrane recruitment in vitro is aided by the Rab GTPase Ypt1. However, these interactions cannot fully explain the distinct localization patterns of Gea1, Gea2, and Sec7, as all three have been shown to be recruited by Ypt1, which is found throughout the Golgi. My work has revealed that in addition to the well-established distinct localization from Sec7, Gea1 also occupies different Golgi compartments from Gea2, so specific signals must exist which help the GEFs decide where to go. My current efforts focus on understanding the roles of the other domains of Gea1 and Gea2, identifying the signals which send them to different parts of the Golgi, and unraveling the different roles they play in vesicle trafficking pathways.

PE-005 **Sequence variation in Archaea through diversity-generating retroelements**

Sumit Handa[1], Blair G Paul[2], Kharissa L Shaw[1], David L Valentine[2], Partho Ghosh[1]
1.-Department of Chemistry and Biochemistry, University of California San Diego, 2.-Marine Science Institute, University of California

Protein diversification is an essential tool for the survival and evolution for various species. Diversity-generating retroelements (DGR) in bacteria is known to generate massive variation in DNA through an error prone reverse transcriptase and retrohoming, which leads to variation in protein sequence. Recent discovery of DGRs in intraterrestrial archaeal systems have opened an opportunity to study this massive sequence variation in third domain of life (Paul BG, et al. Nat. Comm.) Here, we present the first crystal structure of variable protein from archaea with ligand-binding pocket is surface exposed. Also, it has conserved C-type lectin (CLec) fold, as shown by previous work on variable proteins, major tropism determinant (Mtd) and Treponema variable protein A (TvpA) which bind ligands through the CLec fold. Despite weak sequence identities (10-15%) among these variable proteins, CLec fold was found to be conserved. This variable ligand-binding site for archaea variable proteins can potentially generate 1013 variants.

PE-006 **Studies of JMJD4-catalyzed oxidative modifications of eukaryotic release factor 1**

Suzana Markolovic[1], Ivanhoe K. H. Leung[2], Mathew L. Coleman[3], Timothy D. W. Claridge[1], Sarah E. Wilkins[1], Christopher J. Schofield[1]

1.-Chemistry Research Laboratory, Department of Chemistry, University of Oxford, 2.-School of Chemical Sciences, University of Auckland, 3.-School of Cancer Sciences, University of Birmingham

Protein synthesis is a dynamic process mediated by a variety of proteins and enzymes. Recent studies have shown that hydroxylation is a key post-translational modification involved in translation termination. In particular, the Fe(II)- and 2-oxoglutarate- dependent oxygenase, Jumonji domain-containing 4 (JMJD4), regulates translation termination via the carbon 4 hydroxylation of an invariant lysine residue, K63, of the eukaryotic release factor, eRF1. In eukaryotes, translation termination is mediated by a release factor complex that includes eRF1. eRF1 is comprised of three domains, and it is responsible for recognizing stop codons in mRNA transcripts before triggering polypeptide release from the ribosome. The lysine residue hydroxylated by JMJD4 falls within the N-terminal domain and more specifically within the highly conserved NIKS motif. This motif has been identified by cross-linking and mutagenesis studies to play an essential role in stop codon recognition. While hydroxylation of K63 by JMJD4 has been found to increase translational termination efficiency, the exact molecular mechanism by which hydroxylation influences termination remains unclear. This work aims to understand how hydroxylation of eRF1 affects translation termination by exploring the effect of hydroxylation on the structure, dynamics, stability, and binding of the N-terminal domain of eRF1 (eRF1-N) using mass spectrometry, protein NMR spectroscopy, circular dichroism and differential scanning fluorimetry. In our efforts to understand the effect of hydroxylation, an additional JMJD4-catalyzed modification, characterized by a +30 Da mass shift on K63, was identified in vitro. The effect of this modification on eRF1 was similarly explored. Our findings suggest that hydroxylation has no effect on the in-solution NMR structure of eRF1-N, which experiences chemical shift changes localized to the target lysine residue. Correspondingly, there are no significant differences in secondary structure content between wild type and hydroxylated eRF1-N. Hydroxylation was also found to have no effect on protein stability or dynamics. Interestingly however, the +30 Da modification appears to cause more significant chemical shift changes dispersed beyond the NIKS motif. This suggests a more global effect on the in-solution NMR structure despite the little differences observed in protein dynamics and secondary structure content. The +30 Da modification was also found to have a destabilizing effect on eRF1-N. Neither hydroxylated nor +30 Da modified eRF1-N exhibited differences in rRNA binding. While hydroxylation of eRF1 was found to have little effect on protein structure, dynamics, stability, or binding, the +30 Da modification has marked effects on protein structure and stability. Such differences suggest that this modification has the potential to play an important role in translation.

PE-007 **Functional and structural analysis of a GH20 ß-N-acetylglucosaminidase from the marine bacterium Vibrio harveyi**

Piyanat Meekrathok[1], Arthur T. Porfetye[2], Marco Bürger[2], Ingrid R. Vetter[2], Wipa Suginta[1]
1.-Biochemistry-Electrochemistry Research Unit, Suranaree University of Technology, 2.-Max Planck Institute of Molecular Physiology

Vibrio harveyi β-N-acetylglucosaminidase (so-called VhGlcNAcase) is a new member of the GH20 glycoside hydrolase family responsible for the complete degradation of chitin fragments, with N-acetylglucosamine (GlcNAc) monomers as the final products. However, the 3D structure of GlcNAcase is still unknown. In this study, crystal structure and function of GlcNAcase were investigated based on protein crystallography. Size-exclusion chromatography and the Native-PAGE were employed to verify the protein state of GlcNAcase in a native form and the acidic active-site residues were mutated using site-directed mutagenesis method. The effects of mutations on the binding and hydrolytic activities were studied by enzyme kinetics. To provide a structural basis of GlcNAcase, the wild-type enzyme was crystalized at 293 K using a solution containing 0.1 M sodium acetate pH 4.6 and 1.3 M sodium malonate and recorded X-ray data. The wild-type enzyme was crystallized within 3 days in the monoclinic crystal form, belonging to space group P21, with unit-cell parameters a = 90.2, b = 130.7, c = 98.5 Å. The crystal structures of V. harveyi GlcNAcase were solved and refined to highest resolution of 2.4 Å. Structural investigation revealed that GlcNAcase comprises three distinct domains, designated as the N-terminal carbohydrate-binding domain, the α+β topology domain and the TIM-barrel catalytic domain. The substrate binding groove of GlcNAcase is a small pocket, which is suitable to accommodate a short-chain chitooligosaccharide. Kinetic analysis revealed that a group of the adjacent D303-H373-E438 showed a significantly decreased activity as compared with the wild-type enzyme, and these residues might be important for enzyme catalysis.

PE-008 **Silencing the molecular timekeeper in human cancer**

Alicia Michael[1], Stacy Harvey[1], Patrick Sammons[1], Amanda Anderson[2], Hema Kopalle[1], Alison Banham[2], Carrie Partch[1]

1.-University of California - Santa Cruz, 2.-University of Oxford

The circadian clock coordinates temporal control of physiology by regulating the expression of at least 40% of the genome on a daily basis.1 Disruption of circadian rhythms through environmental stimuli (e.g. light at night) or genetic means can lead to the onset of diseases such as: diabetes, cardiovascular disease, premature aging and cancer.2–5 The circadian clock orchestrates global changes in transcriptional regulation via the bHLH-PAS transcription factor CLOCK:BMAL1. Pathways driven by other bHLH-PAS transcription factors have a homologous repressor that modulates activity on a tissue-specific basis, but none have been identified for CLOCK:BMAL1. We discovered that the cancer/testis antigen PASD1 fulfills this role to suppress circadian rhythms. PASD1 is evolutionarily related to CLOCK and interacts with the CLOCK:BMAL1 complex to repress transcriptional activation. Furthermore, deletion of one region, highly conserved with CLOCK Exon 19, alleviates repression by PASD1 to suggest that it utilizes molecular mimicry to interfere with CLOCK:BMAL1 function. Structural and biochemical studies of the direct interaction of PASD1 with the CLOCK:BMAL1 complex using recombinant protein expression and biophysical techniques are currently underway. As a cancer/testis antigen, expression of PASD1 is natively restricted to gametogenic tissues but can be upregulated in somatic tissues as a consequence of oncogenic transformation. Reducing PASD1 in human cancer cells significantly increases the amplitude of transcriptional oscillations to generate more robust circadian rhythms. Our work suggests that mechanisms to suppress circadian cycling can be hard-wired in a tissue-specific manner and our data show that they can be co-opted in cancer cells to attenuate clock function. 1. Zhang, R., Lahens, N. F., Ballance, H. I., Hughes, M. E. & Hogenesch, J. B. A circadian gene expression atlas in mammals: Implications for biology and medicine. Proc. Natl. Acad. Sci. 111 , 16219–16224 (2014). 2.Filipski, E. & Lévi, F. Circadian disruption in experimental cancer processes. Integr. Cancer Ther. 8, 298–302 (2009). 3. Jeyaraj, D. et al. Circadian rhythms govern cardiac repolarization and arrhythmogenesis. Nature 483, 96–99 (2012). 4. Kondratov, R. V, Kondratova, A. A., Gorbacheva, V. Y., Vykhovanets, O. V & Antoch, M. P. Early aging and age-related pathologies in mice deficient in BMAL1, the core componentof the circadian clock. Genes Dev. 20, 1868–1873 (2006). 5. Marcheva, B. et al. Disruption of the clock components CLOCK and BMAL1 leads to hypoinsulinaemia and diabetes. Nature 466, 627–631 (2010).

PE-009 **New insights into the interaction between IQGAP1 and Rho family proteins**

Kazem Nouri[1], Mohammad Reza Ahmadian[1]
1.-Medical faculty of the Heinrich-Heine University

The scaffolding protein IQGAP1 participates in various cellular functions such as cell-cell adhesion, cell polarization and migration, neuronal motility, and tumor cell invasion by binding to target proteins, including Rac1 and Cdc42, two members of the Rho family. To better understand the molecular basis of these interactions, we utilized in this study a novel time-resolved fluorescence spectroscopy to determine individual rate constants for IQGAP1 interaction with fourteen different Rho proteins. The results indicated that IQGAP1 binds among Rho proteins selectively to Rac- and Cdc42-like proteins only in a GTP-dependent manner. Moreover, the interaction of Rho proteins with the C-terminal half of IQGAP1 (GRD-C), shorter fragment contains GRD-GBD, only the GRD and also GRD-GBD with single and double phosphomimetic mutations S1441E and S1443D was performed. Obtained results showed that, when both GRD and GBD are existing, fluorescence changes is detected but for GRD alone or in the case of S1443D or S1441E/S1443D no change was observed, suggesting that GBD and specifically, cysteine 1443 is critical for this interaction. Furthermore, fluorescence polarization results showed that the GRD-C interact with Cdc42 and Rac1 but not with RhoA, and interestingly the GRD domain showed similar behavior, but with 10 to 15 folds lower affinity as compared with the GRD-C. Consistent with this, a GDP-bound form of Cdc42 showed interaction with both GRD and the GRD-C in quiet comparable affinities. At last, competition experiments utilizing interacting partners of Rac1, e.g. Tiam1, p50RhoGAP, Plexin-B1, p67phox, PAK1 and RhoGDIα, along with structural analysis, revealed two negative charged areas on the surface of Rho- and Rnd-like proteins, which might explain their inaccessible interaction with IQGAP1. The overlapping binding site of Cdc42 and Rac1 on the surface of IQGAP1 together with the kinetic details of the selective interaction of IQGAP1 with Rac- and Cdc42-like proteins suggests that these interactions are most likely mediated via the same mechanism.

PE-010 **Structural Characterization of the Tumor Suppressor ING5 as a Bivalent Reader of Histone H3 Trimethylated at Lysine 4**

Georgina Ormaza Hernandez[1], Jhon Alexander Rodríguez[1], Alain Ibáñez de Opakua[1], Nekane Merino[1], Maider Villate[1], Tammo Diercks[1], Pietro Roversi[2], Adriana L. Rojas[1], Francisco J. Blanco[1,3]

1.-CIC bioGUNE, Structural Biology Unit, 2.-Oxford University, Department of Biochemistry, 3.-IKERBASQUE, Basque Foundation for Science

The INhibitor of Growth (ING) family of tumor suppressors consists of five homologous proteins that regulate the transcriptional state of chromatin by recruiting histone acetyl transferase and histone deacetylase complexes to sites with histone H3 trimethylated at K4 (H3K4me3)1. This modification is recognized by the Plant HomeoDomain (PHD) present at the C-terminus of the five ING proteins2. ING4 dimerizes through its N-terminal domain, with a symmetric antiparallel coiled-coil structure3, making it a bivalent reader of the H3K4me3 mark. ING5 is highly homologous with ING4, but forms part of a different histone acetyl transferase complex1. Here, we show that ING5 is also a dimer and thus a bivalent reader of the H3K4me3 mark. However, the crystal structure of the N-terminal domain of ING5 shows an asymmetric dimer, different from the homologous ING4 domain. Our NMR data (backbone assignment and paramagnetic relaxation effects) and SAXS data indicate that the structure of the N-terminal domain of ING5 in solution is similar to ING4, suggesting that the crystal structure of ING5 is likely a crystallization artifact. Three point mutations in the N-terminal domain of ING5 have been described in oral squamous cell carcinoma: Q33R, I68V, and C75R4. We have found that the N-terminal domains of the three mutants are dimeric coiled-coils but with different stability, as measured by thermal denaturation. While the Q33R mutant is as stable as the wild type, the I68V and C75R mutants are strongly destabilized, suggesting a role in cancer development at least for these two mutants. References: [1] Y. Doyon et al. (2006) Mol Cell 21, 51-64. [2] P.V. Peña et al. (2006) Nature 442, 100-3. [3] S. Culurgioni et al (2012) J. Biol. Chem. 287(14),10876-84 [4] B. Cengiz eta al (2010) Int J Cancer 127(9), 2088-2094.

PE-011 **ABELSON TYROSINE KINASE, A NEW ENZYME TARGET FOR ALZHEIMER'S DISEASE: EXPLORING MULTIPLE E-PHARMOACOPHORE MODELING, VIRTUAL SCREENING, SELECTIVITY ASSESSMENT FOR POTENTIAL INHIBITORS**

Ravichand Palakurti[1], Ramakrishna Vadrevu[1]
1.-Department of Biological Sciences, BITS-PILANI HYDERABAD CAMPUS

Efforts so far, to combat Alzheimer's disease (AD) have focused predominantly on inhibiting the activity of enzyme(s) that are responsible for the production of the main causative beta amyloid forming peptide. However, the inherent complexity associated with the network of pathways leading to the progress of the disease may involve additional targets for designing effective therapies. Recent experimental findings have identified Abelson's Tyrosine Kinase (c-Abl), a non-receptor kinase involved in a variety of cellular functions as a new target for AD. In the present study we employed energy optimized multiple pharmacophore modeling strategy from multiple c-Abl structures bound with ligands in the inactive ATP binding conformation. Virtual screening followed by docking of molecules from ChemBridge_CNS database, and Maybridge databases resulted in the identification of 15 best scoring molecules. Based on docking score and selectivity assessment and druggability parameters, four out of the 15 molecules are predicted to show increased specificity for c-Abl in comparison to closely related kinases. Given the implied role of c-Abl not only in AD but in Parkinson's disease, the identified compounds may serve as leads to be developed as effective neurotherapeutics.

PE-012 **The Role of Syndecans in Melanocortin Signaling and Energy Balance**

Rafael Palomino[1], Glenn Millhauser[2], Pietro Sanna[2]
1.-University of California Santa Cruz, 2.-The Scripps Research Institute

The central melanocortin system is recognized as a key regulator of energy balance and appetite. The hypothalamic melanocortin receptor, MC4R, is a G-protein coupled receptor that is antagonized by the peptide ligand, agouti-related peptide (AgRP), leading to increased feeding and weight gain. While much research has gone into how this ligand exerts its effects at the receptor, less is known regarding non-melanocortin components of the pathway. Syndecan-3, a heparan sulfate proteoglycan, has previously been implicated in potentiating AgRP antagonism, however details of this interaction are unclear. This work aims to investigate the role of syndecans at both a molecular level and in vivo. We hypothesize that AgRP binds the glycosaminoglycan (GAG) components of syndecans, and that this interaction increases the local concentration of the peptide near MC4R. Furthermore, we have previously shown that designed positive charge mutations to AgRP lead to increased in vivo efficacy that is independent of MC4R activity, and we hypothesize that this is due to greater affinity for the negatively charged GAGs. Using isothermal titration calorimetry we have shown tight binding between AgRP and heparan sulfate, the major GAG component of syndecan-3, and this affinity is strengthened by additional peptide positive charge. Through NMR, we see that both positively charged and polar residues are necessary for binding various heparan sulfate polymers. These data implicate a specific region of AgRP that is not required for MC4R binding as being necessary in its role as a heparan sulfate binding protein. Expanding on these findings, we are now using a syndecan knockout mouse line to explore the mechanism of differential feeding in our designed mutants. Preliminary results indicate a reduction in weight gain in knockouts compared to their wildtype littermates post peptide administration. Collectively, these data show that the physiologically relevant form of AgRP, previously considered unable to interact with syndecans, is indeed a heparan sulfate binding protein. Furthermore, our designed mutants have differential affinities for GAGs, with increased affinity correlating to increased feeding potency. Finally, as the MC4R pathway is thought to be a viable target for wasting disorders such as cachexia, we are interested in leveraging this data to improve the potency and stability of our designed AgRP mutants. Taken together, this work aims to develop new insights and probe the therapeutic potential of a critical metabolic pathway.

PE-013 **Evidence of a proteolytic phenomenon in the starch binding domain of the α-amylase from Lactobacillus amylovorus**

Zaira Esmeralda Sánchez Cuapio[1], Alejandra Hernández Santoyo[2], Sergio Sánchez Esquivel[1], Romina Rodríguez Sanoja[1]1.-Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, 2.-Instituto de Química, Universidad Nacional Autónoma de México

α-amylases are glycoside-hydrolases that catalyze the hydrolysis of internal α-1,4 glycosidic bonds in starch and glycogen generating smaller oligosaccharides (1). These multidomain proteins contain a catalytic barrel (β/α)8 and, in some cases, one or more non-catalytic domains whose function is generally described as carbohydrate binding module (CBM) and particularly as starch-binding domains (SBD). The SBD can bind granular starch increasing the local concentration of substrate at the active site of the enzyme and may also disrupt the structure of the starch surface (2). The α-amylase from Lactobacillus amylovorus has a structure that consists of a catalytic domain (CD) and an unusual carboxy-terminal starch-binding domain with 5 identical CBMs (belonging to family 26) in tandem (3). Each repeat acts as an independent fixing module with an additive or synergic effect between the units (4). When we stored pure SBD from L. amylovorus we found multiple forms of low molecular weight with a constant pattern, which does not correspond to random degradation. Interestingly, when the protein is stored at pH close to 5 and EDTA is added, such proteolysis appears to decrease. So far, there is little information about the proteolytic process of amylases and the nature of it. Here we show that divalent ions induce a proteolytic cleavage of the SBD, raising the possibility of an autoproteolytic activity. Acknowledgments This work is supported by grants PAPIIT IN222113-3 and CONACyT 131149. Sánchez Cuapio Z is supported by a personal grant from Consejo Nacional de Ciencia y Tecnología, México. References 1. Tibbot B., Wong D., Robertson G. & G. (2002). Biologia Bratislava. 11:229- 238. 2. R. Rodríguez-Sanoja,* B. Ruiz, J. P. Guyot, and S. Sanchez (2005). Appl Environ Microbiol. 71(1): 297–302. 3. Rodríguez Sanoja, R., Morlon-Guyot, J., Jore, J., Pintado, J., Juge, N. & Guyot, J.P. (2000). Appl. Environ. Microbiol. 66: 3350-3356. 4. Guillén, D., Santiago, M., Linares, L., Pérez, R., Morlon, J., Sánchez, S. and Rodríguez-Sanoja, R. (2007) Appl. Environ. Microbiol. 73:3833-3837.

PE-014 **In Situ Membrane Protein Structure and Function Analysis using Site-Specific Unnatural Amino Acid Incorporation and Spectroscopy Methods**

Changlin Tian[1]

1.-University of Science and Technology of China

Unnatural amino acid and related methods provided a special mechanism to implement site-specific spectroscopy active probe incorporation in a specific membrane protein in cells. The site specific incorporation resulted in a single signal during acquisition, resulting in unambiguous signal assignment. The protein specific labeling makes it possible for in situ membrane protein analysis using NMR or fluorescence detection. The 19F containing unnatural amino acid incorporation has been applied for dynamic studies of transporters in native lipid membrane, and the phosphorylation quantification analysis for Tyrosine kinase in native lipid membrane with the aid of lipodisc. The fluorescent unnatural amino acid incorporation enabled the site-specific channel responses analysis upon ligand binding in a single cell.

PE-015 **Theoretical Volume Profiles as a Tool for Probing Protein Folding Kinetics**

Heather Wiebe[1], Noham Weinberg[1,2]
1.-Department of Chemistry, Simon Fraser University, 2.-Department of Chemistry, University of the Fraser Valley

The mechanism by which conformational changes, particularly folding and unfolding, occur in proteins and other biopolymers has been widely discussed in the literature. Molecular dynamics (MD) simulations of protein folding present a formidable challenge since these conformational changes occur on a time scale much longer than what can be afforded at the current level of computational technology. Transition state (TS) theory offers a more economic description of kinetic properties of a reaction system by relating them to the properties of the TS, or for flexible systems, the TS ensemble (TSE). The application of TS theory to protein folding is limited by ambiguity in the definition of the TSE, although the experimentally observed first-order kinetics for folding of small single-domain proteins lends itself to interpretation by this theory. The pressure dependences of the folding rate constant can be used to obtain activation energies and activation volumes, which are rationalized as the properties of the folding TSE. The large amount of activation volume data in the literature has gone largely uninterpreted at the quantitative level. We propose to utilize this data in conjunction with MD-calculated volumetric properties to identify the TSE for protein folding. The effect of pressure on reaction rates is expressed in terms of logarithmic pressure derivatives, known as activation volumes. According to TS theory, activation volumes can be identified as the difference in volume between the TS and reactant species:

$-RT(\partial \ln k / \partial P)T = \Delta V‡ = VR$

Activation volumes $\Delta V‡$ have been experimentally determined for the folding of several proteins. The concept of activation volume can be extended to that of a volume profile, $\Delta V(y)$, which describes how the volume of a system changes along reaction coordinate y. If the position $y‡$ of the TS along the reaction coordinate is unknown, it can be found by locating $\Delta V‡$ on the volume profile:

$\Delta V(y‡) = \Delta V‡.$

Such volume profiles can be built using our recently developed MD-based displacement volume method.* Using this method, volumes of single molecules can be calculated by taking the difference between the volume of pure solvent and solvent containing the desired solute. This method takes into account the strength and type of solvent-solute interactions as well as the geometrical configuration of the solute. In this work, we present the successful application of this method to several conformationally flexible systems.

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PE-016 **Structure of the P15PAF/PCNA complex and implications for clamp sliding on the DNA during replication and repair**

Alfredo De Biasio[1], Alain Ibáñez de Opakua[1], Gulnahar Mortuza[2], Rafael Molina[3], Tiago Cordeiro[4], Francisco Castillo[5], David Gil-Cartón[1], Pau Bernadó[4], Guillermo Montoya[2], Francisco Blanco[1,6]

1.-Structural Biology Unit, CIC bioGUNE, Derio, Spain, 2.-Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, 3.-Structural Biology and Biocomputing Programme, CNIO, Madrid, Spain, 4.-Centre de Biochimie Structurale, Université Montpellier 1 and 2, France, 5.-Department of Physical Chemistry, Universidad de Granada, Spain, 6.-IKERBASQUE, Basque Foundation for Science

The intrinsically disordered protein p15PAF is overexpressed in cancer and regulates DNA replication and repair by binding to the proliferating cell nuclear antigen (PCNA) sliding clamp [1,2,3]. We have characterized the structure of the human p15PAF/PCNA complex by NMR, crystallography, and computational modeling [4]. The central PCNA interacting protein motif (PIP-box) of p15PAF is tightly bound to the canonical PIP-box binding groove on the PCNA front face. In contrast to other PCNA interacting proteins, however, p15PAF also contacts the inside of, and passes through, the PCNA ring. The mostly disordered p15PAF chain termini thus emerge at opposite faces of the ring, but remain protected from degradation by the 20S core proteasome. We also unveil a novel DNA binding activity of p15PAF, both free and bound to PCNA, which is mainly mediated by its conserved histone-like N-terminal tail. Molecular modeling shows that a ternary complex with a duplex DNA inside the PCNA ring is energetically feasible and our electron micrographs show increased density inside the ring. We propose that p15PAF acts as a flexible drag that regulates PCNA sliding along the DNA, and may facilitate the switch from replicative to translesion synthesis polymerase binding upon DNA damage. Acknowledgements: This work has been mainly sponsored by MINECO grant CTQ2011-28680 and Juan de la Cierva-2010 contract to Alfredo De Biasio. References: [1] Alfredo De Biasio, et al. p15(PAF) Is an Intrinsically Disordered Protein with Nonrandom Structural Preferences at Sites of Interaction with Other Proteins. Biophys J 106, 4, 865-74, 2014. [2] Chanlu Xie et al. Proliferating cell unclear antigen-associated factor (PAF15): A novel oncogene. Int J Biochem Cell Biol 50, 127-31, 2014. [3] Alfredo De Biasio and Francisco J. Blanco. Proliferating cell nuclear antigen structure and interactions: too many partners for one dancer? Adv Protein Chem Struct Biol 91, 1-36, 2013. [4] Alfredo De Biasio et al. Structure of p15(PAF)-PCNA complex and implications for clamp sliding during DNA replication and repair. Nat Commun. 12, 6, 6439, 2015.

PE-017 **DHRS7 enzyme – important player in human health and diseases?**

Lucie Zemanova[1], Hana Stambergova[1], Tereza Lundova[1], Rudolf Andrys[1], Jiri Vondrasek[2], Vladimir Wsol[1]

1.-Faculty Of Pharmacy in Hradec Kralove, Charles University in Prague, 2.-Institute of Organic Chemistry and Biochemistry, AS CR

Metabolic syndrome (MetS) is one of the leading causes of the death worldwide; however, exact pathophysiological mechanisms of MetS remain largely unknown. Growing evidence suggests that the increased availability of glucocorticoids at the tissue level play an important in MetS development. One of the major determinants of glucocorticoid local action seems to be the enzyme 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1). This enzyme is a well-known member of the short-chain dehydrogenase/reductase (SDR) superfamily. It is an important carbonyl reducing enzyme that, besides its role fine-tuning of glucocorticoids actions, is involved in the biotransformation of drugs and in the development of lung cancer through metabolism of the tobacco specific carcinogen NNK. The phylogenetically closest relative of 11β-HSD1 is DHRS7 enzyme from the same superfamily. Unlike 11β-HSD1, DHRS7 is poorly characterized however it can be supposed at least partially overlapping function to 11β-HSD1. Moreover its possible association with similar pathological conditions in human as 11β-HSD1 has already been indicated by several studies. The aim of this study is the basic biochemical characterization of DHRS7. The enzyme is a member of cluster 3 of "classical" SDR; such members are considered to be retinoid and steroid metabolizing enzymes, so characterization the enzyme was based on this assumption. DHRS7 was prepared in recombinant form in the Sf9 cell line. It was proved that this enzymes is an integral membrane-bound enzyme localized in the endoplasmic reticulum with luminal orientation, similarly to 11β-HSD1. Known substrates of 11β-HSD1 and related enzymes were tested also as substrates of DHRS7. It was proved that DHRS7 is NADPH-dependent reductase with important substrates as steroid hormones cortisone and androstene-3,14-dione, all-trans-retinal and also xenobiotics as 1,2-naphtoquinone or carcinogen NNK at least in vitro. For better understanding of the catalytic function of DHRS7 its structural model was prepared and it is used also for the identification of additional substrates by ligand virtual screening. DHRS7 enzyme is expressed in several human tissues as adrenals, liver, prostate, small intestine and kidney. These brand new initial results point to the possible involvement of DHRS7 in important cellular processes that deserve further investigation. These results will lay the foundation for an understanding of DHRS7 role in human physiology resp. pathophysiology. This project was supported by Grant Agency of Charles University (677012/C/2012) and UNCE 204026/2012).

PE-018 **Structure-based functional identification of Helicobacter pylori HP0268 as a nuclease with both DNA nicking and Rnase activities**

Bong-Jin Lee[1], Ki-Young Lee[1]
1.-College of Pharmacy, Seoul National University

HP0268 is a conserved, uncharacterized protein from Helicobacter pylori. Here, we determined the solution structure of HP0268 using three-dimensional nuclear magnetic resonance (NMR) spectroscopy, revealing that this protein is structurally most similar to a small MutS-related (SMR) domain that exhibits nicking endonuclease activity. We also demonstrated for the first time that HP0268 is a nicking endonuclease and a purine-specific ribonuclease through gel electrophoresis and fluorescence spectroscopy. The nuclease activities for DNA and RNA were maximally increased by Mn(2+) and Mg(2+) ions, respectively, and decreased by Cu(2+) ions. Using NMR chemical shift perturbations, the metal and nucleotide binding sites of HP0268 were determined to be spatially divided but close to each other. The lysine residues (Lys7, Lys11 and Lys43) are clustered and form the nucleotide binding site. Moreover, site-directed mutagenesis was used to define the catalytic active site of HP0268, revealing that this site contains two acidic residues, Asp50 and Glu54, in the metal binding site. The nucleotide binding and active sites are not conserved in the structural homologues of HP0268. This study will contribute to improving our understanding of the structure and functionality of a wide spectrum of nucleases.

PE-019 **High-fidelity recombinant protein production in a silkworm bioreactor**

Sungjo Park[1], In-Wook Hwang[1], Tatsuya Kato[2], Enoch Park[2], Andre Terzic[1]
1.-Center for Regenerative Medicine, Mayo Clinic, 2.-Laboratory of Biotechnology, Shizuoka University

The domesticated silkworm, Bombyx mori, is an attractive host naturally equipped with a proficient post-translational modification machinery adequate to fulfill stringent demands of authentic recombinant protein production. Silkworm-based protein expression has originally relied on a prototype baculovirus vector system that employs silkworm as a bioreactor in place of more traditional cell lines. Recent development of the silkworm trophic B. mori nucleopolyhedrovirus (BmNPV) bacmid launches a second generation of silkworm-based protein production technology. Introducing the recombinant bacmid DNA into silkworms expedites heterologous protein expression by eliminating prior virus construction and amplification steps. Salient examples of heterologous eukaryotic proteins produced in silkworms are acetyl-CoA carboxylase 2, malonyl-CoA decarboxylase, Spot14/Mig12 heterodimer and α2,6-sialyltransferase with consistent high levels of protein expression. Thus, equipped with a fail-safe post-translational modification machinery, eukaryotic proteins are readily bioengineered using a silkworm-based protein expression platform.

PE-020 **Studies exploring potential applications of synthetic antifreeze proteins in the frozen food industry**

Ho Zee (Charles) Kong[1], Conrad Perera[1], Ivanhoe Leung[1], Nazimah Hamid[2], Viji Sarojini[1]
1.-School of Chemical Sciences, The University of Auckland., 2.-School of Applied Sciences, Auckland University of Technology

In nature, certain species of plants, insects and fish produce a group of antifreeze glycoproteins and polypeptides which enable them to survive the freezing temperatures of their natural habitat. These naturally occurring antifreeze proteins (AFPs) were first discovered in polar fishes such as antarctic notothenioids and winter flounder. These AFPs have the ability to bind to ice crystals and restrict their size and morphology; decrease the freezing point of water and inhibit the ice recrystallization processes. Ice crystal formation is of primary concern to the frozen food industry, as ice crystal formation during freezing can be disruptive to and cause damage to the cellular structures in food. The unique properties of AFPs can be developed into a potential solution to minimize freeze-thaw damage to frozen food. A number of tailor made synthetic analogues based on the naturally occurring AFPs were successfully designed and synthesized. Antifreeze activity studies of the AFPs were carried out using the Clifton nanoliter osmometer attached with a microscope. The AFPs exhibited thermal hysteresis as well as modification of ice crystal morphology, confirming their antifreeze activity in vitro. The ability of these synthetic AFPs in preserving the texture and structure of frozen food was evaluated using the techniques of scanning electron microscopy. The AFPs showed great potential to preserve the cellular structures of frozen food samples during freeze-thaw process. Additionally, secondary structure analysis of the AFPs was carried out using circular dichroism. This presentation will summarize our current results on the design, synthesis and anti-freeze activity analysis of the synthetic AFPs.

PE-021 **Development of Fungal-Specific Calcineurin Inhibitors Based on Molecular Structure and Dynamics**

Ronald Venters[1], Leonard Spicer[1,2], Joseph Heitman[3], William Steinbach[4], Praveen Juvvadi[4], Maria Schumacher[2]

1.-Duke University NMR Center, 2.-Duke University Biochemistry Department, 3.-Duke University Department of Molecular Genetics and Microbiology, 4.-Duke University Department of Pediatrics

Invasive fungal infections remain a leading cause of death in immunocompromised patients. Current antifungal agents have a host of issues including limited efficacy, host toxicity and an alarming increase in resistance. Current research in our laboratories is focused on targeting the calcineurin signaling pathway that has been shown to be required for fungal pathogenesis. Calcineurin is a highly conserved serine-threonine-specific Ca2+-calmodulin-activated phosphatase important in mediating fungal pathogenesis and stress responses. It is a key regulator of a signal transduction network required for survival of the most common pathogenic fungi in humans, making it an ideal target for fungal drug development. Calcineurin is a heterodimer of a catalytic (A) and regulatory (B) subunit. Phosphatase activity requires association of the two subunits. Calcineurin is also the target of the immunosuppressant FK506, which functions as an inhibitor by first complexing with the peptidyl-prolyl cis-trans isomerase immunophilin, FKBP12. The FKBP12-FK506 complex subsequently binds to calcineurin in a groove between the A and B subunits and inhibits its activity. Although fungal calcineurins are targeted by FK506, it also targets mammalian calcineurin and is thus immunosuppressive in the host. In order to improve therapeutic efficacy, we have undertaken a unique effort that utilizes both structural biology and molecular mycology in an effort to overcome the fungal versus human specificity barrier. The NMR studies to be presented here have been focused on determining the resonance assignments and solution structures for the FKBP12 proteins from the pathogenic fungi Candida albicans, Candida glabrata and Aspergillus fumigatus. Notably, the X-ray crystallography structures of the wild-type Candida albicans and Aspergillus fumigatus FKBP12 proteins revealed an intriguing intermolecular interaction involving four residues in the 80's loop including Pro104 (in C. albicans) and Pro90 (in A. fumigatus) which are stabilized in the cis conformation. These data suggest that the protein might use itself as an enzyme substrate. In efforts to establish if this interaction remains in a solution environment, we have determined the NMR structure and measured the T2 relaxation rates for the wild-type A. fumigatus FKBP12 protein and for the P90G mutant variant that adopts a dramatically different orientation of the 80's loop and does not form an intermolecular interaction in the crystal structure. The NMR chemical shift data indicate that, while the remainder of the protein structure remains unchanged, the 80's loops in the two variants are indeed different. In addition, the T2 relaxation rates of the residues in this region are dramatically dissimilar in the two variants, but remain identical throughout the rest of the protein. We have also begun inhibitor binding studies of all of the FKBP12 proteins from each of the pathogens by titrating the FK506 inhibitor into native and mutant FKBP12 proteins in order to examine conformational changes associated in the protein upon complex formation. Using this approach we plan to determine the relative Kd values for binding of each inhibitor to the FKBP12 protein from each pathogen for comparison of binding proclivities.

PE-022 **Lupin (Lupinus angustifolius L.) b-conglutin proteins: Structure functional features, catalytic mechanism modeling and cross-allergenicity identification using protein threading and molecular docking methods**

Jose C. Jimenez-Lopez[1,2]

1.-Department of Biochemistry, Cell and Molecular Biology of Plants; EEZ-CSIC, 2.-The UWA Institute of Agriculture; The University of Western Australia

Lupin is an important PULSE, which displays a wide range of benefits in agriculture, particularly these involved in possible plant pathogen suppression. Furthermore, lupin seed proteins promote different positive health aspects, preventing cardiovascular disease, and reduction of glucose and cholesterol blood levels. “Sweet lupine” seeds seem to be promising as a source of innovative food ingredients due to averaged protein content similar to soybean and an adequate composition of essential amino acids. Thus, lupin seeds may be important source of proteins for human and animal consumption. However, and as drawback feature, the number of allergic people to lupin seed proteins is rising, becoming a serious and a growing problem in the Western world, because of the rapid introduction of lupin seeds as new ingredients in traditional and novel foods. The goals of this study are the characterization the structure-functional properties of Lupinus angustifolius L or Narrow leafed lupin (NLL) β-conglutin proteins, with a focus in its catalytic mechanism, and its molecular cross-allergenicity with other legumes, i.e. peanut, by extensive analysis using different computer-aided molecular approaches covering (i) physicochemical properties and functional-regulatory motifs, (ii) sequence analysis, 2-D and 3D structural (threading) modeling comparative study and molecular docking, (iii) conservational and evolutionary analysis, (iv) catalytic mechanism modeling, and (v) sequence, structure-docking based β-cell epitopes prediction, while T-cell epitopes were predicted by inhibitory concentration and binding score methods. β-conglutins (vicilin-like or 7S proteins) are seed proteins typically found in reserve tissues (endosperm and cotyledon). They belong to the Cupin superfamily of proteins, containing a globular domain constituted by a conserved b-barrel. Two barrels were found in all β-conglutin protein isoforms and an additional mobile N-terminal arm constituted bye α-helices. Molecular modeling analysis has shown that one of this barrel contain a semi-conserved metal binding motive (HYX…R), typically found in Oxalate oxidase (OXOX) enzymes. Interestingly, our results revealed considerable structural differences between β-conglutin isoforms, particularly affecting 2-D elements (loops and coils), and numerous micro-heterogeneities are present in fundamental residues directly involved in epitopes variability, which might be a major contributor to the observed differences in cross-reactivity among legumes. We also identified multiple forms of β-conglutins polypeptides ranging from 15-80kDa, with IgE-binding characteristics in atopic patients. Thus, β-conglutins might be considered as major allergen in different species of lupin, including the “sweet lupin” group, since several of these polypeptides were recognized by human IgEs, having the potential to trigger an immune response leading to allergy symptoms. Acknowledgements: This research was funded by the European Research Program MARIE CURIE (FP7-PEOPLE-2011-IOF) under the grant ref. number PIOF-GA-2011-301550.

PE-023 **Intracellular pH and quinary structure**

Rachel Cohen[1], Gary Pielak[1,2,3]

1.-Department of Chemistry, University of North Carolina, 2.-Department of Biochemistry and Biophysics, University of North Carolina, 3.-Lineberger Comprehensive Cancer Center, University of North Carolina

NMR spectroscopy can provide information about proteins in living cells. pH is an important characteristic of the intracellular environment because it modulates key protein properties such as net charge and stability. Here, we show that pH modulates quinary interactions, the weak, ubiquitous interactions between proteins and other cellular macromolecules. We used the K10H variant of the B domain of protein G (GB1, 6.2 kDa) as a pH reporter in Escherichia coli cells. By controlling the intracellular pH, we show that quinary interactions influence the quality of in-cell 15N-1H HSQC NMR spectra. At low pH, the quality is degraded because of the increased number of attractive interactions between E. coli proteins and GB1, which slows GB1 tumbling and broadens its crosspeaks. Our results demonstrate the importance of quinary interactions for furthering our understanding of protein chemistry in living cells.

PE-024 **Advanced analytical tools for monitoring and control in production processes of recombinant hemagglutinin influenza vaccine**

Joanna Szewczak[1], Weronika Surmacz-Chwedoruk[1], Bożena Tejchman-Małecka [1], Jacek Stadnik[1], Grażyna Tronczyńska-Lubowicz [1], Agnieszka Romanik-Chruścielewska [1], Jarosław Antosik[1], Piotr Borowicz[1], Iwona Sokołowska [1], Dorota Stadnik[1],
1.-Institute of Biotechnnology and Antibiotics

Influenza virus is one of the most prevalent pathogens causing respiratory illness which often leads to serious post influenza complications such as pneumonia and myocarditis. Some viruses, as the avian influenza H5N1, are especially dangerous and draw special attention of WHO. This highly pathogenic virus spreads quickly among domestic poultry and wild birds resulting in high mortality. What is more distressing, the H5N1 virus may be transmitted to humans. Because of antigenic drift it is impossible to deliver an effective vaccine against all subtypes of the H5N1 virus. Moreover, traditional egg-based production of influenza vaccines is time- and cost-consuming, what makes it inadequate in case of a pandemic. Hence, we have developed an efficient production process of influenza vaccine based on a recombinant hemagglutinin antigen (rHA). Recombinant vaccines underlay strict regulations and quality requirements. The purpose of this work was to develop a battery of analytical methods that allow to evaluate key quality attributes of rHA on each stage of production. At first, we have focused on rHA structure as a crucial issue for its activity. The primary structure of rHA was confirmed by peptide mapping and TOF/TOF fragmentation (HPLC, MALDI TOF/TOF). Furthermore, FTIR analysis was used to evaluate the secondary structure of the protein. The disulfide bonds, which stabilize the tertiary structure, were assigned by peptide mapping. Additionally, free thiols were measured using Ellman's reagent. Moreover, we have employed RP-HPLC, SEC-MALS and DLS to explore oligomerization of rHA. These techniques appeared to be useful not only to confirm existence of native oligomers, but also to find and discard misfolded fraction, aggregates and truncated forms. In addition, two analytical methods (RP-HPLC and CGE) were developed to assess the purity of rHA as required by ICH guidelines. We also have determined isoelectric point and heterogeneity of rHA by cIEF. Afterward, developed methods were applied in the stability studies that provide a valuable insight into a chemical degradation process and conformational changes of rHA during storage. This work was supported by Innovative Economy Operational Program, Grant No. WND-POIG.01.01.02-00-007/08-00 as a part of project “Centre of medicinal product biotechnology. Package of innovative biopharmaceuticals for human and animal therapy and prophylactics.”

PE-025 **Muscle cell atrophy via HSP gene silencing was counteracted by celastrol-mediated HSP overexpression**

Inho Choi[1], Taesik Gwag[1], Kyoungsook Park[2], Kyoungbong Ha[1], Joo-hee Lee[3], Youn-Kyu Kim[3]
1.-College of Science and Technology, Yonsei University, 2.-Molecular Therapy Research Center, Sungkyunkwan University, 3.-Korea Aerospace Research Institute

Molecular chaperone heat shock proteins (HSP) are known to assist protein quality control under various stresses. Although overexpression of HSP70 was found to promote muscle mass retention in an unloading state, it is unclear whether muscle atrophy is induced by suppression of HSP expression and is counteracted by active HSP overexpression. In this study, we pre-treated Hsp70 siRNA to rat L6 cells for the HSP gene-silencing, and determined myotube diameter, HSP72 expression and anabolic and catabolic signaling activities in the absence or presence of triterpene celastrol (CEL), the HSP70 inducer. Relative to a negative control (NC), muscle cell diameter was reduced by 11% in the siRNA-treated group, increased 1.2-fold in the CEL-treated group and remained at the size of NC in the siRNA+CEL group. HSP72 expression was decreased 65% by siRNA whereas the level was increased 6- to 8-fold in the CEL and siRNA+CEL groups. Expression of FoxO3 and atrogin-1 was increased 1.8- to 4.8-fold by siRNA, which was abolished by CEL treatment. Finally, phosphorylation of Akt1, S6K and ERK1/2 was not affected by siRNA, but was elevated 2- to 6-fold in the CEL and siRNA+CEL groups. These results suggest that HSP downregulation by Hsp gene-silencing led to muscle cell atrophy principally via elevation of catabolic activities. Such anti-atrophic effect was counteracted by CEL-mediated HSP overexpression. This abstract is sponsored by Executive Director Jody McGinness (jmcginness@proteinsociety.org)

PE-026 **A novel in vivo characterization method predicting the physicochemical parameters of an antibiotic efflux pump**

Anisha M Perez[1], Erin L O'Brien[1], Marcella M Gomez[1], Matthew R Bennett[1], Yousif Shamoo[1]
1.-Department of BioSciences

The Centers for Disease Control and Prevention report that at least 2 million people in the United States will become ill due to antibiotic resistant pathogens leading to 23,000 deaths each year. In order to circumvent these resistance mechanisms, it is essential to quantitatively understand how the function of the protein(s) involved relates directly to resistance. Integral membrane efflux pumps are known determinants of single-drug and multi-drug resistance in a wide variety of pathogenic organisms. These transporters are proteins whose characterization typically requires reconstitution in an artificial membrane. Subsequently, these important proteins are difficult to characterize by traditional in vitro studies. My project aims to determine the physicochemical parameters of the efflux pump TetB utilizing molecular biology and mathematical modeling. TetB is composed of 12 transmembrane (TM) alpha-helices and is found within the inner membrane of Gram-negative bacteria. This protein allows for the efflux of tetracycline (TET), doxycycline (DOX), and minocycline (MCN) antibiotics from the cytoplasm into the periplasm. These tetracyclines are a bacteriostatic class of antibiotics that inhibit protein synthesis by binding to the 30S ribosomal, therefore, blocking the binding of aminoacyl-tRNA. For cells grown in tetracyclines, the efflux mechanism of TetB decreases the cytosolic antibiotic concentration allowing for the rate of protein translation to increase. I have inserted a tet(B) expression system into the chromosome of an Escherichia coli lab strain and have determined its growth profile under various concentrations of TET, MCN, and DOX using a high-throughput 96-well plate format. The growth rate profiles correlate with TetB pumping rates for each drug. TetB more readily pumps out TET compared with DOX and MCN and we observe that cells expressing TetB can grow at higher TET concentrations compared with DOX and MCN. The shapes of the growth rate profiles produced in the different drugs give insight into the physicochemical mechanism of TetB. We have built a preliminary mathematical model that can simulate these growth profiles and predict efflux pump physicochemical parameters. We are currently working on understanding how efflux expression effects bacterial growth by testing ribosome binding site (RBS) sequences of varying strengths in our tet(B) expression system. Future work is geared toward modeling more complex efflux pumps such as the tripartite pumps which traverse both bacterial membranes and cause multi-drug resistance. Collectively, this project aims to build an in vivo system which will allow for the characterization of a variety of efflux pumps without the arduous tasks of protein purification and subsequent reconstitution.

PE-027 **Structural Analysis of KCNE1 Transmembrane Mutant Yielding KCNE3-like Function**

Cheryl Law, Charles Sanders

1.-Vanderbilt University Biochemistry Department, 2.-Vanderbilt University Center for Structural Biology, 3.-Vanderbilt University School of Medicine

The KCNE family contains five single transmembrane-spanning proteins that modulate several channels including the voltage-gated potassium channel, KCNQ1. These five proteins provide functional diversity to the KCNQ1 channel. For example, the KCNQ1/KCNE1 complex leads to a slow and delayed opening of the channel while the KCNQ1/KCNE3 complex leads to a rapid opening and constitutively active channel. Mutations in both the KCNQ1 channel and members of the KCNE family have been shown to lead to disease such as long QT syndrome, cardiac arrhythmias, deafness, and sudden death. Melman YF, et al (2007) identified a small transmembrane region of both KCNE1 and KCNE3 that are essential for their unique modulation of the KCNQ1 channel. By swapping a triplet motif in the transmembrane region of KCNE1 and KCNE3, we can flip the primary function of these two proteins. While the key for KCNE1 and KCNE3's unique modulating is believed to lie in this triplet motif, the mechanism and structural changes involved in this modulation is not fully understood. By using NMR spectroscopy, biochemical studies, and computational docking, we aim to look at the structural and conformational differences between KCNE1 and the triple mutant KCNE1 substituted with the three essential KCNE3 residues. We have expressed and purified 15N-labled KCNE1 triple-mutant in sufficient quantities for NMR studies in LMPG detergent micelles and other membrane mimetics, and we have collected 2D NMR spectra using a TROSY-based pulse sequence. Partial backbone assignments of KCNE1 triple mutant have been determined by aligning and transfer assignments of the WT KCNE1 previous determined in our lab. With the structure of KCNE1 triple mutant determined, we aim to computationally dock the triple mutant into a model of the full-length KCNQ1 channel in the open and closed state. Lastly, we will compare the known structure of KCNE1 docked to a model of KCNQ1 to that of the KCNE1 triple mutant to determine key interactions, significant structural and conformational changes, and how the triple motif region gives rise to its specific structural and functional differences. With this information, we can begin to understand the mechanism of the functional diversity of the KCNE family on KCNQ1 potassium channel.

PE-028 **Biochemical characterization of Brassica napus diacylglycerol acyltransferase 1 and its regulatory domain**

Kristian Mark Caldo[1], Rashmi Panigrahi[2], Michael Greer[1], Guanqun Chen[1], M. Joanne Lemieux[2], Randall Weselake[1]

1.-Alberta Innovates Phytola Centre, University of Alberta, 2.-Department of Biochemistry, University of Alberta

Diacylglycerol acyltransferase 1 (DGAT1) is a membrane-bound enzyme catalyzing the final and committed step in the acyl-CoA-dependent triacylglycerol (TAG) biosynthesis. The level of DGAT activity in Brassica napus seeds has a substantial effect on the flux of carbon towards TAG formation. Although membrane-bound DGATs have been investigated for a long time, their modes of action and regulation remain poorly understood due to difficulties encountered during purification. This study aimed to purify and characterize recombinant Brassica napus DGAT1 to increase our understanding of the regulation of seed oil formation. Herein, we describe the purification of active recombinant B. napus DGAT1 (BnaC.DGAT1.a) expressed in Saccharomyces cerevisiae. Purified BnaDGAT1 in n-dodecyl-β-D-maltopyranoside (DDM) micelles behaves as dimers, which can associate further to form tetramers. The acyl donor preference of the major dimeric form with sn-1,2-diolein as acceptor follows the following order: α-linolenoyl-CoA > oleoyl-CoA = palmitoyl-CoA > linoleoyl-CoA > stearoyl-CoA. The first 113 residues of BnaC.DGAT1.a corresponding to a soluble regulatory region was expressed in Escherichia coli and purified. Truncation of this soluble domain reveals that the dimeric interface is located within residues 49-113, while the first 48 residues allow formation of tetramers. This N-terminal region was implicated as an allosteric exosite for acyl-CoAs as revealed by previous Lipidex-1000 binding studies. In the current study, circular dichroism spectroscopy and isothermal titration calorimetry were used to probe the binding kinetics and thermodynamics. DGAT1 appears to shift between two oligomerization states, a phenomenon that may be related to regulation of enzyme activity and mediated by the N-terminal domain.

PE-029 **Alteration of lysine and arginine content as a strategy to modify protein solubility: a test for E. coli proteins**

M. Alejandro Carballo-Amador[1], Jim Warwicker[1], Alan J. Dickson[1]
1.-Faculty of Life Sciences, University of Manchester

Protein aggregation is an undesired physicochemical mechanism whether for biophysical and structural studies or for biopharmaceutical companies, at any scale. In Escherichia coli, protein accumulation in the cytoplasm can result in protein aggregation to form what are known as inclusion bodies (IBs). Several experimental approaches have been undertaken to prevent protein aggregation. However, there is no universal approach or technology that solves protein aggregation. Recently, our group found that the sequence-based property of lysine versus arginine content separated E. coli proteins by solubility. In this study, we investigated solubility alterations for three highly soluble E. coli proteins (thioredoxin-1 [TRX], cold shock-like protein cspB [cspB], and the histidine-containing phosphocarrier protein [HPr]), with varying degree of lysine substitution by arginine. These experiments are predicted to decrease the solubility of the variants, according to our computational calculations. Our findings revealed a significant decrease in solubility for cspB and HPr, which is more evident in variants with low or null lysine content. However, for the expression of TRX variants, solubility only falls under low induction conditions (low temperature and IPTG inducer) compared to WT. This computational and experimental approach is a first step in studying to what extent the lysine:arginine ratio modifies solubility.

PF-001 **Role of C terminal disordered domain of Sesbania mosaic virus RNA dependent RNA polymerase in the modulation of its activity and oligomeric status**

Arindam Bakshi[1], Srinivas Sistala[2], Shruthi Sridhar[1], Savithri H S[1]
1.-Indian institute of Science, 2.-Wipro G E Healthcare Pvt Ltd

Sesbania mosaic virus (SeMV) is a single stranded positive sense RNA virus with a genome length of 4148 nucleotides and belongs to the genus sobemoviruses. SeMV RNA dependent RNA polymerase (RdRp) (62 KDa) was previously shown to interact strongly with virus encoded P10 protein (10 KDa). Such an interaction was found to increase the activity of RdRp in vitro. Further, deletion of C terminal 43 amino acid residues also resulted in increase in the polymerase activity that was comparable to the full length RdRp-P10 complex. It was proposed that the conserved C terminal disordered domain of RdRp was responsible for interaction with P10 and modulation of the activity. In the present study, role of the C terminal disordered domain was further investigated by determining the oligomeric status of the complex and the C terminal deletion mutants of RdRp and also by quantitating the RdRp-P10 interaction using surface plasmon resonance. Size exclusion chromatography revealed that RdRp eluted in the void volume of the column whereas a significant fraction of the RdRp-P10 complex eluted at a position corresponding to the size of the 1:1 complex of RdRp and P10 (77KDa). Activity measurements indicated that the heterodimeric complex was more active than the aggregate eluting in the void fraction. Interestingly, the C terminal deletion mutants of RdRp (C del 43 & C del 72 RdRp) were also found to be less aggregated as compared to full length RdRp and some of the protein eluted at a position corresponding to the respective monomers. These monomers were also more active than the aggregate fractions. These results demonstrate that the increase in activity observed either upon interaction with P10 or deletion of the C terminal domain could be due to the change in the oligomeric state of RdRp. In order to further analyze the interaction of RdRp with P10 surface plasmon resonance was used. RdRp and its deletion mutants were immobilized on Biacore sensor surface and P10 protein was used as an analyte. Full length RdRp and C del 43 RdRp were shown to interact with P10 with KD values of 0.6 and 1 uM respectively. However, C del 72 and C del 85 RdRp did not show any binding with P10. These results suggest that the region 43-72 from the C terminus of RdRp is essential for the interaction with P10. Further, the C del 85 RdRp was inactive although C del 72 RdRp continued to be active suggesting that residues 72-85 from the C terminus are crucial for RdRp activity. Further studies are in progress to identify the residues within these motifs that may be essential for the activity or interaction with P10.

PF-002 **Aggregation of androgen receptor in spinal bulbar muscular atrophy is a multistep process**

Giulio Chiesa[1], Bahareh Eftekharzadeh[1], Daniele Mungianu[1,2], Alessandro Piai[2], Jesus Garcia[1], Isabella Felli[2], Roberta Pieratelli[2], Xavier Salvatella[1,3]

1.-Institute for Research in Biomedicine (IRB), 2.-Magnetic Resonance Center and Department of Chemistry, University of Florence, 3.-ICREA

Spinal bulbar muscular atrophy (SBMA) is a member of the polyglutamine (polyQ) expansion diseases, like Huntington disease, and it is caused by a genetic expansion of the polyCAG tract in exon 1 of androgen receptor (AR) that codes for the polyQ region. SBMA is a late onset disease, which involves a progressive degeneration of the motor neurons and consequent muscular atrophy. There is still no treatment available for this disease. AR is a nuclear receptor that responds to testosterone and that regulates the expression of the masculine phenotype. It is composed of an intrinsically disordered N-terminal domain (NTD) that bears the polyQ tract, a DNA binding domain and a ligand binding domain. Aggregates of AR protein with an extended polyQ are observed in the motor neurons of SBMA patients. In vitro studies showed that aggregation of Androgen Receptor takes place only in presence of testosterone1 and that the cleavage of the protein by caspase 3 is a crucial event for cytotoxicity. However, there is no clear knowledge of the mechanism of aggregation, for this protein. An increasing body of evidence supports the hypothesis that the aggregation of these proteins is controlled by regions flanking the polyQ tract, by regulating the rate of aggregation depending on their secondary structure. We have applied nuclear magnetic resonance (NMR) and circular dichroism for generating information on the secondary structure of the N-terminal cleavage product of AR by caspase 3 and we have studied its aggregation with a set of biophysical methods, like dynamic light scattering, an HPLC sedimentation assay and transmission electron microscopy. We have found that the polyQ tract of AR presents a high degree of helicity. We attribute this conformation to the N-terminal flanking region, characterized by high helicity and we have tested this hypothesis by performing mutations. We have also observed that the rate of the first step of oligomerization is not dependent on the number of glutamine repeats, but instead is due to self interactions of a region N-terminal to and far from the polyQ. Its progression to fibril is dependent to the number of glutamines in the tract. We have therefore identified two steps in the aggregation process of AR, where a motif far from the polyQ at its N-terminal drives the early oligomerization, followed by the interaction of the polyQ chains that stabilize it and determine the progression to fibrils. These findings shed a light for possible interventions on the AR oligomerization process, thus suggesting a different strategy to study the onset of the disease in SBMA patients.

PF-003 **Destabilizing the Transient Helical Conformation of Islet Amyloid Polypeptide Hastens Peptide Self-Assembly and Potentiates Cytotoxicity**

Carole Anne de Carufel[1], Phuong Trang Nguyen[1], Alexandre Arnold[1], Isabelle Marcotte[1], Steve Bourgault[1]

1.-University of Quebec in Montreal, Department of Chemistry

Amyloidogenic polypeptides can be divided into two different structural classes: those that are intrinsically disordered and those that show a well-defined structure in their monomeric soluble state. Natively folded proteins, such as transthyretin, have to unfold (or misfold), at least partially, to form amyloids. In contrast, intrinsically disordered polypeptides, such as the islet amyloid polypeptides (IAPP) and Abeta peptide, need to undergo conformational rearrangements allowing the formation of locally ordered structure(s) to initiate the amyloidogenic process. Studies have shown that IAPP and Abeta adopt an alpha-helix conformation in the initial steps of amyloidogenesis. This intermediate is believed to be on-pathway to fibril formation, although this hypothesis is still the matter of debate. In this study, we designed human IAPP (hIAPP) derivatives in which alpha-helix destabilizing substitutions were incorporated into the putative helical segment of IAPP to probe the initial structural event in amyloid formation. Using trifluoroethanol titration, we observed by CD spectroscopy that strategic incorporation of D-amino acids at positions 15 and 16 leads to an IAPP derivative (dIAPP) that cannot fold into a helix. In homogeneous solution, hIAPP and dIAPP show similar kinetics of fibrillization, as measured by Thioflavin T fluorescence. Although their amyloid fibrils display different characteristics by AFM, IAPP and dIAPP are able to self-associate to form amyloids when mixed together and when seeded with one another. Studies in heterogeneous environment, notably in presence of glycosaminoglycans and model membranes of DOPC/DOPG (7:3), showed a helical intermediate for hIAPP while only a beta-sheet secondary structure was apparent for dIAPP. While the rate of amyloid fibril formation was increased for both peptides, dIAPP was drastically affected by these anionic biomolecules with an absence of lag phase. The incapacity of adopting a transient helical conformation accentuates cell toxicity, supported by the caspase 3/7 activation level and the increase in intracellular calcium level. Overall, this study indicates that the helical intermediate is off-pathway to IAPP amyloid formation and offers novel mechanistic insights for the development of molecular identities modulating peptide self-assembly and IAPP-induced cytotoxicity.

PF-004 **Towards in vivo NMR: Putting prions in context**

Kendra Frederick[1], Robert Griffin[2], Susan Lindquist[1,3],
1.-Whitehead Institute for Biomedical Research, 2.-Francis Bitter Magnet Lab and Department of Chemistry, MIT, 3.-Howard Hughes Medical Institute, Department of Biology, MIT

For an organism to survive, its proteins must adopt complex conformations in a challenging environment where macromolecular crowding can derail even robust biological pathways. The situation is perilous: many diseases arise from improper folding of just a single protein. To cope, cells employ a repertoire of molecular chaperones and remodeling factors that usher unfolded proteins into active conformations, sequester them, or target them for degradation. Yet, not all aggregated proteins are the result of mis-folding. Yeast prions are self-templating protein-based mechanisms of inheritance that rely upon chaperones for their propagation. The best studied of these is the prion domain (NM) of Sup35, which forms an amyloid that can adopt several distinct conformations (strains) that produce distinct phenotypes. Using genetic, biochemical, spectroscopic, and solid state NMR techniques, we investigated the structural and dynamic underpinnings of Sup35 amyloids and found that prion strains differ in both their atomic structure as well as their dynamic motions. Interestingly, these mobility differences correlate with differences in the interaction with molecular chaperones in vivo. Limitations on the specificity and sensitivity of biophysical techniques typically restrict structural investigations to purified systems at concentrations that are orders of magnitude above endogenous levels. Therefore, I developed an approach to apply a sensitivity-enhancement technique for NMR, dynamic nuclear polarization (DNP), to investigate interactions between Sup35 and molecular chaperones at endogenous concentrations in their native environments. Critically, I found that the cellular environment induced structural changes in a region of Sup35 that is intrinsically disordered in purified samples but known genetically to influence prion propagation from one generation to the next. This approach enables structural and mechanistic investigation of proteins in biologically relevant contexts.

PF-005 **Genetic instability within regions encoding repetitive proteins as a driver of adaptation**

Stephen Fuchs[1]
1.-Tufts University

More than ten percent of all eukaryotic proteins contain within them a region of repetitive amino acid sequence. These repetitive domains range from short stretches of a single amino acid to multiple copies of longer, heterogeneous amino acid sequences and generally show lack of defined structure. They play diverse roles in cells including acting as structural proteins, promoting cell-cell interactions, and mediating the assembly of molecular machines. Tandem repeat proteins are known to be variable in length within cellular populations although the mechanisms dictating this variability have not been elucidated. Here we describe work uncovering specific features within the coding sequences of repetitive proteins that contribute to tandem repeat instability in yeast. Furthermore, we demonstrate that cells will expand and/or contract repetitive regions in order to adapt to environmental stresses and describe a role for DNA repair proteins in this process. Lastly, we demonstrate how these mechanisms are likely conserved in higher eukaryotes, including humans. This study uncovers the molecular basis for an important aspect of natural protein evolution and describes a novel mechanism for adaptation in response to environmental changes.

PF-006 **A Proline-Tryptophan turn in the intrinsically disordered domain 2 of NS5A protein is essential for Hepatitis C virus RNA replication**

Marie Dujardin[1], Vanesa Madan[2], Roland Montserret[3], Puneet Ahuja[1], Isabelle Huvent[1], Helene Launay[1], Ralf Bartenschlager[2], François Penin[3], Guy Lippens[1], Xavier Hanoulle[1]

1.-CNRS UMR 8576, UGSF, Lille University, 2.-Department of Infectious Diseases, Molecular Virology, University of Heidelberg, 3.-CNRS UMR 5086, IBCP, LabEx Ecofect, Lyon 1 University

Hepatitis C virus (HCV) nonstructural protein 5A (NS5A) and its interaction with the human chaperone cyclophilin A (CypA), a peptidyl-prolyl cis-trans isomerase (PPIase), are both targets for highly potent and promising antiviral drugs that are in late stage of clinical development [1,2]. Despite its high interest in the development of drugs to counteract the worldwide HCV burden, NS5A is still an enigmatic multifunctional protein poorly characterized at the molecular level. NS5A is required for HCV RNA replication and is involved in viral particles formation and regulation of host pathways. Thus far, no enzymatic activity or precise molecular function has been ascribed to NS5A that is composed of a highly structured domain 1 (-D1), as well as two intrinsically disordered domains 2 (-D2) and 3 (-D3). NS5A-D1 structure has been solved by X-ray crystallography and NS5A-D2 and -D3 have been characterized by NMR spectroscopy. These two last domains do not adopt a stable 3D structure but rather exist as an ensemble of highly dynamic conformers. Using NMR spectroscopy, HCV NS5A-D2 has been shown to establish a direct interaction with the human CypA and to be a substrate for the enzymatic PPIase activity of CypA [3]. The CypA interaction site in NS5A-D2 is composed of nearly 15 residues that correspond to the most conserved region of the domain, with 3 Proline residues being strictly conserved among all HCV genotypes. Whereas NS5A-D2 is mainly disordered, some of its NMR resonances, corresponding to residues in the CypA binding site, display unexpected 1H and 15N NMR chemical shifts for an intrinsically disordered domain. Thus we have further characterized this region by NMR spectroscopy. A short structural motif in the disordered NS5A-D2 has been identified and we solved its NMR structure. In a cellular assay, we showed that this structural motif, a minimal Pro314-Trp316 turn, is essential for HCV RNA replication. We demonstrated that this Pro-Trp (PW) turn is required for proper interaction with the host CypA and influenced its enzymatic PPIase activity on residue P314 of NS5A-D2. This work provides a molecular basis for further understanding of the function of the intrinsically disordered domain 2 of HCV NS5A protein. In addition, our work highlights how very small structural motifs present in intrinsically disordered proteins can exert a specific function.

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PF-007 **Solution structure and celullar functional studies of bovine cathelicidin Bt-6 (BMAP-27)**

Anna Hastings[1], Manuel Ruether[2], H. Paul Voorheis[1], Ken H. Mok[1,3]

1.-Trinity College Dublin, School of Biochemistry and Immunology, 2.-Trinity College Dublin, School of Chemistry, 3.-TCD, Centre for Research on Adaptive Nanostructures and Nanodevices (CRANN)

Solution structure and celullar functional studies of bovine cathelicidin Bt-6 (BMAP-27) Anna Hastings1, Manuel Ruether2, H. Paul Voorheis1 and K. H. Mok1,3 1 Trinity College Dublin, Trinity Biomedical Sciences Institute (TBSI), School of Biochemistry and Immunology, Dublin 2, Ireland 2 Trinity College Dublin, School of Chemistry, Dublin 2, Ireland 3 Trinity College Dublin, Centre for Research on Adaptive Nanostructures and Nanodevices (CRANN), Dublin 2, Ireland

Trypanosoma brucei, the causative agent of African sleeping sickness in humans and nagana in cattle, has been shown to be killed by a bovine antimicrobial peptide, Bt-6 (BMAP-27) [1-2]. This 27-residue peptide also shows toxicity towards mammalian cells but at higher concentrations, suggesting its possible usefulness as a treatment for trypanosomiasis. Here we present the peptide's relative cytotoxicity for bloodstream and procyclic forms of T. brucei and for mammalian cells, the fate of the peptide in T. brucei using fluorescently-labelled Bt-6, and its three dimensional structure using NMR spectroscopy. Minimum inhibitory assays confirmed the peptide's selective toxicity towards both bloodstream and procyclic forms of T. brucei, demonstrating its potential to serve as a starting point for a trypanocidal drug. Fluorescence spectrophotometric experiments, carried out using fluorescein labelled Bt-6, show that the peptide is released from the external surface of the parasite into the suspending medium under de-energized conditions but retained in energized cells. Heteronuclear and homonuclear biomolecular NMR experiments (TOCSY, NOESY, 1H-13C-HSQC,1H-15N-HSQC, etc) folowed by structural calculations (chemical-shift based as well as simulated annealing techniques) in the free state indicate that this peptide is mostly unstructured in aqueous solution, suggesting that there is a major conformational change upon binding to T. brucei that is required for uptake. We suggest that the evolutionary pressure that selected for the intrinsically disordered structure of this peptide was the advantage it conferred upon the host to bind to many different surface structures throughout the microbiological world. References [1] Haines, L. R. et al. (2003) Vector Borne Zoonotic Dis. 3: 175-186. [2] Haines, L. R. et al. (2009) PLoS Negl. Trop. Dis. 3: e373.

PF-008 **Engineered binding proteins to amyloidogenic intrinsically disordered proteins**

Hamed Shaykhalishahi[1,2], Ewa Mirecka[1], Aziz Gauhar[1], Clara Grüning[1], Michael Wördehoff[1], Sophie Feuerstein[2], Matthias Stoldt[1,2], Torleif Härd[3], Dieter Willbold[1,2], Wolfgang Hoyer[1,2]

1.-Physikalische Biologie, Heinrich Heine University, 2.-Structural Biochemistry (ICS-6), Research Centre Jülich, 3.-Chemistry and Biotechnology, Swedish University of Agricultural Sciences (SLU)

The misfolding and amyloid formation of proteins featuring intrinsically disordered regions is a pathological hallmark of several neurodegenerative diseases, including Alzheimer's disease and Parkinson's disease. Engineered binding proteins targeting amyloidogenic proteins aid in the elucidation of the aggregation mechanism and suggest therapeutic strategies. We have constructed phage display libraries enriched in binders to amyloidogenic intrinsically disordered proteins, using ZAb3, a protein with high affinity for the amyloid-beta peptide, as a scaffold. Binding proteins selected from these libraries are termed beta-wrapins (beta-wrap proteins). The beta-wrapins AS69 and HI18 exhibit nanomolar affinity for monomeric alpha-synuclein or islet amyloid polypeptide, respectively. AS69 and HI18 potently inhibit in vitro amyloid formation and toxicity at substoichiometric concentration ratios, indicating that they interfere with the nucleation and/or elongation of amyloid fibrils. The NMR structures of the beta-wrapin:target complexes reveal beta-hairpin motifs in alpha-synuclein and islet amyloid polypeptide which are stabilized by coupled folding and binding. In the case of alpha-synuclein, the beta-hairpin is formed in the sequence region 35-59 which contains the beta-strand segments b1 and b2 of amyloid fibril models and most disease-related mutations. We show by disulfide engineering, biophysical techniques, and cell viability assays that intramolecular tertiary interactions between the b1 and b2 segments of alpha-synuclein interfere with its aggregation, and moreover inhibit aggregation of amyloid-beta peptide and islet amyloid polypeptide. Our results reveal a common preference of different amyloidogenic proteins for formation of beta-hairpin motifs and demonstrate a critical role of hairpin conformers in the control of amyloid formation.

PF-009 **Interaction Profiling through Proteomic Peptide Phage Display**

Cecilia Blikstad[1], Moon-Hyeong Seo[2], Norman Davey[3], Roland Arnold[2], Sachdev S Sidhu[2], Philip M Kim[2], Ylva Ivarsson[1]

1.-Department of Chemistry - BMC, 2.-Donnelly Centre

A considerable part of the human proteome is intrinsically disordered. The disordered regions are enriched in short motifs serving as docking sites for peptide binding domains. Domain-motif interactions are crucial for the wiring of signaling pathways. These interactions are typically transient and difficult to capture through most conventional high-throughput methods. We therefore developed a novel approach for the large-scale profiling of domain-motifs interactions called Proteomic Peptide Phage Display (ProP-PD) (1). In ProP-PD we combine bioinformatics, oligonucleotide arrays, peptide phage display and next-generation sequencing. This allows the interrogation of domain-motif interactions on a proteome-wide scale and the de novo motif discovery. In our pilot experiment we generated two distinct phage libraries, one displaying all human C-terminal sequences and one displaying C-termini of known virus proteins. We used the ProP-PD libraries to identify interactions of human postsynaptic density 95/discs large/zonula occludens-1 (PDZ) domains. We successfully identified novel PDZ domain interactions of potential relevance to cellular signaling pathways and validated a subset of interactions with a high success rate. Recently, we created a ProP-PD library that displays peptides representing the disordered regions of the human proteome. We validate our disorderome library against a range of peptide binding domains, which provides novel insights into their binding preferences and suggest interactions of potential biological relevance as will be presented here. ProP-PD can be used to uncover protein-protein interactions of potential biological relevance in high-throughput experiments and provides information that is complementary to other methods. ProP-PD is scalable and can be developed to any target proteome of interest. 1. Ivarsson, Y., Arnold, R., McLaughlin, M., Nim, S., Joshi, R., Ray, D., Liu, B., Teyra, J., Pawson, T., Moffat, J., Li, S., Sidhu, S. S., & Sidhu, S. S. Large-scale interaction profiling of PDZ domains through proteomic peptide-phage display using human and viral phage peptidomes. (2014) Proc Natl Acad Sci U S A 111, 2542-2547.

PF-010 **Biophysical characterization of phosducin and its complex with the 14-3-3 protein**

Miroslava Kacirova[1,2], Jiri Novacek[3], Petr Man[1,4], Alan Kadek[1,4], Veronika Obsilova[2], Tomas Obsil[1,2]

1.-Faculty of Science, Charles University in Prague, 2.-Institute of Physiology, Czech Academy of Sciences, 3.-Masaryk University, CEITEC – Central European Institute of Technology, 4.-Institute of Microbiology, Czech Academy of Sciences

Phosducin is a 30 kDa phosphoprotein that regulates visual signal transduction by interacting with the Gtβγ; subunit of the retinal G-protein transducin. The function of Pdc is regulated by phosphorylation at Ser54 and Ser73 in a process that involves the binding of phosphorylated Pdc to the regulatory 14-3-3 protein, but the molecular mechanism of the regulation by 14-3-3 protein is still unknown. Pdc was also suggested to be involved in transcriptional control, the regulation of transmission at the photoreceptor-to-ON-bipolar cell synapse, and the regulation of the sympathetic activity and blood pressure [1-3]. Here, the solution structure of Pdc and its interaction with the 14-3-3 protein were investigated using small angle X-ray scattering, circular dichroism, quenching of tryptophan fluorescence, analytical ultracentrifugation, hydrogen-deuterium exchange coupled to mass spectrometry and nuclear magnetic resonance. We show that the 14-3-3 protein interacts with and sterically occludes both the N- and C-terminal Gtβγ binding interfaces of phosphorylated Pdc, thus providing a mechanistic explanation for the 14-3-3-depedent inhibition of Pdc function. The 14-3-3 protein dimer interacts with Pdc using surfaces both inside and outside its central channel. The N-terminal domain of Pdc, where both phosphorylation sites and the 14-3-3 binding motifs are located, is intrinsically disordered protein which remains likely highly flexible when bound to 14-3-3 indicating the fuzzy-like character of this complex. In addition, it has been speculated that the 14-3-3 protein binding decreases the rate of Pdc dephosphorylation after a light stimulus through its interaction with phosphorylated Ser54 and Ser73, thus lengthening the time that Pdc remains phosphorylated after a light exposure. Pdc is dephosphorylated in vivo by protein phosphatases 1 (PP1) and 2A (PP2A). Our dephosphorylation experiments with PP1 revealed that the 14-3-3 protein does slow down the dephosphorylation of doubly phosphorylated Pdc in vitro. 1. R. Gaudet, A. Bohm, P. B. Sigler, Cell 87, (1996), 577-588. 2. B. Y. Lee, C. D. Thulin, B. M. Willardson, J. Biol. Chem. 279, (2004), 54008-54017. 3. L. Rezabkova, M. Kacirova, M. Sulc, P. Herman, J. Vecer, M. Stepanek, V. Obsilova, T. Obsil, Biophysical J. 103, (2012), 1960-1969. This work was supported by the Czech Science Foundation (Project P305/11/0708), Grant Agency of Charles University in Prague (Project 793913); and Czech Academy of Sciences (Research Projects RVO: 67985823 of the Institute of Physiology).

PF-011 **Prion-like proteins sequester and suppress the toxicity of huntingtin exon 1**

Can Kayatekin[1], Kent Matlack[1], William Hesse[2], Yinghua Guan[3], Sohini Chakrabortee[1], Gregory Newby[2], Jenny Russ[4], Erich Wanker[4], Jagesh Shah[3], Susan Lindquist[1,2,5]

1.-Whitehead Institute For Biomedical Research, 2.-Massachusetts Institute of Technology, 3.-Harvard Medical School, 4.-Max Delbrück Center For Molecular Medicine, 5.-Howard Hughes Medical Institute

Huntington's disease (HD) is a devastating neurodegenerative disorder caused by an increase in the length of a polyglutamine repeat (polyQ) in the protein huntingtin. At least nine other proteins are also known to cause neurodegenerative disease in a polyglutamine-length dependent manner. Despite intense study, the molecular basis of polyQ toxicity in HD or any of the other diseases has only partially been elucidated and potential routes to therapeutic intervention are sparse. The use of genetically tractable model organisms to identify the cellular pathologies caused by mutant huntingtin expression is essential to our understanding of the disease pathology in humans. In eukaryotes, many of the protein folding homeostasis pathways are highly conserved and yeast cells expressing a glutamine-expanded fragment of huntingtin exon 1 exhibit a polyQ length-dependent toxicity that recapitulates many of the basic protein folding defects associated with polyQ diseases in neurons. Taking an unbiased approach, we screened an overexpression library of the entire yeast genome for suppressors and enhancers of polyQ toxicity and identified seven proteins with prion-like, Q-rich domains that are strong suppressors in yeast. Intriguingly, the Q-rich domains of these proteins, and several other Q-rich domains, suppress toxicity when expressed in isolation. These suppressors are also efficacious in mammalian cells and, strikingly, one suppressor was independently shown to alleviate polyQ-expanded ataxin-3 toxicity in a Drosophila model. In yeast, the suppressors co-aggregated with an otherwise highly toxic 103-glutamine expanded huntingtin exon 1 protein (Htt103Q), resulting in a non-toxic aggregate and eliminating populations of diffusible oligomeric species. Using a transcriptional sensor for protein co-aggregation, we determined that yeast and human proteins that normally co-aggregated with Htt103Q did not co-aggregate with these hetero-aggregates. Thus, these Q-rich domains may suppress Htt103Q toxicity by two complementary mechanisms: trapping potentially toxic oligomers in larger aggregates and by limiting the interactome of the larger Htt103Q aggregates.

PF-012 **Structuring disorder: the case of the intrinsically disordered Unique domain of c-Src**

Mariano Maffei[1]

1.-BioNMR lab - Faculty of Organic Chemistry - University of Barcelona

About two thirds of eukaryotic proteins contain large intrinsically disordered regions. They represent a change of paradigm from “structure-function” to “information-function” (Uversky, 2011; Babu et al., 2011). Structured proteins are information rich, but the current challenge is to discover how information is stored in disordered protein. Regulation of c-Src activity, the first discovered oncoprotein, by its intrinsically disordered N-terminal region has been recently demonstrated (Perez et al., 2013). Functional studies have revealed that mutations in the ULBR cause strong phenotypes when introduced in full-length c-Src and expressed in Xenopus laevis oocytes (Perez et al., 2013) or in human SW620 colorectal cancer cells (unpublished). However, the connection with the classical regulatory mechanisms is still missing. c-Src domain structure consists of four “Src-homology” domains: SH4, SH3, SH2 and SH1, arranged in this order from the N-terminus to the C-terminus, with the intrinsically disordered “Unique” domain separating the SH4 and SH3 domains. Classically, the SH3 and SH2 domains are involved in regulation and the SH4 domain is the membrane anchoring site. We will present our recent results showing that the Unique domain is part of a long loop closed by the interaction of the SH4 and SH3 domains (Maffei et al., 2015). The conformational freedom of this disordered region is further restricted through direct contacts between the RT-loop of the SH3 domain and, primarily, residues located within the recently discovered Unique lipid binding region (ULBR). The interaction between the Unique and SH3 domains is allosterically modulated by a poly-proline ligand binding to the canonical binding site of the SH3 domain (Maffei et al., 2015). These results demonstrate a direct connection between classical c-Src regulation involving the SH3 domain and the new regulation mechanisms involving the intrinsically disordered regions and provide new evidence of the functional importance and the underlying mechanism behind regulation of signalling pathways by intrinsically disordered domains.

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PF-013 **The Yeast GRASP Grh1 displays features of an Intrinsically Disordered Protein**

Raquel Fonseca-Maldonado[1], Felipe Mendes[1], Luana Meleiro[1], Assuero Garcia[1], Antonio Costa-Filho[1]

1.-Departamento de Física, Universidade de São Paulo-FFCLRP, 2.-Departamento de Química, Universidade de São Paulo-FFCLRP

In mammalian cells, the Golgi reassembly and stacking proteins (GRASP55 and GRASP65) are involved in the stacking of Golgi apparatus cisternae and in the formation of the Golgi ribbon. Since GRASPs have been identified in many organisms, other roles for GRASPs have already been pointed out, such as chaperoning and transport of other proteins, involvement in cell apoptosis, cell migration, unconventional secretion, and in mitosis. In Saccharomyces cerevisiae, it is observed that only 40% of the Golgi cisternae are in stacks and do not form ribbon structures. This build yeast contains a single GRASP, called Grh1, that is analogue to GRASP65. The structural differences of the Golgi apparatus and the functional repertoire of GRASPs suggest a structural dynamic of these proteins. Here, we used a combination of biophysical/biochemical methods to investigate the behavior of Grh1. Bioinformatics and circular dichroism (CD) analyses of Grh1 indicated a high percentage of either flexible regions or extended loops. The partial unfolded Grh1 structure in solution folded into more ordered structures under temperature increasing, dehydration onto a surface and nonaqueous solvents as reported also by CD. Hydration of the dehydrated folded protein is a reversible process that is accompanied by unfolding. Furthermore, Grh1 showed slow migration in SDS–PAGE, high susceptibility to proteases and low cooperativity of the chemical-induced unfolding process. Fluorescence of Trp residues along with CD data showed Grh1 preserves a considerable amount of residual secondary structure, and the unfolding transition monitored by Trp presented higher cooperativity. Another cooperative transition was also reported by the extrinsic hydrophobic fluorescence probe ANS upon chemical denaturation. These set of experiments indicate that Grh1 behaves as a protein containing intrinsically disordered regions (IDRs), characterized by unstructured regions of high polypeptide mobility experiencing many conformations. These findings suggest that an IDP-like behavior may be the solution found by Nature to account for Grh1 functional need for interactions with several different partners in the cell.

PF-014 **Conformational changes governing dengue virus capsid protein function and its inhibition by pep14 23**

André F. Faustino[1], Gabriela M. Guerra[1], Roland G. Huber[2], Axel Hollmann[1], Peter J. Bond[2], Miguel A.R.B. Castanho[1], Andrea T. Da Poian[3], Fábio C.L. Almeida[3], Nuno C. Santos[1], Ivo Martins[1]

1.-Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, 2.-Bioinformatics Institute, A*STAR, 3.-Instituto De Bioquímica Médica, Universidade Federal Do Rio De Janeiro

ABSTRACT Dengue virus (DENV) infection affects millions of people and is becoming a major global disease for which there is no specific treatment available. The interaction of DENV capsid (C) protein with host lipid droplets (LDs) is essential for viral replication. pep14-23, a peptide designed based on a DENV C intrinsically disordered conserved region, inhibits this crucial interaction. Combining bioinformatics and biophysics we determined pep14-23 structure and ability to bind different phospholipids, in the context of DENV C function. pep14-23 becomes α-helical upon binding to anionic phospholipids. Structure prediction of DENV C N-terminal intrinsically disordered region reveals orientations that alternatively shield or expose DENV C hydrophobic pocket, supporting a novel autoinhibitory role for this region. These findings pave the way for similar studies to understand disordered proteins and improved peptidomimetics drug development strategies against flaviviruses. TOPICS Intrinsically Disordered Proteins Protein-Lipid Interactions

PF-015 **Developing mechanistic insight into modulators of tau aggregation**

Eri Nakatani-Webster[1], Hannah Baughman[1], Shaylin Higgins[1], Abhinav Nath[1]
1.-Department of Medicinal Chemistry, University of Washington

The pathological self-association of microtubule-associated protein tau is implicated in a range of neurodegenerative disorders collectively called tauopathies, perhaps the most prominent of which are Alzheimer's disease (AD) and chronic traumatic encephalopathy (CTE). Tau aggregation in vitro shares many features in common with fibril formation by other amyloid-forming proteins: a nucleation-dependent polymerization reaction progressing via oligomeric intermediates into β-sheet-rich fibrillar aggregates, characterized by a distinctive sigmoidal kinetic. Over the years, many investigators have advanced our understanding of how these time-courses might best be characterized and interpreted. In particular, elegant analytical and numerical approaches have been developed that supersede the empirical sigmoidal equations typically used to fit fibril formation traces. These modern approaches have enabled more rigorous insight into the mechanism of amyloid formation, and into how small molecules, protein chaperones, and other binding partners can modulate the process. An understanding of a modulator's effects on amyloid formation mechanism is necessary in order for us to predict and engineer its effects on amyloid pathology in a biological context. A given modulator may affect rates of primary or secondary nucleation, elongation, or fibril fragmentation to different extents. Each of these perturbations, individually or in combination, can alter the kinetics of aggregation, the final state of the amyloid fibrils, and the sampled ensemble of oligomeric intermediates. Unfortunately, fitting of mechanistic models to amyloid formation kinetics is an example of an "ill-posed problem", in that dramatically different combinations of elementary parameters can nevertheless generate very similar sigmoidal kinetic traces. This has typically necessitated global analysis of amyloid kinetic traces collected over a broad range of protein concentrations – a substantial expenditure of time, effort and material that must then be repeated in the presence of a modulator in order to gain insight into its effects. We propose an alternative approach: to fit amyloid formation traces to a large distribution of parameter sets, and determine how various aggregation modulators affect the distribution of parameters. This so-called "parameter distribution analysis" enables the inference of mechanistic effects from measurements at a single protein concentration. Parameter distribution analysis based on numerical modeling has been made tractable by advances in computer hardware and software, and can be easily extended to include additional mechanisms or phases relevant to a protein or modulator of interest. Here, we illustrate how parameter distribution analysis, complemented by fluorescence correlation spectroscopy (FCS), electron microscopy (EM) and other biochemical techniques, can shed light on fundamental aspects of tau amyloidogenesis. We examine the disparate effects that natural products, pharmacotherapies and protein chaperones can have on the mechanism of aggregation, and also discuss the effects of heparin (widely used as an inducer of tau aggregation). These insights demonstrate the value of parameter distribution analysis as applied to amyloid formation and other ill-posed biochemical problems.

PF-016 **New insights into amyloidogenesis of Tau protein induced by enantiomers of polyglutamic acid**

Bartosz Nizynski[1,2,3,4], Hanna Nieznanska[2], Krzysztof Nieznanski[2], Wojciech Dzwolak[4]
1.-College of Inter-Faculty Individual Studies in Mathematics and Natural Sciences, 2.-Department of Biochemistry, Nencki Institute of Experimental Biology, 3.-Institute of High Pressure Physics, 4.-Department of Chemistry, Biological and Chemical Research Centre

New insights into amyloidogenesis of Tau protein induced by enantiomers of polyglutamic acid. Bartosz Nizynski1,2,3,4, Hanna Nieznanska2, Wojciech Dzwolak3,4, Krzysztof Nieznanski2
1. College of Inter-Faculty Individual Studies in Mathematics and Natural Sciences, University of Warsaw, Warsaw, Poland; 2. Department of Biochemistry, Nencki Institute of Experimental Biology, Warsaw, Poland; 3. Institute of High Pressure Physics, Warsaw, Poland; 4. Department of Chemistry, Biological and Chemical Research Centre, University of Warsaw, Warsaw, Poland.

Amyloidogenesis of Tau protein leads to the formation of amyloid fibrils (ordered fibrillar protein aggregates) which are accumulated in neurons of central nervous system during the course of neurodegenerative diseases called tauopathies. Studying Tau (a typical intrinsically disordered protein) amyloidogenesis has been challenging for many reasons. Positive charge on the Tau molecule must be compensated (e.g. in the presence of polyanions) in order to initiate the process. Heparin (glycosaminoglycan) has been the most intensively studied charge-compensating agent in this context. On the other hand induction of Tau aggregation by polyglutamic acid is poorly characterized. Mechanisms responsible for the propagation of Tau conformations has become an interesting research objective. Prion–like features of Tau amyloid can be studied in vitro also in the seed-induced regime of aggregation. Tau amyloid seeds can act as nuclei for amyloidogenesis. Such seeds can be obtained by fragmentation of amyloid fibrils by means of sonication. Given that amyloidogenesis can proceed through various assembly pathways resulting in distinct amyloid 'strains' (self–propagating structural variants of amyloid) we have used poly–L–glutamic acid (PLGA) and poly–D–glutamic acid (PDGA) to direct Tau onto different amyloidogenic pathways. We have hypothesized that the chirality of the inducers could lead to fibril polymorphism. In our studies, we have used a recombinant human 2N4R Tau isoform. We have been using transmission electron microscopy (TEM), sedimentation and kinetic measurment. Firstly, we have characterized unseeded PLGA–/PDGA–induced Tau aggregation to find out that corresponding kinetics were significantly different. Secondly, we have used sonicated fibrils to characterize the kinetics of seeded processes. Both PLGA–/PDGA–induced amyloid seeds were able to efficiently seed Tau aggregation in the presence of PLGA, whereas in the presence of PDGA the aggregation was much less effective. Surprisingly, we found that PDGA–induced amyloid seeds were able to catalyze fibrillogenesis of Tau more clearly in the presence of soluble PLGA than in the presence of PDGA – the primary inducer. We could not induce aggregation of Tau in the absence of polyglutamic acids which indicates that positive charge on Tau molecules must be unconditionally compensated in order to promote amyloidogenesis. Thirdly, using TEM we have characterized different morphologies of Tau amyloid fibrils generated in unseeded and seeded processes. Finally, to further characterize properties of the fibrils we have performed sedimentation experiments. Fibrils induced by PLGA, PDGA and heparin revealed different sedimentation properties. Heparin-induced fibrils underwent sedimentation more readily than PDGA-induced fibrils, whereas PLGA-induced fibrils remained in the supernatant. These results indicate distinct physicochemical properties of these fibrils. We believe that our findings will contribute to the current understanding of the molecular dynamics of Tau amyloidogenesis.

PF-017 **Self-organizing structures of alpha-synulceins and its aggregates by a coarse-grained Monte Carlo simulation**

Ras Pandey[1], Peter Mirau[2], Barry Farmer[2]
1.-University of Southern Mississippi, 2.-Air Force Research Laboratory

Alpha-synuclein (ASN) consisting of 140 residues, an intrinsically disordered protein, is linked to such neurodegenerative diseases as Parkinson's disease (PD) and Alzheimer disease via toxic clumping into amyloid fibrils. We investigate the structure and dynamics of an ASN chain as a function of temperature by a coarse-grained approach where a residue is represented by a node. In our coarse-grained approach, a residue is represented by a node. The basic idea is borrowed from the 'united atom' approach in polymer chain modeling that has been used extensively where the benefits and pitfalls of the method is explored for decades. Such coarse-grained method has also been used protein chain modeling in recent years (e.g. AIP Advances 5, 092502 (2015)). Although the atomic scale structural resolution is sacrificed its specificity is captured via a set of unique knowledge-based residue-residue interactions matrix (e.g. classic Miyazawa-Jernigan matrix, Macromolecules 18, 534 (1985)). A number of local and global physical quantities are analyzed such as contact map, neighborhood and mobility profiles, mean square displacement of protein, its radius of gyration and the structure factor. Based on the mobility profile, we are able to identify three distinct segment of ASN along its contour, i.e. sluggish N-terminal (1-60) and C-terminal (96-140, least mobile) separated by the central region (61-95), the non-amyloid component (NAC) with higher mobility. Contact profile shows that the probability of intra-chain residue aggregation (clumping) is higher in the N-terminal region than the C-terminal with least aggregation in the NAC region. We find that the radius of gyration (Rg) decays monotonically with the temperature, consistent with the finding of Allison et al. (JACS, 131, 18314 (2009)). From the detail analysis of the structure factor we are able to predict the variation of the spatial mass distribution with the temperature as the residues in ASN chain organize and disperse by evaluating its effective dimension D. We find the protein conforms to a globular structure (D~3) at the low temperatures and to a random coil (D~2) at high temperatures which is consistent with the estimates of Uversky et al. (J. Biol. Chem. 277, 11970 (2002)). In addition, we provide the estimates of D ($3 \geq D \geq 2$) for the intermediate structures as the protein chain makes a transition from globular to random coil. Questions under-investigation includes what are the effects of mutations (e.g. β- and γ-synuclein), how does the structure of an isolated ASN chain change in presence of many interacting protein chains, and how do they organize over the multiple length scales? Attempts will be made to address some of these issues as the data become available.

PF-018 **Tear down the wall: dismantling the biofilm scaffold of E.coli**

Cesyen Cedeno[1], Nani Van Gerven[1], Wim Jonckheere[1], Imke Van den Broek[1], Han Remaut[1], Peter Tompa[1]

1.-VIB, Structural Biology Research Center

CsgA is the major subunit of the so-called curli fiber system. This is an amyloid structure formed in the outer membrane on E.coli and acts as a scaffold for the biochemical machinery/matrix in the extracellular milieu (biofilms). Extracellular matrices of this nature are robust platforms helping bacteria colonization; in this context CsgA becomes a key target in order to break the architecture within bacterial biofilms. Chaperones are molecular machines able to stabilize misfolding prone proteins or even retrieve proteins trapped in non-physiological states. Here we show how ERD14 acts as a molecular chaperone inhibiting the formation of CsgA amyloid fibers in vitro. This work illustrates an alternative approach towards biofilm treatment at a molecular level.

PF-019 **Coupled folding and binding of transcription factors**

Sarah Shammas[1], Alexandra Travis[1], Jane Clarke[1]
1.-Department of Chemistry, University of Cambridge

Intrinsic protein disorder is ubiquitous in transcription, particularly within transcription factors, which frequently fold into structures upon binding to partner molecules (DNA or protein). The coupled folding and binding reactions that take place between individual transcription factors and the key hub co-activator proteins are crucial in determining the expression profile of the cell, and hence its phenotype. These interactions have been well studied by structural and equilibrium methods. Here we present mechanistic insights into the process, gained through complementary kinetics experiments, for the binding of five separate transcription factors to a single prototypical co-activator (CBP KIX). The transcription factors investigated belong to cellular (cMyb, MLL, CREB, E2A) and viral (HTLV-1 bLZ) classes. These reactions are remarkably fast; after removing the effect of long-range electrostatic rate enhancement the association rate constant is still approximately 2 x 10^7 M-1s-1, which is just above the typically quoted upper limit for diffusion-limited reactions between pairs of proteins (10^5 – 10^6 M-1s-1), and is also the highest such value we have found reported. This, combined with the apparent insensitivity of the association rate to residual structure within the unbound state, indicates that binding preceeds folding (induced fit mechanism). Interactions between KIX and its transcription factors are additionally modulated by allostery between its two binding sites. We investigate the basis for this, finding it to be mediated by changes in protein flexibility.

PF-020 **Alternative hit finding strategies for intrinsically disordered proteins, exemplified by forkhead-box transcription factors**

Harm Jan (Arjan) Snijder[1], Maria Saline[1], Tomas Jacso[1], Frank Janssen[1], Mattias Rohman[1], Tyrrell Norris[1]

1.-Astrazeneca R&D, Discovery Sciences, SE-431 83,Pepparedsleden 1

Forkhead box O (FOXO) proteins are emerging as key transcription factors in insulin and glucose metabolism, regulation of immune responses, and to balance cell proliferation, apoptosis and senescence. FOXO proteins are predicted to be intrinsically disordered proteins (IDPs); IDPs are largely unstructured and often function as hubs mediating multiple interactions. IDPs are considered to be largely evasive from classical small molecule interference and lead-generation approaches, as they lack defined binding pockets. The available methods for addressing these targets have been lagging behind and needs to be developed to assess tractability of this target class. Here we have evaluated the tractability of fragment screening on various domains of a Forkhead box O member. We could confirm the intrinsically disordered character of FOXO and used NMR screening to identify fragments that interact with FOXO. One of these fragments was subsequently confirmed as a direct FOXO binder in 2D HSQC-NMR spectroscopy and this fragment showed an effect in a FOXO reporter gene assay. These results demonstrate that fragment screening may be a valuable approach for intrinsically disordered proteins although challenges remain to expand these fragments into more potent hits in the absence of detailed structural data.

PF-021 **SDS-PAGE analysis of Aß oligomers is disserving research into Alzheimer´s disease: a call for ESI-IM-MS**

Sílvia Vilaprinyó-Pascual[1], Rosa Pujol-Pina[1], Roberta Mazzucato[1], Annalisa Arcella[2], Marta Vilaseca[3], Modesto Orozco[3], Natàlia Carulla[1]

1.-Institute for Research in Biomedicine (IRB Barcelona), 2.-Joint IRB-BSC Research Program in Computational Biology, 3.-Mass Spectrometry Core Facility, IRB Barcelona, 4.-Department of Biochemistry and Molecular Biology, University of Barcelona

The characterization of amyloid-beta peptide (Abeta) oligomer samples is critical to advance in the field of Alzheimer´s disease (AD). Here we report a critical evaluation of two methods used for this purpose, namely sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), extensively used in the field, and electrospray ionization ion mobility coupled to mass spectrometry (ESI-IM-MS), an emerging technique with great potential for oligomer characterization. To evaluate their performance, we first obtained pure cross-linked Abeta40 and Abeta42 oligomers of specific order. Analysis of these samples by SDS-PAGE revealed that SDS affects the oligomerization state of Abeta42 oligomers, thus providing flawed information on their order and distribution. In contrast, ESI-IM-MS provided accurate information, while also reported on the chemical modifications and on the structure of the oligomers. Our findings have important implications as they challenge scientific paradigms in the AD field built upon the SDS-PAGE characterization of Abeta oligomer samples.

PF-022 **Coarse-grained simulation of protein association: application to rate prediction and implication for association mechanisms**

Yinghao Wu[1],
1.-Systems and Computational Biology, Albert Einstein College of Medicine

The kinetics of protein binding is of paramount importance for understanding cellular functions. For instance, the binding kinetics between membrane receptors and their ligands control the speed of signal transduction after cells are exposed to stimulation. The experimentally measured association rates of protein binding span ten orders of magnitude, a range that was divided into two regimes. It was proposed that a fast association regime is limited by protein diffusion, while the other side of the spectrum is controlled by conformational changes. Consequently, all previous simulation methods neglected conformational changes when calculating the association rate of a diffusion-limited regime. However, the most updated theory of protein binding suggests that a protein remains in a pre-existing equilibrium of unbound conformations. Binding shifts the equilibrium toward its bound state. This highlights the importance of conformational factors for regulating protein binding. Enlightened by this conformational selection model, we hypothesize that the conformational flexibility of protein structures regulates association more widely than previously anticipated. We develop a new coarse-grained model to simulate the process of protein association via the kinetic Monte Carlo (KMC) algorithm. Each residue in this model is represented by its Cα atom and a side-chain functional site. A simple physically based potential is used to guide the relative diffusion of two interacting proteins. Given the size of the simulation box and the length of the simulation, the association rate constant can be derived by counting the frequency of dimerization among a large number of simulation trajectories. We further designed a prediction strategy that accounts for both the conformational and energetic factors of binding. Our method is able to predict rates of protein association that are highly correlated with experimentally measured values. Due to the coarse-grained feature, our model was further applied to several special cases of protein association. In one example, we studied the binding kinetics of proteins with flexible linkers. The interaction between thrombin and its functional inhibitor, rhodniin, was used as a testing system. We captured the conformational changes of flexible linkers from the all-atom molecular dynamic simulations. We found that the association with full-length flexible rhodniin was faster than its two individual domains and that their dissociation was more difficult, supporting a “flycasting” mechanism in which partial structures of an intrinsic disordered protein (IDP) dock to the target first, while the remaining segments undergo conformational searches and sequentially coalesce around the target. In another example, we studied the binding kinetics of membrane receptors from cellular interfaces. The interaction between membrane proteins CD2 and CD58, cell adhesion molecules known to mediate the activation of T cells and natural killer cells, was used as a testing system. The diffusive properties of these proteins on lipid bilayer were captured from all-atom molecular dynamic simulations. We showed that both 3D and 2D association rates could be simulated quantitatively with our method. The calculated values were close to the experimental measurements. We also provided detailed analysis of how molecular diffusions and membrane fluctuations affected 2D association.

PF-023 **(Un)structure-function relationships on the UreG enzyme in the nickel-dependent urease system**

Barbara Zambelli[1], Francesco Musiani[1], Stefano Ciurli[1]
1.-University of Bologna, Dept. of Pharmacy and Biotechnology

Urease is an essential enzyme for many pathogens and soil microorganisms. Its activity relies on the presence of nickel in the active site (1). The incorporation of this metal ion into the enzyme requires the formation of a supra-molecular chaperone involving four accessory proteins, named UreD, UreF, UreG and UreE. UreE is a metallo-chaperone involved in nickel binding and delivery into the enzyme active site. UreG is a GTPase essential for providing energy to the process of nickel site assembly. UreF and UreD form a complex that regulates the GTPase activity of UreG. The present work focuses on UreG, which exists in solution as an ensemble of inter-converting conformations (2). This observation made this protein the firstly discovered natural enzyme with an intrinsically disordered behavior, possibly allowing it to interact with different protein partners, such as UreE (3,4) and UreF (5) and cofactors, such as metal ions (6), in the urease activation network. UreG folding was studied perturbing protein conformation with temperature and denaturants, and investigating its folding response using circular dichroism, NMR and fluorescence (7). A combination of light scattering, calorimetry, mass spectrometry, and NMR spectroscopy shed light on the effect of metal ion binding onto the conformational equilibrium of UreG ensemble (8). The results suggest that metal binding and solution conditions modulate affect the protein-protein interactions and enzymatic activity of UreG. (1) Zambelli et al., Acc. Chem. Res. (2011), 44 (7) pp. 520-30 (2) Zambelli et al. J. Biol. Chem. (2005), 280(6):4684-4695 (3) Merloni et al. BBA - Proteins and Proteomics (2014), 1844(9):1662-74 (4) Yang et al. J. Biol Chem. 2015, in press (5) Fong et al. Plos Biol. 2013, 11(10), e1001678 (6) Zambelli et al. Proteins (2009), 74(1):222-239 (7) Zambelli et al. Mol. Biosyst. (2012), 8: 220-228 (8) D'Urzo et al. J. Biol. Inorg. Chem. (2014), 19(8): 1341-1354

PF-024 **Molecular insights into the VPg-Pro interaction from Pepper Vein Banding Virus: Implication in protease activity**

Pallavi Sabharwal[1], Rashmi Panigrahi[1], Srinivas Sistala[2], Savithri H S[1]
1.-Indian institute of Science, 2.-Wipro G E Healthcare Pvt Ltd

Nuclear Inclusion protein A- protease (NIa-pro) is a protease involved in processing of Pepper vein banding virus (PVBV) encoded polyprotein to generate various intermediates and mature proteins at different stages of the viral life-cycle. NIa-Pro has two domains- N-terminal Viral protein genome linked (VPg) and the C-terminal protease domain (Pro).VPg belongs to the group of proteins that are intrinsically disordered, but attain stable structures upon interaction with other globular proteins. Such protein- protein interactions have a regulatory role on the function of the interacting partners. Previously, the influence of VPg domain on the activity of Pro was studied and it was shown that there was a substantial increase in the protease activity upon interaction with VPg (both in cis and in trans). In the present investigation, several deletion mutants of VPg and NIa were constructed with a view to delineate the domain of VPg involved in interaction with Pro. It was observed that deletion of residues from N-terminus of VPg resulted in a decrease in the activity of Pro in cis and in trans probably because of the abrogation of interaction between the two domains. Interaction studies using SPR (Surface Plasmon Resonance) and ELISA confirmed that the N-terminal 22 residues of VPg are important for interaction with Pro. The N-terminal 22 residues of VPg are a part of the disordered region of VPg and their deletion resulted in the change in the secondary structure of the VPg and its oligomeric state. The Ser 129 and Trp 143 residues of Pro domain were shown to be important both for the interaction of the two domains and for the activity of protease by mutational analysis earlier. These residues were identified to be a part of WC loop (W143-C151) which relay the conformational changes to the active site catalytic triad (His 46, Asp 81 and Cys 151) leading to activation. However, mutations of these residues did not completely abolish the protease activity as well as the interaction with VPg. Therefore, in the present study H142 and H167 which are observed to interact with Trp143 and C151 (via non-covalent interactions) were mutated to alanine and the H142A and H167A mutants showed a drastic reduction in the activity of Protease. Molecular dynamics simulations of the wild type pro and the mutants revealed that Trp 143 – His 142 – His 167 – Cys 151 interaction pathway of the wild type Pro was disrupted in the mutants and additional residues were involved in the interaction pathway, such alterations in the network of interactions could be responsible for the loss of activity. However, a change in the oligomeric status of these mutants was also observed as compared to the wild-type Pro, suggesting that these residues are important for both the structural and functional integrity of Pro and its interaction with VPg. Thus, these results provide a molecular insight into the VPg-Pro interactions and the modulation of their structure and function upon mutation of residues that are part of the interaction interface.

PF-025 **A novel mutant that prevents tetramerization of amyloidogenic transthyretin protein involved in family cardiac amyloidosis (FAC)**

Priscila Ferreira[1], Carolina Andrade[1], Antonio Neves[4], Herbert Pereira[2], Fernando Palhano[1], Marcia Cruz[3], Debora Foguel[1]

1.-Institute of Medical Biochemistry , Federal University of Rio de Janeiro (UFRJ), 2.-Phisics Institute of São Carlos, Universitiy of São Paulo (USP),, 3.-University Hospital CFF, Federal University of Rio de Janeiro (UFRJ), 4.-Department of Microbiology, Fiocruz Pernambuco

Transthyretin (TTR) is one of many proteins that are capable of forming amyloid fibrils in vivo. This protein is associated with two distinct amyloidosis: Familial Cardiac Amyloidosis (FCA) that causes a restrictive cardiomyopathy and Familial Amyloid Polyneuropathy (FAP) that affect peripheral nerves, they are hereditary and caused by mutations in the TTR gene. The non mutated protein can also aggregate in cardiac tissue in advanced age patients. The diagnosis was established at University Hospital since 2008 due to a collaborative between our group and the center of Amyloidosis Antônio Rodrigues de Mello (CEPARM). The only mutation found in Brazil was V30M in 3 patients diagnosed in France. Our group discovered 5 new mutation not described in Brazil and a novel mutation not described yet A19D. The diagnosed patients are registered in Transthyretin Amyloidosis Outcomes Survey (THAOS). The novel mutation A19D causes a severe restrictive cardiomyopathy that is certainly related to a higher profile of aggregation observed for this mutant if compared to others amyloidogenic mutants of TTR. Structural predictions using a bioinformatics tool called FoldX showed that the insertion of the mutation cause a electrostatic clash that facilitates the dissociation and aggregation of protein. This mutant was purified heterologously and biophysical studies revealed that this protein is a dimer and not a tetramer as commonly the TTR structure. The crystallographic structure indicates that this mutant is structurally identical to wild type. Biophysical studies revealed that this protein is a dimer and not a tetramer as commonly the TTR structure. The thermodynamic stability of A19d is lower than the wild type TTR. The aggregation profile showed us that this protein can aggregate in a higher manner and with a fast kinetic to that observed for others amyloidogenic mutants of TTR, forming fibers in two hours of aggregation. Heterotetramers of A19D and WT are able to aggregate in the same fiber structure. The analysis of interface interaction of this mutant using the PDBSum showed modifications in the profile of hydrogen bonds and non bonded contacts. In addition the oligomers of A19D are toxic for primary culture of cardiomyocytes from murine heart. The amyloidogenic profile displayed by this new mutant can be directly correlated with the aggressiveness observed in the disease developed by the identified patient. Furthermore the recent consolidation of TTR diagnosis in our university hospital led to the identification of the rare A19D variant in a Brazilian patient, suggesting that other new, uncharacterized mutants could be identified in the coming years.

PF-027 **Multiple cellular proteins interact with LEDGF/p75 through a conserved unstructured consensus motif**

Petr Tesina[1, 2, 3], Kateřina Čermáková[4], Magdalena Hořejší [3], Milan Fábry[3], Frauke Christ[4], Jonas Demeulemeester[4], Zeger Debyser[4], Jan De Rijck[4], Václav Veverka[1], Pavlína Řezáčová[, 3],
1.-IOCB AS CR, 2.-IMG AS CR, 3.-Faculty of Science, Charles University in Prague, 4.-KU Leuven

LEDGF/p75 protein is an epigenetic reader crucial for HIV integration and MLL1 fusion-driven leukemia development. Its interactions with HIV integrase (HIV IN) and MLL1 are considered an attractive therapeutic target for drug development [1]. The LEDGF/p75-MLL1-Menin complex was structurally characterized, but only partially [2]. Using NMR spectroscopy, we identified and mapped a novel MLL1-LEDGF/p75 interface. Colony forming assays in MLL1-AF9+ leukemic cells expressing MLL1 interaction-defective LEDGF/p75 mutants revealed that this additional interface is essential for leukemic transformation. Interestingly, the newly defined interface overlaps with the binding site of known LEDGF/p75 interactor, the HIV integrase [1]. While the pathophysiological interactions of LEDGF/p75 are intensively studied, its physiological role remains unclear. Since LEDGF/p75 contributes to HIV integration and leukemic transformation and has become a new therapeutic target for drug development, it is crucial to study its physiological interactions. In addition to HIV IN and MLL1-Menin, the LEDGF/p75 integrase binding domain (IBD) also interacts with several other proteins [3,4]. Our recent data (manuscript accepted in Nat. Commun.) revealed structural details of LEDGF/p75 interactions with physiological binding partners. The interaction with the LEDGF/p75 IBD is maintained by an intrinsically disordered IBD-binding motif (IBM) common to all known cellular partners. Based on the knowledge of this motif, we identified and validated IWS1 as a novel LEDGF/p75 interaction partner. The detailed mapping of physiological interaction interfaces on the IBD revealed a notable overlap with the region involved in interaction with HIV IN. Utilizing the structural information, we explained how HIV IN outcompetes the cellular proteins. Finally, the similar binding modes of LEDGF/p75 interaction partners represent a new challenge for the development of selective interaction inhibitors.

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PF-028 **Exploring Anti- amyloidogenic Attributes of Lantibiotic Nisin**

Deovrat Begde[1]
1.-Department of Biochemistry & Biotechnology, , Dr. Ambedkar College, Deekshabhoom

Amyloid fibrils result from misassembly of peptides either formed during conventional or aberrant intracellular protein processing events. β-amyloid peptides which nucleate Amyloid fibrils formation are well known to be responsible for neurodegenerative disorders like Alzheimer's. Surprisingly, mammalian system has an inbuilt program for critical regulation of synthesis of amyloid fibrils during formation of melanosomes. Similarly, pathogenic bacteria like E. coli and Salmonella are known to produce extracellular curli fimbrae, which has all the hallmarks of a typical amyloid and presumably necessary for host colonization and virulence. Here we investigate a unique mechanism of virulence attenuation employed by Lantibiotic, Nisin, which is a autoinducer pheromone peptide produced by a probiotic bacterium Lactococcus lactis to selectively exterminate other competitive Gram-positive bacteria. The preliminary study results gave promising evidences about the exceptional ability of nisin to interfere with cell-cell communication of selected Gram negative opportunistic pathogens, whereby it appeared to create a chaos perhaps attributing towards swarming inhibition and biofilm disruption. Nisin caused a disturbance in expression of virulence factors in Proteus mirabilis, Pseudomonas aeruginosa and Escherichia coli, possibly through abolishment in expression of Gram negative signaling molecule, homoserine lactone (HSL). Nisin demonstrated an unusual competitive inhibition of E. coli curli assembly besides downregulation of HSL production which regulates curli biogenesis in E. coli population. When curli positive E. coli clinical isolates were treated with nisin their ability to form curli fimbrae was significantly reduced. An in silico docking analysis revealed remarkable similarity in the interaction motifs involved in curli nucleator protein, Csg B interaction with nisin compared to those required for curli structural protein, Csg A and Csg B interaction. Based on our results we hypothesize that nisin might block the access of Csg A to the amyloidogenic domain of Csg B by interacting with the residues necessary for Csg A interaction with Csg B. Although our prediction needs validation, the present investigation provides sufficient evidences to demonstrate high anti-virulent potential of nisin against Gram negative bacteria which are known to be eluded from its anti-microbial spectrum. Keywords: Lantibiotics, Nisin, Curli, Amyloid, Anti-virulence, Homoserine lactone

PF-029 **Amyloidogenic lysozyme accumulates in the endoplasmic reticulum tangling with GRP78/BiP and evokes ER stress**

Yasushi Sugimoto[1], Yoshiki Kamada[1], Yusuke Nawata[1], Takahiro Kusakabe[2]
1.-Kagoshima University, The United Graduate School of Agri. Sci., 2.-Kyushu University, Graduate School of Agri. Sci.

Naturally occurring single mutants, I56T, F57I, W64R and D67H of lysozyme in human, have been known to form abnormal protein aggregates (amyloid fibrils) and to accumulate in several organs, including liver, spleen and kidney, resulting in familial systemic amyloidosis. These human pathogenic lysozyme variants are considered to raise subtle conformational changes compared to the wild type. Here we examined the effects of the aberrant mutant lysozymes I56T, F57I,W64R and D67H, each of which possesses a point mutation in its molecule, on a cultured human cell line, HEK293, in which the genes were individually integrated and overexpressed. Western blot analyses showed lesser amounts of these variant proteins in the medium compared to the wild type, but they were abundant in the cell pellets, indicating that the modified lysozyme proteins were scarcely secreted into the medium but were retained in the cells. Immunocytochemistry revealed that these proteins resided in restricted regions which were stained by an endoplasmic reticulum (ER) marker. Moreover, the overexpression of the mutant lysozymes were accompanied by marked increases in XBP1s and GRP78/BiP, which are downstream agents of the IRE1_ signaling pathway responding to the unfolded protein response (UPR) upon ER stress.RNAi for the mutant lysozymes' expression greatly suppressed the increases of these agents. Next, we addressed the interaction between amyloidogenic lysozyme and GRP78/BiP as the former proteins were obtained by immunoprecipitation with the latter protein as well as colocalization of both proteins in the ER. Lysozyme composes of α-domain rich in helices and β-domain rich in sheet. Two helices of α1 and α2 in the N-terminal region arrange in parallel and face to face where hydrophobic amino acids at the 3F, L8, L12, L15, L25 and L31 allocate with equal interval there. In the back of dock, there is a core region of amyloid fibril formation, of which the side chain of I56 is exposed on the protruding. Probably, these hydrophobic amino acids might be crucial for lysozyme folding. Although mutated lysozymes undergo folding by GRP78/BiP in such environment, the dissociation of the GRP from lysozyme by failure of folding is likely inhibited and both proteins remain bound to, resulting in staying to the ER. A part of aberrant lysozymes seem to remain bound to GRP78/BiP during folding and insolubilize with aggregation, thus accumulate in the ER accompanied with ER stress. Lysozyme amyloidosis might be caused by long-term accumulation in the endoplasmic reticulum of the abnormal protein.

PF-030 **Structural characterization of toxic oligomers that are kinetically trapped during alpha-synuclein fibril formation**

Serene W. Chen[1], Srdja Drakulic[2], Emma Deas[3], Myriam Ouberai[4], Francesco A. Aprile[1], German Rivas[5], Andrey Y. Abramov[3], Jose Maria Valpuesta[2], Christopher M. Dobson[1], Nunilo Cremades[1]
1.-Department of Chemistry, University of Cambridge, CB2 1EW, 2.-Department of Macromolecular Structure, Centro Nacional de Biotecnologia, 28049, 3.-Department of Molecular Neuroscience, University College London, WC1N 3GB, 4.-Nanoscience Centre, Department of Engineering, University of Cambridge, CB3 0FF, 5.-Department of Cellular and Molecular Biology, CIB-CSIC

The accumulation of abnormally aggregated proteins within the body is a common feature of several medical disorders, such as Alzheimer's disease, Parkinson's disease and diabetes mellitus type 2. While the specific protein found to be the major component of such deposits varies from one disease to another, the formation of the pathological aggregates seems to occur via a common process of misfolding and self-assembly of a normally soluble polypeptide chain into a series of oligomeric intermediates and, ultimately, into insoluble amyloid fibrils that accumulate within specific organs and tissues. Increasing evidence indicates that certain oligomeric protein species generated during the self-assembly of specific proteins into ordered fibrillar aggregates can be highly cytotoxic and are likely to be key players in the initiation and spreading of neurodegenerative diseases. However, little detailed structural information is currently available for these oligomeric species due to their often transient nature and, more importantly, because of their variability in terms of size and structure. We report here the isolation and detailed characterization of an ensemble of stable toxic oligomers of alpha-synuclein, the protein whose deposition is the hallmark of Parkinson's disease. By defining and minimizing the degree of heterogeneity of these isolated alpha-synuclein oligomers which have accumulated during the process of amyloid formation, we have identified distinct subgroups of oligomers and determined their structural properties and three-dimensional molecular architectures. This characterization has been achieved by the application of a set of complementary biophysical techniques, including a variety of spectroscopic techniques along with analytical ultracentrifugation, atomic force microscopy, and electron microscopy. Although these oligomers exist in a range of sizes, with different extents and nature of beta-sheet content and exposed hydrophobicity, all the oligomeric subgroups possess hollow cylindrical architectures with marked similarities to amyloid fibrils. This suggests that these types of oligomers are kinetically trapped during protein self-assembly and that the accumulation of at least some forms of amyloid oligomers is likely to be a consequence of very slow rates of rearrangement of their beta-sheet structures. Our findings reveal the inherent multiplicity of pathways of protein misfolding and the key role the beta-sheet geometry acquired in the early stages of the self-assembly process plays in dictating the rates of structural conversions, and thus the kinetic stabilities and pathological nature of different amyloid oligomers. The results of this study provide the basis for a more complete understanding of the nature of the self-assembly of polypeptides into beta-sheet rich amyloid aggregates, and potentially contributes to efforts to identify specific targets for drug discovery.
Chen SW, et al. (2015) Proc Natl Acad Sci USA 112(16):E1994-E2003

PF-031 **Metal ions modulate the conformation of Starmaker-like protein from Oryzias latipes**

Mirosława Różycka [1], Magdalena Wojtas[1], Natalie Mutter[2], Benjamin Schuler[2], Jacek Gapiński[3,4], Andrzej Ożyhar[1]

1.- Wrocław University of Technology, Department of Biochemistry, 2.-University of Zurich, Department of Biochemistry, 3.-Molecular Biophysics Department, Faculty of Physics, Adam Mickiewicz University, 4.-NanoBioMedical Center, Adam Mickiewicz University

Fish otoliths and mammalian otoconia, biominerals composed of calcium carbonate and organic matrix, are involved in the functioning of the inner ear, the sensory organ that plays an important role in hearing and balance [1]. However, their developmental origins, growth, and the role of the matrix, especially the protein component, are still poorly understood. It has been shown that proteins involved in the formation of biominerals are usually very acidic. They often belong to the group of intrinsically disordered proteins (IDPs), a class of proteins devoid of a rigid tertiary structure [2, 3]. The shape and polymorph selection of calcium carbonate otolith in Danio rerio is controlled by the Starmaker (Stm) protein [4]. Recently, a gene was identified encoding the Starmaker-like (Stm-l) protein from Oryzias latipes, a putative homologue of Stm. It has been suggested that Stm-l has a similar function as Stm, although there is no sequence similarity between Stm and Stm-l [5]. Several methods, such as size exclusion chromatography, CD spectroscopy and analytical ultracentrifugation demonstrated that Stm-l is an coil-like IDP, with the tendency to form locally ordered structures [6]. Because Stm-l was suggested to play a crucial role in calcium carbonate mineralization, it is possible that calcium ions may influence its conformation, as was previously shown for Stm [7]. However, other ions may also be involved in this process. The aim of this study was to investigate the effect of mono and divalent metal ions on the conformation of Stm-l. We used single molecule Förster resonance energy transfer (smFRET) and fluorescence correlation spectroscopy (FCS), which have shown that calcium ions compacts the proteins most efficiently, followed by magnesium and the monovalent ions. The difference in the effect of monovalent and divalent ions on the protein dimensions is likely to result from the different properties of the ions, like charge density and radius. CD experiments have shown that a high excess of calcium ions caused the formation of ordered secondary structure in Stm-l, which may be crucial for the formation of calcium carbonate crystals, when the ratio of building ions to protein is high. This work was supported by the statutory activity subsidy from the Polish Ministry of Science and Higher Education for the Faculty of Chemistry of the Wroclaw University of Technology. The cost of participation was covered by Wroclaw Centre of Biotechnology, programme The Leading National Research Centre (KNOW) for years 2014-2018.

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PF-032 **Intrinsically disordered recombinant 57K fragment of human DMP1 influences the in vitro crystallization of CaCO3**

Aleksandra Porebska[1], Andrzej Ozyhar[1], Piotr Dobryszycki[1]
1.-Wroclaw University of Technology, Department of Biochemistry

Biomineralization refers to a wide range of processes by which living organisms form mineral crystals. Usually those crystals are formed and deposited for different purposes within the organic matrix and vesicles. Otoliths in bony fishes and otoconia in mammals are composite crystals consisting of calcium carbonate. These biominerals are part of the gravity and linear acceleration detection system of the inner ear. CaCO3 from otoconia of different species have various morphology and crystalline structure and different protein composition what underlines the importance of proteins for the proper otoconia formation. Dentin matrix protein 1 (DMP1) is a noncollagenous protein of extracellular matrix predominately expressed in bone and dentin. DMP1 plays an important role in proper phosphate homeostasis and mineralization. It has been demonstrated that DMP1 is proteolytically processed into fragments, including 37K N-terminal region and 57K C-terminal region. As many proteins characterized to be engaged in biomineralization, DMP1 and its fragments belong to the group of intrinsically disordered proteins (IDPs). It has been suggested that DMP1 and its fragments can take a part in otoconia mineralization, as the protein is present in mouse otoconia, but the role of DMP1 and its fragments in the mineralization of calcium carbonate has not been examined until now. To determine the influence of the DMP1 fragments for otoconia development, 57K DMP1 protein was expressed in bacterial expression system, purified and used in in vitro biomineralization test of calcium carbonate. In particular, immobilized metal anion affinity chromatography (IMAC) was applied as a first step of purification procedure. Because of high content of acidic amino acids, ion exchange chromatography with a Mono Q column was used as a next step. Pure protein was tested in in vitro mineralization test which demonstrated that 57K DMP1 influences size and shape of calcium carbonate crystals. Raman spectroscopy revealed the calcite polymorph of crystals obtained in the presence of 57K DMP1. Circular dichroism spectra showed the intrinsic disorder of 57K protein. Stokes radius estimated based on size exclusion chromatography of protein suggested an extended conformation of protein. Thus, 57K DMP1 may play a role in biomineralization of otoconia.
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PF-033 **Structural analysis of the C-terminal domain of Drosophila melanogaster Methoprene tolerant protein (Met)**

Marta Kolonko[1], Katarzyna Ożga[1], Rafał Hołubowicz[1], Andrzej Ożyhar[1], Beata Greb-Markiewicz[1]
1.-Wroclaw University of Technology

The development of insects is regulated by the combined action of ecdysteroids and juvenile hormones (JH). Pulses of 20-hydroxyecdysone (20E) initiate each step of metamorphosis, while JH modulates its action and prevents precocious differentiation. The biological and molecular mechanism of 20E action is well described. In contrary, the way of the JH activity is still poorly understood. In 1986 Wilson and Fabian [1] reported that Drosophila melanogaster mutants lacking Met are resistant to toxic doses of JH and its analogue methoprene. It has been proved, that Met binds JH at physiological conditions. Therefore Met is believed to be a putative JH receptor. Met may also be involved in a cross-talk between two hormonal signalling pathways, involving 20E and JH. The detailed structure of Met is still unknown. Therefore our main aim is to characterize structural properties of Met. In silico analysis performed on a full-length Met suggested, that N-terminal part of Met contains three conserved domains characteristic for bHLH-PAS transcription factors, whereas C-terminal part is most probably unstructured. For further structural analysis, we divided Met into two fragments: the first one containing N-terminal part with bHLH and PAS domains and the second one encompassing C-terminal part of Met. In this presentation, the results of experiments executed for C-terminal part of Met (MetC) are presented. The expression of MetC in a E. coli cells, at a low temperature, enabled to obtain high amount of a soluble protein. After four steps of purification, the samples of protein with purity suitable for further analysis were collected. CD spectroscopy analysis indicated, that MetC is highly disordered. After incubation with TFE (tetrafluoroethylene) it acquires secondary structure elements consisting dominantly of α-helices. Analysis of the hydrodynamic properties of MetC performed by analytical size-exclusion chromatography and analytical ultracentrifugation proved that MetC is a highly elongated protein with a disordered structure. In conclusion, the results strongly suggest that C-terminus of Met shows the characteristics typical for intrinsically disordered proteins. The disordered tails of transcription factors are often involved in interactions with other partners by serving as a platform for multiple interactions. It can be hypothesised that the lack of defined structure of MetC enables this protein to play an important role in signalling pathways. This work is supported by a statutory activity subsidy from the Polish Ministry of Science and Higher Education for the Faculty of Chemistry of Wrocław University of Technology. Attending in the Conference is supported by Wroclaw Centre of Biotechnology, programme The Leading National Research Centre (KNOW) for years 2014-2018. [1] Wilson T., Fabian J.: ‘A Drosophila melanogaster mutant resistant to a chemical analog of juvenile hormone’; Dev Biol. 1986 Nov;118(1):190-201

PF-034 **Designed cross-amyloid inhibitors of amyloid self-assembly**

Eleni Malideli[1], Erika Andreetto[1], Li-Mei Yan[1], Michael Kracklauer[1], Karine Farbiarz[1], Marianna Tatarek-Nossol[2], Aphrodite Kapurniotu

1.-Division of Peptide Biochemistry, Technische Universität München, 2.-Institute of Biochemistry and Molecular Cell Biology, RWTH Aachen University

Designing inhibitors of amyloid self-assembly of intrinsically disordered polypeptides and proteins is a difficult task. Cross-interactions between amyloidogenic polypeptides have lately emerged as important modulators of protein self-assembly and similar surfaces are often involved in both self- and hetero-association. We have earlier identified a high affinity interaction between non-fibrillar and non-toxic species of Abeta and IAPP, two intrinisically disordered, key amyloidogenic polypeptides in Alzheimer's disease (AD) and type 2 diabetes (T2D) (Yan et al., Angew. Chem. Int. Ed. (2007); Andreetto, Yan et al., Angew. Chem. Int. Ed. (2010)). The Abeta-IAPP interaction results in formation of non-fibrillar and non-cytotoxic Abeta-IAPP hetero-oligomers thus suppressing cytotoxic self-association of both polypeptides. More recently, we have identified short IAPP segments as "hot segments" of both IAPP self- and its hetero-association surface with Abeta (Andreetto, Yan, et al., Angew. Chem. Int. Ed. (2010)). Capitalizing on self- and cross-amyloid interactions, we designed highly effective, peptide-based inhibitors of amyloid self-assembly of Abeta and IAPP. Due to their favourable properties the designed peptides are promising leads for targeting protein aggregation in AD, T2D or both diseases while the inhibitor design strategy should be applicable to other amyloidogenic polypeptides and proteins as well.

PF-035 **Preparation of homogenous recombinant FKBP39 protein from Tribolium castaneum**

Aneta Tarczewska[1], Małgorzata Kozłowska [1], Andrzej Ożyhar [1]
1.-Department of Biochemistry, Faculty of Chemistry, Wrocław, University of Technolo

Juvenile hormone (JH) and ecdysteroids regulate a wide variety of developmental and physiological processes in insects. In contrast to ecdysteroids, the exact role and mechanism of action of JH has not been understood. Also the cross - talk between JH and ecdysteroids remains a puzzle. Recently, common 29bp regulatory element (JHRE) in promoter regions of some JH-responsive genes in Drosophila melanogaster, was identified. Two proteins, immunophilin FKBP39 and calponin like Chd64 were found to bind JHRE and interact with each other as well as other nuclear proteins including ecdysteroid receptor, ultraspiracle nuclear receptor and methoprene tolerant protein. This suggest that FKBP39 and Chd64 play important role in cross – talk between JH and ecdysteroid. Researches on FKBP39 from D. melanogaster are already carried out in our laboratory and we decided to extend our studies to distantly related species - the red flour beetle Tribolium castaneum. Tribolium has become an important model organism for comparative to Drosophila studies of insect development and growth. It shows classical developmental response to JH. To facilitate the exploration of the structure function relationship of FKBP39 we developed and optimised a protocol for its efficient expression and purification. To express FKBP39 recombinant plasmid vectors pQE80L-XH, pQE80L-SX, pQE80L-SXH in fusion with His-tag (XH), Strep-tag (SH) and both of those tags (SXH) were prepared. Solubility analysis revealed that all expressed recombinant proteins remain in the soluble fraction of bacterial proteins. Proteins fused with His-tag and Strep-tag were able to bint TALON® and StrepTactin resin. Finally we elaborated a two-step purification procedure for the homogenous FKBP39 using affinity chromatography (TALON® resin) and gel filtration. The molecular mass value (40221.86 ± 1 Da) was determined using electrospray ionisation mass spectroscopy. The value is compatible with theoretical value (40223.7 Da). TcFKBP39 can further be used for structure – function relation studies. ACKNOWLEDGMENTS: The work was supported by the National Science Centre grant (2012/05/B/NZ1/00659). Conference fee was covered by Wroclaw Centre of Biotechnology, programme The Leading National Research Centre (KNOW).

PG-001 **Live-cell Measurements of the Conformational Rearrangements in Bax at the Initiation of Apoptosis**

Robert Gahl[1], Yi He[1], Shiqin Yu[1], Nico Tjandra[1]
1.-Biochemistry and Biophysics Center, NHLBI, NIH

Apoptosis, the process of programmed cell death, must be carefully regulated in multi-cellular organisms to ensure proper tissue homeostasis, embryonic development and immune system activity. The Bcl-2 family of proteins regulates the activation of apoptosis through the mitochondria pathway. Dynamic interactions between pro- and anti-apoptotic members of this family keep each other in check until the proper time to commit to apoptosis. The point of no return for this commitment is the permeabilization of the outer-mitochondrial membrane (OMM). Translocation of the pro apoptotic member, Bax, from the cytosol to the mitochondria is the molecular signature of this event. Molecular interactions and conformational changes associated with this event have been difficult to obtain due to challenges associated with taking subtle measurements in the complex environment of live cells. To circumvent these challenges, we developed a novel method to reliably detect Förster Resonance Energy Transfer (FRET) between pairs of fluorophores to identify intra-molecular conformational changes and inter-molecular contacts in Bax as this translocation occurs in live cells. In the cytosol, our FRET measurements indicated that the C-terminal helix is exposed instead of tucked away in the core of the protein. This coincided with measurements using fluorescence correlation spectroscopy (FCS) that showed that cytosolic Bax diffuses much slower than expected, suggesting possible complex formation or transient membrane interaction. We propose that this exposed helix allows for this contact to occur. Cross-linking the C-terminal helix (α9) to helix α4 reduced the instances of these interactions while at the same time yielded FRET measurements that are consistent with the α9 helix tucked into the core of the protein. After translocation, our FRET measurements showed that Bax molecules form homo-oligomers in the mitochondria through two distinct interfaces involving the BH3 domain (helix α2) and the C-terminal helix. These findings provide insight into the molecular architecture that may involve possible contacts with other Bcl-2 proteins to permeabilize the OMM, which would also be necessary for the regulation of apoptosis.

PG-002 **Bacterial cell division in super resolution**

Jie Xiao[1], Carla Coltharp[1], Jackson Buss[1], Xinxing Yang[1]
1.-Department of Biophysics and Biophysical Chemistry, Johns Hopkins School of Medicine

Current advances in fluorescence-based superresolution imaging have illustrated great details of cellular structures that are invisible to conventional fluorescence light microscopy. The significantly improved spatial resolution is especially advantageous for bacterial cells because of their small sizes. In the past few years the spatial organization and dynamics of a variety of bacterial cellular structures and protein macromachineries have been revealed with unprecedented details. As the field matures, it is now time to focus on the functional aspect of the observed spatial organizations and dynamics. Are they essential in carrying out a specific cellular function? Do they play a regulatory role in controlling the on and off of a certain cellular process? In this work I will present a few examples from our laboratory that examine the spatial and functional organization of macromolecules involved in bacterial cell division.

PG-003 **Mapping transcription factors dynamics and interactions by advanced fluorescence microscopy techniques**

Martin Stortz[1], Luciana Bruno[2], Paolo Annibale[3], Enrico Gratton[3], Adali Pecci[2], Valeria Levi[4,5]
1.-IFIByNE-Conicet, 2.-IFIBA-Conicet & Dept. of Physics, University of Buenos Aires, 3.-LFD-University of California, 4.-Dept. of Biological Chemistry, University of Buenos Aires, 5.-IQUIBICEN-Conicet

Transcription factors (TF) exert their function by interacting with other proteins and binding to DNA. The nucleus is a compartmentalized space, and the spatial organization of TFs and their partners represents other step of gene expression regulation. We used the glucocorticoid receptor (GR) as a model of TF's mechanism of action. GR is a ligand-activated TF with a relevant role in physiology and a great variety of effects. It can be recruited to specific response elements on DNA or interact with other TFs. Also, the activity of GR is modulated by different co-regulators, e.g. TIF2/GRIP1. GR and TIF2 do not distribute homogeneously within the nucleus but accumulate in distinctive clusters. The functional role of this particular intranuclear organization remains unknown. We used advanced fluorescence microscopy techniques to study the dynamics of GR and TIF2 in the nucleus of living cells with high spatial and time resolution. GR and TIF2 fused to fluorescent tags were transiently expressed in newborn hamster kidney (BHK) cells and visualized by a confocal microscope. Fluorescence correlation spectroscopy (FCS) experiments were carried on to measure the intranuclear mobility of both proteins. The method is based on the analysis of fluorescence intensity fluctuations due to the movement of fluorescent molecules in and out the confocal volume. The data could be fitted with a model that considers a free diffusion of TIF2 and GR in the nucleus and their binding to fixed targets. We also studied the dynamics of different GR mutants in the presence of different ligands and our results suggest that the binding depends on DNA. Both GR and TIF2 autocorrelation curves reveal an increase in the bound population upon GR activation by its agonist dexamethasone (Dex). A cross-correlation analysis showed that, as expected, Dex-stimulus increases the population of GR-TIF2 complexes. Without hormone, GR shows a homogeneous distribution and TIF2 forms large clusters in the nucleus. Upon Dex-binding, GR accumulates in the nucleus, is rapidly recruited to TIF2 foci and there is an important re-distribution of both proteins, that co-localize in the same pattern of small intranuclear clusters. The dynamics of GR and TIF2 molecules at these clusters were studied by performing orbital-scanning measurements, tracking the clusters position in silico and analyzing the intensity fluctuations of the clusters along time. A positive cross-correlation between both channels indicates that Dex-bound GR and TIF2 interact at these foci and dissociate from them forming TIF2-GR hetero-complexes. In conclusion, advanced fluorescence microscopy methods allowed obtaining a dynamical map of GR distribution and function in the nucleus of mammalian living cells.

PG-004 **Assembly of membrane pores as a mechanism for amyloid cytotoxicity by the bacterial prionoid RepA-WH1**

Cristina Fernández[1], Rafael Núñez-Ramirez[1], Mercedes Jimenez[1], Germán Rivas[1], Rafael Giraldo[1]

1.-Centro de Investigaciones Biológicas, CSIC

Amyloid fibril formation is associated with human neurodegenerative diseases. Prefibrillar oligomers formed during the fibril assembly process, rather than mature fibrils are known to be central to disease and may be responsible for cell damage. A commonly proposed mechanism for the toxicity of small oligomers is their interaction with the lipid bilayer of cell membranes, leading to loss of membrane integrity [1]. Recent studies from our laboratory have shown that RepA-WH1, a winged-helix domain from a bacterial plasmid replication protein, can assemble into amyloid fibrils in vitro. When expressed in Escherichia coli RepA-WH1 functions as a cytotoxic protein that shares features with the mammalian amyloid proteinopathies. These features have proved RepA-WH1 to be a suitable synthetic model system to study protein amyloidosis [2,3,4]. In this work, using the RepA-WH1 bacterial model system, we have studied the interaction between the protein and model membranes (large and giant unilamellar lipid vesicles, LUVs, and GUVs respectively). RepA-WH1 shows association and aggregation to membranes composed of anionic phospholipids. Protein association in GUVs did not result in lysis of the vesicles, suggesting the assembly of discrete protein pores as the mechanism for RepA-WH1 membrane damage. To investigate the formation of pores we analyzed by electron microscopy the aggregation of RepA-WH1 in the presence of a pre-formed E. coli lipid monolayer. The EM images show the presence of pore-like particles on the monolayer. Amyloid pores formation explains the permeabilization effect of RepA-WH1 in vesicle models and is in agreement with observations for human amyloidogenic proteins. The approaches presented here provide a deeper insight into amyloid cytotoxicity towards membranes and will make possible the assay of inhibitors and effectors of amyloidosis under controlled conditions. References [1] Butterfield and Lashuel. Angew Chem Int Ed. 2010; 49:5628-5654. [2] Giraldo, et al. Prion 2011; 5:60-64. [3] Gasset-Rosa, et al. Mol Microbiol. 2014; 91:1070-1087. [4] Fernández-Tresguerres, et al. Mol Microbiol. 2010; 77:1456-1469. Sponsored by Jody McGinness (jmcginness@proteinsociety.org)

PH-001 **Investigation of allosteric communication pathways in human ß2-adrenergic receptor**

Basak Akdas[1], Ozge Kurkcuoglu[2], Pemra Doruker[1], Demet Akten[3]
1.-Department of Chemical Eng. and Polymer Research Center, Bogazici University, 2.-Department of Chemical Engineering, Istanbul Technical University, 3.-Department of Bioinformatics and Genetics, Kadir Has University

β2-adrenergic receptor (β2AR) is a member of G protein-coupled receptors, which represent the single largest family of cell surface receptors involved in signal transduction. β2AR recognizes a variety of ligands and communicates with cytoplasmic G-proteins by transmitting signals through the cellular membrane. Thus, investigation of communication pathways for β2AR may give important insights for understanding its allosteric mechanisms and identifying new target sites for more specific and efficient drug molecules to be used in the treatment of pulmonary and cardiovascular disease. In this study, various conformations from 2 μs molecular dynamics (MD) simulations and available crystal structures of human β2AR were investigated to reveal alternative signaling pathways between its extra and intracellular regions. Specifically, shortest communication paths connecting key residues (more than 35 Å apart) at the orthosteric ligand binding site (D113, S203, T286, F289, N312) to either L266 or S329 located near the G-protein binding site were investigated. The conformers from previous MD simulations[1] include the intracellular loop 3 (ICL3), which especially affects the transmembrane collective dynamics but is lacking in x-ray structures. The protein was described as a graph composed of nodes linked by edges. Nodes were placed at the alpha-carbon atoms and the edges were calculated based on the number of atom-atom interactions within a cut-off distance 4.5 Å for each residue pair. Twenty shortest pathways were revealed using k-shortest path algorithm[2] on the coarse-grained network. Our results indicated that distinct signaling paths progressed most frequently on TM6 but alternative paths were also present, which passed partially through TM5, TM7, TM3 or TM2 depending on the conformation. Among the critical residues that transmitted the signal between distant sites, F282 and N318 were detected, whose functional roles were reported in previous experimental studies. Pathway shifting was observed depending on the open-to-closed transition of ICL3 during MD simulations.

[1] Ozcan, O., Uyar, A., Doruker, P., Akten, E.D. Effect of intracellular loop 3 on intrinsic dynamics of human β2-adrenergic receptor. BMC Structural Biology, 2013; 13:29.
[2] Yen, J. Y. Finding the k shortest loopless paths in a network. Management Science, 1971; 17, 712.

PH-002 **Neonatal diabetes and congenital hyperinsulinism mutations change molecular interactions in SUR1 NBD1**

Claudia Alvarez[1,2], Marijana Stagljar[2], Voula Kanleis[1,2,3]

1.-Department of Chemistry, University of Toronto, 2.-Department of Chemical and Physical sciences, University of Toronto, 3.-Department of Chemical and Physical sciences, University of Toronto

The sulfonylurea receptor 1 (SUR1) is an ATP binding cassette (ABC) protein that forms the regulatory subunit in KATP channels found in the pancreas and the brain. MgATP binding and hydrolysis at the two cytosolic nucleotide binding domains (NBD1 and NBD2) in SUR1 control gating of the KATP channel pore.1,2 Proper regulation of KATP channel gating by SUR1 is critical.2 Over 100 mutations that lead to diabetes, hyperinsulinism and developmental delay have been identified in different domains of SUR1, including the NBDs.3 Therefore, molecular-level understanding of the structure and function of the NBDs is essential for designing improved treatments for SUR-related diseases. Here we present biophysical and biochemical studies aimed at understanding the effect of disease-causing mutations on the conformation and nucleotide binding of SUR1 NBD1. Specifically, we are investigating SUR1 NBD1 mutations that cause neonatal diabetes (R826W and H863T) or congenital hyperinsulinism (C717 Δ, G716V, R824G, R837 Δ and K890T).3 Our nuclear magnetic resonance (NMR) data shows that the hyperinsulinism mutation K890T causes chemical shift changes throughout the spectrum of NBD1, implying overall changes in protein conformation that may affect MgATP binding and inter-domain interactions with other domains in the SUR1 protein. Size-exclusion data show that the other hyperinsulinism mutations (C717 Δ, G716V, R824G, R837 Δ) produce mostly aggregated protein, likely as a result of misfolding of NBD1. Misfolding of NBD1 may be the underlying cause of reduced KATP trafficking seen with these mutations and hence decreased KATP channel gating observed in hyperinsulinism. In contrast to the K890T mutations, the congenital diabetes-causing mutations (R826W and H863T) cause few NBD1 NMR spectral changes. However, the congenital diabetes mutation R826W decreases the affinity of NBD1 for MgATP, which is unexpected for congenital diabetes mutations. Our fluorescence, circular dichroism and microscale thermophoresis data corroborate the results that we have obtained by NMR spectroscopy. Our data provide molecular-level details on the effects of disease causing mutations in human SUR1.

1. Campbell, J.D. et al.(2003) Embo Reports 4(11): 1038-104

2. Nichols, C. G. (2006). Nature 440(7083): 470-476 3. Lang, V. et al. (2010) Pharmacogenomics and Personalized medicine 3: 145-161

PH-003 **Glycosylation of EGFR Extracellular Domain Induces Receptor Stability**

Maryam Azimzadeh Irani[1,2], Chandra Verma[1,2]

1.-Bioinformatics Institute (A*-STAR), Singapore, 2.-School of Biological Sciences, Nanyang Technological University

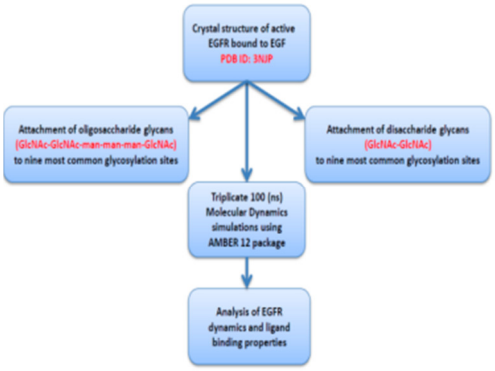
Background

The Epidermal Growth Factor Receptor (EGFR), a tyrosine kinase glycoprotein, is involved in maintaining several cellular processes and is implicated in several cancers when mutated or over expressed. Receptor activation is dependent on ligand binding and dimerization of the extracellular domain which leads to dimerization of the intracellular kinase domain, autophosphorylation and regulation of downstream signaling pathways. The EGFR extracellular domain is heavily glycosylated with oligosaccharides. Several experimental studies have suggested different roles for glycosylation on EGFR activation, dimerization, ligand binding and tyrosine kinase activity.

Objective

In this study we apply molecular modelling/simulations to study the effects of glycosylation on EGFR extracellular domain.

Methods



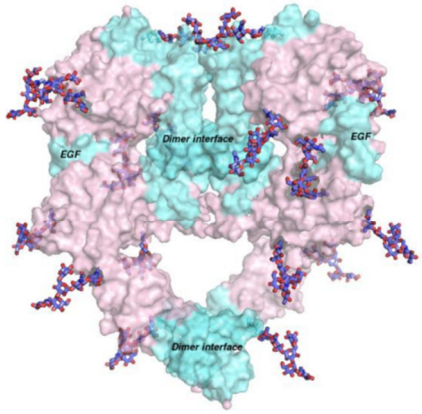


Fig 1. Fully glycosylated dimeric EGFR.

EGFR Increased stability

RMSF of the CA atoms during the MD simulations suggest that glycosylation is associated with dampened motions, suggesting that the glycans stabilize the structure. Subdomain III is the most stabilized while subdomain I is stabilized largely in the proximity of the ligand. Both dimer interfaces including the dimerization arm from domain II and the tip of domain IV fluctuate less upon glycosylation.

Hydrogen bonding; persistent interactions seen for protein-glycan

In the disaccharide-containing system, we observed three highly occupied hydrogen bonds between the glycans and domain III and IV of EGFR. Hydrogen bonds of domain III involve the residue Asp323 in which a sidechain oxygen interacts with oxygen atoms of the N-acetylglucoseamine linked to Asn328. In domain IV a hydrogen bond is seen between the Cys 515 backbone amide and the oxygen atom of N-acetylglucosamine linked to Asn 504. In the oligosaccharide-containing system hydrogen bonds observed between the glycan attached to Asn 172 and domain II. These hydrogen bonds form between the Gln193 sidechain oxygen atom and Cys 191 backbone oxygen atom and the Mannose linked to Asn 172. The reduction in the mobility of these amino acids suggests that hydrogen bonds impart stability to both the sugars and to the interacting EGFR.

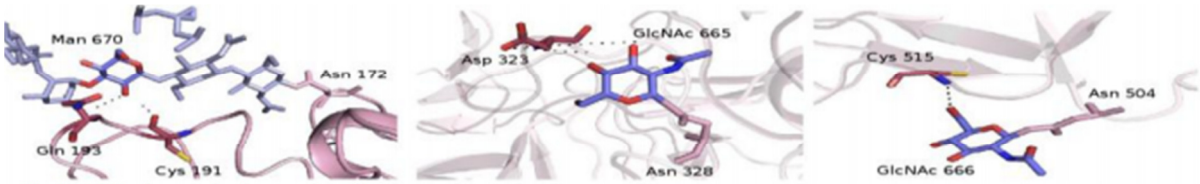


Fig 2. EGFR-glycans hydrogen bonding.

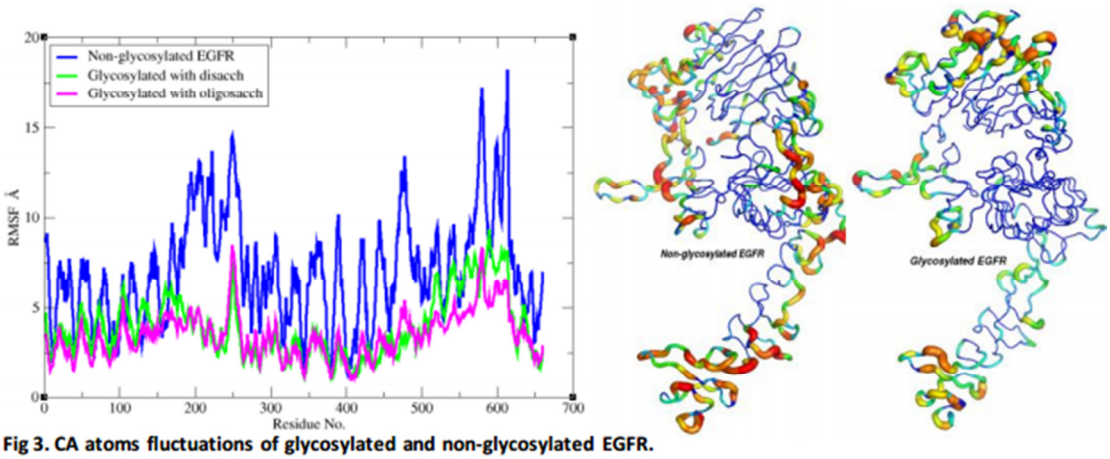


Fig 3. CA atoms fluctuations of glycosylated and non-glycosylated EGFR.

Packing interactions of Glycans Attached to Asn 151 and Asn 328

An interesting feature is seen in our simulations. The glycans attached to Asn 151 and Asn 328 move towards and interact with each other, forming one unit. In this process, the glycan attached to Asn 151 moves towards EGF and the ligand binding site and makes frequent contacts with Asn 91 from subdomain I and Lys 322 from subdomain III. The glycan attached to Asn 328 forms the scaffold of this arrangement by making several contacts with Glu 320, Ser 324, Leu 325, Ser 326 and Thr 330 from subdomain III and Asn 91 and Ser 92 from subdomain I. Electrostatic potential and per-residue free energy decomposition calculations from our MD simulations suggest that favorable electrostatic interactions appear to modulate this interaction. Hence this novel conformational rearrangement leads to the stability of the structure of domains I and III and appears to associate in maintaining the EGF binding pocket.

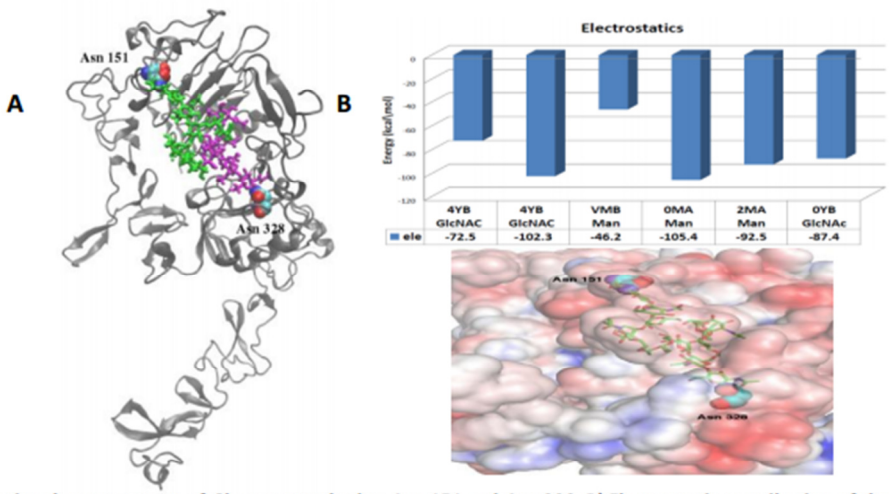


Fig 4. A) Conformational arrangement of Glycans attached to Asn 151 and Asn 328. B) Electrostatic contribution of the monoosaccharide residues from the two interacting glycans.

Conclusions

-Increased stability of glycosylated EGFR arises from hydrogen bond Interactions between subdomains II, III and IV and the attached glycans.
- Packing of Asn 151 and Asn 328 attached oligosaccharides stabilizes the ligand binding pocket by a network of Intermolecular contacts and favorable electrostatic interactions between the two glycans and ligand-binding subdomains of EGFR.
- Stabilization of the ligand-binding subdomains under the influe nce of glycosylation can elongate the half life of the dimeric active state of EGFR.

PH-004 **Dynamic protein complexes mediate reactivity and specificity of complement-like immunity in Anopheles gambiae**

Richard Baxter[1]

1.-Yale University Dept. of Chemistry, Dept. of Molecular Biophysics & Biochemistry

Insects possess a complement-like immune response utilizing thioester-containing proteins, or TEPs. The only arthropod TEP of known structure is Anopheles gambiae TEP1, which is a key component in the natural immunity of this mosquito to malaria parasites (genus Plasmodium). Unlike vertebrate complement factors, AgTEP1 does not contain an anaphylatoxin domain which acts to regulate a massive conformational change accompanying activation of the protein. The mechanism of AgTEP1 must therefore involve an alternative mechanism for allosteric regulation of thioester activation. In place of a small internal domain, a large, heterodimeric complex of two leucine-rich repeat (LRR) proteins, LRIM1 and APL1C, have been shown to specifically bind and stabilize the active conformation of AgTEP1. I will present my group's most recent work in this area. We have shown that different alleles of TEP1, which are known to influence the vectoral capacity of wild mosquitoes, differ significantly in their susceptibility to thioester hydrolysis. Allelic variation is centered on residues at the protein-protein interface within TEP1 containing the thioester bond. The LRIM1/APL1C heterodimer is shown to form an extended and flexible ensemble in solution. Two closely-related genes to APL1C, APL1A and APL1B, can also form a complex with LRIM1, and APL1B LRR domain can form a homodimer. We propose that a flexible and heterogeneous group ensemble of LRIM1/APL1 dimers interact with the active conformation of TEP1, thereby producing an array of immune complexes to protect mosquitoes from a diverse set of pathogens.

PH-005 **Conformational Changes of the Ribose ABC Transporter Studied by EPR Spectroscopy**

Satchal Erramilli[1], Michael Simon[2], Matthew Clifton[3], Cynthia Stauffacher[1]
1.-Purdue University, 2.-Washington University At St. Louis, 3.-Beryllium

Bacterial ATP-Binding Cassette (ABC) transporters are vital for nutrient uptake. Structural and functional studies have identified two distinct types (I and II) of importers based primarily upon mechanistic differences. The ribose transporter in E. coli is a tripartite ABC importer consisting of a cytoplasmic ATP-binding cassette protein with dual fused nucleotide-binding domains (NBD), a transmembrane domain (TMD) homodimer, RbsC, and a periplasmic substrate binding protein (SBP), RbsB. Results from this study demonstrate that the ribose transporter shares structural and functional features with both type I and type II systems. ATP hydrolysis in the NBD is stimulated by the presence of substrate-loaded SBP, typical of type I systems. Paradoxically, substrate is released upon association of ribose-loaded-RbsB and RbsC, as revealed by equilibrium dialysis experiments. EPR experiments demonstrate RbsB samples open and closed conformations when bound to RbsC, suggesting a pathway for substrate release. The presence of additional ribose, in turn, has a destabilizing effect on the RbsB-RbsC interaction, a type II-like behavior. ATP-bound RbsA then associates with the RbsBC complex, with hydrolysis subsequently leading to SBP dissociation. RbsC promotes a closed conformation of RbsA upon association, promoting ATP hydrolysis. Quantitative measurements from EPR experiments show hydrolysis then results in the open conformation of RbsB and destabilizes its interaction with RbsC. RbsA then dissociates from RbsC following ADP release, atypical for ABC transporters of either type. Co-purification studies and EPR experiments show that both the nucleotide and magnesium are required to maintain the complex interactions of RbsC and RbsA. Finally, transport is accomplished by hydrolysis at a single consensus site, with the second site rendered degenerate by mutations of conserved amino acids; such functional asymmetry is not uncommon in ABC exporters, but is heretofore unobserved in importers. Taken together, the ribose transporter appears to function by a distinct mechanism, and offers an opportunity to gain insight into non-canonical systems.

PH-006 **The Catalytic Cycle of hFEN1 Requires Protein and DNA Conformational Changes, but Are They Rate-Limiting?**

L. David Finger[1], Ian A. Bennet[1], Andrea Hounslow[2], Jack C. Exell[1], Nicola J. Baxter[2], Jon P. Waltho[3], Jane A. Grasby[1]

1.-Centre for Chemical Biology, Department of Chemistry, University of Sheffield, 2.-Molecular Biology and Biotechnology, University of Sheffield, 3.-Manchester Institute of Biotechnology, University of Manchester

Human Flap Endonuclease-1 (hFEN1) is an essential metallo-nuclease involved in Okazaki Fragment maturation and long-patch base excision repair. During these processes, bifurcated nucleic acid intermediates with ssDNA 5'-flaps are generated by polymerase strand displacement synthesis and then cleaved one nucleotide into the downstream duplex by FEN1 to create a nicked-DNA that is a suitable substrate for ligase. Until recently, how hFEN1 achieves tremendous catalytic power (rate enhancements >10exp17) and exquisite selectivity for the scissile phosphate had been understood poorly (1). In 2011, the Grasby and Tainer labs solved the structures of hFEN1 in complex with product and substrate. This study revealed that scissile phosphate selectivity is largely due to the substrate DNA undergoing a novel Di-Nucleotide Unpairing (DNU), which places the scissile phosphate diester in contact with the requisite divalent metal ions. In addition, by comparing the structures of hFEN1 alone (2) and in complex with substrate and product DNAs (3), Grasby and Tainer proposed a model, whereby protein conformational changes occur upon binding substrate resulting in placement of key basic residues that position and/or electrophillically catalyse hydrolysis of the scissile phosphate diester. Further work using a CD-based assay showed that metals are absolutely required for DNU, whereas the key basic residues in the active site are not. Surprisingly, perturbations to the protein structure that are much more distant from the FEN1 active site (i.e., helical cap) prevent DNA unpairing, implying that the FEN1 protein actively participates in the unpairing process (4,5); however, how it does remains a mystery. The maximal multiple turnover rate of hFEN1 reaction is rate-limited by enzyme product release, whereas hFEN1 kinetics under substrate-limiting conditions ([E]<[S]<Km) suggest that the enzyme is approaching catalytic perfection (i.e., almost diffusion controlled - 10exp7 M-1s-1) (6). Previous work has shown that a paralogue of hFEN1 (i.e., T5 FEN1) is not rate-limited by chemistry under single turnover (ST) conditions, but rather some physical limitation such as conformational change (6). To ascertain whether the protein and/or DNA conformational changes of hFEN1 mentioned above are rate limiting under ST conditions, we have initiated kinetic and NMR studies to determine the role of conformational dynamics in the catalytic cycle of hFEN1. References 1. Grasby, J.A. et al. (2011) Trends Biochem. Sci. 37, 74-84. 2. Sakarai, S. et al. (2005) EMBO J. 24, 683-693. 3. Tsutakawa, S.E. et al. (2011) Cell 145, 198-211. 4. Finger, L.D. et al. (2013) Nucl.Acids Res., 41, 9839-9847. 5. Nikesh Patel et al. (2013) J. Biol. Chem., 288, 34239-34248. 6. Finger, L.D. et al. (2012) Subcell. Biochem. 62, 301-326.

PH-007 **Dynamical structure changes in binding of pharmaceutical target proteins**

Hideaki Fujitani[1]

1.-Research Center for Advanced Science and Technology, The University of Tokyo

Owing to the latest advance in the computational technologies of microprocessor, high-speed inter-processor connection, and parallelization algorithm, all-atom molecular dynamics (MD) simulations of microsecond time scale are getting popular to study the pharmaceutical target proteins. An antibody binds to its antigen with structure changes. A small molecule moves around the target protein and finds an entrance to get into the binding site. These phenomena can be observed in microsecond simulations, but the vital issue is whether the adopted force field is enough accurate to correctly describe the phenomena. We are developing a force field (FUJI) based on general AMBER atom types and AMBER94 van der Waals parameters in order to describe arbitrary organic molecules in a unified manner including proteins and nucleic acids. We use the first principle theoretical method to refine the force field in contrast to common empirical fittings to experimental data. The dihedral torsion parameters of protein backbone were determined to agree with the torsion energy profiles calculated by high-level quantum mechanical theory for the model systems of protein backbone. Conformational preferences of dipeptides in water were measured by vibrational spectroscopy. Comparing with the experimental distribution of Ramachandran angles of dipeptides, how accurately molecular calculations predict the conformation distribution of dipeptides were investigated for various force fields and semiempirical quantum methods. FUJI force field gave the best prediction score among the various methods (Tzanov et al, 2014). In this work we examine dynamical structure behaviors of pharmaceutical target proteins by microsecond MD simulations with FUJI force field. Epiregulin (EPR) is a transmembrane protein with 140 residues belonging to the epidermal growth factor family. Mature EPR (46 residues) binds to epidermal growth factor receptor (EGFR), which stimulates the proliferative signaling in cancer cells. Our EPR antibody has a proline at the residue 103 in the third complementarity-determining region (CDR) of the heavy chain, which has cis peptide bond in free state and trans one in the bound state with EPR according to X-ray crystallography analysis. In order to clarify the structure changes in binding we performed extensive MD simulations of our antibody (fab; 436 residues) and EPR. The total simulation time was about 240 microseconds. We also performed MD simulations for a protein kinase and a small molecule. We show how the complex system reaches thermal equilibrium states in simulations from the stiff X-ray crystal structure. The calculations are compared with experiments such as X-ray crystal structures and thermodynamic quantities measured by isothermal titration calorimetry (ITC) and surface plasmon resonance (SPR). This research has been supported by MEXT SPIRE Supercomputational Life Science (hp130006, hp140228) and FIRST Kodama project in Japan.

PH-008 **Structure-based recombination of drug resistance enzymes: structural and functional tolerance to new dynamics in artificially-evolved enzymes**

Sophie M.C. Gobeil[1,2], Maximillian C.C.J.C. Ebert[1,2], Jaeok Park[1,4,5], Donald Gagné[1,5,6], Christopher M. Clouthier[1,3], Jürgen Pleiss[7], Nicolas Doucet[1,5,6], Albert M. Berghuis[1,4,5], Joelle N. Pelletier[1,2,3]

1.-PROTEO, 2.-Department of Biochemistry, U. of Montreal, 3.-Department of Chemistry, U. of Montreal, 4.-Department of Biochemistry and Department of Microbiology and Immunology, McGill, 5.-GRASP, 6.-INRS–Institut Armand-Frappier, U. du Quebec, 7.-Institute of Technical Biochemistry, University Stuttgart

Our understanding of the contribution of protein dynamics to function is still emergent. In a protein engineering context, do we need to take into account the dynamics in order to maximize the fitness and function of the resulting proteins? Using high resolution crystal structures, NMR relaxation dispersion and µs molecular dynamics simulations, we compare two naturally evolved homologous class A β-lactamases, TEM-1 and PSE-4 which share a high degree of structural and functional conservation. We observed a conservation of dynamics on a catalytically relevant timescale. This is consistent with dynamics being an evolutionarily conserved feature. However, laboratory-engineered chimeric enzymes obtained by recombination of the two homologs exhibit striking dynamic differences, despite the function and structure being conserved. The laboratory-engineered chimeras are thus functionally and structurally tolerant to modified dynamics on the timescale of the catalytic turnover. This tolerance of β-lactamases to dynamic changes could be linked to the high fitness of the naturally evolved proteins and implies that maintenance of native-like protein dynamics may not be essential when engineering functional proteins.

PH-009 **Conformations of the RNA polymerase clamp throughout the transcription cycle studied by single-molecule FRET**

Sarah Sarah[1], Andreas Gietl[1], Philip Tinnefeld[1], Finn Werner[2], Dina Grohmann[1,3]

1.-Physikalische Chemie - NanoBioSciences, Technische Universität Braunschweig, 2.-Institute of Structural and Molecular Biology, University College London, 3.-Institute of Microbiology - Single-Molecule Biochemistry, University Regensburg

Transcription is one of the most fundamental processes in biology and every living organism depends on it. RNA polymerases (RNAP) are at the heart of the transcriptional machinery catalyzing the synthesis of RNA in a DNA-dependent fashion. This intrinsically dynamic process requires the highly coordinated interplay of the RNAP with multiple nucleic acids and transcription factors through the initiation, elongation and termination phase of transcription in order to coordinate the movement of the RNAP along the DNA template and the catalytic action of the enzyme. Conformational rearrangements are a central theme in the process of transcription and the clamp domain is the main mobile element of RNA polymerases. Even though structures of eukaryotic RNAPs are available, it has been extremely difficult to decipher the conformational space sampled by the RNAP clamp at different stages of transcription. Combining the highly tractable recombinant archaeal transcription system with single-molecule fluorescence resonance energy transfer (FRET) measurements allowed us to resolve long-standing questions about the conformational state of the clamp of archaeal-eukaryotic RNAPs in solution. An immense advantage of our recombinant system is the possibility to perturb it by introducing unnatural amino acids for subsequent labelling with fluorescent dyes or to omit subunits from the multisubunit RNAP. Comparing free, nucleic acid- and factor-bound RNAP complexes we found that the position of the clamp is modulated during the transcription cycle in a fashion exceeding the simplified closed-open picture drawn from crystallographic studies. We show that the clamp adopts at least two distinct conformations in both initiation and elongation complexes. Moreover, we were able to link conformational states to the catalytic activity of the RNAP. Transcription factors TFE and Spt4/5, which both bind to the RNAP clamp domain and stimulate the activity of the enzyme, shift the equilibria towards one of the main conformations suggesting that both prompt an allosteric switch that influences the structure of the RNAP. Hence, our data provide a mechanistic rationale for the function of these factors and provide new insights into the complex dynamic behaviour of the transcriptional machinery.

PH-010 **Solvent models for protein simulations – the good, the bad and the applications**

Duy Hua[1], Amitava Roy[1], He Huang[1], Carol Post[1]
1.-Purdue University

The solvent environment plays a crucial role in determining the structure, dynamics and function of a biomolecule. In order to utilize molecular dynamics (MD) simulations to examine the structural changes of biomolecules, it is imperative that the solvent environment be accurately modeled. Implicit solvent models (ISMs), in which water molecules are absent and the solvent effect is estimated using an energy function, offer a low-cost approach to describe the solvent environment in MD simulations. ISMs have been used for a variety of biological studies; yet, the performance of implicit solvation methods in capturing the structural characteristics of proteins has not been rigorously demonstrated. In this work, three ISMs, namely GBMV II, FACTS, and SCPISM, were evaluated for their abilities to emulate the solvent environment and its effect on the structures, dynamics and electrostatic interactions of the Src SH2 domain and the Lyn kinase domain. Structural properties such as phi-psi distribution, average positional fluctuations, ion pair distance distribution, and all-against-all rms deviation were used to compare the performance of ISMs to the TIP3P explicit solvent model. Our study shows that the Src SH2 domains solvated with TIP3P, GBMVII and FACTS sample similar secondary structures as well as global conformations. Additionally, GBMVII and FACTS perform relatively well in modeling solvent-accessible electrostatic interactions that would typically require the presence of water molecules. These charge-charge interactions, however, are not adequately described in the SCPISM solvent model. Overall, FACTS is computationally efficient and is a reliable alternative solvent model for the studies of globular proteins. On the other hand, for non-globular proteins with complex structures, FACTS does not accurately describe the solvent effect on the sampling of local interactions and global conformations due to the possibilities of water-mediated interactions. Our assessment of ISMs in terms of structural features in folded proteins expands previous studies that have utilized hydration energy as the metric for comparison. Our work reveals that ISMs show poor performance for non-sphrerical, multi-lobal proteins and the best use of ISMs remains limited to small, globular proteins.

PH-011 **Dissection of the water cavity of yeast Thioredoxin 1: the effect of a hydrophobic residue in the cavity**

Anwar Iqbal[1], Fabio C. L. Almeida[1], Catarina Miyomoto[2], Ana P Valente[3], Francisco Gomes Neto[4]

1.-National Center of Nuclear Magnetic Ressonance, Insti of Medical Biochemistry, 2.-Faculdades Integradas de Três Lagoas-AEMS,, 3.-Center of Structural Biology and Bioimaging (CENABIO), UFRJ, 4.-Laboratory of Toxicology, Instituto Oswaldo Cruz, Fiocruz

Thioredoxins (Trx) are ubiquitous proteins that play a key role in redox state regulation of the cell. They interact with multiple targets in the cell. Our group solved the structure of S. cerevisiae (yTrx1) and measured its dynamics in different timescales (pico to seconds). It is well established that the Asp24 is the proton acceptor in the reduction process of the target protein. We proposed that Asp24 also works to couple hydration and motion of the interacting loops. We studied conformational motions of the residues of water cavity and interacting loops. The water molecules in water cavity of the protein play a vital role in the redox activity of thioredoxin. The degree of mutational frustration explains in a great deal the multiple timescales observed in the protein dynamics in yTrx1 and the mutant D24A. The yTrx1 displays a complex equilibrium between the ground state and several excited hydration states. This excited states are more permeable to water and it is key to understand the proton transfer and activation of Cys33. In this current study, we examined the mutant D24A, and observed that this small hydrophobic residue induces conformational exchange in residues exposed to the water cavity, such as Cys33. Along with molecular dynamics simulations and calculation of mutational frustration levels, we were able to describe conformational details of the water cavity. It is formed by three independent contiguous lobes which we called lobes A, B and C. Lobe B is the central lobe that contains the catalytic important residues Cys33 and Asp24. It closed upon mutation D24A. Lobe A and C remains open upon mutation, meaning that their hydration are independent. The NMR structures for the oxidized and reduced state of the Mutant D24A has been studied.

PH-012 **In-vitro and in-silico studies of ligand binding to the nuclear receptor PPARgamma using FRET and MD**

Narutoshi Kamiya[1], Gert-Jan Bekker[1], Takuma Shiraki[2], Haruki Nakamura[1]
1.-IPR, Osaka University, 2.-Kinki University

The nuclear receptor PPARgamma is a ligand-dependent transcription factor which regulates gene expression related to glucose homeostasis and insulin sensitization. It is a potential therapeutic target for metabolic syndrome, cancer, and inflammatory diseases. Crystal structures have revealed that its ligands bind deep inside the pocket. However, how the ligand recognizes PPARgamma and penetrates inside the pocket remains unclear. We have measured the binding kinetics using the FRET method and have calculated potential dissociation paths via molecular dynamics (MD) simulations. The binding kinetics were measured using FRET between the ligand, C8-BODIPY, which is a fluorescent pigment structurally similar to one of its agonists 15-oxo-eicosatetraenoic acid, and cyan fluorescent protein (CFP) which is fused to PPARgamma. Fluorescence of C8-BODIPY is observed when it is close to CFP when CFP is excited by light at 410nm. The ligand was simultaneously added to the PPARgamma-CFP solution using the stopped-flow method. Time-resolved fluorescence signals of C8-BODIPY and CFP were also monitored. Two time constants were observed by the FRET experiment, which correspond with the landing and docking processes. We used our new MD program, psygene-G [1], which utilizes the GPU for acceleration of the non-bonded interactions, such as electrostatic interaction which uses our original zero-dipole summation method [2, 3]. Previously we were able to attain similar thermodynamics with respect to the particle mesh Ewald method in membrane proteins and DNA-water-ion systems [4, 5] and have applied it to the thermodynamic study of dynein [6]. We have introduced random accelerated MD (RAMD) into the psygene-G program to predict a ligand's dissociation path from the initial crystal structure (PDB ID: 2zk6). In RAMD, an external random-directional constant force is added to the ligand. We executed 96-RAMD simulations with different random seeds for the initial pull directions. Twelve RAMD trajectories managed to dissociate within 1 ns, while the remaining simulations did not manage to disassociate within this time-frame. In all twelve cases the ligand went past R288, which is located at the surface of PPARgamma. Interestingly, a somatic mutant (R288H) is related to colon cancer. We concluded that R288 plays a role of a gate keeper to assist ligand binding. References [1] Mashimo T. et al. J. Chem. Theory Comput. 9, 5599 (2013). [2] Fukuda I. et al. J. Chem. Phys. 134, 164107 (2011). [3] Fukuda I. et al. J. Chem. Phys. 140, 194397 (2014). [4] Kamiya N. et al. Chem. Phys. Lett. 568, 26 (2013). [5] Arakawa T. et al. PLoS ONE 8, e76606 (2013). [6] Nishikawa Y. et al. J. Mol. Biol. 426, 3232 (2014).

PH-013 **Atomic Insight into kinetic mechanism for sumoylation of UBC9 with substrate motif (Ψ-K-x-D/E) by molecular dynamic simulation**

Mooseok Kang[1], Wookyung Yu[1], Juhwan Lee[1], Iksoo Chang[1]
1.-Department of Brain Sciences, Daegu Gyeongbuk Institute of Science and Technolog

SUMOylation is a post-translational modification involved in various cellular processes, such as nuclear-cytosolic transport, transcriptional regulation, apoptosis, protein stability, response to stress, and progression through the cell cycle. SUMOylation is a conjugation of glysine residue of SUMO protein with lysine residue in target(RanGAP1) protein through the influence of enzyme E2(Ubc9) and E3(RanBP2). SUMO proteins modify the function of target protein in the cell. But the kinetic mechanism of SUMOylation is not well-understand. We traced out the full kinetic process of SUMOylation of the SUMO1-Ubc9 -RanGAP1-RanBP2 complex in atomic detail via molecular dynamic simulation. We uncovered the kinetics of SUMOylation mechanism and verified the important residues which play a key role in the SUMOylation process. The comparison of our results with the experimental one are also discussed.

PH-014 **Bending over Backwards for Water: How KCNE3 Helix Curvature and Flexibility Influence a Human Potassium Channel's Conduction Profile**

Brett Kroncke[1,2], Wade Van Horn[0], Jarrod Smith[1,2], David Nannemann[2,6], Jens Meiler[2,6], Carlos Vanoye[7], Charles Sanders[1,2]

1.-Department of Biochemistry, Vanderbilt University, 2.-Center for Structural Biology, Vanderbilt University, 3.-Department of Chemistry and Biochemistry, Arizona State University, 4.-Biodesign Institute, Arizona State University, 5.-Center for Personalized Diagnostics, Arizona State University, 6.-Department of Chemistry, Vanderbilt University, 7.-Northwestern University Feinberg School of Medicine

Human potassium channel KCNQ1 is expressed in several tissues including inner ear, heart muscle, lung, intestine, and stomach, each requiring a unique current profile for proper function. To tune its output current, KCNQ1 complexes with several accessory proteins from the KCNE family, each KCNE family member modulating KCNQ1 distinctly: KCNE1 causes the channel to delay opening and become more conductive in the open state, KCNE3 causes the channel to be constitutively conductive, and KCNE4 closes the channel. The purpose of this study is to uncover the atomic-scale, mechanical mechanism of how KCNE3 modulates KCNQ1. To do this we modeled the interaction between KCNQ1 and KCNE3 with a hybrid experimental-computational approach. Our strategy is to determine the NMR structure of KCNE3 alone in bilayer-mimicking bicelles, build a homology model of the open-state KCNQ1, and dock the structure of KCNE3 onto the model of KCNQ1 using electrophysiology-based restraints to validate and refine the resulting model complexes. Paramagnetic Relaxation enhancement, residual dipolar coupling and sequence analysis suggest the single span transmembrane helix of KCNE3 is significantly curved. Hydrogen-deuterium exchange, nuclear Overhauser effect cross peaks, and AMBER simulation suggest KCNE3 carries a significant amount of water in the C-terminal side of the transmembrane helix. The 1H-15N two dimensional nuclear magnetic resonance spectra of a known phenotype-changing mutation in the center of the transmembrane helix, V72T, suggests the helix adopts multiple conformations when the extra side-chain hydroxyl group is present. A docking funnel corroborates a binding pocket suggested by the in vivo electrophysiology data where KCNE3 wedges between a helix near the potassium pore and two helices in the voltage sensitive element of the channel. Within this pocket, we believe the conformational sampling and structural rigidity—or flexibility, in the context of the V72T mutant—of the accessory protein KCNE3 directly influence the modulation profile.

PH-015 **Watching conformational changes in proteins by molecular dynamics simulations**

Kresten Lindorff-Larsen[1]
1.-Department of Biology, University of Copenhagen

Proteins are dynamical molecules and their ability to adopt alternative conformations is central to their biological function. Examples include motions that underlie allosteric regulation or ligand binding, or protein dynamics in enzymes that can modulate the overall catalytic efficiency. Protein motions can often be described as an exchange between a dominant, ground state structure and one or more minor states. The structural and biophysical properties of these transiently and sparsely populated states are, however, difficult to study, and an atomic-level description of those states is challenging. In an attempt to determine how well molecular dynamics simulations can capture slow, conformational changes in protein molecules we have studied two different protein systems which are known to undergo conformational exchange on the millisecond timescale, and for which structural information is available for both major and minor states. Using enhanced-sampling all-atom, explicit-solvent molecular simulations, guided by structural information from X-ray crystallography and NMR, we show that current force fields and sampling methods allow us to sample experimentally-determined alternative conformations with surprisingly high accuracy. In particular, we find that we can reversible sample both the ground state and minor state, and that the simulations capture the structure of the minor states also. Our simulations enable us to calculate the conformational free energy between the two states, and comparison with relaxation dispersion NMR experiments demonstrates a high accuracy. Thus, we show for two distinct proteins that we can map the free energy landscapes and that both the structure and energetics are in excellent agreement with NMR experiments. Our simulations provide insight into the structural and biophysical properties of transiently populated minor states, and help reinterpret previous experimental measurements. Further, our results demonstrate that, at least in the two cases we have studied, modern simulation methods enable us to examine these otherwise “invisible” states of proteins and describe their structural, functional and thermodynamic properties. Our results in this way help demonstrate how simulations can now add to our knowledge of transiently formed, “hidden” states of proteins.

PH-016 **Coupling Conformational and Energetic Changes in G Protein Signaling**

Alyssa Lokits[1], Julia Koehler Leman[2], Kristina Kitko[1,3], Natha Alexander[4], Heidi Hamm[1,5], Jens Meiler[1,5,6]

1.-Neuroscience, Vanderbilt University Medical Center, 2.-Chemical and Biomolecular Engineering, Johns Hopkins University, 3.-Engineering, Vanderbilt University Medical Center, 4.-Pharmacology, Case Western Reserve University, 5.-Pharmacology, Vanderbilt University Medical Center, 6.-Chemistry, Vanderbilt University Medical Center

Cell signaling is a fundamental process for all living organisms. G protein-coupled receptors (GPCRs) are a large and diverse group of transmembrane receptors which convert extracellular signals into intracellular responses primarily via coupling to heterotrimeric G proteins. In order to integrate the range of very diverse extracellular signals into a message the cell can recognize and respond to, conformational changes occur that rewire the interactions between the receptor and heterotrimer in a specific and coordinated manner. By interrogating the energetics of these interactions within the individual proteins and across protein-protein interfaces, a communication network between amino acids involved in conformational changes for signaling, is created. To construct this mapping of pairwise interaction energies in silico, we analyzed Rhodopsin GPCR coupled to a Gαi1β1γ1 heterotrimer. The structure of this G protein complex was modeled in the receptor-bound and unbound heterotrimeric states as well as the activated, monomeric Gα(GTP) state. From these tertiary structural models, we computed the average pairwise residue-residue interactions and interface energies across ten models of each state using the ROSETTA modeling software suite. Here we disseminate a comprehensive analysis of all critical interactions and create intra-protein network communication maps. These networks represent nodes of interaction necessary for G protein activation.

PH-017 **Structure and dynamics of the polymyxin-resistance-associated response regulator PmrA in complex with the promoter DNA**

Yuan-Chao Lou[1], Yi-Fen Kao[1], Tsi-Hsuan Weng[2], Yi-Chuan Li[2], Chwan-Deng Hsiao[2], Chinpan Chen[1]

1.-Institute of Biomedical Sciences, Academia Sinica, 2.-Institute of Molecular Biology, Academia Sinica

PmrA, an OmpR/PhoB family response regulator (RR), takes part in the two-component system that manages genes for polymyxin resistance through a phosphorylation-dependent regulation. Phosphorylation of OmpR/PhoB RR induces the formation of a two-fold symmetric dimer in the N-terminal receive domain (REC), promoting 2 C-terminal DNA-binding domains (DBDs) to recognize tandem repeat DNA sequences on the promoter to elicit adaptive responses. Recently, Narayanan et al. presented the complex structure of active-like KdpE in complex with DNA, which reveals a unique asymmetric REC-DBD interface that is necessary to form stable complexes for transcription activation. In this study, we report the 3.2 Å resolution crystal structure of BeF3--activated PmrA in complex with promoter DNA as well as its dynamics in solution by NMR. Unlike the case of KdpE, PmrA-DNA complex structure reveals a different asymmetric REC-DBD interaction. Interestingly, NMR assignments and dynamics study suggest that REC and DBD do not form stable contacts in solution; instead, 2 domains tumble separately in the absence or presence of DNA. Relaxation dispersion experiments on methyl groups further show that several REC-DBD interfacial residues exhibit similar slow dynamics in the presence of DNA. It is hence highly possible that the slow dynamics observed on these interfacial methyl groups are related to the formation of asymmetric REC-DBD interaction, which reduces the mobility of PmrA-DNA complex and promotes crystallization. In solution, two domains tumble independently and have diverse orientations, which together with the DBD-DBD interface may facilitate searching best interactions with RNA polymerase holoenzyme for transcription activation.

PH-018 **Time-resolved X-ray Observations of Nano-scale Protein Assembly Networks**

Yufuku Matsushita[1], Hiroshi Sekiguchi[2], Noboru Ohta[2], Keigo Ikezaki[1], Yuji Goto[3], Yuji Sasaki[1,3]
1.-The University of Tokyo, Graduate School of Frontier Science, Advanced Materials Science, 2.-SPring-8, 3.-Osaka University, Institute For Protein Research

Protein aggregation and network formation in diverse protein species had been studying in various experimental approaches such as absorbance spectrophotometry, dynamical light scattering and X-ray scattering. However, these conventional methods are only able to observe averaged information on bulk conditions and also these techniques have a limitation of time resolved observations. Recently, development of single molecular observation techniques are remarkable. Diffracted X-ray Tracking (DXT) is one of the notable single molecular observation methods to capture detailed intramolecular dynamics of an individual single protein molecule by the labeling X-ray method. In this study, we present an innovative method for observing the protein assembly networks of nano-scale size on the bulk solution using a DXT that possess pico-meter scale positional accuracy and micro-second time resolution. This method is founded by detecting angular rotational displacement of a coexisted and dispersed single gold nanocrystal (approximately 100 nm) on target solution. For target sample, we choose a crystal precursor metastable state of 20 mg/ml lysozyme solution (Hen egg white lysozyme: 14331.20 g/mole on 0.2 M sodium acetate 3 – 4 w/v NaCl, pH 4.7 buffer) and 10 mg/ml of lysozyme solution such as stable condition. For reference sample, we prepare 20 mg/ml and 10 mg/ml of ribonuclease A solution (Ribonuclease A (Bovine): 13708.40 g/mol, 0.2 M sodium acetate 3 – 4 w/v NaCl, pH 4.7 buffer). The experiment was carried out at SPring-8 BL40XU. From DXT analysis, we obtained logarithmic rotational velocity distribution of 20 mg/ml, 10 mg/ml lysozyme solution and 20 mg/ml, 10 mg/ml of Ribonuclease A solution during 1000 μs and we processed regression analysis of Gaussian fitting in each distribution. From this result, 10 mg/ml of lysozyme solution (stable) and 20 mg/ml, 10 mg/ml (reference) have definitely regressed by single peak normal Gaussian distribution that are positional peaks at 3.66 mrad and 1.4 mrad and 1.2 mrad, respectively. In contrast, 20 mg/ml of lysozyme solution consisted of double peak logarithmic distribution at 3.42 and 9.54 mrad. This peak separation tendency from DXT are typically observed in a supersaturated condition of the sodium acetate solution which coexisting nano-scale solute networks (DXT and Small Angle X-ray Scattering (SAXS) experiment). From this study, DXT measurement results and detailed analysis process for a protein solution such as crystal precursor metastable state of lysozyme solution are concerned that this technique is a powerful tool for observing nano-scale protein network's existence and its local dynamics in bulk solution. At the present time, we will demonstrate the detailed analysis and processing from DXT raw data and the conformity of the results from another experiment confirmation such as SAXS and Darkfield microscopy. Finally, we present a detailed protein network model of lysozyme metastable solution from DXT result.

PH-019 **Functional implications of co-evolving residue sectors in the Ribonuclease A family**

Chitra Narayanan[1,2], Kimberly Reynolds[3], Rama Ranganathan[3], Nicolas Doucet[1,2,4]

1.-INRS-Institut Armand-Frappier, Université du Québec, 531 Boulevard des Prairies, 2.-GRASP, Groupe de Recherche Axé Sur la Structure des Protéines, 3649 Promenade S, 3.-Green Center for Systems Biology, University of Texas Southwestern Medical cent, 4.-PROTEO, Québec Network for Research on Protein Function, Structure and Engineeri

Increasing evidence suggests that conformational fluctuations experienced by proteins play a key role in promoting function. However, the experimentally derived dynamic behavior of discrete protein systems has yet to provide clear evidence on the evolutionary conservation of motional events between structural and functional protein homologues. In this work, we used a statistical coupling analysis (SCA) approach to analyze the role of co-evolving amino acids in protein function and flexibility. SCA facilitates the identification of distinct residue sectors and provides an understanding of the correlation between amino acid co-evolution and biological function. As a model system, we used the Ribonuclease A (RNase A) family, which includes canonical members that are structurally similar, but perform diverse activities such as angiogenesis, anti-pathogenicity and neurotoxicity, while preserving the common ribonucleolytic function. Analysis of 1922 sequences with SCA 6.0 allowed the determination of five independent components (ICs) corresponding to amino-acid sectors with distinct functional, structural and/or dynamical roles within the family. While most ICs correspond to residues critical for protein stability and catalytic function, IC4 correlates with residues experimentally determined to be highly dynamic on the catalytic timescale in several RNase A homologues, as we confirmed by NMR 15N-CPMG experiments. Additionally, IC5 residues form a hydrogen-bonding network shown to allosterically modulate and coordinate catalytically productive motions. These results provide a direct observation between the role of residue sectors in stability and motions relevant to function in the RNase A family. This study highlights the role of concerted action of multiple residues towards a common function, which can guide experiments for engineering proteins to enhance function such as catalysis.

PH-020 **Effects of KCl on the Dynamics and Catalytic Mechanism of a Halophilic Enzyme - Dihydrofolate Reductase (hvDHFR) from Haloferax volcanii**

Sivanandam V. N.[1], Ana Laín[1], Óscar Millet[1]
1.-Structural Biology Unit, CICbioGUNE

Halophilic archea are a type of extremophiles that thrive in hypersaline conditions. In order to avoid the osmotic shock, their cytoplasm is maintained with higher ionic strength up to 4-6M. Such a high ionic strength of intracellular environment protects the cells from desiccation or salting out. However, one wonders how such high salt concentrations keep the cell functional and active throughout all the cellular reactions (i.e. enzyme catalysis). The answer lies in the evolution of the halophiles that has left the halophilic proteins with a certain preference for amino acids composition [1] (prefers more acidic residues and overall decrease in hydrophobic side-chains). It's also known that the salt concentration can affect the activity and the underlying mechanism [2] of the halophilic enzymes. In order to further understand the haloadaptation, we have chosen to study a model system, dihydrofolate reductase (DHFR) from Haloferax volcanii (hvDHFR). DHFR is a very well understood enzyme and known to bound variety of ligands that can modulate the dynamics and the energy landscapes of the enzyme [3]. We have probed the μs-ms dynamics of hvDHFR bound to the cofactor NADPH (holoenzyme) and other substrates (DHF, THF) using relaxation dispersion NMR. In order to understand the salt contribution to the dynamics and catalytic mechanism, the experiments were done in the increasing order of salt concentration, shedding light on the conformational ensembles involved in the catalytic mechanism. The NMR relaxation dispersion analysis of different enzyme complexes in the presence of 3M and 2M KCl has shown more conformational fluctuations than compared to the enzyme with 1M KCl. This result suggests that the enzyme preferably is highly active & samples more flexible conformers in the hypersaline environment. Further, quantification of these conformers/invisible-states and their salt-dependence will provide new insights into the mechanism of the enzyme-catalysis. Also, it will facilitate designing novel enzymes that can be used in the bioprocess industrial set-up under extreme conditions.

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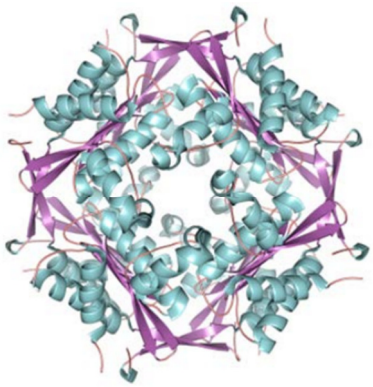
PH-021 **Structure and dynamics of the octameric iron-, heme- and cobalamin-binding protein HbpS from the soil bacterium Streptomyces reticuli**

Dario Ortiz De Orue Lucana[1], Matthew Groves[2], Ina Wedderhoff[1]
1.-University of Osnabrueck, 2.-University of Groningen

We have identified the extracellular protein HbpS in the cellulose degrader Streptomyces reticuli and shown that it specifically binds iron ions, heme and aquo-cobalamin. Based on 3D crystal structures, structural alignments, sequence comparisons, mutagenesis, and comparative biochemical investigations, we identified the coordination sites for iron, heme and aquo-cobalamin, and binding kinetics were elucidated [1,2]. HbpS interacts with the membrane-embedded sensor kinase SenS. While under non-stressed conditions HbpS inhibits SenS autophosphorylation, under oxidative-stressing conditions activates it. SenS in turn phosphorylates the response regulator SenR that activates the transcription of anti-oxidative genes [3]. We crystallized HbpS and solved its 3D crystal structure that revealed an octomeric assembly which is required for interaction with SenS [4].



Using mutagenesis, FRET, CD spectroscopy, fluorescence spectroscopy and site-directed spin labelling combined with pulse electron paramagnetic resonance spectroscopy, we demonstrated that iron-mediated oxidative stress induces both secondary structure and overall intrinsic conformational changes within HbpS. We additionally showed that HbpS is oxidatively modified, leading to the generation of highly reactive carbonyl groups and tyrosine-tyrosine bonds [5]. We concluded that these molecular events are responsible for the HbpS-mediated activation of the sensor kinase SenS. This presentation will focus on the HbpS structure and dynamics within the protein.

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PH-022 **A centrosomal protein FOR20 regulates microtubule assembly through a direct interaction with tubulin**

Dulal Panda[1], Shalini Srivastava[1], Ilina Bareja[1]
1.-Indian Institute of Technology Bombay

FOR20 (FOP-related protein of 20 kDa), a centrosomal protein, is conserved across all ciliated eukaryotes. It has been shown to be involved in ciliogenesis in RPE cells and in the S-phase progression in HeLa cells. It localizes to the pericentriolar satellite and at the centrosome. The localization of FOR20 at the centrosome has been found to be throughout the cell cycle. In Paramecium, FOR20 is involved in docking of the basal body to the cell membrane and in the assembly of the transition zone. We found that FOR20 colocalizes with ciliated microtubules in NIH3T3 cells. Since, the localization of FOR20 to the pericentriolar satellite is dependent on microtubules and the cilium itself is a microtubule-based structure; we sought to investigate the role of FOR20 in microtubule assembly. The over-expression of GFP-FOR20 in HeLa cells led to the depolymerization of microtubules while only the GFP expressing HeLa cells showed typical microtubule network. In addition, the microtubules of GFP-FOR20 expressing HeLa cells were found to be more sensitive towards nocodazole, a microtubule-depolymerizing agent, suggesting that FOR20 destabilizes microtubules. The effect of FOR20 on the polymerization of tubulin was monitored by light scattering, sedimentation assay and electron microscopy. In vitro, FOR20 inhibited the rate and extent of polymerization of tubulin. For example, 5, 10, 15 and 20 μM FOR20 inhibited the polymerization of purified tubulin by 11, 20, 39 and 52%, respectively. Further, the sedimentation assay suggested that FOR20 inhibited the polymerization of tubulin. In addition, electron microscopic analysis of the assembly mixture showed that FOR20 inhibited microtubule formation in vitro. The binding of FOR20 to purified tubulin was monitored by several complimentary techniques. Using size exclusion chromatography, FOR20 was found to be co-eluted with tubulin indicating that FOR20 binds to tubulin. Further, the interaction between FOR20 and tubulin was analyzed by surface plasma resonance technique and the result showed that FOR20 binds to tubulin with a modest affinity. The data together suggested that FOR20 regulates microtubule assembly through a direct interaction with tubulin.

PH-023 **How amide hydrogens exchange in native proteins**

Filip Persson[1], Bertil Halle[1]
1.-Biophysical Chemsitry, Lund University

Amide hydrogen exchange (HX) is widely used in protein biophysics even though our ignorance about the HX mechanism makes data interpretation imprecise. Notably, the open exchange-competent conformational state has not been identified. Based on analysis of an ultra-long molecular dynamics trajectory of the protein BPTI, we propose that the open (O) states for amides that exchange by subglobal fluctuations are locally distorted conformations with two water molecules directly coordinated to the N–H group. The HX protection factors computed from the relative O-state populations agree well with experiment. The O states of different amides show little or no temporal correlation, even if adjacent residues unfold cooperatively. The mean residence time of the O state is ~100 ps for all examined amides, so the large variation in measured HX rate must be attributed to the opening frequency. A few amides gain solvent access via tunnels or pores penetrated by water chains including native internal water molecules, but most amides access solvent by more local structural distortions. In either case, we argue that an over-coordinated N–H group is necessary for efficient proton transfer by Grotthuss-type structural diffusion.

PH-024 **Differences in redox reactions with NADP+/H between ferredoxin-NADP+ oxidoreductases from Bacillus subtilis and Rhodopseudomonas palustris**

Daisuke Seo[1], Hidehiro Sakurai[2], Pierre Sétif[3], Takeshi Sakurai[1]

1.-Graduate School of Natural Science and Technology, Kanazawa Univ., 2.-Research Institute for Photobiological Hydrogen Production, Kanagawa University, 3.-CEA, iBiTecS

Ferredoxin-NAD(P)+ oxidoreductase (FNR) is a ubiquitous enzyme that catalyze the redox reaction between soluble small iron-sulfur protein ferredoxin and NADP+/H. FNRs from photosynthetic green sulfur bacterium Chlorobaculum tepidum (CtFNR), low-GC content gram-positive bacterium Bacillus subtilis (BsFNR) and purple non-sulfur bacterium Rhodopseudomonas palustris (RpFNR) are homo-dimeric proteins containing one FAD prosthetic group per subunit. Crystal structure analyses of Ct- and BsFNRs have revealed their significant structural homology to NADPH-thioredoxin reductase from Eschelicia coli, which is distinct from the monomeric FNRs from plastids of higher plants, cyanobacteria, γ-proteobacteria and putidaredoxin reductase. Previous studies on steady-state reaction of Bs- and RpFNRs with NADP+/H by diaphorase assay have demonstrated that NADPH oxidation rates of BsFNR and RpFNR were comparable to those of the FNRs from plastid and cyanobacteria. But affinities toward NADP+/H and rates for oxidation of NADPH differed significantly between BsFNR and RpFNR, despite their high amino acid sequence homology. In this work, we report pre-steady state reactions of BsFNR and RpFNR with NADP+/H by a stopped-flow spectrophotometry. Mixing oxidized BsFNR with NADPH yielded a rapid formation of charge transfer complexes (CTCs) followed by a reduction of the enzyme. BsFNR was almost fully reduced at equilibrium. Mixing photochemically reduced BsFNR with NADP+ also rapidly provided an absorption spectrum of CTC but followed reoxidation of reduced BsFNR was very slow. The amount of oxidized BsFNR after equilibrium depended on NADP+ concentration. Kinetic analyses indicated that the rate-determining steps were the hydride-transfer reactions in both directions and the rate for the forward direction was much faster than that for the reverse direction. Mixing oxidized RpFNR with NADPH exhibited a rapid CTCs formation followed by a slower reduction of the enzyme. Increase in NADPH concentration reduced the observed rate, suggesting redox potential of RpFNR was similar to that of NADP+/H couple in the presence of excess NADPH. Mixing photochemically reduced RpFNR with NADP+ rapidly provided CTCs. The decay corresponding to the reoxidation of reduced RpFNR with NADP+ involved two distinctive kinetic phases, which would be due to the similarity in hydride transfer rates of forward and reverse directions, and the presence of excess amount of NADP+. Kinetic analyses indicated that the rate-determining steps were the hydride-transfer reactions in both directions and the rate for the forward direction was close to that for the reverse direction. Obtained data suggested BsFNR and RpFNR are optimized for different direction of the reaction and may play different physiological roles.

PH-025 **The internal dynamics of fibrinogen and its implications for coagulation and adsorption**

Stephan Köhler[1,2], Friederike Schmid[1], Giovanni Settanni[1,3]

1.-Institute of Physics, Johannes Gutenberg University Mainz, Germany, 2.-Graduate School Materials Science in Mainz, 3.-Max Planck Graduate Center with the Johannes Gutenberg-University Mainz

Fibrinogen is a serum multi-chain protein which, when activated, aggregates to form fibrin, one of the main components of a blood clot. Fibrinolysis controls blood clot dissolution through the action of the enzyme plasmin, which cleaves fibrin at specific locations. Although the main biochemical factors involved in fibrin formation and lysis have been identified, a clear mechanistic picture of how these processes take place is not available yet. This picture would be instrumental, for example, for the design of improved thrombolytic or anti-haemorrhagic strategies, as well as, materials with improved biocompatibility. Here, we present extensive molecular dynamics simulations of the fibrinogen complex which reveal large bending motions centered at a hinge point in the coiled-coil region of the complex. This feature, likely conserved across vertebrates according to our analysis, suggests an explanation for the mechanism of exposure to lysis of the plasmin cleavage sites on fibrinogen coiled-coil region. It also explains the conformational variability of fibrinogen observed during its adsorption on inorganic surfaces and it is supposed to play a major role in the determination of the hydrodynamic properties of fibrinogen. In addition the simulations suggest how the dynamics of the D region of fibrinogen may contribute to the allosteric regulation of the blood coagulation cascade through a dynamic coupling between the a- and b-holes, important for fibrin polymerization, and the integrin binding site P1.

PH-026 **Membrane curvature – the assembler of proteins**

Mijo Simunovic[1,2], Gregory Voth[1], Patricia Bassereau[2]
1.-Chemistry Department, The University of Chicago, 2.-Physico-Chimie Curie, Institut Curie

Many biological phenomena require the membrane to change its shape. This process is often mediated by curvature-generating proteins, most notably by those containing one of many BAR domains. At the same time, membrane curvature controls the way proteins interact with one another and so it acts as a vital signaling mechanism in the cell. We combine theoretical modeling with microscopy imaging techniques to study the driving force underlying the reshaping of biological membranes induced by N-BAR proteins. In particular, we employ a combination of coarse-grained molecular dynamics with field-theoretical simulation methods to study the assembly of proteins on the membrane at molecular resolution. This approach allowed us to elucidate the precise mechanism by which cell membranes rapidly and from large distances recruit proteins to membrane-reshaping sites. It also let us identify a surprising role of membrane tension in directing the strength and geometry of protein-protein interactions. By complementing our simulations with fluorescence and atomic force microscopies, we study the way molecular assembly of proteins affects the membrane morphology at a more macroscopic level. We demonstrated how large-scale ordering of the proteins on the membrane is mediated by a strikingly long-range interaction that is driven by membrane fluctuations. In sum, our combined theoretical and experimental approach gives vital clues on how the mechanical properties of the membrane may regulate protein dynamics in living cells.

PH-027 **Transmission of rigidity at a distance - new insights into allosteric signalling in G-Protein Coupled Receptors**

Adnan Sljoka[1], Alexandr Bezginov[2]
1.-Kyoto University, 2.-University of Toronto

Understanding how a protein functions depends in critical ways on predicting which parts are rigid and which are flexible. Using rigidity-theoretical techniques, a number or programs such as FIRST, ProFlex or Kinari can rapidly decompose a protein into flexible and rigid regions. In this study we extend this technique and develop a novel computational approach for detecting protein allosteric interactions. It is widely believed that the binding of a ligand at the allosteric site triggers a conformational change that is transmitted through the protein to cause a rearrangement and alteration of the shape of the active site. Allostery was first described more than 50 years ago; however, the underlying allosteric mechanism is still not well understood. We will introduce a rigidity-based allosteric mode of communication together with an algorithm which can detect transmission of rigidity and shape changes between two (or more) distant binding sites in allosteric proteins. Our algorithm is also used to predict and identify regions in the protein that are critical for the coupled communication between distant sites (i.e. allosteric pathways). Starting with a set of known GPCR 3-dimensional structures, we apply our methods and show how binding of an activating ligand (i.e. agonist) triggers small rigidity changes which propagate to the critical G-protein binding regions. In contrast, in the inactive GPCR structures no such rigidity allosteric communication is observed. Detailed predictions and analysis on activated (agonist-bound) and inactive adenosine receptors is discussed and results are compared with experimental evidence. These results show that rigidity-based allosteric model and algorithm is a powerful new tool for detecting allostery in GPCRs.

PH-028 **Comparing the intrinsic dynamics of multiple proteins using elastic network models reveals global similarities based on their overall shape**

Sandhya Tiwari[1,2], Nathalie Reuter[1,2]

1.-Department of Molecular Biology, University of Bergen, 2.-Computational Biology Unit, Department of Informatics, University of Bergen

With increasing protein structural data, we find ourselves with the opportunity to study the dynamical similarities within large ensembles in order to understand the role of protein dynamics at various levels. Is there evolutionary pressure to conserve dynamics? Is this necessary to retain functionality, as it has been suggested? We performed strategic comparative analysis using the elastic network model, which has proven to be highly efficient and informative (Fuglebakk et al., BMC Bioinformatics, 2014) to investigate how the dynamics of related proteins change when their functional or oligomeric state shifts, and if it is conserved within protein families. In our work, we have found that proteins with different ligand-binding states, as in adenylate kinase (Tiwari et al., BMC Bioinformatics, 2014), or oligomeric states, as in the PyrR family (Perica et al., Science, 2014), can be classified based on the global similarity of their intrinsic dynamics. Moreover, our recent analysis on functionally distinct protein families with the TIM barrel fold indicates that the overall similarity in shape dictates similarity in intrinsic dynamics regardless of sequence and functional conservation or evolutionary links. We suggest that since shape dictates a large part of dynamical similarity in proteins, the changes in local flexibility play a strong role in differentiating various functional states.

PH-029 **Oxygen-Affinity and Cooperativity of Hemoglobin (Hb) are Regulated by 4D Structural Changes (Protein Dynamics), rather than 3D Structural Changes**

Takashi Yonetani[1], Kenji Kanaori[2]

1.-University of Pennsylvania, Department of Biochemistry & Biophysics, 2.-Kyoto Institute of Technology, Department of Bio-Molecular Engineering

Introduction: The widely-held mechanism of allostery of Hb has been that the changes from the R-quaternary/tertiary structures of oxy-Hb to the T-quaternary/tertiary structures of deoxy-Hb exert certain constraints on the coordination structure of the heme group, leading to a lower O2-affinity of the hemes and thus that of Hb [1]. However, we found that there is no causal correlation between the static T/R-quaternary structures and the low/high O2-affinities of Hb, respectively [2]. We explore an alternative mechanism of the regulation of the ligand-affinity and cooperativity in Hb. Results and Discussion: The O2-affinities of free Fe[II]-protoporphyrin IX-nitrogenous base complexes in organic solvents are very low (P50 > 102 ~ 103 Torr), whereas the apparent O2-affinities of these metalloporphyrins, which are incorporated in apo-myoglobin, apo-Hb, serum albumin, etc., increase substantially to P50 < 10-1 ~ 101Torr, though their coordination structures are apparently unchanged [3]. Such substantial increases in the apparent ligand-affinities of metalloporphyrin-containing proteins are accomplished by preventing/inteferring with the dissociation of the ligand by protein matrix, since the interior of globin is nearly fully packed by protein matrix. In Hb, the dissociation process of the ligand proceeds through the “caged” state [4-6], which can be produced by cryogenic photolysis of the ligated-states at 4.2K and in which the metal-ligand bond is broken and the un-bonded ligand is trapped near the bonding site within the globin moietiy. This “caged” state has spectral features distinct from those of either deoxy- or ligated states of the respective hemoproteins. The apparent ligand-affinities of Hb are regulated by heterotropic effectors without detectable changes in either static quaternary/tertiary structures of the globin moiety or the coordination/electronic structures of the metalloporphyrin moiety and thus the ligand-affinity of the metalloporphyrins themselves [7-9]. The reduction of the apparent ligand-affinities of Hb may be caused by increases in the migration rate of ligands through globin matrix from the “caged” state to solvent, resulting from the effector-linked, enhanced high-frequency thermal fluctuations which increase the transparency of the globin matrix toward small diatomic ligands [7-9]. Conclusion: The ligand-affinity of Hb is regulated through protein dynamics by heterotropic effectors, rather than static quaternary/tertiary structural changes. Thus, the “caged” state of Hb acts as a critical transition state in regulation of the affinity for small diatomic ligands in Hb [9].

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PH-030 **On the Role of Metal Ions in Synaptic Proteins Assembly**

Rafal Jakubowski[1], Jakub Rydzewski[1], Wieslaw Nowak[1]
1.-Faculty of Physics, Astronomy and Informatics, Nicolaus Copernicus University

The role of metal ions in the regulation of life processes is extremely important. They act as signal transducers, protein configuration stabilizers, enzymatic cofactors, oxygen transport supporters and many others. For example, subtle perturbations in calcium homeostasis may lead to mental disabilities and are linked to diseases such as Autism Spectrum Disorders (ASD). In this study we focus on complex protein systems, mainly those present in the brain. We search for dimers mediated by the presence of metal ions, and determine the impact of the presence or absence of the latter on the structure and energetic properties of the complex in the protein-protein interface. We investigate ions' influence on the interface stability using classic molecular dynamics methods (MD), including Steered MD. Moreover, we apply a novel suite of enhanced MD-based methods recently developed by our team (Rydzewski & Nowak) to explore ion diffusion pathways in protein fragments of the synapses. Finally, we describe specific inter-protein ion binding motifs with the most important interactions, collating them with various structures deposited in the Protein Data Bank [1]. This research was supported by "Krok w przyszlosc – 5 edycja" scholarship from the Marshal of Kuyavian-pomeranian voivodeship (RJ). [1] H.M. Bermanet al., NAR, 2000, 28, 235-242

PH-031 **The role of the Mg(II) ion on integrin-collagen interactions: regulating affinity through conformational fluctuations**

Ana Monica Nunes[1,2], Jie Zhu[1,2], Jackie Jezioro[1,2], Conceição Minetti[1], David Remeta[1], Samir Hamaia[3], Richard Farndale[3], Jean Baum[1,2]

1.-Department of Chemistry & Chemical Biology, Rutgers University, 2.-Center of Integrative Proteomics Research, Rutgers University, 3.-Department of Biochemistry, University of Cambridge

The binding of integrins to collagen plays a critical role in numerous cellular adhesion processes including platelet activation and aggregation, a key process in clot formation. Collagen is an unusually shaped ligand, and its mechanism of recognition and role in selectivity and affinity are unique, and at this stage not well understood. The I-domain of the integrin protein binds to collagen specifically at multiple sites with variable affinities, however the molecular mechanism of integrin I-domain (αI) regulation remains unknown. Using NMR, along with isothermal titration calorimetry, mutagenesis, and binding assays we are developing a novel integrated picture of the full recognition process of the integrin α1I binding to collagen. The adhesion of the α1β1 integrin receptors to collagen is cation-dependent with collagen binding a Mg(II) ion that is located at the top of the extracellular integrin α1I-domain (α1I). Our results show evidence for a regulatory effect of the Mg(II) ion on α1I affinity, by inducing allosteric ms-μs motions of residues distant from the binding site. We propose a novel model of α1I recognition to collagen, comprising a two-step mechanism: a conformational selection step, induced by Mg(II) coordination, and an induced-fit step caused by collagen binding. Hydrogen-deuterium exchange experiments show that the induced-fit step is facilitated by the reduced local stability of the C-terminus. We propose that the conformational selection step is the key factor that allows discrimination between high and low affinity collagen sequences.

PH-032 **Effect of Membrane Composition on the Structure of Membrane-Attached Cytochrome P450 3A4**

Veronika Navratilova[1], Marketa Paloncyova[1], Michaela Kajsova[1], Karel Berka[1], Michal Otyepka[1]
1.-RCPTM, Department of Physical Chemistry, Faculty of Science, Palacky University

Cytochromes P450 (CYP) are heme containing enzymes involved in the metabolism of endobiotics and xenobiotics, such as drugs or pollutants.[1] In humans, CYPs are attached to the biological membranes of endoplasmic reticulum or mitochondria by N-terminal transmembrane anchor and they are partially immersed by their catalytic domain to different level.[2] Generally, the composition of lipid membrane may significantly affect behavior of protein embedded in respective membrane e.g. the cholesterol in membrane alters membrane properties such as: thickening of the membrane, changing the stiffness or enhancing ordering of the membrane. Furthermore, the increasing amount of cholesterol in membrane may also alter interaction with membrane proteins and affect solute partitioning between membrane and water molecules.[3] Cholesterol is also known to noncompetitively inhibit the most typical drug-metabolizing CYP - CYP3A4,[4] however the mechanism was unknown. For this reason, we prepared the set of simulations of CYP3A4 embedded in DOPC lipid bilayers with various cholesterol concentrations (0, 3, 6, 20 and 50% wt; Figure 1) and the 200ns+ long MD simulations were carried out. MD simulations showed the formation of funnel-like shape of the lipids close to the catalytic domain of CYP. In addition, the cholesterol molecules have tendency to accumulate in the vicinity of membrane-attached F/G loop. The catalytic domain sunk deeper into the membrane with cholesterol and also the number of amino acids in contact with membrane was bigger than in the pure DOPC bilayer. In contrast, the presence of higher amount of cholesterol affected the pattern of channel opening effectively blocking the access to the active site from the membrane, which in turn may affect the substrate preferences and catalytic efficiency.[5] Finally, we study the effect of different lipid types on membrane-attached CYP3A4.

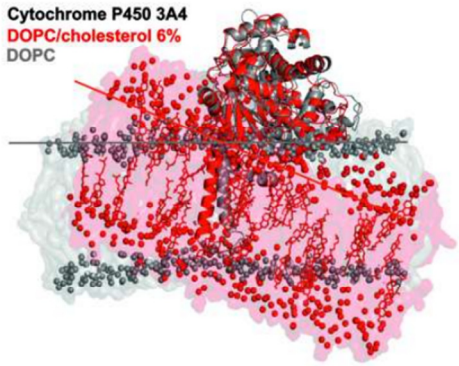

Figure 1: Difference in orientation and position of CYP3A4 on lipid bilayers with and without high amount of cholesterol.

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Acknowledgement: IGA_PrF_2015_027, NPU LO1305, GACR P208/12/G016

PH-033 **Generation of single-chain Fv antibody against (4-hydroxy-3-nitrophenyl)acetyl and analysis of its structural dynamics**

Yusui Sato[1], Yusuke Tanaka[1], Hiroshi Sekiguchi[2], Satomi Inaba[1], Takahiro Maruno[3], Yuji C. Sasaki[4], Yuji Kobayashi[3], Takachika Azuma[5], Masayuki Oda[1]

1.-Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, 2.-Japan Synchrotron Radiation Research Institute, 3.-Graduate School of Engineering, Osaka University, 4.-Graduate School of Frontier Sciences, The University of Tokyo, 5.-Research Institute for Biological Sciences , Tokyo University of Science

Anti-(4-hydroxy-3-nitrophenyl)acetyl (NP) antibodies are one of the most widely analyzed type of antibodies, especially with respect to affinity maturation [1-3]. Affinity maturation is a process in which B cells produce antibodies with increased affinity for the antigen during the course of an immune response, and is like “evolution” in term of increasing antigen-binding affinity. During the course of affinity maturation, the structural dynamics of antibodies, which are closely correlated with the binding function, can change. To analyze the structural dynamics at atomic resolution and the single-molecule level, we tried to express and purify single-chain Fv (scFv) antibodies against NP. Using scFv antibodies, we can also analyze the effects of key residues on affinity maturation via site-directed mutagenesis. As the first step, we have succeeded in generating a sufficient quantity and good quality of scFv of affinity-mature anti-NP antibody, C6, with a linker composed of four repeats of GGGS. The scFv protein was expressed in the insoluble fraction of E. coli, and solubilized using 8 M urea, followed by refolding by step-wise dialysis to decrease the urea concentration. The final step of purification using an antigen column indicated that approximately 2% of the solubilized protein was correctly refolded and possessed antigen-binding ability. The analytical ultracentrifugation (AUC) analysis showed that the purified C6 scFv exists in the monomeric state with little oligomeric contamination. The secondary structure and thermal stability of C6 scFv were analyzed using circular dichroism (CD). The far-UV CD spectra of C6 scFv indicated typical β-sheet-rich structures. Upon antigen binding, the far-UV CD spectrum remained unchanged, but the thermal stability increased by approximately 20oC. The antigen-binding function of C6 scFv was analyzed using a surface plasmon resonance (SPR) biosensor, Biacore. The binding affinity and kinetics of C6 scFv for NP conjugated to bovine serum albumin immobilized on the sensor chip were similar to those of intact C6. Taken together, the results of AUC, CD, and SPR indicated that C6 scFv could be refolded successfully and would possess its functional structure. Next, to analyze the structural dynamics of C6 scFv in the absence or presence of antigen, experiments involving diffracted X-ray tracking (DXT) were performed [4]. C6 scFv with an N-terminal His-tag was immobilized on substrate surfaces using tag chemistry, and Au-nanocrystals were labeled on the surface of scFv as tracers. The motions of C6 scFv were analyzed in two rotational directions representing tilting (θ) and twisting (χ) Mean square displacement (MSD) analysis from more than 200 trajectories showed that the slope for C6 scFv without antigen, especially in the θ direction, was greater than that for C6 scFv with antigen, suggesting that the motion of scFv was suppressed on antigen binding. [1] Furukawa et al., Immunity 11, 329, 1999. [2] Sagawa et al., Mol. Immunol. 39, 801, 2003. [3] Murakami et al., Mol Immunol. 48, 48, 2010. [4] Sekiguchi et al., Sci. Rep. 4, 6384, 2014.

PH-034 **Antibiotic binding drives catalytic activation of aminoglycoside kinase APH(2'')-Ia**

Shane Caldwell[1], Albert Berghuis[1]
1.-McGill University

The antibiotic resistance enzyme APH(2'')-Ia confers antimicrobial resistance to aminoglycoside antibiotics in staphylococci and enterococci. This kinase phosphorylates aminoglycosides such as gentamicin and kanamycin, chemically inactivating the compounds.

We have determined multiple structures of the enzyme in complex with nucleoside and aminoglycoside substrates and cofactor magnesium. Introduction of aminoglycoside to crystals of APH(2'')-Ia induce gross conformational changes in crystallo, illustrating several important stages of the catalytic cycle of the enzyme. An interaction between nucleoside triphosphate and an amino acid residue on a conserved loop has also been identified that appears to govern a conformational selectivity and modulates the enzyme activity when no substrate is present.

Comparisons between multiple protein molecules both within and between crystal structures allow us to infer functional states of the enzyme as it carries out catalysis. These structures collectively highlight an enzymatic flexibility that not only allows the binding of diverse aminoglycosides, but also appears to transition from a stabilized, inactive enzymatic state to a catalytically active enzyme with an active site geometry identical to distantly-related eukaryotic protein kinases. Mechanistic insight gained from these studies begin to demystify a widespread staphylococcal resistance factor, and provide a starting point for the development of anti-infectives toward this important antimicrobial resistance machine.

PH-035 **Disease Related Mutation Effects on Conformations and Dynamics of the Zinc-Finger NEMO**

Ryan Godwin[1], William Gmeiner[2], Freddie Salsbury[1]
1.-Wake Forest University - Department of Physics, 2.-Wake Forest University Health Sciences - Department of Cancer Biology

The zinc-finger of the NF-κB Essential Modulator (NEMO) is a ubiquitin binding domain, and an important regulator of various physiological processes including immune/inflammatory responses, apoptosis, and oncogenesis. The nominally functioning 28 residue monomer (2JVX) is represented by a ββα motif, with a CCHC active site coordinating the zinc ion. Here, we investigate the effects of a single point mutation that has been linked to the disease states associated with ectodermal dysplasia. The single mutation of the last binding cysteine (residue 26) to a phenylalanine (2JVY) distorts the available conformation and dynamics of the protein, as shown via microsecond, GPU-accelerated Molecular Dynamics simulations. We examine these two proteins in various states of zinc-binding and coordinating cysteine protonation. In addition to destabilization of the alpha-helix induced by the cysteine to phenylalanine mutation, prominent conformations show the β-sheets turned perpendicular to the alpha-helix, providing a possible mechanism for the induced disease state.

PH-036 **Structural characterization of the binding of HIV-1 integrase to its cellular co-factor Ku70**

Ekaterina Knyazhanskaya[1], Andrey Anisenko[2], Marina Gottikh[3], Timofei Zatsepin[1]

1.-Moscow State University, Chemistry department, 2.-Moscow State University, Department of bioengineering and bioinformatics, 3.-Moscow State University, Belozersky institute of physico-chemical biology

Ku is a heterodimer complex composed of two DNA-binding subunits, Ku70 and Ku80, displaying essential functions for human cell survival. At the same time, Ku has been identified as a cellular factor important for HIV-1 replication. It is known that the levels of viral replication are significantly lowered in cells depleted of Ku70. Reportedly, Ku complex may act at different stages of the HIV-1 cycle, such as the formation of 2-LTR circles, integration and transcription of the integrated provirus. Remarkably, Ku may also be incorporated into virions during viral propagation. However, the precise impact of Ku on HIV-1 expression remains unclear and requires further examinations. A putative role of Ku in the HIV-1 integration is that its Ku70 subunit protects viral integrase (IN) from degradation by a direct interaction. Thus, the inhibition of Ku70-IN interaction might affect viral replication. A detailed structure of Ku70/IN complex would greatly facilitate the inhibitor design. In our work we have proven the existence of a stable complex between purified Ku70 and HIV-1 IN with a Kd ~ 70 nM. To gain insights on the structure of Ku70/IN complex, we performed a systematic analysis of subdomains within IN that are required for the complex formation. We used both pull-down and SPR-technique to elucidate interactions of full-length Ku70 with IN fragments. N-His6-tagged HIV-1 IN separate domains (N-terminal (1-50 aa), catalytic (51-220 aa) and C-terminal (220-270 aa)) were expressed in E. coli. Several truncated IN variants containing amino acids 1-160, 1-220, 51-160 and 51-280 were also prepared. A full-size Ku70 with a GST-tag on its N-terminus was purified from E. coli. All the experiments performed showed that neither N-terminal nor C-terminal domains of HIV-1 IN are essential for its binding with Ku70 despite a weak binding capacity retaining to the C-terminal domain. The catalytic core (51-220 aa) as well as the mutant lacking C-terminal domain (1-220) both demonstrated affinity to Ku70 comparable to the affinity of the full-size IN, whereas its truncated variant (51-160 aa) bound to Ku70 protein only weakly. We also expressed a C-terminal HA-tagged full-length IN and its 1-220 variant in HEK 293T cells together with a WT Ku70-3FLAG and showed that both IN variants are stabilized by co-expression with Ku70 by approx. twofold. We hypothesize that the binding surface within IN lies in the region from 160 to 230 a.a. that is a long α-helix. We have shown that a homologous integrase from prototype foamy virus that lacks this structural element does not bind to Ku70. It is worth noting that Ku70 does not affect the interaction of IN with its major cellular partner – LEDGF/p75 as well as its interaction with the DNA substrate.

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PH-037 **Structural characterization of calmodulin bound to the intracellular calmodulin binding domain of Kv7.2 channels by NMR**

Ganeko Bernardo Seisdedos[1,2], Álvaro Villarroel[2], Oscar Millet[1]
1.-CIC-Biogune, 2.-Unidad de Biofísica (CSIC-UPV/EHU)

Mammalian KCNQ genes encode five Kv7 potassium channel subunits (Kv7.1-Kv7.5). Kv7.2 and Kv7.3 are expressed in the nervous system, being the principal molecular components of the slow voltage gated M-channel, which exert a strong control in neuronal excitability. Calmodulin (CaM) binds to two sites named helix A and B within the intracellular C-terminus, mediates inhibition of Kv7.2 channels and is required for the channels to exit the endoplasmic reticulum. Whereas the understanding of its regulation and its electrophysiological properties has increased dramatically in the last years, still little is known about Kv7 potassium channels structure, mainly because obtaining the large amounts of purified protein required for crystallography or NMR has proven to be very challenging. Here, we present a structural characterization of the cytosolic domains of Kv7.2, as studied by NMR spectroscopy. Due to the large size of the protein, we have devised a strategy where fragments of increasing size have been structurally characterized in complex with CaM. The dynamic and functional properties of the CaM-Kv7.2 cytosolic domains are discussed.

PH-038 **Cytochrome P450 Oxidoreductase Simulations: Cofactors Movement and Structural Changes**

Martin Srejber[1], Veronika Navratilova[1], Michal Otyepka[1], Karel Berka[1]
1.-RCPTM, Department of Physical Chemistry, Faculty of Science, Palacky University

The NADPH-dependent Cytochrome P450 Oxidoreductase (CYPOR) is large 677 amino-acid long microsomal multidomain enzyme responsible for electron donation to its redox partner cytochrome P450 (CYP) involved in drug metabolism. Electron transfer (ET) chain is mediated by two riboflavin-based cofactors – flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) within their respective domains and nicotinamide adenine dinucleotide phosphate (NADPH). During this electron transfer CYPOR undergoes several structural changes in open and closed state of both domains in different degree of contact. In spite of the fact that CYP-CYPOR complexes play a key role in drug metabolism, the atomistic mechanism of structural rearrangements during complex electron transfers is still lacking. Here, we present the results of our study on structural changes during CYPOR multidomain complex movement between individual electron transfers using classical molecular dynamics (MD) and metadynamics (MTD) simulations with cofactors of NADPH, FAD and FMN in resting state. Homology model of human CYPOR in both forms (opened and closed) were embedded into pure dioleoylphosphatidylcholine (DOPC) bilayer. After system equilibration (Figure 1), structural changes of protein, anchor and cofactor movement were studied. We were able to select possible CYPOR-membrane orientation which would allow interaction with cytochrome P450. In addition, spontaneous closing of open CYPOR was observed. However structural changes between crystal structures and structures obtain from MD simulations lead us to the use of metadynamics in order to speed up the process. FMN and FAD cofactor remained in close van der Waals contact during the 100-ns long simulation stabilized by π stack interaction of FAD with Trp676, whereas continual movement of NADPH continually weakens its π stack interaction with FAD. After 100 ns of classical MD additional metadynamics simulations were performed in order to investigate internal motion of cofactors during electron transfer. Atoms C4N (NADPH) and N5 (FAD) which are responsible for ET were able to move closer to the distance of 3 Å after adding biasing potential. This distance is more than sufficient for electron transfer to occur. After switching back to classical MD cofactors got into resting positions (8 Å) again. Our results show that CYPOR undergo several structural changes and internal motions of cofactors in order to transfer electrons to its redox partner - CYP.

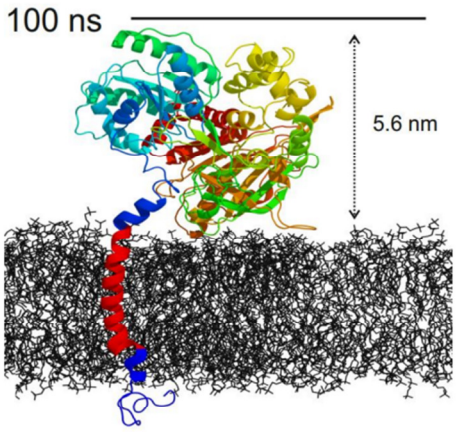

Figure 1: CYPOR equilibrated membrane structure model in closed conformation

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PH-039 **Single Molecule Motion Map of Pentameric Ligand Gated Ion Channel by Diffracted X-ray Tracking**

Hiroshi Sekiguchi[1], Yufuku Matsushita[2], Yuri Nishino[3], Keigo Ikezaki[2], Atsuo Miyazawa[3], Tai Kubo[4], Christele Huron[5], Jean-Pierre Changeux[5], Pierre-Jean Corringer[5], Yuji Sasaki[2]
1.-Research & Utilization Div., JASRI/SPring-8, 2.-Grad. School Frontier Sci., Univ. Tokyo, 3.-Grad. Sch. Sci., Univ. Hyogo, Japan, 4.-National Institute of Advanced Industrial Science and Technology, Japan, 5.-Pasteur Institute

Pentameric ligand-gated ion channels (pLGICs) are a major family of membrane receptors that open to allow ions to pass through the membrane upon binding of specific ligands. pLGICs are made up of five identical (homopentamers) or homologous (heteropentamers) subunits surrounding a central pore. Structural information about their multiple allosteric states, carrying either an open or a closed channel, has become available by recent studies by X-ray crystallography. However, dynamic information are needed to understand their mechanism of gating, notably the long-range allosteric coupling between the agonist binding site and the ion channel gate. Here we used the diffracted X-ray tracking (DXT) method (1) to detect the motion of the extracellular and transmembrane domain two pLGICs: the nicotinic acetylcholine receptor (nAChR) and a proton-gated bacterial ion channel from Gloeobacter called GLIC. DXT is a powerful technique in biological science for detecting atomic-scale dynamic motion of allosteric proteins at the single molecular level and at tens of micro seconds timescale resolution. The dynamics of a single protein can be monitored through trajectory of a Laue spot from a nanocrystal which is attached to the target protein immobilized on the substrate surface (2,3). DXT detects two kinds of rotational motions of nanocrystal, tilting and twisting, based on X-ray incident beam axis. DXT analysis with 0.1ms/f time resolution showed that tilting motion of the transmembrane domain of GLIC and both tilting and twisting motions of the extracellular domain of GLIC and nAChR were enhanced upon application of agonists (lowering the pH for GLIC, and binding of acetylcholine for nAChR). The detailed dynamic information, including size effect of gold nanocrystal to the motion of them, is discussed.

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PH-040 **Evolutionary hinge migration sheds light on the mechanism of green-to-red photoconversion in GFP-like proteins**

Rebekka M. Wachter[1], S. Banu Ozkan[2], Hanseong Kim[1], Taisong Zhou[2]

1.-Department of Chemistry and Biochemistry, Arizona State University, 2.-Center for Biological Physics, Department of Physics, Arizona State University

Proteins possess unique structure-encoded dynamics that underlie their biological functions. Here, we provide experimental evidence for an evolutionary mechanism driven solely by long-range dynamic motions without significant backbone adjustments, catalytic group rearrangements, or changes in subunit assembly. Crystallographic structures were determined for several ancestral GFP-like proteins that were reconstructed based on posterior sequence predictions, using members of the stony coral suborder Faviina as a model system. The ancestral proteins belong to the Kaede-type class of GFPs, a group of proteins that undergoes irreversible green-to-red photoconversion and is therefore frequently employed in superresolution microscopy. Surprisingly, we find that the structures of reconstructed common green ancestors and evolved green-to-red photoconvertible proteins are very similar. Therefore, we analyzed their chain flexibility using molecular dynamics and perturbation response scanning. We find that the minimal number of residue replacements both necessary and sufficient to support light-induced color conversion provide for increased fold stiffness at a region remote from the active site. At the same time, the allosterically coupled mutational sites appear to increase active site conformational mobility via epistasis. These data suggest that during evolution, the locations of fold-anchoring and breathing regions have been reversed by allosteric means. Therefore, we conclude that the green-to-red photoconvertible phenotype has arisen from a common green ancestor by migration of a knob-like anchoring region away from the active site diagonally across the beta-barrel fold. Based on titration experiments, we estimate that at pH 6, 0.1% of the protein population harbors neutral side chains for His193 and Glu211, residues that form an internal salt bridge near the chromophore. We propose that this reverse-protonated subpopulation constitutes the catalytically competent state. In the electronically excited state, light-induced chromophore twisting may be enhanced, activating internal acid-base chemistry that facilitates backbone cleavage to enlarge the chromophore. In this way, a softer active site appears to be coupled to a mechanism involving concerted carbon acid deprotonation and beta-elimination. Dynamics-driven hinge migration may represent a more general platform for the evolution of novel enzyme activities by tuning motions in the active site. References: 1. Kim, H., Zou, T., Modi, C., Doerner, K., Grunkemeyer, T. J., Chen, L., Fromme, R., Matz, M. V., Ozkan, B., Wachter, R. M. (2015). A Hinge Migration Mechanism Unlocks the Evolution of Green-to-Red Photoconversion in GFP-like Proteins. Structure 23, 34-43. 2. Kim, H., Grunkemeyer, T. J., Modi, C., Chen, L., Fromme, R., Matz, M. V., Wachter, R. M. (2013), Acid-Base Catalysis and Crystal Structures of a Least-Evolved Ancestral GFP-like Protein Undergoing Green-to-Red Photoconversion. Biochemistry 52, 8048-8059.

PH-041 **Quercetin effect on the stability and regeneration of the G-protein-coupled receptor rhodopsin**

Maria Guadalupe Herrera Hernández[1,3], Xiaoyun Dong[1], Cecylia S. Lupala[2], Juan J. Perez[2], Pere Garriga[1]

1.-GBMI, Centre de Biotecnologia Molecular, Universitat Politecnica de Catalunya, 2.-GBMI, ETSEIB, Universitat Politècnica de Catalunya, 3.-Unidad de Biotecnología. Campo Experimental Bajío (INIFAP)

G-protein coupled receptors (GPCRs) are transmembrane heptahelical receptors that constitute a large and widespread family of signal transduction proteins. A number of extracellular ligands, ranging from small molecules to GPCR-binding proteins, have been proposed as good candidates for drug design. The binding of an agonist to a GPCR causes a conformational change in the receptor that leads to its activated functional state. Rhodopsin, the membrane receptor responsible for photoreception in the vertebrate retina, is a prototypical GPCR and has been extensively used in structural, biochemical and biophysical studies of this class of receptors. Different small molecules have been described to be capable of binding to rhodopsin. In addition, mutations in rhodopsin have been associated with retinal diseases and efforts have been carried out in order to find potential ligands that can offset the effect of these mutations. Cyanidins, a group of flavonoids within the larger family of polyphenols, have been reported to stimulate chromophore regeneration of rhodopsin by means of the formation of regeneration intermediates. The aim of the current study was to evaluate the effect of the flavonoid quercetin on the conformational properties of both native bovine rhodopsin and heterologously expressed recombinant rhodopsin. Rhodopsin was purified from bovine retinas by immunoaffinity chromatography, and photobleaching, thermal stability, metarhodopsin II decay and chromophore regeneration assays were carried out in the absence or in the presence of 1μM quercetin. For recombinant rhodopsin, a plasmid encoding wild-type opsin was transfected into mammalian COS-1 cells, in the absence or in the presence of 1μM quercetin, harvested, regenerated with 11-cis-retinal, or 9-cis-retinal, and subsequently purified in dodecyl maltoside solution. No differences in photobleaching behavior, upon illumination, could be detected in the purified quercetin-containing samples compared to those in the absence of this flavonoid. In the case of rhodopsin, and the recombinant wild-type protein regenerated with 11-cis-retinal, quercetin did not significantly alter the thermal stability and rate of regeneration of the purified proteins under our experimental conditions. However, a two-fold increase in the thermal stability and a 40% increase in chromophore regeneration were observed for the recombinant wild-type protein regenerated with 9-cis-retinal in the presence of quercetin. In contrast, the presence of quercetin did not alter the electrophoretic and basic spectroscopic properties of rhodopsin, or those of the recombinant wild-type protein, suggesting no important structural alterations as a result of quercetin binding to the receptor. The positive effect of quercetin on the stability, and chromophore regeneration of rhodopsin, could be potentially used to counteract the effect of naturally-occurring misfolding mutations in rhodopsin. Thus, quercetin could help stabilizing rhodopsin mutants associated with retinal diseases such as retinitis pigmentosa. Furthermore, docking of the ligand, carried out on the crystallographic structure of rhodopsin (entry 1GZM), reveals several favorable sites for quercetin binding. One of this would be compatible with 9-cis-retinal suggesting a complementary binding to the receptor of this isomer which would not be compatible with 11-cis-retinal binding.

PH-042 **Identification of prospective allosteric sites of p38 by computational methods**

Patricia Gomez-Gutierrez[1], Juan Jesus Perez[1]
1.-Departament d'Enginyeria Química (ETSEIB), Universitat Politècnica de Catalunya

Identification of prospective allosteric sites of p38 by computational methods Patricia Gomez-Gutierrez and Juan J. Perez Grup de Biotecnologia Molecular i Industrial, Centre de Biotecnologia Molecular Departament d'Enginyeria Química (ETSEIB), Universitat Politècnica de Catalunya, Barcelona, Spain. Tel: 93 4016679 Protein function is intrinsically associated with structural flexibility, so that understanding the functional properties of proteins requires going beyond the static picture produced by X-ray diffraction studies. Structural flexibility can also be interpreted as a dynamic exchange between different conformational states with low energy barriers at room temperature. Allosterism is a mechanism to regulate protein function associated with the plasticity exhibited by proteins. Allosteric sites can be considered transient cavities that can be occupied by a small molecule with the subsequent modulation of the protein plasticity. Occupation of these sites may modify the affinity of the protein for its native substrate that can be positive when the affinity increases or negative when the affinity decreases. Allosterism can be used for the design of non-competitive ligands as new therapeutic agents. This mechanism of activity modulation is particularly interesting for those targets that use a common substrate for activation, like in the case of kinases to search for selective compounds. Proteins can be viewed in solution as an ensemble of diverse energy accessible conformations. Binding of an allosteric ligand produces a redistribution of the population of the diverse conformational states, which at the end modulate the affinity of the native substrate. Allosteric sites can be characterized using computational methods by ensemble docking. It consist of characterize a set of structures that represent the accessible conformations of a protein that can then be used to perform virtual screening. In the present work we have studied prospective allosteric sites of p38 using computational methods. The protein is a member of the mitogen-activated protein kinases (MAPKs), a highly regulated group of enzymes that control a variety of physiological processes, including mitosis, gene expression, apoptosis and metabolism movement among others. The conformational profile of p38 was assessed using a 4 us trajectory of accelerated molecular dynamics as sampling technique in explicit solvent. We used as starting structure the apo- form of p38 in its inactive conformation (entry 1P38). The conformational features of the protein were assessed through the analysis of the variance of the most flexible regions of the protein using principal component analysis. The snapshots of the trajectory were projected onto the two principal components. Subsequent cluster analysis permitted us to select a few structures for further studies. Specifically, prospective biding sites were identified using a hydrophobic probe as implemented in the SiteMap program. The results show previously described regulatory sites and some new prospective ones.

PH-043 **Hydrogen/deuterium exchange-mass spectrometry provides clues on the mechanism of action of Min E**

Maria T. Villar[1], Kyung-Tae Park[2], Joe Lutkenhaus[2], Antonio Artigues[1]
1.-Department of Biochemistry and Molecular Biology, 2.-Department of Microbiology, Molecular Genetics & Immunology

Cell division in most bacteria is initiated by the formation of the Z ring, an essential cytoskeletal element that serves as a scaffold for the cytokinesis machinery, at the mid body of the cell. In E coli the spatial location of the Z ring is regulated by the Min protein system, comprised by three major proteins: MinC, MinD and MinE. The dynamic interaction between these proteins results in the formation of an oscillating protein gradient between the poles of the cell. This oscillation determines the position of the formation of the Z ring. Many aspects of this simple mechanism are beginning to be understood. In particular, the conformational changes associated with the interaction of the three Min proteins between them and with the cell membrane, are of especial interest. Hydrogen/deuterium exchange mass spectrometry (HDX MS) is a sensitive technique for the detection of changes in protein conformation and dynamics. The main advantages of this methodology are the ability to study native proteins in solution, the requirement for low protein concentrations, the potential to discriminate multiple coexisting conformations, and the lack of an upper limit to the size of protein to be analyzed. Here we use HDX MS to analyze the dynamics of the wild type MinE and of its inactive double mutant D45A D49A. Our results show significant differences in the rates of exchange and in the total amount of deuterium exchanged at the end of the reaction between these two forms of MinE. The wild type protein exchanges most of the amide hydrogen during the first few seconds of initiation of the exchange reaction. On the other hand, the mutant protein exchanges only 50 % of the total amide hydrogen atoms during the first seconds of initiation of the exchange, and the remaining 50% amide hydrogen atoms are exchanged more slowly during the next few minutes of the reaction. Our data are consistent with the existence of a highly flexible structure for the wild type protein and the coexistence of at least two rigid conformations for the double mutant that are undergoing a cooperative transition. Interestingly, the central β-sheet forming the interface between the two subunits is protected against exchange on both proteins. These results provide insights into the conformational changes that MinE undergoes during its interaction with MinD.

PH-044 **Biased signalling and heteromization of the Dopamine D2 receptor in Schizophrenia and Parkinson's disease**

Pablo Herrera Nieto[1], James Dalton[1], Jesús Giraldo[1]
1.-Universidad Autónoma de Barcelona

Biased signalling and heteromization of the Dopamine D2 receptor in Schizophrenia and Parkinson's disease As a significant component of dopamine signalling in the brain, the dopamine D2 receptor (D2R), a member of the Class A GPCR family, is an important target in the treatment of neurological conditions such as schizophrenia and Parkinson's disease. D2R shows a variety of signalling pathways through G proteins, including adenylyl cyclase inhibition, Gβγpotentiation of adenylyl cyclase 2, and ERK kinase activation, in addition to β-arrestin recruitment,. These pathways are differentially activated by some agonists and it has been suggested that D2R ligands with Gαi/o antagonist and β-arrestin agonist activity may have anti-psychotic behavioural activity with reduced extra-pyramidal side effects. D2R has also been found to form homodimers or higher-order hetero-oligomers with other GPCRs,, which may modulate D2R conformation and activity, thus constituting an additional form of allosteric receptor regulation. Based on these findings, we have computationally modelled the full-length structure of D2R, including its long intracellular loop 3 (ICL3) that is 130+ residues in length and absent in all homologous GPCR crystal structures. Using state-of-the-art tools, such as ROSETTA for ab initio protein folding and ACEMD for micro-second+ molecular dynamics (MD) simulations we have successfully de novo folded ICL3, which primarily consists of extensions to transmembrane helices (TMH) 5 and 6 and an intervening disordered histidine/proline-rich region, which is highly flexible. The latter is observed to interact with other receptor intracellular loops (ICL1 and ICL2) and appears to restrict access to the G-protein binding-site. In addition, we have docked a structurally diverse collection of 14 ligands (biased agonists, antagonists and allosteric modulators) into our D2R model and observed characteristic binding patterns suggestive of different biased signalling mechanisms. Finally, through protein-protein docking with ROSETTADOCK, we have generated a complete heterodimer model of D2R with the Adenosine A2A receptor (AA2AR), where a mutual interface is formed between their respective TMHs 4 and 5, as well as an association between the C-terminus of AA2AR and ICL3 of D2R. This may be a particularly relevant biological complex in the treatment of Parkinson's disease where antagonists of AA2AR have been shown to ameliorate disease effects, potentially through direct interaction with D2R.

PH-045 **Bis-ANS as a tool to monitor conformational changes upon assembly of binary and ternary complexes of eIF4E, 4E-BP1 inhibitory protein, and the mRNA 5'cap**

Anna Modrak-Wojcik[1], Monika Wisniewska[1], Ryszard Stolarski[1]
1.-Division of Biophysics, Faculty of Physics, University of Warsaw

Specific recognition of the mRNA 5' terminal cap structure by the eukaryotic initiation factor eIF4E is the first and rate-limiting step in the cap-dependent translation. Small 4E-binding proteins, 4E-BP1, 4E-BP2, and 4E-BP3, inhibit the translation initiation by competing with eIF4G initiation factor for the same binding site, and by blocking the assembly of the translation machinery [1]. Our recent studies revealed intricate cooperativity between the cap and 4E-BP1 binding sites of eIF4E [2]. Here, we applied a fluorescent dye, 4,4'-dianilino-1,1'-binaphthyl-5,5'-disulfonate (bis-ANS) to investigate conformational changes upon assembly of binary and ternary complexes composed of human eIF4E, 4E-BP1, and the mRNA 5'cap analogue, m7GTP. The fluorescence quantum yield of bis-ANS increases significantly upon binding to hydrophobic sites of proteins, making the probe a convenient tool to determine the accessibility to hydrophobic surfaces, and to monitor structural reorganisation of macromolecules [3]. We characterised the interaction of bis-ANS with eIF4E and 4E-BP1 by fluorescence titration. The association processes takes up to several hours until the saturation of the fluorescence signal is achieved, reflecting high flexibility of the protein structures. The association constants Kas of eIF4E/bis-ANS complexes are very high for the non-specific interaction. The Kas values for eIF4E/bis-ANS and eIF4E/4E-BP1/bis-ANS are similar (~10^7 M^-1), whereas the presence of m7GTP results in ca. 5-fold weaker binding of the probe to eIF4E. The affinity of bis-ANS for 4E-BP1 is ~10-fold lower than that for eIF4E. We found no effect of either m7GTP or 4E-BP1 on the fluorescence of bis-ANS in complex with eIF4E, thus indicating lack of conformational changes around the probe on eIF4E/m7GTP or eIF4E/4E-BP1 complex formation. It also testifies that bis-ANS does not bind to the cap-binding site, despite the hydrophobic nature of this eIF4E region. On the contrary, addition of m7GTP to the eIF4E/4E-BP1/bis-ANS complex causes an increase of the probe fluorescence, which indicates differences in the structural reorganisation in the binary, m7GTP/eIF4E, compared with the ternary, m7GTP/eIF4E/4E-BP1, complexes, and confirms the spatial cooperation between the cap and 4E-BP1 binding sites. We also observed an increase of fluorescence for bis-ANS bound to 4E-BP1 in the presence of eIF4E, pointing out that 4E-BP1 partially folds upon association with eIF4E. In summary, our results provide a deeper insight into the structural aspects of the molecular interaction at early stages of the cap-dependent translation. Acknowledgements: This work was supported by the BST 170000/BF project from University of Warsaw [1] N. Sonenberg, A.G. Hinnebusch, Cell 136 (2009) 731-745 [2] A. Modrak-Wojcik, M. Gorka, K. Niedzwiecka, K. Zdanowski, J. Zuberek, A. Niedzwiecka and R. Stolarski., FEBS Letters 587 (2013) 3928–3934 [3] A. Hawe, M. Sutter, and W Jiskoot, Pharmaceutical Research 25 (2008) 1487-1499

PH-046 **Mapping of Thrombin - Beta2Glycoprotein I Interaction Sites**

Laura Acquasaliente[1], Simone Tescari[1], Daniele Peterle[1], Giulia Pontarollo[1], Vittorio Pengo[2], Vincenzo De Filippis[1]

1.-Department of Pharmaceutical and Pharmacological Sciences, University of Padua, 2.-Department of Cardiac, Thoracic and Vascular Sciences, University of Padua

Background: Beta2-Glycoprotein (B2GpI) is a protein abundantly present in human plasma and highly conserved in all mammals. B2GpI has been identified as the major antigen in the antiphospholipid syndrome (APS), a severe thrombotic autoimmune disease. Despite its importance in the pathogenesis of APS, the physiological role of B2GpI is still elusive. In a previous work we have demonstrated that B2GpI significantly prolongs the clotting time in fibrin generation assays, and inhibits aggregation of gel-filtered platelets (IC50=0.36uM), either isolated or in whole blood, by inhibiting cleavage of PAR1 on intact platelets (IC50=0.32uM) and in solution. Importantly, B2GpI does not alter the ability of thrombin (FIIa) to generate the anticoagulant protein C, with or without thrombomodulin added. Hence, we concluded that B2GpI inhibits the key procoagulant properties of FIIa, without affecting its unique anticoagulant function. We also proposed that B2GpI, together with other more efficient anticoagulant pathways such as thrombomodulin- FIIa -protein C and antithrombin III- FIIa, may function as a mild anticoagulant in vivo especially in those compartments were the efficacy of thrombomodulin is limited, as in the large vessels, or is even absent, as in the brain vasculature. Aims: Lacking the threedimensional structure of B2GpI-thrombin complex, the aim of this work is to identify the peptide regions either on thrombin and B2GpI involved in complex formation. Results: Data obtained by fluorescence and surface plasmon resonance (SPR) indicated that B2GpI interacts whit FIIa whit physiological affinity (Kd=43±4nM). Kd values calculated by reverting the interacting systems are very similar to each other (Kd=98±9nM), suggesting that B2GpI in the mobile phase has a conformation which is competent for the binding to immobilized FIIa. The affinity of FIIa for immobilized B2GpI is markedly decreased by increased ionic strength (i.e. Kd increases by 50-fold going from 0.1 M to 0.4 M), suggesting the electrostatic interactions play a key role in FIIa - B2GpI recognition. Filling/inactivation or perturbation of FIIa active site does not alter the affinity of FIIa for immobilized B2GpI, confirming that the active site is not involved in the interaction. Mapping of thrombin binding sites with specific exosite-directed ligands (i.e. hirugen, GpIbalpha, HD1 aptamer) and thrombin analogues having the exosites variably compromised (i.e. prothrombin, prethrombin-2, alpha-thrombin), reveals that the positively charged exosite-II of FIIa plays a key role in B2GpI binding. From the docking model of the bB2GpI-thrombin complex, we identified a highly negatively charged segment 219-232 in domain V of B2GpI interacting with positively charged pathes in thrombin exosite II. The synthetic peptide B2GpI(219-232) was able to bind to FIIa with an affinity (Kd=38±9nM) comparable to that of full-length B2GpI, deduced from fluorescence or SPR measurements and to compete in SPR measueremnts with the binding of full-length B2GpI to thrombin. Hence, combining experimental and theoretical data, we obtained a reliable model of the B2GpI-thrombin complex.

PH-047 **Dynamical Variability in the Clan MA of Metalloproteases**

Henrique F. Carvalho[1,2], Ana Cecília A. Roque[1], Olga Iranzo[3], Ricardo J. F. Branco[1]
1.-UCIBIO, REQUIMTE, Faculdade de Ciências e Tecnologia, Universidade Nova Lisboa, 2.-ITQB António Xavier, Universidade Nova de Lisboa, 3.-Aix Marseille Université, Centrale Marseille, CNRS, iSm2 UMR 7313

Metalloproteases are one of the most diverse types of proteases, presenting a wide range of folds and catalytic metal ions. In the case of the MEROPS MA clan, where most of the known metalloproteases are grouped based on the consensus HEXXH sequence motif, a single catalytic zinc ion and common fold architecture [1]. Despite these common features, members from distinct families present distinct domain composition and topology. Given our interest in developing new tailor-made metalloproteases for bioengineering applications, an in-depth understanding of the factors governing their function is required. Protein internal dynamics includes the space of functionally-relevant structural changes occurring during an enzymatic reaction, and there is an increasing understanding on how it relates with protein sequence and structure evolution. Therefore, we have recently assessed how the structural heterogeneity of metalloproteases relates with the similarity of their dynamical profiles [2]. First, the dynamical profile of the clan MA type protein thermolysin, derived from the Anisotropic Network Model, was evaluated and compared with those obtained from principal component (PC) analysis of a set of 112 crystallographic structures and essential dynamics (ED) analysis of a 20 ns molecular dynamics simulation trajectory [3]. A close correspondence was obtained between normal modes (NM) derived from the coarse-grained model and experimentally-observed conformational changes (RMSIP between NM1-NM3 and PC1 of 0.81), corresponding to functionally-relevant hinge bending motions that were shown to be encoded in the internal dynamics of the protein (cumulative overlap of ED1-ED3 and PC1 of 0.85). Next, dynamics-based comparison methods that employ a related coarse-grained model (β-Gaussian Elastic Network Model) was made for a representative set of 13 MA clan members [4], allowing for a quantitative description of its structural and dynamical variability. Although members are structurally similar (87% pairs with DaliLite Z-score > 2.0), they nonetheless present distinct dynamical profiles (69% of pairs with ALADYN P-value > 0.02), with no identified correlation between structural and dynamical similarity. For cases where high dynamical similarity was observed, the respective modes corresponded to hinge-bending motions encompassing regions close to the active site. Further inspection of the produced alignments indicates that for MA clan metalloproteases, conservation of internal dynamics has a functional basis, namely the need for maintaining proper intermolecular interactions between the protein and respective substrate. Previously unnoticed dynamical similarity between clan members Botulinum Neurotoxin Type A, Leishmanolysin and Carboxypeptidase Pfu was also found. Together, these results suggest that distinct selective pressure mechanisms acted on metalloprotease structure and dynamics through the course of evolution. This work shows how new insights on metalloprotease function and evolution can be assessed with comparison schemes that incorporate additional information of protein dynamics. [1] Rawlings ND, Waller M, Barrett AJ, Bateman A. Nucleic Acids Res. 2014; 42: D503–9. [2] Carvalho HF., Roque ACA., Iranzo O., Branco, RJF, submitted. [3] Bakan A, Meireles LM, Bahar I. Bioinformatics. 2011; 27: 1575–7. [4] Potestio R, Aleksiev T, Pontiggia F, Cozzini S, Micheletti C. Nucleic Acids Res. 2010;38: W41–5.

PH-048 **X-ray crystallographic analysis of cold-adapted and thermostable glucokinase**

Tokuro Oda[1], Naoki Fuchita[1], Hiroyuki Motoshima[1], Keiichi Watanabe[1],
1.-Department of Applied Biochemistry and Food Science, Saga University

Glucokinase from Antarctic psychrotroph Pseudoalteromonas sp. AS-131 (PsGK) has a higher specific activity at low temperatures and a higher thermal stability than its mesophilic counterpart from E. coli (EcGK). In order to elucidate the structural basis for cold–adaptation and thermal stabilization of PsGK, we have determined the crystal structure of PsGK at 1.69 Å and compared it with the EcGK structure. PsGK is a homodimer of the subunit of 328 amino acid residues. Each subunit consists of two domains, a small α/β domain (residues 7–125 and 314–328) and a large α + β domain (residues 126–313). The active site is located in a cleft formed between the two domains. The identity of amino acid sequence between PsGK and EcGK was 36%, but three dimensional structures of them are very similar to each other, having the conserved catalytic residues and substrate–binding residues. The analysis of the main–chain temperature factors revealed that the regions of small domain and the hinge region connecting two domains of PsGK showed higher temperature factors with a lower number of intramolecular hydrogen bonds and ionic interactions than the corresponding regions of EcGK. However, the large domain regions of PsGK showed lower temperature factors with a higher number of intramolecular hydrogen bonds than EcGK. Furthermore, the atomic temperature factors of catalytic Asp112 on the small domain were higher, but those of glucose-binding Glu169, His172, and Glu199 on the large domain were lower than EcGK. These results suggest that highly flexible hinge region and the catalytic residue on the small domain of PsGK may contribute to its cold-adaptation, namely higher activity at low temperatures, whereas a more rigid structure of the large domain of PsGK stabilizes its overall structure more strongly than EcGK.

PI-001 **Genetic engineering of new formate dehydrogenases for cofactor regeneration**

Anastasia Alekseeva[1,2], Irina Dolina[2,3], Ivan Kargov[2,3], Svyatoslav Savin[2,3], Vladimir Tishkov[1,2,3]
1.-A.N. Bach Institute of Biochemistry, Russian Academy of Sciences, 2.-Innovations and High Technologies MSU Ltd, 3.-Chemistry Faculty, M.V. Lomonosov Moscow State University

Nowadays non-waste technologies in synthetic chemistry become more and more popular. Such processes are often carried out using different enzymes. Dehydrogenases represent the large group of enzymes, which are widely used in synthesis of chiral compounds and other useful molecules. Such enzymes need NADH or NADPH as a cofactor and due to high cost of reduced coenzymes a cofactor regeneration system is an obligate part in such kind of processes. It was shown that formate dehydrogenase (FDH, EC 1.2.1.2.) is one of the best enzymes for NAD(P)H regeneration. FDH catalyses the reaction of formate oxidation to carbon dioxide coupled with reduction of NAD(P)+ to NAD(P)H. The main advantages of FDH are the irreversibility of catalyzed reaction, low price of formate ion and wide pH optimum of activity. Our laboratory has the largest collection of formate dehydrogenases from different sources. Many FDH genes from bacteria, yeasts and plants were cloned and enzymes were expressed in active and soluble forms. Mutant formate dehydrogenases from bacterium Pseudomonas sp.101 show the highest thermal stability as well as activity in comparison with other reported formate dehydrogenases. Now we have focused on eukaryotic genes. The recombinant enzymes from soya Glycine max (SoyFDH), Arabidopsis thaliana (AthFDH), moss Physcomitrella patens (PpaFDH) and yeast Ogataea parapolymorpha (OpaFDH) were obtained by genetic engineering methods. It was revealed, that SoyFDH has the best Michaelis constants among all known FDHs, but it's less thermally stable compared to other FDHs. New mutant forms of SoyFDH with excellent catalytic characteristics and high thermal stability were obtained by protein engineering. Other enzymes (AthFDH, PpaFDH and OpaFDH) are comparable in their stability with majority of bacterial enzymes (but not with PseFDH), so all the new obtained FDHs can be successfully used for cofactor regeneration. This work was supported by grant of Russian President MK-2304.2014.4 and Russian Foundation for Basic Research (grant RFBR 14-04-01665 A and14-04-01625 A).

PI-002 **Mutations of a conserved tryptophan residue of the TEM-1 ß-lactamase**

F Ece Altinisik[1], F Gizem Avci[1], Berna Sariyar Akbulut[1], Elif Özkirimli Ölmez[3], Didem Vardar Ulu[2], Ipek Karacan[1], Duygu Sentürk[1]

1.-Marmara University, 2.-Wellesley College, 3.-Bogaziçi University

Antibiotics are essential therapeutic drugs widely used in the treatment of bacterial infections. Unfortunately, misuse of these drugs resulted in the development of bacterial defense mechanisms. B-lactamase synthesis is among these mechanisms that renders β-lactam antibiotics ineffective. Understanding the dynamic behavior of this enzyme is an important step in controlling its activity. In a former study, the importance of highly conserved W229 in modulating the hinge type H10 motion was reported. In the light of this information, mutant TEM-1 β-lactamase enzymes with W229A, W229F and W229Y substitutions were constructed. Wild-type and mutant TEM-1 β-lactamases purified with Ni2+-affinity chromatography were subjected to enzyme assay using CENTA as the substrate. With W229F and W229Y mutations, the remaining activity was approximately 10 % of the initial activity. However with the W229A mutation, activity was totally lost. Structural studies of the W229A mutant with CD and florescence spectroscopy indicated that there was no major change in the overall structure. However this mutation disrupted the interactions of W229 which resulted in an increase in the flexibility of this region of the protein. This project was supported by TÜBİTAK project no 113M533.

PI-003 **Light-switchable Zn2+ binding proteins to study the role of intracellular Zn2+ signaling**

Stijn Aper[1], Maarten Merkx[1]
1.-Eindhoven University of Technology

Zn2+ plays an important catalytic and structural role in many fundamental cellular processes and its homeostasis is tightly controlled. Recently, free Zn2+ has also been suggested to act as an intracellular signaling molecule. To get increased understanding of the signaling role of Zn2+ we are developing light-switchable Zn2+ binding proteins to perturb the intracellular Zn2+ concentration using light. These protein switches consist of two light-responsive Vivid domains and the Zn2+ binding domains Atox1 and WD4, linked together with flexible peptide linkers. In the dark, Zn2+ is tightly bound in between the two Zn2+ binding proteins. Light-induced dimerization of the Vivid proteins disrupts this interaction and thus results in Zn2+ release. The fluorescent proteins Cerulean and Citrine were attached to the Vivid domains to allow the different conformational states of the protein switch to be monitored using FRET. Zn2+ titrations revealed a 3-fold decrease in Zn2+ affinity going from dark- to light-state for the initial design, which was further improved to 10-fold by optimizing the linkers between the protein domains. In addition, the Zn2+ affinities of both states were tuned to be optimal for intracellular applications. Switching between the high affinity dark-state and the low affinity light-state was found to be reversible for at least two light-dark cycles. Following the in vitro characterization, we are currently assessing the performance of this genetically encoded 'caged' Zn2+ in mammalian cells.

PI-004 **Proteins as supramolecular building blocks: engineering nanoscale structures**

Helen Ashmead[1,2,3], Leonardo Negron[1], Jack Sissons[6], Kyle Webster[6], Vic Arcus[2,4], Juliet Gerrard[1,2,5]

1.-Callaghan Innovation, 2.-Biomolecular Interaction Centre, University of Canterbury, 3.-School of Biological Sciences, University of Canterbury, 4.-Faculty of Science & Engineering, University of Waikato, 5.-School of Biological Sciences, University of Auckland, 6.-School of Biological Sciences, Victoria University

Proteins hold great promise in forming complex nanoscale structures which could be used in the development of new nanomaterials, devices, biosensors, electronics and pharmaceuticals. The potential to produce nanomaterials from proteins is well supported by the numerous examples of self-assembling proteins found in nature. We are exploring self-assembling proteins for use as supramolecular building blocks, or tectons, specifically the N-terminal domain of a DNA binding protein (Nterm-Lsr2) and a typical 2-cys peroxiredoxin (hsPrx3). Non-native forms of these proteins have been designed undergo self-assembly into supramolecular structures in a controllable manner. Self-assembly of Nterm-Lsr2 is initiated via proteolytic cleavage, thereby allowing us to generate supramolecular assemblies in response to a specific trigger. We will show that the degree of oligomerisation can be controlled by variations in environmental conditions such as pH and protein concentration. Furthermore, via protein engineering, we have introduced a new “switch” for oligomerisation via enteropeptidase cleavage. The new construct of Nterm-Lsr2 can be activated and assembled in a controlled fashion and provides some ability to alter the ratio of higher ordered structures formed. hsPrx3 has been shown to oligomerise into dimers, toroids, stacks and tubes in response to specific triggers such as pH and redox state. In this work we have utilised the histidine tag to further control the assembly of this versatile protein tecton. We will show that minute variations in pH can induce oligomersation of hsPrx3 toroids into stacks and tubes. Furthermore, by utilising the histidine tag as a ligand we can bind divalent metals to these supramolecular structures. This not only drives the formation of higher ordered oligomers but also provides a facile route which may facilitate the functionalisation of these protein nanoscale structures after they have been assembled.

PI-005 **A Structure Based Approach to Engineering Contraceptive Vaccine Antigens**

Danielle Basore[1,2], Rajesh Naz[5], Scott Michael[6], Sharon Isern[6], Benjamin Wright[3], Katie Saporita[1], Donna Crone[1], Christopher Bystroff[1,2,4]

1.-Biological Sciences, Rensselaer Polytechnic Institute, 2.-CBIS, Rensselaer Polytechnic Institute, 3.-Chemical and Biological Engineering, Rensselaer Polytechnic Institute, 4.-Computer Science, Rensselaer Polytechnic Institute, 5.-Obstetrics and Gynecology, West Virginia University, 6.-Biological Science, Florida Gulf Coast University

Unintended pregnancy is a worldwide public health concern, with 85 million pregnancies being classed as unintended in 2012 . The magnitude of this number clearly indicates an unmet need in terms of contraception. Methods that are currently available are effective, but exhibit many problems. Side effects, ease of use, cost, and availability are all concerns. We propose a contraceptive vaccine that would be safe, effective, long-lasting, cheap, and reversible. Our vaccine would prevent pregnancy by targeting sperm with antibodies raised in the woman's body. Several approaches have been taken to developing a contraceptive vaccine in recent years. The most successful so far has been using human chorionic gonadotropin (hCG), a hormone produced during pregnancy, as an antigen . The hCG vaccine progressed to phase 2 clinical trials, but only displayed an 80% efficacy, which is insufficient for a contraceptive. Our lab uses a structure based approach to the design of an anti-sperm antigenic protein. We believe this will raise a more vigorous immune response that will produce a longer lasting titer. The CatSper complex is a heterotetrameric calcium channel found in the tail region of sperm . Each subunit of the complex contains an exposed loop known as the P-loop. The P-loop is unique on the surface of sperm because it is not glycosylated, allowing antibodies to potentially recognize and bind it. YLP12 is a twelve residue peptide that mimics the glycans in the glycocalyx of sperm . YLP12 is a member of the FliTRX library , and in mice, produced protective titers that were reversible both voluntarily and involuntarily. Our designs will introduce these two potential antigens into a loop of the L1 protein of Human Papilloma Virus. L1 spontaneously assembles into virus like particles , and will aid in the production of a robust immune response.

PI-006 **Protein carriers for passage of the Blood–Brain Barrier**

Sinisa Bjelic[1]
1.-Department of Chemistry and Biomedical Sciences, Linnaeus University

Medical solutions that help protein therapeutics accumulate into the brain are crucial for future treatment of neurological disorders. Biodrugs have a tremendous potential to treat disorders of the nervous system, but their efficiency has been severely restricted. To reach the brain all drugs must traverse the blood–brain barrier (BBB) — a permeable wall that separates blood from the brain — whose main function is to protect the nervous system from environmental influences of bacteria and toxins. Unfortunately the BBB is also the culprit that effectively blocks access to therapeutics required for treatment of neurological diseases. A way to boost exposure of therapeutics across the BBB is to piggyback onto the transferrin receptor, a multidomain protein anchored in the membrane, which is involved in the physiological facilitation of iron uptake. Here I present research that aims at successfully developing potent protein carriers for transferrin receptor-mediated passage of the BBB by using computational protein design in combination with yeast display methodology for hit validation and optimization. The long-term goal is to couple therapeutics — as for example drugs against Alzheimer's — to the designed carriers to increase the brain uptake and cure neurological disorders.

PI-007 **Medium-throughput multistep purification of coagulation factor VIIa**

Jais R. Bjelke[1], Gorm Andersen[1], Henrik Østergaard[1], Laust B. Johnsen[1], Anette A. Pedersen[1], Tina H. Glue[1]

1.-Global Research Unit, Novo Nordisk

There is a need of medium-to-high throughput purification of low-titre recombinant protein variants for screening to identify the final biopharmaceutical lead. Such proteins include coagulation factors to be used for treatment of haemophilia and other bleeding disorders. At Novo Nordisk we have established a platform for production of recombinant coagulation factor VIIa variants, which include a spectrum of single-point mutations to large domain insertions. The variants were produced using transiently transfected HEK293F, HKB11 or CHOEBNALT85 (QMCF Technology) suspension cells. Harvest cultivations were typical in the range of 0.3- to 1L. A 3-step continuous, multistep purification method was implemented on ÄKTAxpress systems (GE Healthcare). The interlinked process steps include capture using an immunoaffinity column, polish, concentration and buffer exchange using an anion-exchange column and proteolytic activation of the zymogen variant forms using a coagulation factor Xa-immobilized column. Buffers were designed such that elution from the capture column was aligned with binding conditions on the polish column to avoid a desalting step in-between. The following and final enzymatic activation was optimized with regards to flow rate to ensure full conversion while minimizing unwanted secondary cleavages in factor VIIa. The final products were fractionated in sharp chromatographic peaks ready for characterization. HPLC and SDS-PAGE analyses showed a solid quality of the produced variants and more than 800 variants have been produced in sub mg scale using the outlined method.

PI-008 **Biomimetic Sequestration of CO2: reprogramming the B1 domain of protein g through a combined computational and experimental approach**

Esra Bozkurt[1], Ruud Hovius[1], Thereza A. Soares[2], Ursula Rothlisberger[1]
1.-École Polytechnique Fédérale de Lausanne, 2.-Federal University of Pernambuco

Protein engineering is a powerful tool to generate highly specific enzymes for biomimetic production of chemicals. Among many applications, the development of enzymes to accelerate carbon dioxide fixation is a possible route to limit CO2 emission. In this project, we are inspired by the ancient enzyme carbonic anhydrase which efficiently catalyzes the reversible hydration of carbon dioxide in the presence of a zinc ion active site.1 To create an efficient biocatalyst, the engineered GB1 domain2 containing a His3Cys Zn (II) binding site was used as a starting point.3 In subsequent work, B1 domains comprising of His3Wat Zn (II) binding sites have been rationally designed to produce carbonic anhydrase mimics. The re-engineering was accomplished through a series of mutations to orient the zinc bound reactive species to form a hydrogen bond network in the active site while retaining the native secondary structure. We performed classical molecular dynamics (MD), quantum mechanics/molecular mechanics (QM/MM) simulations and metadynamics, with the aim to explore potential catalytic roles of the re-engineered B1 domains and to elaborate the reaction mechanism. Briefly, we introduced novel Zn (II) binding sites into thermostable B1 domain. In parallel, experiments are underway. Wild-type protein was expressed and purified. Structural and mutagenesis studies are ongoing. The results emphasize the power of theoretical work to enable the mimicking of Nature's enzymes for desired catalytic functions.

PI-009 **The roles of entropy and packing efficiency in determining protein-peptide interaction affinities**

Diego Caballero[1,2], Corey O'Hern[1,2,3,4], Lynne Regan[2,5,6]

1.-Physics, Yale University, 2.-Integrated Graduate Program in Physical and Engineering Biology, Yale University, 3.-Mechanical Engineering and Materials Science, Yale University, 4.-Applied Physics, Yale University, 5.-Molecular Biophysics and Biochemistry, Yale University, 6.-Chemistry, Yale University

Despite many recent improvements in computational methods for protein design, we still lack a quantitative and predictive understanding of the driving forces that control protein stability, for example, we do not know the relative magnitudes of the side-chain entropy, van der Waals contact interactions, and other enthalpic contributions to the free energy of folded proteins. In addition, we cannot reliably predict the effects of point mutations on enzyme specificity or sequence tolerance in ligand binding sites. The tetratricopeptide repeat (TPR) motif is a common and versatile protein system that has been used as a model to study protein-protein interactions. For example, recent studies have experimentally measured the binding affinity and specificity for different TPR binding pockets and peptide ligands and generated a ranking of the protein-peptide pairs with the highest affinity. To gain a fundamental understanding of the interplay between atomic close packing and fluctuations of side-chain conformations in protein-peptide binding pairs, we performed all-atom Langevin Dynamics simulations of key residues near the binding interface of TPR proteins and their cognate peptides. The Langevin Dynamics simulations enabled us to calculate the entropy and potential energy of side chain conformations in the presence of backbone fluctuations for each protein-peptide pair. We compile rankings of the stability and affinity of mutant TPR-peptide structures to those obtained from experimental studies. This research has enhanced our ability to rationally manipulate protein-peptide interfaces. Advances from this research will enable the design of TPR modules that specifically recognize biologically important proteins.

PI-010 **Monitoring protein-protein interactions using tripartite split-GFP complementation assays**

Stéphanie Cabantous[1], Hau B. NGuyen[3], Jean-Denis Pedelacq[2], Faten Koraichi[1], Anu Chaudhary[3], Kumkum Ganguly[3], Meghan A. Lockard[3], Gilles Favre[1], Thomas C. Terwilliger[3], Geoffrey S. Waldo[3]

1.-Cancer Research Center of Toulouse, 2.-CNRS- IPBS , UMR 5089, 205 Route De Narbonne, 3.-Los Alamos National Laboratory, Los Alamos NM

Protein-fragment complementation assay (or PCA) is a powerful strategy for visualizing protein-protein interactions in living cells. Previously described split-GFP based sensors suffer from the poor solubility of individual PCA fragments in addition to background signal originating from their spontaneous self-assembly (1). We developed a new encoded genetic reporter called “tripartite split-GFP” for visualizing protein-protein interactions in vitro and in living cells. The assay is based on tripartite association between two twenty amino-acids long split-GFP tags, GFP10 and GFP11, fused to interacting protein partners, and the complementary GFP1-9 detector. When proteins interact, GFP10 and GFP11 self-associate with GFP1-9 to reconstitute a functional GFP (2). Using coiled-coils and FRB/FKBP12 model systems we characterize the sensor in vitro and in Escherichia coli. We extended our studies to mammalian cells and examine the FK-506 inhibition of the rapamycin-induced association of FRB/FKBP12. The small size of these tags and their minimal effect on fusion protein behavior and solubility should enable new experiments for monitoring protein-protein association by fluorescence and for screening modulators of complex formation in cell-based assays. References: [1] Cabantous S., T. C. Terwilliger, et al. (2005). Protein tagging and detection with engineered self-assembling fragments of green fluorescent protein. Nat Biotechnol 23(1): 102-107. [2] Cabantous S, Nguyen HB, Pedelacq JD, Koraichi F, Chaudhary A, Ganguly K, Lockard MA, Favre G, Terwilliger TC, Waldo GS. A new protein-protein interaction sensor based on tripartite split-GFP association. Sci Rep 2013;3:2854.

PI-011 **Role of residues Cys301 and Cys303 in the active site of human ALDH2.**

Luis Francisco Calleja Castañeda[1], José Salud Rodríguez Zavala[1]
1.-Instituto Nacional de Cardiología 'Ignacio Chávez'

Aldehyde dehydrogenases (ALDHs) catalyze the oxidation of aldehydes to their corresponding acids using NAD(P)+ as coenzyme. These enzymes are responsible for the detoxification of lipid peroxidation products, which have been involved in the etiology and pathogenesis of different diseases involving increments in oxidative stress. Recent data from our group, showed that ALDH3A1 is resistant to inactivation by lipid peroxidation products, even at concentrations 50-100 times higher than those required to inactivate ALDH1A1 and ALDH2. The amino acids sequence of the aldehyde-binding site of the three enzymes was analyzed, and it was found that the enzymes susceptible to the effect of lipid peroxidation products (ALDH1A1 and ALDH2), have Cys residues flanking the reactive Cys (position 302), based on this criteria and considering that these aldehydes react preferentially with cysteine, a mutant of ALDH2 was generated changing the Cys residues adjacent to Cys302. The mutant ALDH2-Cys301Thr-Cys303Val, was resistant to the inactivation by acrolein and 4-HNE, even at concentrations 1000-fold higher than those required to inactivate ALDH2. However, the mutant presented values of Km 2, 5 and 50-fold higher for acrolein, propionaldehyde and acetaldehyde, respectively, compared to the wild type enzyme, but showed a catalytic efficiency similar to the parent enzyme. These data revealed that Cys residues near to the reactive Cys in ALDH2 are important in the inactivation process induced by lipid aldehydes, but also participate in determining the specificity for the substrates in this enzyme.

PI-012 **Small molecule-assisted shutoff: A widely applicable method for tunable and reversible control of protein production**

H. Kay Chung[1], Conor Jacobs[1], Yunwen Huo[2], Jin Yang[3], Stefanie Krumm[4], Richard Plemper[4,5], Roger Tsien[0], Michael Lin[3]

1.-Department of Biology, Stanford University, 2.-Department of Pediatrics, Stanford University, 3.-Department of Pharmacology, University of California San Diego, 4.-Department of Pediatrics, Emory University, 5.-Institute for Biomedical Sciences, Georgia State University, 6.-Department of Chemistry and Biochemistry, University of California San Diego, 7.-Howard Hughes Medical Institute, University of California San Diego, 8.-Department of Bioengineering, Stanford University

The ability to quickly control the production of specific proteins would be useful in biomedical research and biotechnology. We describe Small Molecule-Assisted Shutoff (SMASh), a technique in which proteins are fused to a self-excising degron and thereby expressed in a minimally modified form by default. Degron removal is performed by a cis-encoded hepatitis C virus (HCV) protease, so that applying clinically available HCV protease inhibitors causes degron retention on subsequently synthesized protein copies and suppresses further protein production. We find that SMASh allows reversible and dose-dependent shutoff of various proteins with high dynamic range in multiple cell types, including yeast. We also successfully use SMASh to confer drug responsiveness onto a RNA virus for which no licensed drug inhibitors exist. As SMASh does not require permanent fusion of a large domain, it should be useful when control over protein production with minimal structural modification is desired. Furthermore, as SMASh only uses a single tag and does not rely on modulating protein-protein interactions, it should be easy to generalize to multiple biological contexts.

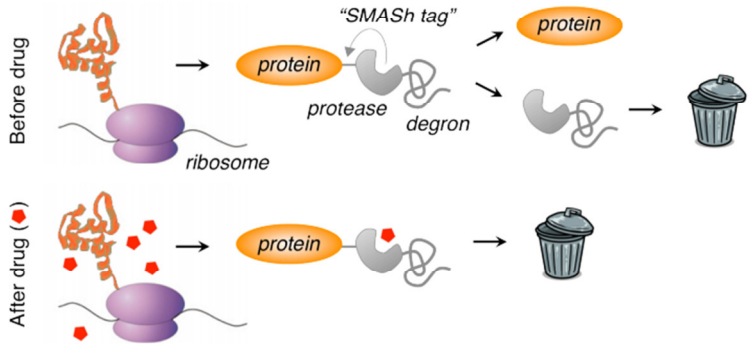

Figure 1. Small Molecule-Assisted Shutoff (SMASh) concept and development. **(a)** SMASh concept. Top, a protein of interest is fused to the SMASh tag via a HCV NS3 protease recognition site. After protein folding, the SMASh tag is removed by its internal NS3 protease activity, and is degraded due to an internal degron activity. Bottom, addition of protease inhibitor induces the rapid degradation of subsequently synthesized copies of the tagged protein, effectively shutting off further protein production.

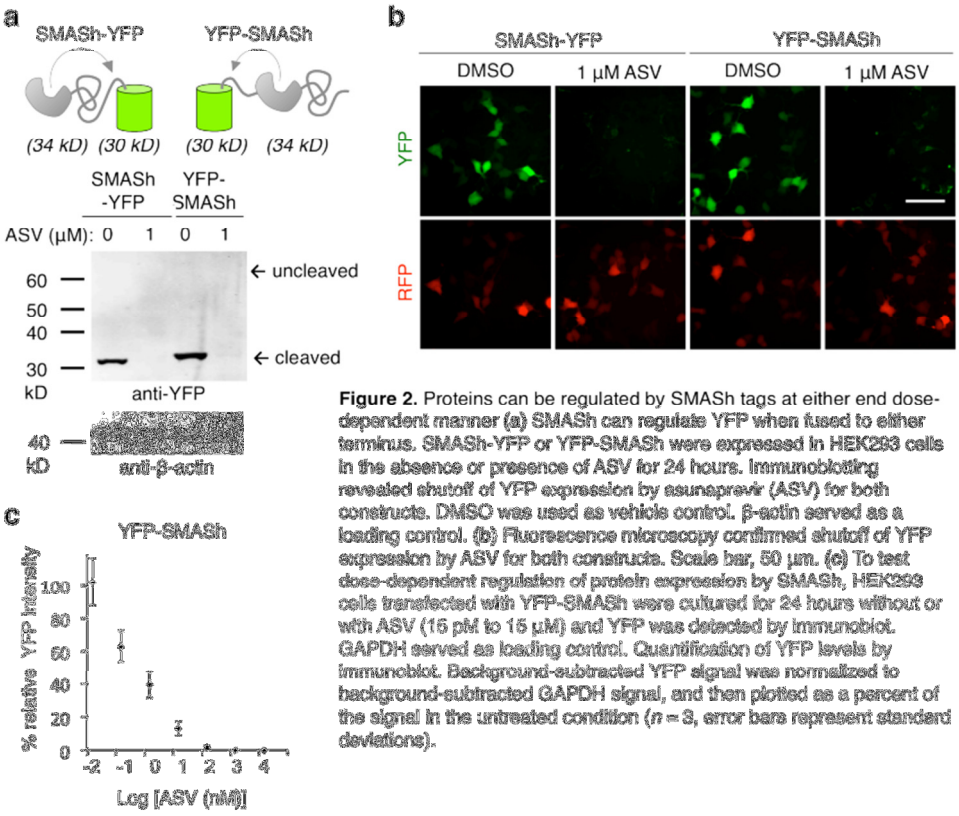

Figure 2. Proteins can be regulated by SMASh tags at either end dose-dependent manner **(a)** SMASh can regulate YFP when fused to either terminus. SMASh-YFP or YFP-SMASh were expressed in HEK293 cells in the absence or presence of ASV for 24 hours. Immunoblotting revealed shutoff of YFP expression by asunaprevir (ASV) for both constructs. DMSO was used as vehicle control. β-actin served as a loading control. **(b)** Fluorescence microscopy confirmed shutoff of YFP expression by ASV for both constructs. Scale bar, 50 μm. **(c)** To test dose-dependent regulation of protein expression by SMASh, HEK293 cells transfected with YFP-SMASh were cultured for 24 hours without or with ASV (15 pM to 15 μM) and YFP was detected by immunoblot. GAPDH served as loading control. Quantification of YFP levels by immunoblot. Background-subtracted YFP signal was normalized to background-subtracted GAPDH signal, and then plotted as a percent of the signal in the untreated condition ($n = 3$, error bars represent standard deviations).

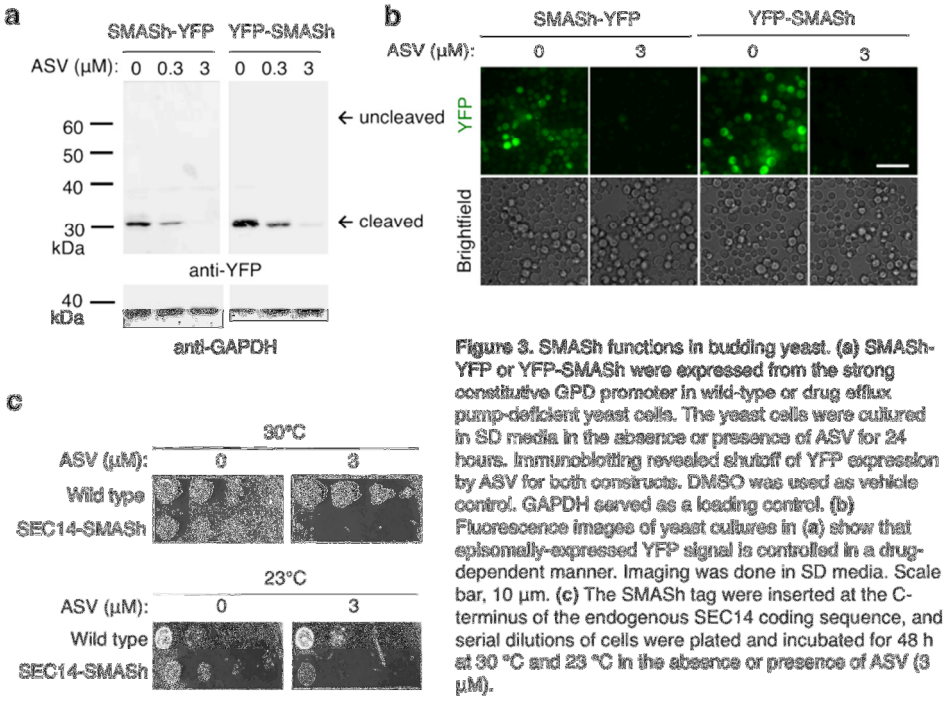

Figure 3. SMASh functions in budding yeast. **(a)** SMASh-YFP or YFP-SMASh were expressed from the strong constitutive GPD promoter in wild-type or drug efflux pump-deficient yeast cells. The yeast cells were cultured in SD media in the absence or presence of ASV for 24 hours. Immunoblotting revealed shutoff of YFP expression by ASV for both constructs. DMSO was used as vehicle control. GAPDH served as a loading control. **(b)** Fluorescence images of yeast cultures in (a) show that episomally-expressed YFP signal is controlled in a drug-dependent manner. Imaging was done in SD media. Scale bar, 10 μm. **(c)** The SMASh tag were inserted at the C-terminus of the endogenous SEC14 coding sequence, and serial dilutions of cells were plated and incubated for 48 h at 30 °C and 23 °C in the absence or presence of ASV (3 μM).

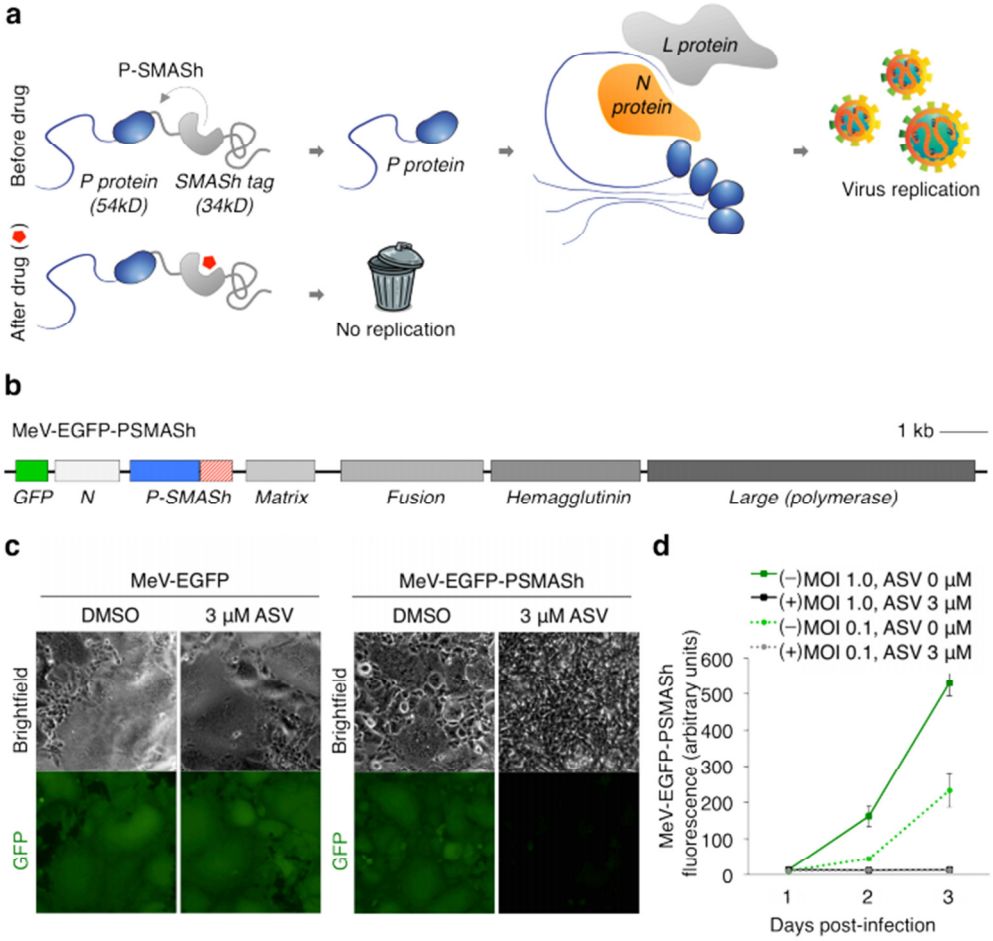

Figure 4. Generation of a drug-controllable "SMAShable" Measles vaccine virus. **(a)** Concept of controlling MeV replication with P-SMASh. In the absence of the drug, essentially unmodified phosphoprotein (P, blue) is released and this traceless P protein can successfully form replication complex with with nucleocapsid (N, orange) and large (L) protein. **(b)** Genome organization of MeV-EGFP-PSMASh. Scale bar is 1 kilobase. **(c)** Regulation of MeV-EGFP-PSMASh by drug. Vero cells infected with MeV-EGFP or MeV-EGFP-PSMASh at multiplicity of infection (MOI) of 1 were grown for 72 hours in the absence or presence of ASV. Drug inhibited syncytium formation and GFP expression in MeV-EGFP-PSMASh-infected but not MeV-EGFP-infected cells. Scale bar, 50 μm. **(d)** Quantification of fluorescence from Vero cells infected with MeV-EGFP-PSMASh at MOI 1 and 0.1 in the absence or presence of 3 μM ASV ($n = 3$, error bars are standard deviation).

PI-013 **Proof of principle for epitope-focused vaccine design**

Bruno Correia[1], John Bates[2], Rebecca Loomis[3], Chris Carrico[4], Joseph Jardine[5], David Baker[6], Roland Strong[7], James Crowe[3], Phillip Johnson[4], William Schief[1,6,7]

1.-Institute of Bioengineering, Swiss Federal Institute of Technology Lausanne, 2.-Department of Biochemistry, University of Washington, 3.-The Vanderbilt Vaccine Center, Vanderbilt University Medical Center, 4.-The Children's Hospital of Philadelphia Research Institute, 5.-Division of Basic Sciences, Fred Hutchinson Cancer Research Center, 6.-Department of Immunology and Microbial Science, The Scripps Research Institute, 7.-IAVI Neutralizing Antibody Center, The Scripps Research Institute

Novel strategies for the development of efficacious vaccines are currently needed for several serious global health threats (e.g. HIV, Flu, Ebola, Dengue, etc). A new class of protein based immunogens for vaccine development has emerged, epitope-focused immunogens, but in the past these have failed to deliver the expected outcome. Here, we employed a new computational design methodology (Rosetta Fold From Loops or FFL) to design epitope-focused immunogens. FFL was devised to insert structurally defined functional sites into protein scaffolds. Throughout the FFL stages the structure of the scaffold is folded and its sequence designed to stabilize the desired functional conformation of the inserted site. We used FFL to design epitope-focused immunogens for the Respiratory Syncytial Virus (RSV), for which despite the intense research we are still lacking an approved vaccine. We designed three-helix bundles harboring an RSV epitope, that was previously co-crystallized with the neutralizing antibody motavizumab. The designs were thermodynamically stable (Tm > 100˚C) and showed extremely high affinities to motavizumab (KD ≈ 30 pM). Structural characterization through x-ray crystallography of antibody-bound and unbound scaffolds showed good agreement to the computational models in the overall structure (rmsd - 1.2 Å) and exquisite mimicry of the epitope region (rmsd - 0.4 Å), when compared to the peptide-epitope in complex with motavizumab. The designed immunogens were used to immunize non-human primates (NHP), and approximately 75% of the cohort developed RSV neutralizing activity, in some instances with high potency. To evaluate the therapeutic relevance of the elicited neutralization activity, we compared the NHP neutralization titers to those of human sera after natural RSV infection, which generally yields protective levels of antibodies. The neutralization potency of the best NHP responders was comparable to that of the human sera. To better understand the features of the antibodies elicited, we isolated several rhesus monoclonal antibodies (RhmAbs) from the animal that exhibited the most potent neutralization. Two of the RhmAbs bound to the immunogen with very high affinity (KD ≈ 3 pM) and were potent RSV neutralizers. Interestingly, these RhmAbs were approximately 10 fold more potent than the FDA-approved prophylactic antibody Palivizumab. Our results provide the first proof-of-principle for epitope-focused vaccine design, and demonstrate the power of the FFL computational methodology. We anticipate that FFL will be useful for a variety of other challenges in the computational design of functional proteins.

PI-014 **Designed repeat proteins as templates for photoactive molecules and fluorescent nanoclusters**

Sara H. Mejias[1,2], Antonio Aires[1,2], Javier López-Andarias[3], Pierre Couleaud[1,2], Begoña Sot[1,2], Carmen Atienza[3], Nazario Martín[1,3], Aitziber L. Cortajarena[1,2]

1.-IMDEA Nanoscience, c/ Faraday, 9, Ciudad Universitaria de Cantoblanco 28049, 2.-CNB-CSIC-IMDEA Nanociencia Associated Unit "Unidad de Nanobiotecnología", 3.-Departamento de Química Orgánica I, Facultad de Química, Universidad Complutense

Self-assembly of biological molecules into defined functional structures has a tremendous potential in nanopatterning, and the design of novel bionanomaterials and functional devices. Molecular self-assembly is a process by which complex three-dimensional structures with specified functions are constructed from simple molecular building blocks. We present first the study and characterization of the assembly properties of modular repeat proteins, in particular designed consensus tetratricopeptide repeats (CTPRs), and their application as building blocks in order to generate functional nanostructures and biomaterials. CTPR proteins can be assembled into self-standing thin films,1 and thin nanometer fibers in solution.2 In this work, we show the use of the designed consensus repeat proteins as scaffolds to template: (1) photoactive organic molecules, and (2) fluorescent nanoclusters. 1. We explore the potential of CTPR proteins to arrange donor-acceptor pairs for electro-active materials. In particular, porphyrin rings arranged by CTPRs in a defined distance and orientation for favoring face-to-face orientation which should lead to an improvement in the optoelectronic properties. Our results confirm the successful ability of CTPR proteins to be used as scaffold for ordering organic chromophores, while preserving their structure. The unique self assembly properties of CTPR scaffolds have been exploited to generate ordered conductive films of the protein-porphyrin conjugates. These results open the door to fabricate hybrid protein-based solid devices. 2. We show results on the ability of CTPR to encapsulate and stabilize fluorescent gold nanoclusters. We investigated the influence of the protein sequence in the final properties of the nanoclusters. The structural and functional integrity of the protein template is critical for future applications of the protein-cluster complexes. Therefore synthetic protocols that retain the protein structure and function have been developed. As a proof of concept, a CTPR module with specific binding capabilities has been successfully used to stabilize nano clusters. References: 1. Grove TZ, Regan L, Cortajarena AL. Nanostructured functional films from engineered repeat proteins. J R Soc Interface. 2013; 10(83):20130051. 2. Mejías SH, Sot B, Guantes R, Cortajarena AL. Controlled nanometric fibers of self-assembled designed protein scaffolds. Nanoscale. 2014 Oct 7;6(19):10982-8. doi: 10.1039/c4nr01210k.

PI-015 **Engineering of proteins to develop biomimetic hematite-based biohybrid materials**

Greta Faccio[1], Krisztina Schrantz[2], Linda Thöny-Meyer[1], Artur Braun[2], Julian Ihssen[1],
1.-Laboratory For Biointerfaces, Swiss Federal Laboratories For Materials Science and Technology, CH, 2.-Laboratory For High Performance Ceramics, Swiss Federal Laboratories For Materials Science and Technology, CH

Biohybrid photoelectrochemical cells have been developed by functionalizing the hematite photoanode with the light-harvesting cyanobacterial protein C-phycocyanin (PC) yielding a substantial enhancement of the photocurrent density. Photoelectrochemical cells combining light-harvesting proteins and inorganic semiconductors have potential for the use in artificial photosynthesis. In this work we present processing routes for the functionalization of hematite photoanodes with PC, including in situ co-polymerization of PC with enzymatically-produced melanin and using a recombinantly produced PC 2. Moreover, recombinant forms of the light-harvesting protein C-phycocyanin from Synechocystis sp. PCC6803 were engineered to carry a peptide with affinity for hematite. Similarly, a bacterial laccase was engineered to acquire affinity for hematite. Results obtained from the different approaches to hematite functionalization and the advantages offered by protein engineering will be presented. 1 J. Ihssen, A. Braun, G. Faccio, K. Gajda-Schrantz, L. Thöny-Meyer, Light harvesting proteins for solar fuel generation in bioengineered photoelectrochemical cells; Current Protein and Peptide Science, Accepted for publication. 2 Faccio G, Schrantz K, Ihssen J, Boudoire F, Hu Y, Mun BS, Bora DK, Thöny-Meyer L, Braun A Charge transfer between photosynthetic proteins and hematite in bio-hybrid photoelectrodes for solar water splitting cells, Nano Convergence, Accepted Nov 2014.

PI-016 **Correcting free energy expressions for thermal motion**

Martin Goethe[1], Ignacio Fita[2], J. Miguel Rubi[1]
1.-Department of Fundamental Physics, University of Barcelona, 2.-Molecular Biology Institute of Barcelona (ibmb-CSIC)

Minimizing a suitable free energy expression is arguably the most common approach in (ab initio) protein structure prediction. The achieved accuracy depends crucially on the quality of the free energy expression in use. Here, we present corrections to existing free energy expressions which arise from the thermal motion of the protein. We (i) devise a term accounting for the vibrational entropy of the protein, and (ii) correct existing potentials for 'thermal smoothing'. (i) Vibrational entropy is almost always neglected in free energy expressions as its consideration is difficult. This practice, however, may lead to incorrect output because distinct conformations of a protein can contain very different amount of vibrational entropy, as we show for the chicken villin headpiece explicitly [1]. For considering vibrational entropy, we suggest a knowledge based approach where typical fluctuation and correlation patterns are extracted from known proteins and then applied to new targets. (ii) At ambient conditions, time-averaged potentials of proteins are considerably smoothened due to thermal motion where the strength of this effect varies strongly between atoms. Distinguishing these inhomogeneities by introducing new atom species regarding their locale environment can therefore increase the precision of time-averaged potentials [2]. * Department of Fundamental Physics, University of Barcelona ** Molecular Biology Institute of Barcelona (ibmb-CSIC) [1] M. Goethe, I. Fita, and J.M. Rubi, Vibrational Entropy of a Protein: Large Differences between Distinct Conformations, J. Chem. Theory Comput. 11, 351 (2015). [2] M. Goethe, I. Fita, and J.M. Rubi, in preparation.

PI-017 **Tertiary structural propensities reveal fundamental sequence/structure relationships**

Fan Zheng[1], Craig Mackenzie[2], Jian Zhang[3], Gevorg Grigoryan[1,3]

1.-Department of Biological Sciences, Dartmouth College, 2.-Institute For Quantitative Biomedical Sciences, Dartmouth College, 3.-Department of Computer Science, Dartmouth College

Extraction of general principles from the continually growing Protein Data Bank (PDB) has been a significant driving force in our understanding of protein structure. Atomistic or residue-level statistical potentials, secondary-structural propensities, and geometric preferences for hydrogen bonding are among the classical insights that arose from observations in the PDB. Given the magnitude of structural data available today, it is likely that many quantitative generalizations remain to be made. Here we hypothesize that the PDB contains valuable quantitative information on the level of local tertiary structural motifs (TERMs), with TERM statistics reflecting fundamental relationships between sequence and structure. We define a TERM to be the structural fragment that captures the local secondary and tertiary environments of a given residue, and put our hypothesis through a series of rigorous tests. First, we show that by breaking a protein structure into its constituent TERMs, and querying the PDB to characterize the natural ensemble around each, we can estimate the compatibility of the structure with a given amino-acid sequence through a metric we term “structure score.” Considering submissions from recent Critical Assessment of Structure Prediction (CASP) experiments, we find a strong correlation (R = 0.69) between structure score and model accuracy, with poorly predicted regions readily identifiable. This performance exceeds that of leading atomistic statistical energy functions. Next, we show that by considering the TERMs of a structure that are affected by a given mutation, and mining the PDB to characterize sequence statistics associated with each, we are able to predict mutational free energies on par with or better than far more sophisticated atomistic energy functions. Finally, we ask whether TERM statistics are sufficient to enable the design of proteins de-novo. We demonstrate that given a native backbone conformation, TERM considerations alone with no input from molecular mechanics correctly predict roughly the same fraction of amino acids from the corresponding native sequence as state-of-the-art computational protein design methods. Knowledge-based energy functions have already put PDB statistics to good use by parsing structural environments into geometric descriptors, generally assuming their conditional independence. Our results suggest that it may now be possible to instead consider local structural environments in their entirety, asking questions about them directly. If this is the case, then the PDB is an even larger treasure trove of information than it has been generally known to be, and methods of mining it for TERM-based statistics should present opportunities for advances in structure prediction and protein design.

PI-018 **De novo design of an ideal TIM-barrel scaffold**

Po-Ssu Huang[1,2], Kaspar Feldmeier[3], Fabio Parmeggiani[1,2], D. Alejandro Fernandez Velasco[4], Birte Höcker[3], David Baker[1,2,5]

1.-Department of Biochemistry, University of Washington, 2.-Institute for Protein Design, University of Washington, 3.-Max Planck Institute for Developmental Biology, 4.-Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad Universita, 5.-Howard Hughes Medical Institute, University of Washington

Comprehensive understanding of a protein fold is intertwined with successful design. Recent advances in designing de novo structures have shown that proteins can be designed for a few globular and helical folds. However, designing all-β structures and barrels remains challenging because loops and intricate long range interactions that are important in these topologies are difficult to control. For designing novel catalysts, the (α/β)8 -barrel (or TIM-barrel) fold is one of the most important examples, for it is the most common topology for enzymes. For almost 30 year, attempts in designing de novo TIM barrel structures have all resulted in poorly folded proteins. Here we describe the successful design of a 4-fold symmetrical (α/β)8 barrel directly from geometrical and chemical principles. 22 designed variants with a wide range of stabilities from being molten globules to cooperatively folded proteins were experimentally characterized, and the results revealed the importance of sidechain-backbone hydrogen bonding for defining the characteristic α/β-barrel. The 184 residue TIM barrel structure is among the smallest TIM-barrels and has a fully-reversible melting temperature of 88 °C. The X-ray crystal structure shows atomic-level agreement with the design model. Despite this structural similarity, PSI-BLAST searches do not identify sequence similarities to known TIM-barrel proteins. More sensitive profile-profile searches suggest that the design is sufficiently distant from other native TIM-barrel superfamilies to be in a superfamily of its own, further implying that Nature has only sampled a subset of the sequence space available to the TIM-barrel fold. The ability to de novo design TIM-barrels opens new possibilities for custom-made enzymes.

PI-019 **Directed evolution of fluorescent protein function**

Felix Vietmeyer[1], Premashis Manna[1,2], Kevin Dean[3], Amy Palmer[3], Ralph Jimenez[1,2]
1.-JILA, University of Colorado and NIST, 2.-Dept. of Chemistry & Biochemistry, University of Colorado, 3.-University of Texas Southwestern Medical Center, 4.-BioFrontiers Institute, University of Colorado

Creation of new molecular sensors and actuators based on fluorescent proteins relies on methods for identifying complex photophysical phenotypes and subsequently performing separations on cell populations. We developed a microfluidic flow cytometry approach tailored to interrogating the performance of genetically-encoded fluorophores and present the results of studies employing this technology. The system screens cell-based libraries on the basis of multiple photophysical parameters relevant to imaging, including brightness, photostability, and excited-state lifetime (i.e. a proxy for fluorescence quantum yield) at a rate of up to 180 cells/sec. In a first generation of experiments, molecular dynamics-guided design was used to create a library of mCherry mutants that was screened with this system, resulting in the identification of a variant with a higher stability β-barrel and improved photostability but with a decreased brightness due to reduction in the fluorescence quantum yield. To avoid inadvertent decreases in this important performance criterion, subsequent rounds of selection were performed on the basis of both photostability and excited-state lifetime as sorting criteria. In these second generation selections, mutations were designed to target pathways of oxygen access through the bottom of the β-barrel in addition to a position that directly interacts with the chromophore. Furthermore, subsequent rounds of screening were used to improve folding and maturation. The multiparameter sort identified multiple clones with up to 8-fold improved photostability and up to double the excited-state lifetime of the parent mCherry fluorescent protein. The best mutant we identified produces one order of magnitude more photons before photobleaching compared to mCherry, at excitation conditions characteristic of confocal fluorescence microscopy. Our results demonstrate the utility of combining molecular-dynamics-guided library design with technology for photophysics-based selections. We anticipate that the new fluorescent proteins obtained in this work will find use in low-copy-number and long-duration imaging live cell imaging applications in cell-lines created by genomic editing techniques.

PI-020 **Targeted protein degradation achieved through a combination of degrons from yeast and mammalian ornithine decarboxylase**

Rushikesh Joshi[1], Ratna Prabha C.[1]
1.-The Maharaja Sayajirao University of Baroda

Targeted protein degradation achieved through a combination of degrons from yeast and mammalian ornithine decarboxylase Targeting the over accumulated protein in the cell for degradation using specific degrons is an emerging research area. The degradation of the vast majority of cellular proteins is targeted by the ubiquitin-proteasome pathway. But in the case of ubiquitin independent protein degradation, ODC/AZ system is more effective in achieving targeted protein degradation than other types of degradation1. Ornithine decarboxylase (ODC) is key regulatory enzyme in the biosynthesis of polyamines. The protein has two domains namely, N terminal α/β barrel domain and C-terminal β-sheet domain. Degradation of ODC is mediated by polyamine inducible protein, antizyme (AZ). Antizyme interacts with ODC on N-terminal region, which results in degradation of ODC by proteasomes. In mammalian ODC the C-terminal has an unstructured tail of 37 residues, which pulls ODC into proteasome for degradation. It was reported earlier by Coffino's group that the unstructured tail acts as a degron in chimeric fusion with GFP2. In yeast, same function is achieved by N-terminal 44 residues3. Present study focuses on accomplishing targeted protein degradation in Saccharomyces cerevisiae by adding these two degradation signals or degrons of yeast ODC and mammalian ODC as tags to a reporter protein. We have selected two degrons namely, N terminal α/β barrel domain of yeast ODC and C-terminal 37 residues of mouse ODC and grafted them to N and C- terminus of the reporter protein yEGFP. Degradation of yEGFP and yEGFP fusion with degrons of ODC (degron-yEGFP) were monitored by western blot using anti-GFP antibody and fluorescence spectroscopy. Initially, the amount of degron-yEGFP fusion protein was very low compared to control yEGFP. It means that the chimeric protein underwent rapid degradation in the cells. After inhibition of proteasome, increase in the level of degron-yEGFP was observed, confirming that the degrons cause rapid degradation of reporter protein through proteasome. Earlier, we have also tagged ubiquitin from yeast with last 37 residues of mODC and observed enhanced degradation of ubiquitin in Saccharomyces cerevisiae. Therefore, both the degrons of ODC alone and in combination are capable of decreasing stability of reporter protein in the cells. However, the combination of degrons is more effective than either of them in isolation. References: 1Matsuzawa S. et al. 2005, Proc Natl Acad Sci USA 102, 14982-14987. 2Zhang M. et al. 2003, Embo J 22, 1488-1496. 3Godderz D. et al. 2011, J Mol Biol 407, 354-367.

Corresponding author: Department of Biochemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara – 390002, India. e-mail: chivukula_r@yahoo.com; Mobile: +91-9327201349

PI-021 **Short peptides self-assemble in the presence of metals to produce catalytic amyloids**

Caroline Rufo[1], Yurii Moroz[1], Olesia Moroz[1], Olga Makhlynets[1], Pallavi Gosavi[1], Jan Stöhr[2], Tyler Smith[1], Xioazhen Hu[3], William DeGrado[3], Ivan Korendovych[1]

1.-Syracuse University, 2.-Institute for Neurodegenerative Diseases and Department of Neurology, UCSF, 3.-Department of Pharmaceutical Chemistry, UCSF

Enzymes fold into unique three-dimensional structures, which underlie their remarkable catalytic properties. The requirement that they be stably folded is a likely factor that contributes to their relatively large size (> 10,000 Dalton). However, much shorter peptides can achieve well-defined conformations through the formation of amyloid fibrils. To test whether short amyloid-forming peptides might in fact be capable of enzyme-like catalysis, we designed a series of 7-residue peptides that act as Zn2+-dependent esterases. Zn2+ helps stabilize the fibril formation, while also acting as a cofactor to catalyze acyl ester hydrolysis. The fibril activity is on par with the most active to date zinc-protein complex. Such remarkable efficiency is due to the small size of the active unit (likely a dimer of 7-residue peptides), while the protein is at least 15-fold larger in molecular weight. The observed catalytic activity is not limited to ester hydrolysis. We have designed copper binding peptides that are capable oxygen activation. These results indicate that prion-like fibrils are able to not only catalyze their own formation – they also can catalyze chemical reactions. Thus, they might have served as intermediates in the evolution of modern-day metalloenzymes. These results also have implications for the design of self-assembling nanostructured catalysts including ones containing a variety of biological and nonbiological metal ions.

PI-022 **Rational design of the cold active subtilisin-like serine protease VPR with improved catalytic properties and thermal stability**

Kristinn Oskarsson[1], Sigridur Thorbjarnardottir[2], Magnus Kristjansson[1]
1.-Science Institute, University of Iceland, Department of Biochemistry, 2.-Institute of Biology, University of Iceland

Rational design of the cold active subtilisin-like serine protease VPR with improved catalytic properties and thermal stability. Kristinn R. Óskarsson1, Sigridur H. Thorbjarnardóttir2, Magnús M. Kristjánsson1. 1Science Institute, University of Iceland, Department of Biochemistry, Reykjavík, Iceland. 2University of Iceland, Institute of Biology. mmk@hi.is On the basis of research done on the subtilisin-like serine proteinase VPR, from a psychrophilic Vibrio species and its thermophilic structural homologue, aqualysin I (AQUI) from Thermus aquaticus, we set out to design a mutant of VPR which would be more thermostable, but would retain the high catalytic activity of the wild type enzyme. Our starting protein template was a previously stabilized mutant containing two inserted proline residues close to the N-terminus of VPR (N3P/I5P). This VPR_N3P/I5P mutant was shown to have a significantly increased thermal stability but displayed a concomitant tenfold loss of catalytic efficiency. From our previous studies we selected two mutations, one which increased catalytic activity (Q142K) of the enzyme significantly and another which stabilized the protein against thermal denaturation (N15D). The N15D mutation had been shown to introduce a salt bridge into the structure of the cold adapted proteinase, yielding higher stability but without negative effects on activity. The Q142K exchange had been shown to double the turnover number (kcat) to that of the wild type enzyme. Insertions of these selected mutations into the VPR_N3P/I5P mutant were according to predictions; the Q142K increased the kcat tenfold, and the N15D mutation increased the thermal stability. In the combination mutant, VPR_N3P/I5P/N15D/Q142K, thermal stability was increased by 8 °C and 10 °C, in terms of Tm and T50%, respectively. Furthermore, the catalytic activity of the mutant was somewhat higher than that of the wild type enzyme.

PI-023 **Critical peptide stretches may not serve as faithful experimental mimics for protein amyloidogenesis**

Bishwajit Kundu[1], Dushyant Garg[1]
1.-Kusuma School of Biological Sciences, IIT Delhi

Certain amino acid stretches are considered critical to trigger the amyloidogenesis in a protein. These peptide stretches are often synthetically produced to serve as experimental mimics for studying amyloidogenesis of the parent protein. Here we provide evidence that such simple extrapolation may be misleading. We studied the amyloidogenesis of full length bovine carbonic anhydrase II (BCAII) and compared it with those formed by its critical amyloidogenic peptide stretch 201-227 (PepB). Under similar solution conditions and initial monomeric concentrations, we found that while amyloid formation by BCAII followed aggregation kinetics dominated by surface-catalyzed secondary nucleation, PepB followed classical nucleation-dependent pathway. The AFM images showed that BCAII forms short, thick and branched fibrils, whereas PepB formed thin, long and unbranched fibrils. ATR-FTIR revealed parallel arrangement of cross β sheet in BCAII amyloids, while PepB arranged into antiparallel β sheets. Amyloids formed by BCAII were unable to seed the fibrillation of PepB and vice versa. Even the intermediates formed during lag phase revealed contrasting FTIR, far UV CD signature, hydrophobicity and morphology. We propose that for any polypeptide, the sequences flanking a critical region are equally effective in modulating the initial nucleation events, generating prefibrillar and finally fibrillar species with contrasting characteristic. The results have been discussed in light of amyloid polymorphism and its importance in the design of therapeutic strategies targeting such toxic regions.

PI-024 **A systematic exploration of protein uptake and trafficking into intracellular compartments**

Aksana Labokha[1], Ralph Minter[1]
1.-Antibody Discovery & Protein Engineering dpt, MedImmune

All approved biological drugs target extracellular proteins and not the majority of the expressed human genome, which resides within intracellular compartments. Included in the latter category are many important, disease-relevant targets which cannot be easily addressed by small molecule approaches, such as the oncology targets c-Myc and K-Ras. Although bacteria and viruses have evolved strategies to deliver biological material to the cell cytoplasm and nucleus, our ability to engineer recombinant proteins to replicate this is somewhat limited by (i) our nascent understanding of protein uptake and trafficking pathways and (ii) the ability to easily quantify cell delivery to the cytoplasm and cellular organelles. The aim of my project is to address these challenges by developing an effective assay for cytoplasmic uptake and then using it to measure the delivery efficiency of recombinant proteins which mimic natural delivery strategies e.g. cell penetrating peptides fusion, exotoxin mimics, and supercharged proteins (proteins with high surface charge which can enter cells). I also intend to explore the influence of the Rab superfamily, which are the master regulators of protein trafficking, to influence and control both the kinetics and final subcellular destination of exogenous proteins.

PI-025 **Protein engineering: what's next?**

Maria Fatima Lucas[1,2], Víctor Guallar[1,3]

1.-Joint BSC-CRG-IRB Research Program in Computational Biology, 2.-Anaxomics Biotech, 3.-ICREA

With the growing industrial need for engineering enzymes for the deconstruction and transformation of plant biomass in biorefineries, there is a want for the development of new approaches for designing special purpose biocatalysts. Techniques, such as directed evolution, which mimic the natural selection process by evolving proteins towards the improvement of a given property, have unquestionably demonstrated their value and are routinely used in large industrial companies. Nevertheless, the brute force employed in these methods, could significantly gain from an all-atom description of the underlying catalytic mechanisms, to center the efforts on more limited areas of the protein. In the last years, we have developed computational tools, which combine the electronic structure description of QM/MM methods with the potential to model long time scale processes of PELE,1 to study the details of a variety of reactions. Examples, which will be discussed, include rationalizing the selective oxyfunctionalization of steroids using fungal enzymes2 and the study of the effect of point mutations on the oxidation efficiency of laccases.3 These methods have shown their potential not only at the descriptive level but, more importantly, through their high predictive capability that opens many opportunities for their use in biotechnology. In this talk, we will show how recent advances in in silico approaches are setting new grounds for future computer guided directed evolution. This work was done in collaboration with: Instituto de Recursos Naturales y Agrobiología de Sevilla, CSIC; Novozymes A/S; JenaBios GmbH; TU Dresden; Centro de Investigaciones Biológicas, CSIC and was funded by the INDOX (KBBE-2013-7-613549) European Project. 1-Borrelli K., et al. PELE: Protein energy landscape exploration. A novel Monte Carlo based technique. J. Chem. Theory Comp. 1, pp. 1304. 2005. 2-Babot, B., et al. Steroid hydroxylation by basidiomycete peroxygenases: A combined experimental and computational study. Applied and Environmental Microbiology. 2015. Accepted manuscript posted online doi: 10.1128/AEM.00660-15 3-Monza, E., et al. Insights into Laccase Engineering from Molecular Simulations: Toward a Binding-Focused Strategy. Journal Physical Chemical Letters. 6, pp. 1447 - 1453. 2015.

PI-026 **Bottom-up construction of a synthetic carboxysome**

Shiksha Mantri[1], Raphael Frey[1], Marco Rocca[1], Eita Sasaki[1], Donald Hilvert[1]
1.-ETH Zurich, Switzerland, 2.-ETH Zurich, Switzerland, 3.-ETH Zurich, Switzerland, 4.-ETH Zurich, Switzerland, 5.-ETH Zurich

Several orthogonal bioreactions take place simultaneously within membrane bound organelles in eukaryotes and proteinaceous microcompartments in bacteria. These subcellular structures contain sets of enzymes co-involved in metabolic pathways. Towards the goal of creating artificial protein microreactors, we seek to develop an artificial organelle that emulates the metabolic activity of the carbon fixating organelle of autotrophic bacteria, the carboxysome. Here, we show that the two key carboxysomal enzymes, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) and carbonic anhydrase (CA), can be efficiently co-encapsulated using our previously reported encapsulation system which is based on a bacterial capsid formed from the protein lumazine synthase (AaLS-13). Our preliminary results suggest that the enzymes can act in tandem and that the co-encapsulation of CA with RuBisCO in the capsid is necessary for enhanced RuBisCO activity in vitro. We attribute this observation to the high local concentrations of the RuBisCO substrate, CO2, produced by CA within the capsid. We are developing a theoretical model of a minimal carboxysome using the kinetic rate constants of our RuBisCO and CA variants and AaLS-13 as the shell to complement these experiments. Next, we will incorporate our minimal carboxysome within an expression host such as E.coli, opening up the possibility of further optimization through directed evolution.

PI-027 **CXCL10 engineering: novel insights into glycan interactions**

Michael Nagele[1], Martha Gschwandtner[1], Patrick Sorger[1], Andreas J. Kungl[1]
1.-Institute of Pharmaceutical Sciences, University of Graz, Universitaetsplatz 1

In the past targeting and engineering of chemokines has led to several interesting drug candidates. [1] Amongst them, Met-RANTES, a Met-CCL5 with high G protein-coupled receptor (GPCR) affinity but no subsequent signal transduction, as well as mutants addressing the interaction with the so-called glycosaminoglycans (GAGs) seem to be the most promising candidates. Both, GAG knockout as well as GAG affinity matured chemokine isoforms have been considered as anti-inflammatory drug candidates, out of which an IL-8 mutant with 5 modifications reached clinical phase 1 where it was profiled for acute neutrophil-related exacerbation in COPD.[2] CXCL10 (IP-10) is a proinflammatory chemokine released by various cells following stimulation by interferon γ (IFN-γ). It is therefore considered as a late chemokine being responsible for the attraction of different lymphocytes. [3] Any therapeutic indication is consequently related to chronic and multiple applications. We have therefore engineered CXCL10 very conservatively at positions to ultimately generate dominant-negative mutants with a mildly improved GAG-binding affinity and an entire knock off GPCR activity. The first steps of our engineering approach were in silico modelling of the mutants and the establishment of a suitable upstream- and downstream-processing protocol. Next we generated a fluorescently engineered CXCL10 variant for our fluorescence-based affinity studies which was subjected to biocomparability investigations relative to the native, non-fluorescent protein. Compared to the wild type, the fluorescently engineered mutant exhibited similar biological, chemotactic and GAG-binding properties. Next we started to produce sufficient amounts of the members of our nascent mutant library which were tested with respect to their biophysically behavior as well as to their knocked out chemotactic potency on cells. These experiments included gel electrophoresis and Western Blot analysis to determine identity and purity; Circular Dichroism (CD) and chaotrope-induced unfolding to approximate structure; Isothermal Fluorescence Titration (IFT); Surface Plasmon Resonance (SPR) and Isothermal Titration Calorimetry (ITC) to quantify GAG-binding affinity and Boyden Chamber experiments to determine the chemotactic activity. Our results show that we are able to tune the GAG binding strength along with the GPCR activity of human CXCL10 which could lead to therapeutic applications in the future.

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PI-028 **Creating large covalently circularized nanodiscs and their application in studying viral entry and genome translocation**

Mahmoud Nasr[1], Mike Strauss[1], James Hogle[1], Gerhard Wagner[1]
1.-Dep. of Biological Chemistry and Molecular Pharmacology, Harvard Medical School

Nanodiscs are composed of a nanometer-sized phospholipid bilayer encircled by two α helical, amphipathic membrane scaffold proteins (MSPs). These particles provide a unique detergent free lipid bilayer model enabling biochemical and biophysical characterization of membrane proteins in a physiologically relevant medium. Previously, the largest diameter reported of a nanodisc assembled using MSPs was about 16-17 nm. Here we present a method to create large nanodiscs (up to 80nm in diameter) assembled with covalently circularized MSPs (cMSP). We can observe the homogeneity in nanodiscs diameter as a narrow distribution using negative-stain EM. Using our method, we have created 50 nm nanodiscs and used them to study poliovirus (~35 nm diameter) entry and RNA translocation. A 50 nm nanodisc is sufficiently large to accommodate multiple copies of the CD155 receptor (also known as the poliovirus receptor), and has enough surface area to act as a surrogate membrane for the RNA translocation complex during viral uncoating. The 50 nm nanodiscs functionalized with the His-tagged ecto-domain of poliovirus receptor, CD155, were generated by adding lipids derivatized with a NTA nickel-chelating head group to the lipid mixture during nanodisc assembly. CD155 receptor was added to the already assembled nanodiscs and incubated for 30 minutes at room temperature. The receptor-decorated nanodisc complex was purified by size exclusion chromatography. The purified complex was then incubated with poliovirus for 5 minute at 4ºC, and then heated to 37ºC for 15 minutes to initiate receptor-mediated viral uncoating. Virus binding to nanodisc-CD155 complex and subsequent insertion of viral components into and across the membrane were confirmed by negative-stain electron microscopy (Figure 1c). To obtain a high-resolution structure for the RNA translocation complex we conducted single-particle cryo-EM studies using a Polara F30 microscope. Unlike liposomes, generating a reconstruction of samples containing nanodiscs is less complicated since the nanodiscs are more homogenous in size, and allow for thinner ice. Also, the viral RNA can be visualized more easily. The method for making large nanodiscs as well as the negative stain and cryo-EM data will be will be presented and discussed.

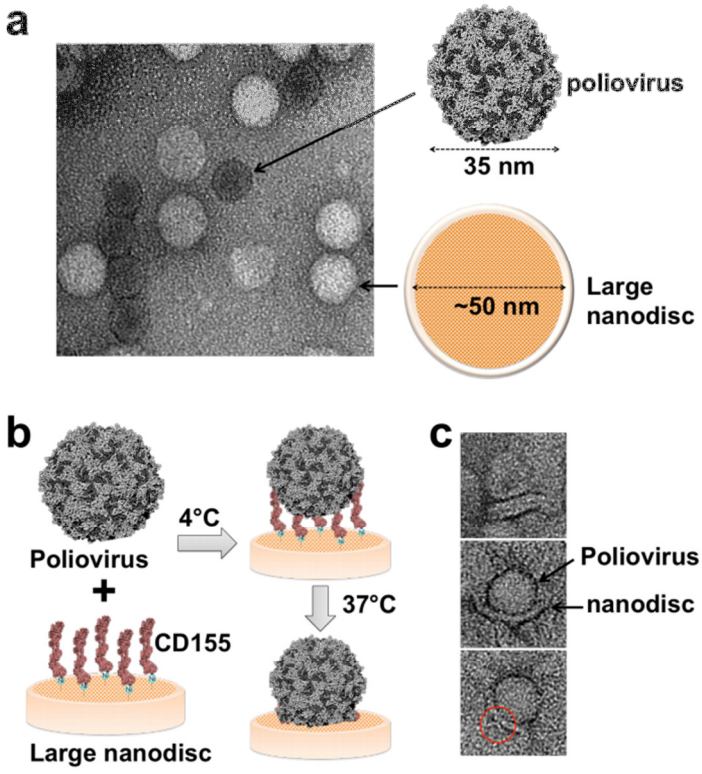

Figure 1. Poliovirus- membrane fusion studies using large nanodisc. (a) Negative stain EM of 50 nm nanodiscs plus poliovirus (control) shows no bridging or fusion. (b) Outline of the procedure for initiating poliovirus bridging and fusion with nanodiscs modified with CD155. CD155 is also known as the poliovirus receptor. (c) Individual viruses tethered to nanodiscs.

PI-030 **Parametric design of alpha-helical barrels and pore-like assemblies with very high thermodynamic stabilities**

Gustav Oberdorfer[1,2,7], Po-Ssu Huang[1,7], Chunfu Xu[1,7], Verena Kohler[2], Xue Y. Pei[3], Brent L. Nannenga[4], Joseph M. Rogers[5], Tamir Gonen[4], Karl Gruber[2], David Baker[1,6,7]

1.-Department of Biochemistry, University of Washington, 2.-Institute of Molecular Biosciences, University of Graz, 3.-Department of Biochemistry, University of Cambridge, 4.-Janelia Research Campus, Howard Hughes Medical Institute, 5.-Department of Chemistry, University of Cambridge, 6.-Howard Hughes Medical Institute, University of Washington, 7.-Institute for Protein Design, University of Washington

Computational design of novel protein structures and enzymes with new functions is a promising tool to create superior biological materials with tailor-made properties, new pharmaceuticals, complex fine chemicals or renewable fuels. It also challenges our understanding of protein folding, protein evolution, molecular recognition and catalysis. Here we present a procedure for designing proteins with backbones produced by varying the parameters in the Crick coiled-coil generating equations [1]. Combinatorial design calculations using the software suite Rosetta identify low energy sequences for alternative helix supercoil arrangements. After that, loop modeling is applied to connect the designs with lowest energy. The extent to which the designed sequences encode the designed structures is evaluated using large-scale structure prediction calculations, as well as symmetric and asymmetric protein-protein docking calculations. Subsequently, synthetic genes are generated for sequences that converge strongly on the designed structure for experimental characterization. We applied this approach to monomeric three and four helical bundle structures as well as a pentameric five-helix bundle structure using idealized coiled-coil geometries [2]. Recently we expanded this approach to higher complexity backbones, which resulted in the de-novo design of monomeric, antiparallel six-helix bundles with untwisted, left- and right-handed geometries. Circular Dichroism (CD), Size-exclusion coupled Multi-Angle Light Scattering measurements (SEC-MALS), negative stain electron micrographs (EM) and Small Angle X-ray Scattering (SAXS) of these designs suggest that they indeed form the designed structures. In addition, we used Rosetta protein-protein interface design functionality to computationally design oligomers out of our previously published three and four helix bundle structures to generate self-assembling pore-like structures with the potential use as channels or transporters. Again, experimental validation of these designs by CD, SEC-MALS, EM and SAXS show that the designs are correct. We are currently undertaking further structural investigation of all these designs by X-ray crystallography. The designs described above can act as templates for protein or small molecule binding, holding a catalytic machinery or for scaffolding enzymes in reaction cascades. Some of these applications are currently under investigation, including a self-sufficient redox system employing two copper-centers, binding of heme-moieties as a prosthetic group and tailoring the pore-like geometries to be used in nanopore sequencing.

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PI-031 **Leucine Zipper fused Fab; Enhancement of active Fab formation in E. coli in vitro and in vivo expression systems**

Teruyo Ojima-Kato[1, 2], Kansuke Fukui[2], Takaaki Kojima[2], Hideo Nakano[2]
1.-Aichi Science and Technology Foundation, 2.-Nagoya University

Background: Recombinant monoclonal antibodies (mAbs) are one of the essential tools in biotechnology. The smaller fragments of antibodies such as fragment of antigen binding (Fab) or single chain variable fragment (scFv) are favorable form for the production in microorganisms. These have been produced in periplasmic space in E.coli, however, the productivity is still limited. On the other hand, cytoplasmic production of these molecules could enlarge their productivity greatly, however, often results in insoluble aggregates. In particular, soluble expression of Fab is challenging because intermolecular disulfide bond between heavy chain and light chain is hardly formed in the E.coli cytoplasm. Purpose: To achieve functional Fab production in E. coli in vitro and in vivo expression system, peptides segment pairs of Leucine zipper that dimerize in parallel were fused to the C-termini of Fab. Results: Mouse anti E. coli O157 mAb obtained from Hybridoma and rabbit anti Listeria monocytogenes mAb generated by single B cell RT-PCR were used as model. These mAbs were not produced in E. coli as active Fab. Leucine zipper (ACID-p1 (LZA)/BASE-p1 (LZB) or c-Jun/c-Fos) were fused to the C-terminal of heavy chain (VH-CH1) and light chain (VL-CL), respectively, to accelerate the association of the heavy chain and light chain of Fab. The modified Fabs were produced in either E. coli in vitro or in vivo expression systems as active proteins. SDS-PAGE analysis of the purified Fab-Leucine zipper conjugates showed absence of disulfide bond between heavy and light chains, indicating they were connected via leucine zipper interaction. These leucine zipper fused Fab(s) had significant binding and specificity toward antigen in ELISA. Conclusions: Leucine zipper fusion to Fab greatly enhanced assembly of heavy chain and light chain to form active Fab.

PI-032 **The road not taken: Exploring repeat protein architectures by computational design**

Fabio Parmeggiani[1], Po-Ssu Huang[1], TJ Brunette[1], Damian Ekiert[2], Gira Bhabha[2], Susan Tsutakawa[3], Greg Hura[3], John Tainer[3], David Baker[1]

1.-University of Washington, 2.-University of California, San Francisco, 3.-Lawrence Berkeley National Laboratory

Repeat proteins are an example of how evolution proceeds by building on existing structures and functions, but also a source of modular protein scaffolds for molecular recognition and biomaterials. However, it is unclear whether the limited number of folds and families that we know today is the result of the intrinsic limitations of polypeptide chains or the consequence of the path followed by evolution. We explored this hypothesis by computational design of repeat proteins based on modular units formed by two alpha helices and two loops of variable lengths, without relying on information from available repeat protein families. The automated sampling of the conformational space resulted in a large number of architectures from which 83 de novo designs were selected for experimental characterization. 66% of the proteins were stable up to 95°C and monodisperse and 42 designs were structurally validated by small angle X-ray scattering. Crystal structures were solved for 15 of them, with root mean square deviation from the models between 0.7Å and 2.5Å. The designs differ from known proteins both at the sequence and structure levels and cover a broader range of geometries than observed in naturally occurring repeat protein families, indicating that existing architectures represent only a small fraction of what can be achieved. Our results show that it is possible to expand the range of repeat protein architectures beyond the naturally occurring families, and that computational design can provide new scaffolds and enable the design of proteins tailored for specific applications.

PI-033 **Design and characterisation of a synthetic serpin with novel folding properties**

Benjamin Porebski[1], Shani Keleher[1], Adrian Nickson[2], Emilia Marijanovic[1], Mary Pearce[1], Natalie Borg[1], James Whisstock[1], Stephen Bottomley[1], Sheena McGowan[1], Ashley Buckle[1]
1.-Department of Biochemistry and Molecular Biology, Monash University, 2.-Department of Chemistry, University of Cambridge

The serpin family of proteins consists of over 1500 members, all with a highly conserved native structure that is metastable (1). Serpins use this metastability to control the activity of proteases, via a specific inhibitory process. The serpin binds to its target protease through specific residues within the reactive centre loop, the protease cleaves the loop and results in a large conformational change causing the protease to become distorted and catalytically inactive whilst the serpin becomes much more stable (1, 2, 3). The metastable nature of AAT is therefore required to facilitate the rapid and gross conformational changes required for its inhibitory function (2, 3). Several disease-causing mutants of AAT have been identified, the most common of them being the Z-variant (4). The Z-variant has an increased propensity to polymerize in the endoplasmic reticulum of hepatocytes leading to cell death and liver damage (4). During the past fifteen years, many groups have unsuccessfully screened a number of serpins and a vast range of solution conditions to identify a combination of serpin and conditions that will enable the folding reaction of a serpin to be characterized. We have now taken an alternative approach and designed a synthetic “model” serpin that folds reversibly to its native state. In order to do this, we used a consensus design approach, analysing a sequence alignment of 212 serpin sequences and determining the prevalent amino acid residue at each position, we termed this serpin conserpin (consensus serpin). Here we present the structural, biophysical and functional characterisation of conserpin. Combined crystallographic and folding studies reveal the characteristics of conserpin that likely dictate its unique stability and folding behaviour, whilst retaining activity as a serine protease inhibitor.

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PI-034 **Computational design of shape-optimized leucine-rich repeat proteins**

Sebastian Rämisch[1], Ulrich Weininger[2], Jonas Martinsson[1], Mikael Akke[2], Ingemar André[1]
1.-Department for Biochemistry & Structural Biology, Lund University, 2.-Department for Biophysical Chemistry, Lund University

The development of enhanced protein binding scaffolds is a key for engineering protein inhibitors and biosensors with advanced characteristics. Utilizing the structural variability and designability of repeat proteins offers a means for designing protein binders where the overall shape is customized to optimally match a target molecule. We developed a computational protocol for the design of repeat proteins with a predefined geometry. By combining sequence optimization of existing repeats and de novo design of capping structures, we designed leucine-rich repeat (LRR) proteins where the building blocks assemble into a novel structure. The suggested design procedure was validated by engineering an artificial donut-like ring structure, which is constructed from ten self-compatible repeats. Characterization of several designed constructs further suggests that buried cysteines play a central role for stability and folding cooperativity in certain LRR proteins. This effect could provide a means for selectively stabilizing or destabilizing specific parts of an LRR-based protein binder. The computational procedure may now be employed to develop repeat proteins with various geometrical shapes for applications where greater control of the interface geometry is desired.

PI-035 **Engineering APOBEC3G enzymes for altered specificity and processivity**

Louis Scott[1], Muhammad Razif[1], Aleksandra Filipovska[1,2], Oliver Rackham[1,2]
1.-Harry Perkins institute of Medical Research, 2.-School of Chemistry and Biochemistry, The University of Western Australia

APOBEC3G (A3G) is a host-encoded protein involved in the defense against HIV-1 and other retroviral infections. A3G is a cytidine deaminase with a 3' to 5' processive nature, causing targeted C to T mutations along a DNA strand. The catalytic and processive activity of A3G leads to the hypermutation of nascent retroviral cDNA, resulting in premature termination codons and dysfunctional proteins. Ultimately, the action of A3G inhibits viral replication. The ability of A3G to jump and slide along a DNA strand, deaminating at targeted sequences, makes it an interesting candidate for protein engineering. Engineered A3G enzymes for increased activity, altered specificity, and altered processivity are attractive options for expanding the DNA modifying enzyme toolbox. Mutation of catalytic residues, residues thought to affect its processive nature and those thought to be involved in target recognition, can create novel A3G enzymes. Using structure guided selection, residues in key functional sites that are amiable to mutation will be chosen. Individuals from the resulting libraries of mutants will be selected by directed evolution for desired characteristics. The resulting A3G enzymes will be examined for the relationship between their structure and function. Such engineered A3G enzymes could be targeted to catalyse the reversion of deleterious genetic mutations. Furthermore, engineered A3G enzymes could be used in mutational studies that call for targeted deamination along a DNA strand, or mutational studies that call for unspecific and high throughput DNA deamination.

PI-036 **Engineering porous protein crystals as scaffolds for programmed assembly**

Thaddaus Huber[1], Luke Hartje[1], Christopher Snow[1]
1.-Colorado State University

A key motivation for nano-biotechnology efforts is the creation of designer materials in which the assembly acts to organize functional domains in three dimensions. Crystalline materials are ideal from the validation perspective because X-ray diffraction can elucidate the atomic structure. Relatively little work has focused on engineering protein crystals as scaffolds for nanotechnology, due to the technical challenges of coaxing typical proteins into crystallizing, and the likelihood of disrupting the crystallization process if changes are made to the monomers. We have circumvented these limitations by installing guest protein domains within engineered porous crystals (~13 nm pore diameter) that have been rendered robust using covalent crosslinks. The retention of the scaffold structure despite changes to the solution conditions and macromolecule uptake can be validated through X-ray diffraction.

We have engineered scaffold crystals for the non-covalent and covalent capture of guest macromolecules. By controlling the reversible loading and release, we can prepare "integrated" crystals with spatially segregated guest loading patterns. As assessed using confocal microscopy, such host-guest crystals are highly stable. Ultimately, the resulting crystals may serve as a robust alternative to DNA assemblies for the programmed placement of macromolecules within materials.

PI-037 **Engineering ultrasensitive protein probes of voltage dynamics for imaging neural activity in vivo**

Francois St-Pierre[1,2], Michael Pan[1,2], Helen Yang[3], Xiaozhe Ding[1,2], Ying Yang[1,2], Thomas Clandinin[3], Michael Lin[1,2]

1.-Department of Bioengineering, Stanford University, 2.-Department of Pediatrics, Stanford University, 3.-Department of Neurobiology, Stanford University

Nervous systems encode information as spatiotemporal patterns of membrane voltage transients, so accurate measurement of electrical activity has been of long-standing interest. Recent engineering efforts have improved our ability to monitor membrane voltage dynamics using genetically encoded voltage indicators. In comparison with electrophysiological approaches, such protein-based indicators can monitor many genetically defined neurons simultaneously; they can also more easily measure voltage changes from subcellular compartments such as axons and dendrites. Compared with genetically encoded calcium indicators, voltage sensors enable a more direct, accurate, and rapid readout of membrane potential changes. However, several challenges remain for in vivo voltage imaging with genetically encoded indicators. In particular, current voltage sensors are characterized by insufficient sensitivity, kinetics, and/or brightness to be true optical replacements for electrodes in vivo. As a first step towards addressing these challenges, we sought to develop new voltage indicators that further improve upon the performance of the fast voltage sensor Accelerated Sensor of Action Potentials 1 (ASAP1). In ASAP1, voltage-induced conformational changes in a natural voltage-sensing domain perturb the fluorescence emission of a covalently linked green fluorescent protein (GFP). Using a structure-based approach to guide mutagenesis, we discovered several amino acids that tune the kinetics and voltage sensitivity of ASAP1. These residues are not only located in the voltage-sensing domain, but also in the fluorescent protein and in the linkers bridging sensing domain and GFP. Our most improved variant, ASAP2, exhibits improved sensitivity to voltage transients such as neuronal action potentials and subthreshold depolarizations. We sought to characterize the ability of these new voltage sensors to monitor neural activity in vivo using laser-scanning two-photon microscopy, a technique that allows imaging with lower autofluorescence and deeper tissue penetration. We report that ASAP sensors were able report stimulus-evoked voltage responses in axonal termini of the fly visual interneuron L2. ASAP sensors enabled voltage imaging with dramatically improved temporal resolution compared to three recently reported calcium and voltage sensors. Overall, our study reports novel voltage indicators with improved performance and highlights how specific amino acids can tune the performance of a protein-based fluorescent sensor. We anticipate that these results will pave the way for further engineering of voltage sensing proteins, and that our new sensor ASAP2 will facilitate current and future efforts to understand how neural circuits represent and transform information.

PI-038 **Assembly of armadillo repeat proteins from complementary fragments**

Erich Michel[1], Randall Watson[1], Martin Christen[1], Fabian Bumback[3], Andreas Plückthun[2], Oliver Zerbe[1]

1.-Department of Chemistry, University of Zurich, 2.-Department of Biochemistry, University of Zurich, 3.-University of Melbourne

Repeat proteins are built of modules, each of which constitutes a structural motif. In Armadillo repeat proteins each module comprises 40 residues and contains three helices arranged in a triangular fashion. These modules pack against each other, resulting in an elongated shape of the protein. We recently demonstrated that complementary fragments of a designed consensus Armadillo repeat protein (ArmRP) recognize each other [1]. The two fragments YM2: MA, in which Y, M and A denote the N-cap, internal repeats and the C-cap, respectively, form a 1:1 complex with a nanomolar dissociation constant, which is essentially identical to the crystal structure of the continuous YM3A protein. We further demonstrate that structurally intact Armadillo repeat protein complexes can be reconstituted from fragments obtained at various split sites – essentially after every repeat but also within repeats. The fragments display variable affinities towards each other, depending on the split site. The low affinity of some complementary pairs can be dramatically increased upon addition of peptide ligands. While a number of proteins are known that can be reconstituted from fragments we believe that the fact that Armadillo repeat proteins can be reconstituted from various complementary fragments is novel and opens new interesting perspectives and applications in biochemistry. Reference: [1] R. Watson, M. Christen, F. Bumback, C. Ewald, C. Reichen, M. Mihajlovic, E. Schmidt, P. Güntert, A. Caflisch, A. Plückthun, O. Zerbe (2014): Spontaneous Self Assembly of Engineered Armadillo Repeat Protein Fragments into a Folded Structure, Structure, 22, 985-995.

PI-039 **Engineering light-controllable kinases and Cas9 endonuclease with photodissociable dimeric fluorescent protein domains**

Xin Zhou[1], Linlin Fan[2], Michael Lin[1,3,4]

1.-Department of Bioengineering, Stanford University, 2.-Department of Chemical Biology, Harvard University, 3.-Department of Pediatrics, Stanford University, 4.-Department of Chemical and System Biology, Stanford University

A reliable method for generating optically controllable proteins would enable researchers to interrogate protein functions with high spatiotemporal specificity. We recently engineered a tetrameric fluorescent protein, Dronpa145N, that undergoes light-induced monomerization, then developed a general architecture for light-inducible proteins based on this light-induced transition. We created proteins whose active sites were blocked by fused Dronpa145N domains in the dark, but would become unblocked by light. Here we present further two extensions to this concept that together enabled the generalization of this method to additional classes of proteins. First, we engineered a photodissociable dimeric Dronpa (pdDronpa) with tunable affinity, faster photoswitching speed, and decreased level of protein aggregation, enabling better performance of fusion proteins. Second, we introduce the concept of caging a protein active site by insertion of Dronpa domains into loops rather than strictly at the protein termini. We use the pdDronpa system to impose optical control on kinases and the Cas9 endonuclease. The resulting light-inducible MEK1 kinase, Raf1 kinase, and Cas9 endonuclease showed high caging efficiency of protein activities in the dark, and robust protein activation upon light illumination. We believe that our efforts on further improving and generalizing this method would bring the power and benefits of light control to a broad community of biologists.

PI-040 **Exploring the evolution of folds and its application for the design of functional hybrid proteins**

Saacnicteh Toledo Patiño[1], Birte Höcker[1]
1.-Max Planck Institute for Developmental Biology

The structural diversity of proteins may appear endless, nevertheless even large protein complexes can be decomposed into protein domains and smaller sub-domain sized fragments. Only recently, we could identify such fragments employing sequence-based comparisons of different folds, as the TIM-barrel and the flavodoxin-like fold (Farias-Rico et al., 2014). As an extension of this work, we compared all α/β proteins and identified several fragments shared by different folds illustrating how nature may have achieved structural and functional diversity from a reduced set of building blocks. Inspired by this combinatorial concept, we searched for homologous fragments bearing active sites to engineer a functional fold-chimera. We extracted the vitamin-B12 binding part from methylmalonyl CoA mutase, which belongs to the flavodoxin-like fold (FL) and used it to replace the corresponding fragment in uroporphyrinogen III synthase, which belongs to the hemD-like fold (HDL). The new hybrid resulted in a stable and well-folded protein whose structure was determined by X-ray crystallography. Moreover, cobalamin-binding function was successfully transferred to the new protein from the FL parent, which shows the advantage of using this approach for the design of new functional proteins. In addition, profile alignments revealed sequence and structural evidence that suggested an evolutionary path for HDL from FL by gene duplication. To test this hypothesis, we expressed a modified C-terminal half of uroporphyrinogen III synthase and solved its structure by NMR spectroscopy, thereby confirming the predicted FL architecture. Altogether, our approach facilitates the detection of common ancestry among different folds contributing to our understanding of protein development. Furthermore, our results show how new complex proteins can be designed using fragments of existing proteins that serve as building blocks in a Lego-like manner. We believe that combining fragments containing existing properties will provide a successful method for the design of novel functionalities in the future. References: Farias-Rico JA, Schmidt S, Höcker B (2014) Evolutionary relationship of two ancient protein superfolds. Nature Chem Biol 10:710-5.

PI-041 **Semisynthesis and initial characterization of sortase A mutants containing selenocysteine and homocysteine**

Lena Schmohl[1], Felix Roman Wagner[1], Michael Schümann[2], Eberhard Krause[2], Dirk Schwarzer[1]
1.-Interfaculty Institute of Biochemistry, University of Tuebingen, 2.-Laboratory of Mass Spectrometry, Leibniz-Institut Für Molekulare Pharmakologie

Sortase A is a bacterial transpeptidase that recognizes and cleaves the sorting motif LPxTG (where x can be any amino acid) at the threonine residue. The intermediate is bond to active site Cys184 as thioester and subsequently ligated to an N-terminal glycine residue of a second peptide [2]. The active site cysteine plays a key role in the reaction mechanism and we investigated this residue in more detail by exchanging this moiety with selenocysteine (Sec) and homocysteine (Hcy). The sortase mutants were generated by semisynthesis using expressed protein ligation (EPL). The resulting Cys-, Sec- and Hcy-sortase enzymes were characterized and showed a moderate 2–3-fold reduction of activity for Sec-sortase. The activity of Hcy-sortase was barely detectable with less than 1% of wildtype activity. The alkylation efficiency of the active site nucleophiles correlated with the expected pKa values of Sec, Cys and Hcy. Analysis of the pH dependency of the transpeptidation reactions showed that the activity optimum of Sec-sortase was shifted towards more acidic conditions. These investigations provide further insights into the reaction mechanism of sortase A and the semisynthetic enzymes may provide new tool for further biochemical studies. [1] Schmohl L., Wagner F. R., Schümann M., Krause E., Schwarzer D., Bioorg. Med Chem. 2015, 15, 2883-9. [2] Frankel B. A., Kruger R. G., Robinson D. E., Kelleher N. L., McCafferty D. G., Biochemistry, 2005, 44, 11188-200.

PI-042 **Directed evolution on FucO – structural explanations for changes in substrate scope**

Käthe M. Dahlström[1], Cecilia Blikstad[2], Mikael Widersten[2], Tiina A. Salminen[1],
1.-Structural Bioinformatics Laboratory, Biochemistry, Åbo Akademi University, 2.-Department of Chemistry, Uppsala University

Propanediol oxidoreductase from Escherichia coli (FucO) uses NADH/NAD+ as cofactors to catalyze the conversion of S-lactaldehyde to S-1,2-propanediol and vice versa. FucO is an attractive enzyme in the search for possible biocatalysts producing α-hydroxy aldehydes, which are important for the synthesis of natural products and synthetic drugs. Enzymes catalyzing these types of reactions are unique in catalytic power and stereoselectivity. The usage of FucO in synthetic industry is limited by the restricted substrate scope, which makes FucO inactive with larger phenyl-substituted alcohols. We used re-engineering and directed evolution to enable FucO to catalyze the regio- and enantioselective oxidation of arylsubstituted vicinal diols, such as phenylpropanediols, into α-hydroxy aldehyde products. We mutated amino acids considered to restrict the entry into the active site, and modeled the mutants that were most active with the substrates phenylacetaldehyde and S-3-phenyl-1,2-propanediol and performed docking studies with them. As expected, our experimental and in silico results show that the mutations enlarge the active site cavity and enable the mutant enzymes to accommodate the new substrates. We also found specific amino acids in the active site, which need to be conserved to allow the substrates to make stabilizing interactions. Interestingly, an asparagine residue makes the mutant enzymes able to discriminate between phenylacetaldehyde and S-3-phenyl-1,2-propanediol. In conclusion, we successfully re-engineered the specialist enzyme FucO to accept also bulkier molecules as substrates, thereby making it more useful for industrial purposes.

PI-043 **Aided-Crystallization of the artificial protein Octarellin V.1 by alpha-Reps and nanobodies**

Maximiliano Figueroa[1], Mike Sleutel[2], André Matagne[3], Christian Damblon[4], Els Pardon[2], Marielle Valerio-Lepiniec[5], Philippe Minard[5], Joseph Martial[1], Cécile Van de Weerdt[1]

1.-GIGA-Research, University of Liège, 2.-Structural Biology Brussels, Vrije Universiteit Brussel, 3.-Laboratoire d'Enzymologie et Repliement des Protéines, University of Liège, 4.-Department of Chemistry, University of Liège, 5.-Institute for Integrative Biology of the Cell, Université de Paris Sud

One way to gain insight into the sequence-structure-function relationship in proteins is to de novo design artificial proteins. Despite impressive successes in de novo protein design, designing a folded protein of more than 100 amino acids still remains a challenge. Using this approach, an idealized (beta/alpha)8 fold protein was designed leading to the production of a protein of 216 amino acids (Octarellin V). This protein showed a low solubility and stability. Through directed evolution we produced a soluble variant, Octarellin V.1. The biophysical characterization of Octarellin V.1 shows a well folded monomeric and thermostable protein with a Tm over 90 °C. However, after several screenings, we could not find crystallization conditions for this protein. As an alternative, we decided to co-crystallize Octarellin V.1 with a protein partner that helps the crystallization process. We used 2 protein partners: alpha-Reps and nanobodies. The first one is characterized to interact through a large surface contact, whereas the second is characterized to recognize an specific small epitope. Crystallization of both complexes was performed successfully by vapor diffusion and the structures were solved. The experimental structures correspond to the first for an artificial protein of this size and it will allow to criticize the computational design of the Octarellin V.

PI-044 **Generation of synthetic antibodies against membrane proteins in nanodiscs for use in structural biology**

Pawel K. Dominik[1], Marta T. Borowska[1], Olivier Dalmas[1,2], Sangwoo S. Kim[1], Dawid Deneka[1], Eduardo Perozo[1], Robert J. Keenan[1], Anthony A. Kossiakoff[1]

1.-Department of Biochemistry and Molecular Biology, The University of Chicago, 2.-Department of Structural Biology

Establishing the link between structure, dynamics, and function of membrane proteins remains elusive. Previous efforts have presented a number of technical challenges because the relevant functional states of membrane proteins are transient, making it difficult to study them using high-resolution biophysical methods. Here, we describe a robust strategy for generating a class of high performance antibody-based affinity reagents that have proven useful in determining the structures of relevant functional states of membrane proteins. These reagents are Fab fragments that are generated by phage display from fully synthetic libraries and are called synthetic antibody fragments, or sABs. We have developed phage display sorting strategies that can trap a desired conformational state, making it accessible to structural analysis, or target a particular epitope on the protein surface. However, to maximize this technology for membrane proteins, several limitations of phage display sorting in detergent formats had to be overcome, the greatest being that using detergents can produce non-native conformational biases. We sought to address these limitations by embedding membrane proteins into nanodiscs, soluble lipid-filled discoidal particles, to better mimic the native membrane environment. Nanodiscs stabilize the membrane protein and allow it to respond to conformation-inducing stimuli such as ligands, ions and pH during phage display selections. We have established and validated an improved protocol using two membrane protein systems: 1) Mj0480, an archaeal membrane protein of unknown function, and 2) CorA, a pentameric magnesium ion channel. Using Mj0480, we compared the nanodisc protocol with the standard method performed in detergent, and as an important byproduct, we characterized the influence of the membrane protein environment on the apparent affinity of sABs to their cognate antigen. Using CorA, we developed a more sophisticated sorting strategy resulting in a variety of sABs specific to either the open or closed conformation of the channel. Finally, using sABs as crystallization chaperones we obtained the structure of Mj0480 at 3.5Å resolution, and crystallized CorA in several new conditions.

PI-045 **A novel drug delivery system for poorly water-soluble anti-tumor drug SN-38 utilizing intravital transporter protein**

Masatoshi Nakatsuji[1], Haruka Inoue[1], Masaki Kohno[1], Mayu Saito[1], Syogo Tsuge[1], Shota Shimizu[1], Osamu Ishibashi[1], Takashi Inui[1]

1.-Graduate School of Life and Environmental Sciences, Osaka Prefecture University

Lipocalin-type prostaglandin D synthase (L-PGDS) is a member of the lipocalin superfamily, and binds a large variety of small hydrophobic molecules. Using this function of L-PGDS, we have already reported the feasibility of L-PGDS as a novel drug delivery vehicle for the poorly water-soluble drugs [1]. SN-38, 7-ethyl-10-hydroxy-camptothecin, is a semi-synthetic analogue of anti-cancer alkaloid camptothecin that targets DNA topoisomerase I. Despite of the potent anti-tumor activity, however, SN-38 was not used directly in a clinical practice due to its poor water solubility. Thus, irinotecan hydrochloride (CPT-11), which is the water-soluble prodrug of SN-38, is used for the cancer treatment. However, CPT-11 shows approximately 0.1% cytotoxic activity of SN-38 against the various cancer cell lines in vitro, and its metabolic conversion rate is 10% of the original volume of CPT-11. Here, we show the development of the drug delivery system utilizing L-PGDS, which enables a direct clinical usage of SN-38. First, we investigated the effect of L-PGDS on the solubility of SN-38. In the presence of 2 mM L-PGDS, the concentration of SN-38 was 1.7 mM, which was 1,130-fold as compared with that in PBS. Then, we carried out isothermal titration calorimetry measurements to investigate the detailed binding mode of SN-38 to L-PGDS. As a result, it was revealed that L-PGDS binds three molecules of SN-38, and the dissociation constant value was 60 ± 4.0 μM. In vitro growth inhibition assay revealed that SN-38/L-PGDS complex showed the high anti-tumor activity against three human cancer cell lines, Colo201, MDA-MB231, and PC-3. The calculated IC50 values of SN-38/L-PGDS complex on the cell growth of Colo201, MDA-MB231, and PC-3 cells were 35 ± 6.5, 900 ± 190, and 10 ± 1.5 nM, respectively. In addition, the anti-tumor activity of SN-38 after the administration of SN-38/L-PGDS-complex was evaluated in the Colo201 human colorectal tumor xenograft model. The intravenous administration of SN-38/L-PGDS complex at doses of 1.0, 2.0 or 2.8 mg/kg/d every other day for 2 weeks showed a pronounced anti-tumor activity, while the administration of CPT-11 at a dose of 4.0 mg/kg/d did not show any anti-tumor activity. Finally, in order to estimate the side effects of SN-38/L-PGDS complex, we performed histopathological analysis in the small intestine. The intestinal mucosa of mice administered with SN-38/L-PGDS complex at a dose of 2.8 mg/kg/d using the same administration schedule in the growth inhibition assay showed the preservation of the villi and the crypt architecture, which was similar to that of PBS administered group. These results indicated that SN-38/L-PGDS complex did not induce the intestinal lesion. In conclusion, L-PGDS could improve the solubility of SN-38, and the intravenous administration of SN-38/L-PGDS complex showed a potent anti-tumor activity on xenograft tumor model. Thus, we believe that L-PGDS is a novel and valid drug delivery vehicle for SN-38.

[1] Fukuhara, A. et al. (2012) J. Control. Release 158, 143-150

PI-046 **Intrinsic Disorder as Biomimetic Strategies for the Introduction of Hill-Type Cooperativity into Biomolecular Receptors**

Anna Simon[1], Alexis Vallée-Bélisle[2], Francesco Ricci[3], Kevin Plaxco[1,4,5]

1.-Biomolecular Science and Engineering Program, UC Santa Barbara, 2.-Département de Chimie, Université de Montréal, 3.-Dipartimento di Scienze e Tecnologie Chimiche, University of Rome, Tor Vergata, 4.-Department of Biochemistry and Chemistry, 5.-Center for Bioengineering

Control over the sensitivity with which artificial biomolecular receptors respond to small changes in the concentration of their target ligand is critical for the proper function of many cellular processes. Such control could likewise be highly useful in artificial biotechnologies in which highly responsive behavior is of value, such as biosensors, genetic logic gates, and "smart" materials and delivery devices. In nature, the control of molecular responsiveness is often achieved using "Hill-type" cooperativity, a mechanism in which sequential binding events on a multivalent receptor are coupled such that the first enhances the affinity of the next, producing a steep, higher-order dependence on target concentration. Here we use an intrinsic-disorder-based mechanism that can be implemented without requiring detailed structural knowledge to rationally introduce this potentially useful property into several normally non-cooperative biomolecules. To do so we fabricate a tandem repeat of the receptor that is destabilized (unfolded) via the introduction of a long, unstructured loop. The loop spatially separates the two sets of the two halves of the binding sites, preventing a complete binding site that enables target molecule binding without prior closure of the loop. Thus, the first binding event requires the energetically unfavorable closing of this loop, reducing its affinity relative to that of the second binding event, which, in contrast occurs at a pre-formed site. Using this approach we have rationally introduced cooperativity into three unrelated aptamers, achieving in the best of these a Hill coefficient experimentally indistinguishable from the theoretically expected maximum. The extent of cooperativity, and thus the steepness of the binding transition, are, moreover, well modeled as simple functions of the energetic cost of binding-induced folding, speaking to the quantitative nature of this design strategy.

PI-047 **Essential and non-essential amino acid species for an ancestral protein**

Satoshi Akanuma[1]
1.-Faculty of Human Sciences, Waseda University

The translation system is an essential element for life because it links genetic information embedded in genes to functional molecules, proteins. The modern genetic code, which encodes the standard 20 amino acids (and three terminations) using 64 triplet codons, is shared by most of the extant organisms on the earth. A number of theories have been proposed for the origin and evolution of the genetic code, and these theories suggest that only a fewer amino acids were used in primitive proteins and later the amino acid repertoire gradually increased up to 20 through the course of evolution. If so, one would wonder how many number of and which types of amino acids were involved in the primitive proteins. I have begun to address this issue experimentally. I first resurrected several ancestral proteins and then restricted the amino acid usage of one of the resurrected proteins. I targeted nucleoside diphosphate kinase (NDK) that catalyzes the transfer of a phosphate from a nucleoside triphosphate to a nucleoside diphosphate. NDK may have arisen early because at least one gene that encodes NDK is present in most extant organisms. The first step in the reconstruction of ancestral NDK sequences is to prepare multiple amino acid sequence alignments using homologous sequences of NDK from extant species. Then, phylogenetic trees were built. Ancestral sequences of NDK that represent the last common ancestors of Archaea and of Bacteria were reconstructed using the information contained in the predictive phylogenetic trees. The reconstructed ancestral kinases are extremely thermally stable [Akanuma et al., 2013]. Then, using the most thermally stable ancestral NDK, Arc1, as the starting molecule, I restricted its amino acid usage. Arc1 does not contain any cysteine residue and therefore consists of 19 amino acid species. I completely replaced one of the 19 amino acid species by other amino acid species and thus created 19 proteins each of which consisted of 18 amino acid species. Then, I evaluated the stabilities and activities of the resulting 19 Arc1 variants to assess the individual contributions of the 19 amino acid species. As the result, I found that the 19 amino acid species do not equally contribute to the stability and activity of Arc1 and that some amino acid species can be easily lacked but others are important or essential for its stability and function. The result clearly shows that the full amino acid species are not necessarily essential and supports the hypothesis that proteins in the early stage of evolution were made from a reduced amino acid set. Akanuma et al., Proc. Natl. Acad. Sci. USA 110, 11067–11072 (2013)

PI-048 **De novo design of protein-protein interaction using hydrophobic and electrostatic interactions**

Sota Yagi[1], Satoshi Akanuma[2], Akihiko Yamagishi[1]
1.-Tokyo University of Pharmacy and Life Sciences, Department of Applied Life Scien, 2.-Waseda University, Faculty of Human Sciences

The protein surface recognition for protein-protein interactions (PPI) is involved in signal transduction, immune reaction, and creation of the nanostructures in living cells. The methods for rational designing of PPI that could provide non-antibody scaffolds and nanostructured materials are required for the therapeutic and nanotechnological applications. Although there have been some successful rational designs with computational methods, it is still difficult to design freely the PPI onto arbitrary proteins. The reason for this limitation is decreased solubility in the designed protein due to the additional hydrophobic residues in order to drive PPI. Another reason is a limited set of design modes by which proteins can interact, because the target proteins have individual surface structures. Therefore, many methods of constructing an interface for numerous target scaffold proteins without loss of their solubility are necessary. Surface exposed α-helices are often observed in natural globular proteins. Moreover, there are many examples for naturally occurring oligomeric proteins where an α-helix from each subunit interacts to form an intermolecule coiled coil. Further, the works related to designing of artificial helical bundle reported by the several other groups have provided information about how to generate and tune the interaction between α-helices. Therefore, a surface exposed α-helix would be a good target for designing a de novo interface onto the scaffold protein. Here we engineered two different proteins, sulerythrin and cys-LARFH, to form the cys-LARFH–sulerythrin dimer–cys-LARFH heterotetramer via an intermolecular helix-helix interaction. Wild-type sulerythrin forms a dimeric eight-helix bundle. Cys-LARFH is a designed monomeric protein that forms four-helix bundle containing interhelical S-S bonds. Both sulerythrin and cys-LARFH are extremely thermostable. To design protein-protein interfaces onto the individual proteins, we first introduced six leucines to the two α-helices of sulerythrin and three leucines to a α-helix of cys-LARFH. As expected, the introduction of the hydrophobic amino acids reduced their solubilities. To recover the solubility, we then introduced six aspartates or glutamates around the hydrophobic surface of the sulerythrin (hereafter referred to as 6L6D or 6L6E). Similarly, three arginines were introduced around the artificial hydrophobic surface of the cys-LARFH (hereafter referred as IV-3L3R). The solubilities of the mutants with the hydrophobic interface and additional charged residues were recovered their solubility. In addition, the sulerythrin mutants 6L6D and 6L6E exist mainly as dimer. The cys-LARFH mutants IV-3L3R, also exists as monomer. We then examined the interaction between 6L6E or 6L6D and IV-3L3R. A pull-down experiment, in which Co2+ beads bound to either His-tagged cys-LARFH and IV-3L3R were used to pull down wild-type sulerythrin, 6L6D, or 6L6E, demonstrates that 6L6D or 6L6E specifically interacts to IV-3L3R. Furthermore, when analysed by size exclusion chromatography, the dominant peaks of the mixture of 6L6D and IV-3L3R appeared at the volume expected for the heterotetrameric complex. Thus we successfully created the de novo PPI by using a very simple concept involving hydrophobic interaction in combination with charge interactions.

PI-049 **In vitro selection of liposome anchoring peptide by cDNA display**

Naoto Nemoto[1], Ryoya Okawa[1], Yuki Yoshikawa[1], Toshiki Miyajima[1], Shota Kobayashi[1]
1.-Graduate School of Science and Engineering, Saitama University

A liposome-anchoring peptide (LA peptide) was selected against liposomes composed of dioleoyl-sn-glycero-3-phosphocholine (DOPC) by in vitro selection using cDNA display method. The selected peptide LA peptide consists of the N-terminal region (hydrophobic) and the C-terminal region (basic) in a characteristic manner. Thus, LA peptide was synthesized chemically and the interactions between LA peptide and particular types of liposomes were investigated and confirmed by confocal laser scanning microscopy.

PI-050 **Designing of a novel platinum-binding amino acid sequence on a protein surface**

Asumi Kaji[1], Hiroya Niiro[1], Satoshi Akanuma[2], Tetsuya Uchida[1], Akihiko Yamagishi[1]
1.-Tokyo University of Pharmacy and Life Sciences, 2.-Waseda University

Designing of a novel interaction between a metal and a protein is a key to create hybrid materials between organic and inorganic materials. For example, in a glucose biosensor, which is widely used for measuring glucose concentration in blood, glucose oxidoreductase molecules are immobilized on a platinum electrode by polyacrylamide gel. A metal-binding tags that is added to the N- or C-terminus of a protein is also used for fix the protein to a metal. However, a technique to create a metal binding site on a desired position of a protein has not been invent. If such a technique would be established, the technique would contribute to developing and improving biosensors and to producing new bionanoelectronic materials. In this study, we created a platinum-binding site on a loop located at a protein surface. We used an artificial protein, LARFH, that had been synthesized by connecting four identical alpha helices originated from the C-terminal segment of the Escherichia coli Lac repressor with three identical loops. We randomized the Ser, Gly, Gln, Gly, Gly, Ser sequence within one of the inter-helical loops and then selected for binding to platinum by a T7 phage display system. Most of the selected LARFH variants contained the Tyr, Lys, Arg, Gly, Tyr, Lys (YKRGYK) sequence in the randomized segment. We then evaluated the affinity of the LARFH variant to platinum by means of Quartz Crystal Microbalance analysis. We found that the variant binds to platinum more strongly than does the original LARFH. In the annual symposium, we will also report about the affinity of the isolated YKRGYK sequence to platinum and about the crucial role of the first tyrosine in binding to platinum.

PI-051 **Engineering of an isolated p110α subunit of PI3Kα permits crystallization and provides a platform for structure-based drug design**

Alexei Brooun[1], Ping Chen[1], Ya-Li Deng[1], Simon Bergqvist[1], Matthew Falk[1], Wei Liu[1], Sergei Timofeevski[1]

1.-Oncology Structural Biology, Worldwide Research and Development, Pfizer Inc

Structural Biology, Worldwide Research and Development, Pfizer Inc, 3.-Oncology Structural Biology, Worldwide Research and Development, Pfizer Inc, 4.-Oncology Research Unit, Worldwide Research and Development, Pfizer Inc, 5.-Oncology Research Unit, Worldwide Research and Development, Pfizer Inc, 6.-Oncology Structural Biology, Worldwide Research and Development, Pfizer Inc, 7.-Oncology Research Unit, Worldwide Research and Development, Pfizer Inc

PI3Kα remains an attractive target for development of anticancer targeted therapy. A number of p110α crystal structures in complex with the nSH2-iSH2 fragment of p85 regulatory subunit have been reported, including a few small molecule co-crystal structures, but the utilization of this crystal form is limited by low diffraction resolution and a crystal packing artifact that partially blocks the ATP binding site. Taking advantage of recent data on the functional characterization of the lipid binding properties of p110α, we designed a set of novel constructs allowing production of isolated stable p110α subunit missing the Adapter Binding Domain (ABD) and lacking or featuring a modified C-terminal lipid binding motif. While this protein is not catalytically competent to phosphorylate its substrate PIP2, it retains ligand binding properties as indicated by direct binding studies with a pan-PI3Kα inhibitor. Additionally, we determined apo and PF-04691502 bound crystal structures of the p110α (105-1048) subunit at 2.65 Å and 2.85 Å respectively. Comparison of isolated p110α (105-1048) with the p110α/p85 complex reveals a high degree of structural similarity, which validates suitability of this catalytically inactive p110α for iterative SBDD. Importantly, this crystal form of p110α readily accommodates the binding of non-covalent inhibitor by means of a fully accessible ATP site. The strategy presented here can be also applied to structural studies of other members of PI3KIA family.

PI-052 **Identification of structural determinants involved in the differential conformational changes of EF-hand modules**

Emma Liliana Arevalo Salina[1], Joel Osuna Quintero[1], Humberto Flores Soto[1], Gloria Saab Rincón[1]

1.-Instituto de Biotecnología, Universidad Nacional Autónoma de México

Identification of structural determinants involved in the differential conformational changes of EF-hand modules Calcium signals are regulated by several proteins, most of which belong to the EF-hand superfamily. The EF-hand motif is formed by a helix-loop-helix that binds calcium through its loop1. These motifs occur in adjacent pairs, forming a single globular domain which is the basic structural and functional Ca2+ binding unit. The proteins in this family can be classified as calcium sensors or modulators, according with their function. The first group undergoes a major conformational change upon calcium binding, while the second one remains practically unchanged1,2. To explain the biophysics behind the different behavior of these proteins upon Ca2+ binding, we have sought to identify structural determinants that could account for these features, especially for the difference in the conformational change. We examined the primary structure from two EF-hand motifs: a sensor EF-hand from chicken troponin C (SCIII) and a modulator EF-hand from bovine Calbindin D9k (ClbN). The main differences were in the binding Ca2+ loop and a group of charged residues in the H2 helix of the modulator EF-hand. Then, we constructed chimeric ClbN motifs containing the loop or the loop and H2 from SCIII motif (H1ClbNSCIII and H1H2ClbNSCIII). These constructs were analyzed using a reporter system that discriminates EF-hand-sensor motifs from signal-modulators at the single-motif level. This reporter is based on the fusion of genes codifying for the EF-hand and the prephenate dehydrogenase from E. coli (TyrA), a protein which is active only as a dimer. Isolated EF-hand motifs have the ability to homo-dimerize and in the fusion can stabilize and activate TyrA. The sensor motif exhibits a conformational change by binding calcium and in doing so, destabilizes the dimeric conformation of TyrA and virtually eliminates its activity. In the modulators, on the other hand, the rather small conformational change only gives rise to a decreased TyrA activity. Both constructed chimeric EF-hand fusions showed a loss of activity upon Ca2+ binding, indicating that the 12 residues connector of the sensor EF-hand from SCIII is sufficient to confer the conformational change. In addition we used CD and extrinsic fluorescence spectroscopies to analyze any conformational change in the H1H2ClbNSCIII and H1ClbNSCIII isolated modules, not finding any difference between the Ca2+ free and Ca2+ bound chimeras, suggesting that the change in activity of the reporter protein is due to a change in the orientation of the helices in the EF-hands induced by calcium. The effect of Ca2+ binding of the chimeras in the context of the entire Calbindin D9k protein is under investigation. 1Nelson, M. R., Chagot, B. & Chazin, W. J. Encyclopedia of Life Sciences. JohnWiley&Sons,Ltd, 2010 2Gifford, J. L., Walsh , M. P. & Vogel , H. J. Structures and metal-ion-binding properties of the Ca2+-binding helix-loop-helix EF-hand motifs. Biochem. J. 405, 199-221. 2007

PI-053 **Mapping side chain interactions at the N- and C-termini of protein helices**

Nicholas Newell[1]
1.-Independent Researcher

Mapping side chain interactions at the N- and C-termini of protein helices Nicholas E Newell, Independent Researcher Interactions involving one or more amino acid side chains near the ends of protein helices stabilize helix termini and shape the geometry of the adjacent loops, contributing to supersecondary structure. Side chain structures that have been identified at the helical N-terminus include the Asx/ST N-caps, the capping box, and hydrophobic and electrostatic interactions. At the C-terminus, capping is often achieved with main-chain polar groups, (e.g. the Schellman loop), but here also particular side chain motifs clearly favor specific loop geometries. Key questions that remain concerning side chain interactions at helix termini include: 1) To what extent are helix-terminal motifs that include multiple amino acids likely to represent genuine cooperative interactions between side chains, rather than chance alignments? 2) Which particular helix-terminal loop geometries are favored by each side chain interaction? 3) Can an exhaustive statistical scan of a large, recent dataset identify new side chain interactions at helix termini? In this work, three analytical tools are applied to answer the above questions for both N- and C-termini. First, a new perturbative least-squares 3D clustering algorithm is applied to partition the helix terminal structures in a large (25,000 example), low-redundancy PDB dataset by loop backbone geometry. The clustering algorithm also generates a set of structural exemplars, one for each cluster, that is used to represent the most important loop geometries at each terminus. Next, Cascade Detection (Newell, Bioinformatics, 2011), an algorithm that detects multi-amino acid cooperativities by identifying overrepresented sequence motifs, is applied to each cluster separately to determine which motifs are most important in each loop geometry. Finally, the results for each motif are displayed in a CapMap, a 3D conformational heatmap that depicts the distribution of motif abundance and overrepresentation across all loop geometries by projecting these quantities onto the structural exemplars generated by clustering. The CapMap reveals the loop conformations most favored by a motif. Actual structures from the clusters corresponding to these favored conformations are then examined in a structure browser to characterize the side chain interaction associated with the motif. This work identifies a 'toolkit' of side chain motifs which are good candidates for use in the design of synthetic helix-terminal loops with specific desired geometries, because they are used in nature to support these geometries. Highlights of the analysis include determinations of the favored loop geometries for the Asx/ST motifs, capping boxes, big boxes, and other previously known and unknown hydrophobic, electrostatic, H-bond, and pi-stacking interactions. A goal of future work is to make these results available in a structurally-addressable database that would enable researchers to immediately retrieve the side chain interactions most compatible with a desired loop geometry.

PI-054 **Generation of fluorescent protein-tagged gp120 mutants to analyze the intracellular distribution of HIV-1 envelope protein**

Shuhei Nakane[1], Zene Matsuda[3]

1.-Green Earth Research Center, Green Earth Institute Co., Ltd., 2.-Res Ctr for Asian Infect Dis, Inst of Med Sci, the Univ of Tokyo, 3.-Lab of Struct Virol and Immunol, Institute of Biophysics, CAS

HIV-1 is a causative enveloped virus of AIDS. Its envelope protein (Env) has two non-covalently associated subunits, gp120 and gp41, which are proteolytically processed from a gp160 precursor. The gp120 subunit is a surface protein and gp41 is a transmembrane protein. The gp120 and gp41 subunits are responsible for the receptor recognition and membrane fusion, respectively. The cytoplasmic tail (CT) of gp41 is about 150 amino acids long and is believed to play a critical role in intracellular trafficking of Env. To visualize dynamic trafficking, the C-terminus of gp41 has been tagged with fluorescent proteins such as GFP. However, tagging of CT may cause a concern to affect the interactions between the CT and cellular proteins that are involved in intracellular trafficking. To avoid this problem, here we tried to insert GFPopt, a GFP variant, into five variable regions of gp120. We have analyzed the phenotypes of Env mutants, such as the cell surface expression, processing of gp160, membrane fusion activity, and virion incorporation. Among 5 variable regions of gp120, the V3 region was most sensitive to insertion. V1/V2 region was less sensitive than V3. Consistent with the recently revealed structure, exteriorly located V4 and V5 were highly tolerant to insertion. We used the mutant with the GFP insertion in the V5 region to analyze the intracellular distribution of Env with and without CT. We found that deletion of CT increased the presence of vesicles colocalized with late endosome markers. This is consistent with the hypothesis that the CT region contains a motif regulating intracellular trafficking. Our results showed that Env with GFPopt insertion in its gp120 subunit is a useful tool for the study of intracellular dynamics of HIV-1 Env. These mutants would also be useful to trace the fate of virus particles during infection.

PI-055 **NGS-guided phage panning: Comparison to conventional panning strategy**

Buyung Santoso[1], Dorain Thompson[1], John Nuss[1], John Dwyer[1]
1.-Ferring Research Institute

Phage display is a powerful tool for generating binders to a target protein. Multiple rounds of panning with conventional phage display strategies typically result in a number of hits, which are then individually screened using in vitro assays. Clones screened at this stage are a combination of specific binders, sequences that are selected due to amplification bias, and non-specific binders. If the number of specific clones is low relative to the non-specific sequences, a larger number of clones have to be screened to ensure sufficient diversity of early leads. With the advent of next generation sequencing (NGS) technology, we aim to test whether we can increase the diversity of specific hits and decrease the number of non-specific sequences. In our experiment, four rounds of conventional panning produced ten peptide binders to target protein. NGS analysis after two rounds of panning was done in parallel, yielding more than ten thousand sequences, ranked by abundance. All ten binders from conventional panning were found in the top 150 most abundant NGS hits. More importantly, additional hits were found in NGS analysis but not in conventional panning, highlighting this strategy as a promising alternative for hit discovery with the significant upside of more diverse and higher affinity leads.

PI-056 **Fully Automated Mini, Midi, and Maxi plasmid prep on the autoplasmid MEA instrument**

Carrie Huynh[1], Lee Hoang[1], Chris Suh[1], Jonathan Grambow[1]
1.-PhyNexus In San Jose

Numerous processes in pharmaceutical development, including construct screening, structural genomics, protein engineering and expression optimization among others, require the use of higher throughput plasmid DNA purification. The majority of issues encountered in Mini, Midi, and Maxiprep purification kits involve flocculate removal following alkaline lysis, and there is currently no easy way to produce large amounts of plasmid DNA without the addition of complicated and time consuming clarification steps. The existence of a hassle-free automated system that is not restricted by sample size would significantly help in cutting time and costs during the initial processing steps of plasmid purification. The AutoPlasmid MEA instrument provides a fully automated solution to traditional problems faced in plasmid purifications, allowing Mini, Midi, and Maxiprep plasmid purifications to be performed on a single instrument. The data presented here on plasmid yield, purity, and suitability for sequencing and transfection/transformation illustrate a new strategy for automated plasmid preps. By eliminating traditional clarification methods, cell culture volumes between 1 - 120 mL can be processed leading to yields ranging from 3 – 1000 μg. This flexible system was developed in order to satisfy a wide variety of concentration and yield requirement, while eliminating the time consuming steps previously needed to obtain similar results. The ability to perform fully automated Mini, Midi, and Maxi plasmid preps on one instrument allows for a customized all-in-one purification system that is not restricted by traditional clarification methods, eliminating manual intervention, and streamlining the purification process.

PI-057 **RE3volutionary computational design of symmetric proteins that biomineralize nano-crystals**

Kam Zhang[1], Arnout Voet[1], Hiroki Noguchi[2], Christine Addy[2], Jeremy Tame[2]

1.-Structural Bioinformatics Team, DSSB, CLST, RIKEN, 2.-Drug Design Laboratory, GSMLS, Yokohama City University

RE3Volutionary Computational Design of Symmetric Proteins That Biomineralize Nano-Crystals Arnout RD Voet (1), Hiroki Noguchi (2), Christine Addy (2), Kam YJ Zhang (1), Jeremy RH Tame (2) (1) Structural Bioinformatics Team, Division of Structural and Synthetic Biology, Center for Life Science Technologies, RIKEN, 1-7-22 Suehiro, Yokohama, Kanagawa 230-0045, Japan (2) Drug Design Laboratory, Graduate School of Medical Life Science, Yokohama City University, 1-7-29 Suehiro, Yokohama, Kanagawa 230-0045, Japan The modular nature of protein architectures suggests that proteins have evolved through duplication and fusion to give rise to modular, often symmetric forms, which later diversified under the influence of evolutionary pressure. We have developed a computational protein design method termed REverse Engineer Evolution (RE3Volution) to create symmetrically self-assembling protein building blocks. We have used this method to design a perfectly symmetric β–propeller protein called Pizza. Subsequently, we have engineered a metal binding site into this Pizza protein. This new Pizza variant carries two nearly identical domains per polypeptide chain, and forms a trimer with three-fold symmetry. The designed single metal ion binding site lies on the symmetry axis, bonding the trimer together. Two copies of the trimer associate in the presence of cadmium chloride in solution, and high resolution X-ray crystallographic analysis reveals a nano-crystal of cadmium chloride, sandwiched between two trimers of the protein. This nano-crystal, containing seven cadmium ions lying in a plane and twelve interspersed chloride ions, is the smallest reported to date. Our results indicate the feasibility of using rationally-designed symmetrical proteins to biomineralize nano-crystals with applications in bio-nanotechnology.

PI-058 **Bacillus licheniformis Trehalose-6-phosphate Hydrolase structures suggest keys to substrate specificity**

Chwan-Deng Hsiao[1], Min-Guan Lin[1], Long-Liu Lin[2], Yuh-Ju Sun[3]

1.-Institute of Molecular Biology, Academia Sinica, 2.-Department of Applied Chemistry, National Chiayi University, 3.-Depaertment of Life Science, National Tsing Hua University

Trehalose-6-phosphate hydrolase (TreA) of the glycoside hydrolase family 13 (GH13) catalyzes the hydrolysis of trehalose-6-phosphate (T6P) to yield glucose and glucose-6-phosphate. Products of this reaction can be further metabolized by the energy-generating glycolytic pathway. Here we present the crystal structures of Bacillus licheniformis TreA (BlTreA) and its R201Q mutant complexed with p-nitrophenyl-α-D-glucopyranoside (R201Q/pPNG) at 2.0 Å and 2.05 Å resolution, respectively. The overall structure of BlTreA is similar to other GH13 family enzymes. However, detailed structural comparisons revealed that the catalytic groove of BlTreA contains a long loop adopting a different conformation from those of GH13 family members. Unlike the homologous regions of Bacillus cereus oligo-1,6-glucosidase (BcOgl) and Erwinia rhapontici isomaltulose synthase (NX-5), the active site surface potential of BlTreA exhibits a largely positive charge, contributed by the four basic residues His281, His282, Lys284 and Lys292. Mutations at these residues resulted in significant decreases of BlTreA enzymatic activity. Strikingly, a 281HHLK284 motif and the Lys292 residue played critical roles in BlTreA substrate discrimination.

PI-059 **Crystal structure of engineered LRRTM2 synaptic adhesion molecule and a model for neurexin binding**

Anja Paatero[1], Katja Rosti[1], Alexander Shkumatov[2], Cecilia Brunello[3], Kai Kysenius[3], Prosanta Singha[1], Henri Huttunen[3], Tommi Kajander[1]

1.-Institute of Biotechnology, University of Helsinki, Helsinki, Finland, 2.-Dept of Pharmaceutical and Pharmacological Sciences, KU Leuven, Leuven, Belgium, 3.-Neuroscience Center, University of Helsinki

Synaptic adhesion molecules are key components in the development of the brain, and in the formation of neuronal circuits, as they are central in the assembly and maturation of the chemical synapses. Several families of neuronal adhesion molecules have been identified such as NCAMs, neurexins and neuroligins, and in particular recently several leucine rich repeat protein families, e.g. Netrin G-ligands, SLITRKs and LRRTMs. The LRRTMs form a family of four proteins. They have been implicated in excitatory glutamatergic synapse function, and were specifically characterized as ligands for neurexins in excitatory synapse formation and maintenance. In addition, LRRTM3 and LRRTM4 have been found to be ligands for heparan sulphate proteoglycans. We report here the crystal structure of a stability-engineered mouse LRRTM2, with a Tm 30 °C higher than the wild type protein, while retaining its function. We localized the neurexin binding site to the concave surface based on protein engineering, sequence conservation and prior information on the ligand interaction with neurexins, allowing us to propose a tentative model for LRRTM:neurexin interaction compex. Cell culture studies and binding experiments show that the engineered protein is functional and capable of forming synapse-like contacts. Small angle X-ray scattering data suggests that the wild type protein forms transient dimers, which may have importance for the function. The structural and functional data presented here provide the first structure of an LRRTM protein, and a model for molecular mechanism of LRRTM function in adhesion.

PI-060 **Computational design of phenylalanine binder**

Olga Khersonsky[1], Gil Benezer[1], Sarel Fleishman[1]
1.-Department of Biological Chemistry, Weizmann Institute of Science

Recently, AbDesign algorithm was developed in our lab for de novo design of antibodies (1). It is guided by natural conformations and sequences, and exploits the modular nature of antibodies to generate an immense space of conformations, which can be used as scaffolds for design of stable high-affinity binders. We have used AbDesign to design a binder of phenylalanine. ~30,000 antibody scaffolds were obtained by splicing H3 and L3 fragments into a template (pdb ID 2brr), and subsequent optimization of VH and VL orientation. Phenylalanine binding site, based on native phenylalanine binders, was introduced into the scaffolds with RosettaMatch (2), and the sequences were subsequently optimized by Rosetta enzyme design protocol (3). ~30 designs were experimentally tested by yeast display for binding of biotinylated phenylalanine ligand. Several designs were found to bind the ligand, and we plan to further characterize this affinity and improve it using directed evolution techniques. In collaboration with the group of Prof. Johnsonn, the resulting phenylalanine binder will be incorporated in a bio-luminescent (LUCID) sensor for phenylalanine (4). Phenylalanine monitoring device would be of primary importance for patients with phenylketonuria, a genetic disease with phenylalanine metabolism problem.

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4. Bioluminescent sensor proteins for point-of-care therapeutic drug monitoring. Griss R. et al, Nat Chem Biol. 2014;10(7):598-603.

PI-061 **Dimer dynamics in a cold-active enzyme: The case of alkaline phosphatase**

Bjarni Ásgeirsson[1], Manuela Magnúsdóttir[1], Jens Hjörleifsson[1], Gaetano Invernizzi[2], Elena Papaleo[2]

1.-Department of Biochemistry, Science Institute, University of Iceland, 2.-University of Milano-Bicocca

Cold-adapted enzymes are interesting because of their higher catalytic activity compared to mesophilic and thermophilic homologues. Alkaline phosphatase (AP) from a psychrophilic Vibrio marine bacteria (VAP) has an unusual large surface loop that extends from each of its monomers to stabilize a homodimeric structure (1). In many cold-adapted enzymes, the loop regions are longer compared to proteins of mesophilic organisms and our aim was to study the functional and structural role of this loop. Three substitutions (R336L, Y346F and F355Y) were introduced within the large surface loop as directed by 1-microsecond molecular dynamics (MD) simulations. With the R336L mutation, two hydrogen bonds were broken that connect the loop to residues on the adjacent subunit, and further two hydrogen bonds broken with the adjacent Q334. As a consequence, R336L displayed a 25% higher kcat compared with wild-type and a slight decrease in the Km value. Overall, the catalytic efficient improved by 45%. The global heat stability (Tm) and the active site sensitivity to heat (T50%) were reduced by 6°C and 13°C, respectively. MD simulations showed that hydrogen bonds to Arg336 are important for long-range communication to the active site. Certain rotamers of two important residues in the catalytic site, Ser65 and Arg129, were favored, presumably toward states more competent for catalysis upon the replacement of Arg336 with Leu. In the Y346F variant, removal of one hydrogen bond between the loop and the other subunit caused a small drop in stability parameters, whereas both kcat and Km were reduced by about half, giving similar kinetic efficiency (kcat/Km) to that of wild-type. Finally, we changed a residue at the root of the large loop (F355Y) such that one new intersubunit hydrogen bond could form. This variant maintained the wild-type characteristics. In conclusion, removing hydrogen bonds connecting the major loop of one subunit to the protein surface of the other subunit in VAP produced higher catalytic activity and this shows functional connections between loop mobility and the active site. Our study also demonstrates that interactions between residues in the large disordered loop and the opposite subunit in the dimeric VAP are determinants of its stability. Thus, we managed to show that loosening of interface contacts between the two VAP subunits by replacement of crucial residues provides a way to orchestrate structural and kinetic dynamics in a productive way. Relevant publications: (1) Helland, R., Larsen, R.L., Ásgeirsson, B. (2009) Biochim. Biophys. Acta.1794, 297-308. (2) Papaleo, E., Renzetti, G., Invernizzi, G., Ásgeirsson, B. (2013) Biochim. Biophys. Acta 1830, 2970-2980. (3) Papaleo, E., Magnúsdóttir, M., Hjörleifsson, J.G., Invernizzi, G., Ásgeirsson, B. (2015). Modulation of activity and stability of a dimeric cold-adapted enzyme acting on a disordered region at the monomer-monomer interface (Under review).

PI-062 **A novel secondary structure element assembly protocol for the design of artificial (??)8-barrel proteins using ROSETTA**

Cristina Elisa Martina[1], Steven Combs[2], Rocco Moretti[2], Maximiliano Figueroa[1], Cecile Van De Weerdt[1], Andre Matagne[1], Jens Meiler[2]
1.-University of Liege, 2.-Vanderbilt University

The de novo design of artificial proteins arises as a stringent test of our understanding of the relationship between sequence, structure, and function. Examples include the design of a four α-helix bundle, a new protein topology called TOP7, and a series of artificial (βα)8-barrels called Octarellins. However, de novo design has proven difficult for larger proteins with more than 100 amino acids. Here we present two methods to generate the backbone and to perform the de novo design of (βα)8-barrel proteins through the use of the software ROSETTA; both have different advantages and limitations. The first method for generating the backbone is knowledge-based, with a first analysis of a non-redundant database of natural (βα)8-barrel proteins in order to obtain statistical analysis on preferred secondary structure element length and amino acidic propensities. With this information we use the ROSETTA CM software to create more than 1000 models which are then ranked in term of ROSETTA energy. The second method is performed with the ParametricDesign package of ROSETTA, in which only geometrical information are requested (number of strands and helices, radius of the β- and α-barrels, degree of inclination, orientation of the side chains, among others). Both methods contain a step of loop refinement and multiple steps of sequence design with the package ROSETTA Design, in order to find low scoring amino acid sequences for each of the starting backbone conformations. Thousands of models will be generated by both methods and then analyzed in term of sequence similarity, secondary and tertiary structure prediction, and stability by molecular dynamics simulations. The 30 best candidate sequences will be selected for the experimental verification. In order to identify a putative successfully design, we added a metal binding site during the design step. All the proteins will be expressed in E. coli. The solubility of the designed proteins inside bacteria will be determined thanks to the fusion to green fluorescence protein (GFP). Solubility, stability, secondary structure, and cooperativity of folding will be assessed for each protein before determination of their three-dimensional structure.

PI-063 **Construction of protein capsule possessing drugs controlled release ability**

Shota Shimizu[1], Masatoshi Nakatsuji[1], Keisuke Yamaguchi[1], Yuya Sano[1], Yuya Miyamoto[1], Takashi Inui[1]

1.-Graduate School of Life and Environmental Sciences, Osaka Prefecture University

Most compounds that exhibit anti-tumor activities are water-insoluble, thus limiting their clinical use. Chemical modification of these compounds and the use of solubilizing agents such as organic solvents, surfactants and pH modifiers improve their solubility. However, chemical modification of compounds decreases their potency, and the use of solubilizing agents causes toxicity in many cases. Thus, drug delivery systems (DDS) for poorly water-soluble anti-tumor drugs which exploit liposomes, cyclodextrins, and lipid nanoparticles have been studied intensely. In these DDS, the controlled release of drugs from the delivery vehicle is one of the most important functions. Selective release in target cells leads to adequate therapeutic efficacy with few side effects. In our laboratory, we have already demonstrated that lipocalin-type prostaglandin D synthase (L-PGDS), an intravital transporter protein, is a novel and valid drug delivery vehicle for SN-38, a poorly water-soluble anti-tumor drug. In this study, we generated L-PGDS-based protein capsules with a controlled-release function by introducing a disulfide bond into the upper part of the drug-binding cavity of L-PGDS. The intracellular concentration of glutathione (0.5~10 mM) is known to be substantially higher than the extracellular concentration (~2 μM). Therefore, it is expected that in the extracellular oxidative environment the disulfide bonds in the protein capsule remain stable, avoiding premature release of the internal drugs during circulation of blood, after reaching the target cells, the disulfide bonds are cleaved in the intracellular redox-environment, and then the internal drugs are released. We generated three kinds of protein capsules which have disulfide bonds in different positions, W54C/W112C, K58C/H111C, K58C/W112C, based on tertiary structure information of human L-PGDS (PDB ID: 3O2Y). Firstly, we performed circular dichroism (CD) measurements to confirm the structure of each capsule. The CD spectra of three protein capsules were similar to that of wild-type L-PGDS in the far-UV region. Therefore, the secondary structures of three protein capsules were not changed from wild-type L-PGDS by introducing the mutations. Quantitative analysis of the free thiol group in the protein capsule by DTNB assay revealed that the intermolecular disulfide bond was formed by H2O2-induced oxidation and cleaved by dithiothreitol-induced reduction. In addition, to investigate the solubility of SN-38 in the presence of protein capsules, we mixed the protein capsule of reduced-form with SN-38 suspension, and stirred at 37ºC for 48 hours. The resulting concentrations of SN-38 in PBS with 1 mM W54C/W112C, K58C/H111C, and K58C/W112C were 374 μM, 194 μM, and 349 μM, respectively. These values were approximately 200-fold higher than without protein capsules. SDS-PAGE analysis showed that the bond formation decreased in a time-dependent manner, and that new intermolecular disulfide bond was not formed in the protein capsules after 48 hours' incubation. From the above, we succeeded in generating drug delivery vehicles possessing openable and closable lids that are responsive in an oxidation-reduction environment.

PI-064 **Formation of Cytochrome cb562 Oligomers by Domain Swapping**

Takaaki Miyamoto[1], Mai Kuribayashi[1], Satoshi Nagao[1], Yasuhito Shomura[2], Yoshiki Higuchi[3,4], Shun Hirota[1]

1.-Graduate School of Materials Science, Nara Institutte of Science and Technology, 2.-Graduate School of Science and Engineering, Ibaraki University, 3.-Department of Life Science, Graduate School of Life Science, University of Hyogo, 4.-RIKEN SPring-8 Center

Domain swapping has been of interest as a mechanism of protein oligomerization, where a secondary structural region or a domain of one protein molecule is replaced with the corresponding region or domain of another protein molecule. We have previously shown that c-type cytochromes and myoglobin form oligomers by domain swapping.1,2 In this study, we show that a four-helix bundle protein cyt cb562, in which the heme of cyt b562 is attached to the protein moiety by insertion of two Cys residues, forms a domain-swapped dimer. Dimeric cyt cb562 was more stable than dimeric cyt b562 at 4 °C, showing that attachment of the heme to the protein moiety stabilizes the domain-swapped structure. Absorption and CD spectra of dimeric cyt cb562 were similar to the corresponding spectra of the monomer, showing that the active site and secondary structures were similar between the dimer and monomer. The redox potential of dimeric cyt cb562 was also similar to that of its monomer. The dissociation temperature of dimeric cyt cb562 was 50 °C, and its ΔH on dissociation to monomers was -13.3 kcal/mol (per dimer). According to X-ray crystallographic analysis, dimeric cyt cb562 exhibited a domain-swapped structure, where the two helices in the N-terminal region (helices 1 and 2) in a protomer and the other two helices in the C-terminal region (helices 3 and 4) of the other protomer interacted between each other. The heme coordination structure of the dimer was similar to that of the monomer. We have previously shown that domain-swapped oligomers of horse cyt c form through intermolecular hydrophobic interaction between the N- and C-terminal α-helices at the early stage of folding.3 It has been suggested that helices 2 and 3 form first at the initial stage of folding in wild-type apo cyt b562.4 Therefore, we propose that cyt cb562 forms a domain-swapped dimer when helices 2 and 3 interact intermolecularly at the initial stage of folding, whereas the intramolecular interaction of helices 2 and 3 results in formation of a monomer.

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PI-065 **A highly buried and conserved tryptophan residue close to the dimer interface in a cold-adapted phosphatase is phosphorescent and important for activity**

Jens Hjörleifsson[1], Bjarni Ásgeirsson[1]
1.-Department of Biochemistry, Science Institute, University of Iceland

A highly buried and conserved tryptophan residue close to the dimer interface in a cold-adapted phosphatase is phosphorescent and important for activity. Jens G. Hjörleifsson and Bjarni Ásgeirsson. Department of Biochemistry, Science Institute, University of Iceland, Dunhagi 3, 107 Reykjavik, Iceland. Alkaline phosphatase (AP) from Vibrio G15-21 is a cold-adapted dimeric enzyme with one of the highest catalytic efficiency reported for known APs. It contains five intrinsic tryptophan (Trp) residues and one additional Trp located on the C-terminal StrepTag used for expression and purification. In this study, we made several single Trp-substitutions to determine the role of each of the Trp in the fluorescence emission spectrum. We also determined their solvent exposure by acrylamide fluorescence quenching. The results indicate that Trp301, Trp460 and Trp475 are mostly responsible for the fluorescence emission. Quenching experiments with acrylamide indicated that all the Trp residues were about equally accessible for quenching, except Trp460 which was shown to be highly buried in the core of the protein. Interestingly, the enzyme was found to be highly phosphorescent at 10 °C, having two phosphorescence lifetimes. The longer lifetime is due to Trp460. Trp460 is located close to the dimer interface and points towards a helix in the active site where His277 binds an active-site zinc ion. In other APs, an aromatic amino acid is conserved in the location occupied by the Trp460 residue. In most cases for cold-adapted APs it is indeed a Trp. Interestingly, the mutation of the Trp460 to a phenylalanine affected both stability and activity of the enzyme. kcat/KM was 10-fold lower than for wild-type. Overall, this study reveals that Trp460 can be used as a phosphorescent probe of local dynamics and could possibly also serve to study the dimer-monomer equilibrium due to proximity to the dimer interface, an area clearly crucial for enzyme activity and stability.

PI-066 **Modulating protein-protein interaction with a molecular tether**

Helen Farrants[1], Oliver Hantschel[1], Kai Johnsson[1]
1.-École Polytechnique Fédérale de Lausanne (EPFL)

High-affinity scaffolds for protein-protein interactions, such as monobodies and DARPins can be engineered in vitro to bind to protein targets. We speculate that the affinity for the target protein can be modulated by incorporating these evolved scaffolds and a synthetic intramolecular tether into protein switches, in a protein construct of composed of SNAP-tag, a monobody and a circular permutated dihydrofolate reductase. The tether, attached to the construct via SNAP-tag, was composed of a linker and trimethoprim, which interacts reversibly with the circular permutated dihydrofolate reductase. We have investigated the affinity between the N-SH2 domain of the phosphatase Shp2 and an evolved monobody in such a protein construct using a FRET assay. When the intramolecular tether was bound the circular permutated dihydrofolate reductase ("closed" conformation), there was an increase in the affinity of the construct to the target N-SH2. In the presence of a small molecule competitor ("open" conformation) the affinity of the monobody construct to its target was reverted to the value reported in the literature. The intramolecular tether in these protein constructs combined with engineered scaffolds for protein-protein interactions may be a general approach towards protein switches.

PI-067 **LIL traptamers: artificial transmembrane proteins with minimal chemical diversity**

Daniel DiMaio[1], Erin Heim[1], Ross Federman[1], Lisa Petti[1], Jez Marston[1]
1.-Yale Unversity School of Medicine

Because most proteins are long polymers of amino acids with twenty or more chemically-distinct side-chains, there are an enormous number of potential protein sequences. Here, we report the construction of biologically active proteins with minimal chemical diversity. Transmembrane domains of proteins can specifically interact with other transmembrane domains to modulate the folding, oligomerization, and function of transmembrane proteins. For example, the bovine papillomavirus E5 protein is a 44-amino acid transmembrane protein that transforms fibroblasts to tumorigenicity by binding directly to the transmembrane domain of the platelet-derived growth factor β receptor (PDGFβR), resulting in ligand-independent receptor activation and cell transformation. These studies showed that a free-standing transmembrane domain could fold properly in cells and act in trans to modulate the activity of a larger transmembrane protein target. Because of the relative chemical simplicity of transmembrane domains and this ability to act even when not linked to more complex soluble protein domains, we reasoned that short transmembrane proteins could be used to define the minimal chemical diversity sufficient to construct biologically active proteins. To accomplish this, we infected cultured mouse cells with a retroviral library expressing 26-amino acid proteins consisting of an initiating methionine followed by a randomized sequence of leucines and isoleucines, two hydrophobic amino acids that differ only by the position of a single methyl group, and selected rare proteins with transforming activity. We isolated numerous proteins consisting of diverse sequences of leucine and isoleucine that cause morphologic transformation, escape from contact inhibition and focus formation, and growth factor independence. Genetic and biochemical analysis of these proteins indicate that like E5 they interact with the transmembrane domain of the PDGFβR to specifically activate the receptor and transform cells. Mutational analysis of individual proteins identified specific leucines and isoleucines required for transforming activity, and insertion of a single isoleucine at a particular position in a stretch of leucines is sufficient for activity. These proteins identify the minimal chemical diversity required to generate a biologically active protein and have important implications for biochemistry, protein evolution, protein engineering and synthetic biology.

PI-068 **Efficient Encapsulation of Enzymes in an Engineered Protein Cage**

Yusuke Azuma[1], Donald Hilvert[1]
1.-Laboratory of Organic Chemistry, ETH Zurich

Virus-like particles that are precisely loaded with functional cargo are an important tool to study the effect of spatial confinement and create novel entities with application in biotechnology and medicine. By genetic fusion to a positively supercharged green fluorescent protein (GFP(+36)), an enzyme retro-aldolase (RA) was efficiently targeted to the negatively charged lumen of an engineered protein cage, Aquifex aeolicus lumazine synthase variant 13 (AaLS-13). The encapsulation is quantitative under mild aqueous condition up to a mixing ratio of 45 guest enzymes per host cages. The chromophoric tag is used for precisely quantifying the enzyme concentration, which allows detailed characterization of the effect of encapsulation on the enzyme activity. The generality of the encapsulation system was examined with 8 structurally different enzymes.

PI-069 **Identification of disease-related antigen-specific human antibodies by a method that combines biopanning and high throughput sequencing from patient-derived scFv antibody library**

Yuji Ito[1], Yurie Enomoto[1], Shuhei Umemura[1], Aiko Fujiyama[1], Ryoko Mieno[1], Yukiko Kato[1], Dai-ichiro Kato[1]

1.-Graduate School of Science and Engineering, kagoshima University

Introduction and purpose: In the immune system, high affinity antibodies are generated by selection of B cells activated by antigen-stimulation followed by additional optimization through somatic hyper mutation of antibody genes. In the artificial antibody libraries, such as phage libraries, selection of specific antibody clones from the library is performed by in vitro selection process called biopanning and the subsequent binding screening. However, in spite of high efficiency of enrichment in biopanning, there is a possibility that we overlook the minor antigen-specific clones in the screening because of the limitation of the number of clones employed for screening. In recent years, high-throughput analysis of DNA sequences by the next-generation sequencer (NGS) has become available not only for genomic analysis of organisms but also repertoire analysis of antibodies. In this presentation, we report the successful isolation of a variety of antigen specific antibodies from patients-derived antibody phage library by a combination method of high throughput sequence analysis on NGS and biopanning. Method: We constructed two kinds of human single chain Fv (scFv) antibody libraries from pooled mRNA of five cancer patients and of a wheat allergy patient, respectively. After biopanning against a cancer antigen or wheat allergy antigen " gluten", the phagemid vector DNA prepared from the pooled phages before or after biopanning was used for PCR amplifications of VH genes, adding the index and adapter sequences for NGS analysis. The high throughput sequencing was performed on Miseq (illumina) using MiSeq Reagent Kits v3. After discarding the short sequences and low quality data, 5'-and 3'-reading sequences were unified by a Merge program. The frequencies (%) of all VH sequences were evaluated using a program based on Usearch 8.0 clustering software and the changes of the frequency (%) of each sequence between before and after panning were assigned as amplification rate. Results and discussion: VH sequences at each round of pooled phages after biopanning against cancer antigen were analyzed on NGS. After three rounds of biopanning, three clusters of antibody sequences were specifically enriched suggesting these are specific binders. To check this, scFv gene were regenerated by PCR using H-CDR3 specific primers and scFv-displaying phages reconstructed were subjected to binding analysis. All three phages showed a clear specific binding to cancer antigen in ELISA. Subsequently, to test the usefulness of this method, we applied it to identify allergen-specific scFv from allergy patient-derived antibody phage library. The phylogenetic tree analysis of VH sequences which showed the amplification rate higher than 2.5 by a single round of biopanning elucidated total eleven clusters of VH sequences. The VH sequences in the two clusters with the highest amplification factor were selected and the regenerated scFv-displayed phages were tested for binding analysis. The prepared scFv-displayed phages and also scFv proteins showed a clear binding ability to allergen. Thus, it is suggested that the analytical method of VH sequences on NGS before and after biopanning is very useful to isolate a variety of disease related antigen-specific novel antibodies quickly with high degree of certainty.

PI-070 **Biochemical analysis of the recognition helix of Z-DNA binding proteins: Roles in conformational specificity**

Yang-Gyun Kim[1], Xu Zheng[1], So-Young Park[1]
1.-Department of Chemistry, Sungkyunkwan University

Conversion of right-handed B-DNA into left-handed Z-DNA is one of the dramatic structural transitions in biological processes including gene regulation and chromatin remodeling. Z-DNA binding motif, Zalpha (Zα), was first discovered from human ADAR1. Subsequently, with sequence and structure similarity to the hZαADAR1, families of proteins including viral E3L, interferon-induced protein DAI (ZBP1) and PKZ has been identified to have Zα domain(s). Interestingly, the Zα domain of the E3L protein from vaccinia virus (vvZαE3L) was confirmed to have the ability of Z-DNA-binding, but it does not have the B-to-Z conversion activity. Here, we showed that the replacement of the α3-helix of vvZαE3L (vvZαE3L-α3) with that of hZαADAR1 results in acquiring the ability to converting B-DNA to Z-DNA. The detailed biochemical analysis of the α3-helix mutants of vvZαE3L further suggested that the contribution of positively charged residues in the C-terminal part of the α3-helix is crucial during the B-to-Z transition. In addition, hydrophobic residues of the N-terminal part of the vvZαE3L-α3 also influence on the B-to-Z conversion activity, possibly through forming a tightly-packed structure. In conclusion, our results revealed the previously-unknown contribution of amino acid residues existed in the α3-helix of the Zα domains to the B-to-Z conversion. Moreover, it strongly implies that such residues may play important roles in initiating conformational changes of DNA structure during the B-to-Z conversion event. [This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and future Planning (No. 2015R1A2A2A01008367).]

PI-071 **Photo switching of protein conformation**

Frank D. Sönnichsen[1], Matthias Lipfert[1], Hauke Kobarg[1], Anne Müller[1], Thisbe K. Lindhorst[1]
1.-Otto Diels Institute for Organic Chemistry, Christian-Albrechts-University

The ability of switching the activity of proteins at will is of great interest from an application point of view. One promising approach utilizes a protein modification with an organic photochromic molecule. Linking two protein side chains with the photochrome that undergoes a light induced conformational change, protein secondary and tertiary structure can be stabilized or destabilized and thus the structure dependent activity can be switched “on” and “off” by light irradiation. For this the photochrome must fulfil several requirements. Foremost, it must possess two states of comparable stability that differ significantly in their geometry. It must further be water soluble and non-toxic, and should not experience fatigue phenomena upon multiple irradiations. There are two classes of molecules that fulfil those requirements: azobenzenes and spiropyrans. We are pursuing two different strategies for the design of photoswitchable proteins. In the first approach we attach an azobenzene compounds to side chains of the alpha-helical antifreeze protein Type I. The end to end distance of the photochromic molecule is sterically compatible with the folded helix only in one form, photoisomerization therefore switches the folding state between an active helical state and an inactive unfolded form. In a second, more general approach we use the Trp-Cage domain as a switching unit. The Trp-Cage is the smallest known folded protein (20 amino acids). Its folding is induced by hydrophobic interactions of a tryptophan side chain in a short helical segment. After modification with a photochromic molecule in appropriate positions, its structure is rendered sensitive to the state of the chromophore. By creating protein chimera of such a Trp-Cage and biologically active peptides with helical propensity, we aim at conferring the light-dependent fold of the cage to the attached peptide moiety.

PI-072 **ADSETMEAS: Automated Determination of Salt-bridge Energy Terms and Micro Environment from Atomic Structures using APBS method, version 1.0**

Arnab Nayek[1], Shyamashree Banerjee[1], Parth Sarthi Sen Gupta[1], Biswa pratap Sur[1], Pratay Seth[1], Sunit Das[1], Nathan A Baker[2], Amal K Bandyopadhyay[1]

1.-Department of Biotechnology, The University of Burdwan, Burdwan, WB, India, 2.-Pacific Northwest National Laboratory

Salt-bridges are electrostatic interactions between groups of opposite charges. Net interaction energy (ΔΔGnet) of a salt-bridge is partitioned into bridge (ΔΔGbrd), desolvation (ΔΔGdsolv) and protein (ΔΔGprot) energy-terms of which estimation of ΔΔGdsolv and ΔΔGprot are only possible by computational means. Thus, general purpose Poisson-Boltzmann Equation solver: "Delphi" (in commercial package of INSIGHT-II) and "APBS" (Open-source) are popularly used to determine these energy-terms. Nevertheless, the computation-method is highly involved one than other uses of these solvers. Moreover, protein-specific salt-bridges, grid-points, center, hydrophobic-isosteres-mediated mutation-files of original charge-radius file and others are to be worked out prior to the computation. This might answer as to why only limited numbers of structure files (≤2% of crystal-structure-database) are worked out till date. At this juncture, an efficient fully automated all-in-one-procedure that could analyze large dataset in a single run would be useful. To the best of our knowledge, such procedure is truly lacking in public domain. At this end, our fully automated all-in-one procedure: ADSETMEAS (available freely at http://sourceforge.net/projects/ADSETMEAS/ along with detailed documentation) uses "APBS" method to compute component as well as net energy-terms of salt-bridges and redirect compact output in excelformat. Further, micro-environments of salt-bridges are also been reported based on the presence of polar, dipolar, acidic, basic and hydrophobic side-chains in their proximity. The procedure provides versatility to users in choosing a] model for computation of energy-terms to-date available in the literature and b] method (default or advanced) for parametric optimization in "APBS" calculations. It works in UNIX like environment including CYGWIN. It processes all proteins present in the working directory with any number of salt-bridges in them. A pre-released version of the procedure was successfully applied for energy-terms on 220 salt-bridges from 22 halophilic proteins. Overall, our ADSETMEAS provides intricate details on salt-bridge energetic from crystal structures and find application in the field of computational structural biology. These and other results will be discussed in the conference.

PI-073 **Next generation analgesics – targeting ion channels with antibody-drug conjugates (ADCs)**

Anna Wojciechowska-Bason[1], Clare Jones[2], Chris Lloyd[3]

1.-Postdoctoral Fellow, ADPE, Medimmune, Cambridge, 2.-RIA, Medimmune, Cambridge, 3.-ADPE

Ion channels are common targets for chronic pain therapies. Small molecule analgesics are widely used therapeutically, but due to poor specificity they often cause a wide range of side effects. As a result, efficacy of existing treatments is very limited. We believe that to achieve the required specificity and efficacy, a novel and innovative approach is required that would combine the potency of the small molecule with the selectivity of an antibody. Therefore, we propose to apply antibody-drug conjugates (ADCs) to deliver small molecules or peptides to ion channels in order to specifically modulate pain signalling pathways. Voltage-gated sodium channel Nav1.7 has a well characterised role in the perception of pain. Here we present the activity of the peptide Huwentoxin-IV (HWTX-IV) and small molecule inhibitors PTC-A, PTC-B and PTC-C on voltage gated sodium channels Nav1.7 and Nav1.6. In novel findings, we report that these inhibitors show little selectivity between the voltage-gated sodium channel family members, Nav1.6 and Nav1.7, and that the IC50 values and the impact on channel biophysics (voltage-dependence of activation and fast inactivation) of the inhibitors are largely similar for both channel types. Therefore, the use of HWTX-IV and other small molecule inhibitors of Nav1.7 for pain therapy could be dose-limited due to side effects mediated by the inhibition of channel Nav1.6. In conclusion, we propose that HWTX-IV and the investigated small molecule inhibitors could be used for the treatment of pain as part of a Nav1.7 antibody-drug conjugate (Nav1.7-ADC), establishing Nav1.7 specificity and minimising side effects.

PI-074 **Semi-synthesis and Evaluation of Parasitic GPI-Anchored Proteins**

Maria Antonietta Carillo[1], Daniel Varon Silva[1]
1.-Max Planck Institute of Colloids and Interfaces, Biomolecular System department

Malaria is one of the most infectious diseases caused by Plasmodium species parasites. The merozoite surface protein 1 (MSP1) is the most abundant protein on the surface of the Plasmodium species merozoite stage, which plays an important role during the erythrocytes invasion process [1]. MSP1 is synthesized as a ~200-kDa glycosylphosphatidylinositol (GPI) anchored protein precursor which is processed at the end of the schizogony into four different fragments. The primary processing step produces a complex of four fragments that are present on the merozoite surface. The secondary processing step at erythrocytes invasion results in the detaching of the complex from the surface, except for the C-terminal 19-kDa domain (MSP119), which remains anchored to the parasite surface by the GPI moiety. In human malarial infections, the GPI is considered to be a toxin that causes the expression of various host genes and induces a pro-inflammatory immune response, making it a valuable candidate for the development of anti-malarial drugs. In order to study the function of the GPI and evaluate the effects, MSP119 fragment has been expressed, purified and anchored to the synthetic GPI molecule using protein trans-splicing strategy based on the split intein method [2]. The role of the GPI moiety will be studied through protein folding experiments and the effect of the anchored protein will be evaluated in vitro in order to understand the function of the GPIs.

PI-075 **Assessment of UCH-L3 Substrate Selectivity using Engineered Ubiquitin Fusions with Varying Linker Lengths**

Peter Suon, Mario Navarro, John Love
1.-San Diego State University, 2.-San Diego State University, 3.-San Diego State University

Assessment of UCH-L3 Substrate Selectivity using Engineered Ubiquitin Fusions with Varying Linker Lengths Peter Suon, Mario Navarro, and John J. Love San Diego State University The Ubiquitin Proteasome System (UPS) is a complex system composed of multiple structural and functional elements that play key roles in cellular processes such as signal transduction, cell cycle regulation, apoptosis, and protein degradation. Proteins destined for degradation are first tagged with the protein, ubiquitin, which is covalently attached to internal lysine residues. Once the target has be degraded by the proteasome; the enzyme Ubiquitin Carboxy Hydrolase L3 (UCH-L3) is believed to prepare ubiquitin for additional rounds of ubiquitination by cleaving small peptides and chemical adducts from the ubiquitin C-terminus. Previously in our laboratory, protein substrates of UCH-L3 were engineered and used to characterize UCH-L3 substrate selectivity. The engineered substrates consisted of N-terminal monoubiquitinated test variants derived from Streptococcal protein G (protein Gβ1) and Staphylococcal protein A (SpAB). The thermal denaturation temperatures (Tm) of the fusion proteins were measured using circular dichroism and span a range of over 60 oC. More importantly, the rate of hydrolysis for the fusion proteins is demonstrated to be directly correlated to the Tm of the test variant fused to the C-terminus of ubiquitin. Previously, the engineered substrates were designed to emulate natural ubiquitin fusions and thus did not contain any 'linker' residues between the C-terminus of ubiquitin and the N-terminus of the test protein. To explore the effects of linker length on UCH-L3 hydrolysis we are engineering new UCH-L3 substrates that contain an unstructured 12 amino acid linker between ubiquitin and the test protein. To further explore the catalytic efficiency of UCH-L3 we will revisit diubiquitin (Ub-Ub), which is not hydrolyzed by UCH-L3, and will make mutations in the hopes of generating a hydrolysable substrate. Using rational design, the new variants will be engineered to destabilize the C-terminal ubiquitin to determine if this results in hydrolysis of the new Ub-Ub construct. The thermal stability of these new fusion protein substrates will be measured using circular dichroism spectroscopy (CD) and UCH-L3 hydrolysis rates will be characterized using existing assays. Our goal is to continue the use of engineered substrates to further explore the catalytic properties of UCH-L3 activity and the potential role in protein trafficking and degradation within living cells.

PI-076 **Beta-hairpins: Molecular Accessories for Helical Peptide Expression**

Melissa Lokensgard[1], John Love[1]
1.-San Diego State Uniersity

We present a biophysical study of a suite of helical proteins that have been modified to contain 12- and 17- amino acid additions on their termini that impart increased resistance to degradation in E. coli recombinant expression systems. The B domain of Staphylococcal Protein A (AB) and the homeobox DNA-binding domain from D. melanogaster Engrailed (En) are small 3-helix bundles. These domains do not appreciably accumulate in the E. coli BL21 (DE3) cytoplasm when expression in a pET vector is chemically induced. This is likely due to host protein degradation/recycling factors that function to efficiently degrade these two proteins. Addition of sequences encoding either of two amino-terminal beta-hairpins to either the N- or C-terminus of AB and En results in the accumulation of large amounts of these new chimeric proteins. Additionally, destabilization of the AB or En sequence does not abolish the expression enhancement effect of the beta-hairpin addition. We have investigated the biophysical origins and effects of the beta-hairpin additions using circular dichroism (CD) spectroscopy, and have determined that the added sequence does not significantly perturb the secondary structure of AB or En, nor does it significantly influence the unfolding temperature (Tm). While investigation into the origin of the accumulation effect is ongoing, we hypothesize that the addition of the sequence is disruptive to recognition events in the native protein degradation machinery in E. coli. Thus, this approach represents both a biotechnological tool for expressing helical peptides recalcitrant to expression, as well as a system well-suited to probing mechanisms of protein recycling and homeostasis.

PI-077 **Development of a semisynthetic method for the cell surface presentation of proteins**

Dorottya Németh[1], Balázs Schäfer[1], Éva Hunyadi-Gulyás[2], Zsuzsanna Darula[2], Csaba Tömböly[1]
1.-Biological Research Centre,Instute of Biochemisty,Laboratory of Chemical Biology, 2.-Biological Research Centre, Laboratory of Proteomics Research

Current Protein Society member: Jody McGiness; jmcginness@proteinsociety.org Cell surface proteins have important biological functions including signal transduction, cell adhesion or antigen presentation. A special class of these proteins are lipidated proteins containing a glycosylphosphatidylinositol (GPI) glycolipid moiety at the C-terminus. The lipid chains of the GPI anchor molecule are responsible for the membrane association of the attached protein. A unique feature of GPI-anchored proteins is that after isolation they can be reinserted into the membrane of recipient cells with the retention of the biological function. Accordingly, the exogenous introduction of fluorescent GPI-anchored protein analogues into cell membranes is a useful method for visualizing the cellular traffic of membrane associated proteins and for engineering cell surfaces. We have recently shown that cholesterol can be applied for anchoring proteins to the plasma membrane of live cells without perturbing the membrane. In order to introduce proteins containing covalent modifications that are not genetically encoded, an enzymatic method was considered and fused with the C-terminal cholesterylation method. The usefulness of the method is demonstrated via the preparation of multimeric model proteins of 40 kDa monomers, that is an appropriate representation of the ligation of domain size proteins.

PI-078 **Transmembrane domain dimerization drives p75NTR partitioning to lipid rafts**

Irmina García Carpio[1], Marçal Vilar[1]
1.-Sociedad de Biofísica de España. SBE

p75 neurotrophin receptor (p75NTR), is best known for its role in mediating neuron cell death during development or after injury but it also regulates cell proliferation, axon guidance or survival. The key to understand its signaling could rely in its structure and conformational states. It has been described that p75 forms disulfide-linked dimmers through the Cys257 in the transmembrane domain which are essential for its NGF mediated signaling. Previous studies have shown that p75 is present in lipid rafts, where it interacts with intracellular adaptors to activate different signaling pathways. We design several p75 mutants in the TM domain that impairs dimerization and study the role of TM domain dimerization in lipid rafts recruitment. Our analysis suggests that p75 TM domain dimerization influences lipid raft partitioning. These results could be a key role to understand its signaling and processing

PI-079 **Bioluminescent sensor proteins for therapeutic drug monitoring of the monoclonal antibody Cetuximab**

Martijn Van Rosmalen[1], Remco Arts[1], Brian Janssen[1], Natalie Hendrikse[1], Dave Wanders[1], Maarten Merkx[1]

1.-Laboratory of Chemical Biology / Institute of Complex Molecular Systems

Therapeutic Drug Monitoring (TDM) – adapting the drug dosage scheme to the individual patient's pharmacokinetic and pharmacodynamic characteristics – is still uncommon for therapeutic monoclonal antibodies, despite preliminary studies showing its potential benefits. One of the factors impairing TDM implementation is the lack of equipment and trained personnel to regularly measure drug concentrations in patients receiving treatment. Point-of-care diagnostic devices which could be used by patients themselves or by their general practitioners would greatly advance the feasibility of TDM. Here we present a biosensor for the therapeutic monoclonal antibody Cetuximab. We developed a series of cyclic peptides that specifically recognize Cetuximab, covering a fourfold range of affinities, and incorporated these cyclic peptide sequences into a set of luminescent sensor proteins. The sensors translate cetuximab concentrations into a change in emission color that can be read out using a mobile phone camera. Together, these sensors can quantify cetuximab levels within the relevant therapeutic concentration range and we propose that they can be used for Therapeutic Drug Monitoring applications.

PI-080 **Genetically encoded biosensor for cell permeability of inhibitors of the p53-HDM2 interaction**

Silvia Scarabelli[1], Thomas Vorherr[2], Kai Johnsson[1]
1.-Ecole Polytechnique Fédérale de Lausanne, 2.-Novartis Institute for BioMedical Research

The evaluation of the permeability across the cellular membrane is a key step in the development of therapeutics, since it affects the distribution and the efficacy of the latters. Reliable and versatile techniques for the determination of structural permeability determinants of molecules and information about the entry kinetics are still missing. We introduced in the past a class of semi-synthetic ratiometric sensor proteins (Snifits) that has been shown to be suitable for the measurement of intracellular metabolites concentrations. Here we describe a totally genetically encoded sensor based on the Snifits modular design for the assessment of the cell permeability of small molecules and peptides inhibitors of the protein-protein interaction between p53 and HDM2. We show that our sensor detects the presence of HDM2-binding stapled peptides in vitro, and, when expressed in mammalian cells, it responds to the perfusion of the known small molecule HDM2 inhibitor Nutlin-3a. Moreover, experiments made with an automated microscope show that the sensor is suitable for measuring and comparing the kinetics of entry of different kinds of inhibitors in the cytosol of living cells. In parallel, we are developing an HCAII-based sensor protein for the sensing of sulfonamides and eventually their peptide derivatives. We show that the sensor responds to the presence of different kinds of HCA-inhibitors in vitro and in perfusion experiments. This second sensor would broaden the range of molecules and peptides whose permeability can be studied with our tools beyond the family of the HDM2-binders. Our sensors overcome the limitations of the already existing techniques for measurements of permeability while offering a simultaneous measurement of the cell permeability and of the binding efficiency of small molecules and peptides of interest.

PI-081 **Archer: Predicting protein function using local structural features. A helpful tool for protein redesign.**

Jaume Bonet[1], Javier Garcia-Garcia[1], Joan Planas-Iglesias[2], Narcis Fernandez-Fuentes[3], Baldo Oliva[1]

1.-Structural Bioinformatics Lab, GRIB, UPF, 2.-Division of Metabolic and Vascular Health, University of Warwick, 3.-IBERS, Abersystwyth University

The advance of high-throughput sequencing methodologies has led to an exponential increase of new protein sequences, a large proportion of which remain unannotated. The gap between the number of known proteins and those with assigned function is increasing. In light of this situation, computational methods to predict the function of proteins have become a valid and necessary strategy. Here we present Archer, a server that exploits ArchDB's hierarchy of super-secondary structures to map GO and Enzyme functions upon protein regions and, thus, infer the function of a protein. The server relies on either the sequence or structure of the protein of interest and returns the mapping of functional subclasses extracted from ArchDB. Moreover, it computes the functional enrichment and significance of each subclass, combines the functional descriptors and predicts the function of the query-protein. Combining the functional enrichment analysis of the super-secondary structures with the structural classification of ArchDB, users can select variants of the target sequence that swap the region of a super-secondary structure by another that putatively fits in the same scaffold minimizing the effect on the global tertiary structure. Only variants that modify the predicted function are offered for selection, thus providing a rational, knowledge-based, approach for protein design and functionalization. The Archer server is accessible at http://sbi.imim.es/archer.

PI-082 **Light-induced interaction of protomers in bacterial phytochrome from Rhodopseudomonas palustris**

Taras Redchuk[1], Evgeniya Omelina[1], Konstantin Chernov[1], Vladislav Verkhusha[1,2]
1.-Dept. of Biochemistry, Faculty of Medicine, University of Helsinki, 2.-Dept. of Anatomy and Structural Biology, Albert Einstein College of Medicine

Phytochromes are natural photoreceptors known to regulate photosynthesis in plants, fungi and bacteria. Phytochromes found in bacteria share common architecture and consist of a PAS-GAF-PHY photosensory core and a C-terminal output module, responsible for biological function. A bacterial phytochrome, BphP1, from Rhodopseudomonas palustris undergoes reversible conversion from the far-red absorbing state (Pfr) to the red-absorbing state (Pr) followed by the conformational change upon 740 nm light irradiation. As most of bacterial phytochromes, BphP1 forms a dimer. It was shown that 740 nm light causes a protomer swapping between the BphP1 dimers; and likely, the output module is involved in this process. However, the mechanism of the light-induced swapping is poorly studied. We tested an ability of the protomer swapping between BphP1 dimers using pull-down biochemical assay. For this, strep-tagged BphP1 was immobilized on Strep-Tactin sepharose beads in the presence of untagged BphP1 fused to mRuby2 at different concentrations. After incubation, the proteins were eluted and visualized in SDS-gel using a zinc-induced fluorescence assay. An amount of the bound to beads protein was estimated by densitometry. It was found that more than 75% of heterodimers (strep-tagged-BphP1 and BphP1-mRuby2) form within 2.5 h of incubation under 740 nm light at 8-fold excess of one of the interacting partners. In darkness, the swapping was much slower. In the similar setup we checked the amount of heterodimers after 15, 30 and 120 min of incubation. No difference was observed for different time points, suggesting that the protomer swapping is relatively fast process. Next, a role of the C-terminal effector domain of BphP1 in the light-induced interaction was studied. For this, kinetics of the Pfr-to-Pr transition was analysed by measuring of absorbance at 680 nm and 740 nm for full-length BphP1 and a BphP1 mutant with the deleted C-terminal domain. While full-length BphP1 showed the normal Pfr-to-Pr transition, absorbance of the mutated BphP1 at 680 nm did not raise. However, 740 nm absorbance changes were similar for both proteins; and surprisingly, the similar dark relaxation kinetics was observed. We propose that the impaired Pfr-to-Pr transition is caused by restricted Pr conformation in the mutant rather than by fast Pr-to-Pfr relaxation. Understanding the mechanisms of the BphP1 light-induced structural changes and the protomer interaction should advance engineering of bacterial phytochromes into fluorescent probes and optogenetic tools.

PI-083 **Luminescent sensor proteins for antibody detection in solution**

Remco Arts[1], Susann Ludwig[1], Marina van Vliembergen[1], Vito Thijssen[1], Stan van der Beelen[1], Ilona den Hartog[1], Stefan Zijlema[1], Maarten Merkx[1]
1.-Eindhoven University of Technology

Antibody detection is an integral part of many diagnostic strategies, most crucially so when infectious diseases are involved. Currently used assays, such as ELISA or SPR, enable detection of antibodies in the laboratory with high sensitivity, yet a translation of these technologies to an application outside of the laboratory setting is far from trivial. Problematically, the burden of disease for many infectious diseases is carried precisely by those countries where access to laboratory facilities is severely limited. We therefore developed a novel, one-step assay that allows the detection of antibodies directly in solution using a luminescent sensor protein. Our strategy is based on the use of a bright luciferase, NanoLuc, tethered to a green fluorescent protein (mNeonGreen) via a semi-flexible linker containing two epitope sequences. Crucially, two small helper domains were fused to the protein termini. These domains keep NanoLuc and mNeonGreen in close proximity in the absence of antibody, enabling efficient Bioluminescence Resonance Energy Transfer (BRET). Binding of antibody to the epitopes in the sensor proteins linker domain pulls the BRET partners apart, effectively changing the color of emission from green to blue. The assay allowed the detection of picomolar amounts of anti HIV1-p17 antibodies directly in solution, both under optimized buffer conditions and in blood plasma. In principle. the modular sensor architecture should allow detection of any antibody with a well-defined epitope of sufficient affinity. To demonstrate this, the HIV-epitopes were substituted for two HA-tag epitopes, yielding a sensor that enabled the detection of picomolar amounts of anti-HA antibodies. The simple optical readout provided by the sensor system allowed us to record the emitted signal with a conventional mobile phone camera. A simple software application that analyzes the image based on RGB values sufficed to interpret the recorded image vis-á-vis the presence of antibody. Bearing in mind the eventually envisioned application in a point-of-care diagnostic setting, this combination of sensor recording and interpretation using nothing more than a mobile phone and a software application holds considerable diagnostic potential. Beyond point-of-care diagnosis of infectious diseases, a simple assay to detect and quantify antibodies directly in solution could also have a substantial impact in other fields. Antibodies are ubiquitous in biotechnology, and this is reflected by the plethora of potential sensor applications, which range from a role in microfluidic circuits or monitoring the biotechnological production of antibodies, including validation of bispecificity, to veterinary applications, diagnosis of autoimmune diseases and monitoring the success of vaccination campaigns.

PI-084 **Tertiary Structural Propensities Reveal Fundamental Sequence-Structure Relationships**

Fan Zheng[1], Jian Zhang[2], Gevorg Grigoryan[1,2]

1.-Department of Biological Sciences, Dartmouth College, 2.-Department of Computer Science, Dartmouth College

The continually growing Protein Data Bank (PDB) has been a key resource for general principles of protein structure. For example, parsing structural observations in the PDB into simple geometric descriptors has given rise to statistical energy functions. Here we present a novel strategy for mining the PDB on the basis of local tertiary structural motifs (TERM). We define a TERM to be the structural fragment that captures all local secondary and tertiary structural environments of a given residue, and query the PDB to obtain quantitative information for each TERMs. First, we show that by breaking a protein structure into its constituent TERMs, we can describe its sequence-structure relationship via a new metric we call “structure score.” Using submissions in recent Critical Assessment of Structure Prediction (CASP) experiments, we find a strong correlation (R = 0.69) between structure score and model accuracy - a performance that exceeds leading atomistic statistical energy functions. Next, we show that querying TERMs affected by point mutations enables the quantitative prediction of mutational free energies. Our simple approach performs on par with state-of-the-art methods Fold-X and PoPMuSiC on ~1300 mutations, and provides superior predictions in certain cases where other methods tend to fail. In all, our results suggest that the data available in the PDB are now sufficient to enable the quantification of much more sophisticated structural observations, such as those associated with entire TERMs, which should present opportunities for advances in computational structural biology techniques, including structure prediction and design.

PI-085 **Exploiting natural sequence diversity for protein crystallization**

Sergio Martínez-Rodríguez[1], Valeria Risso[1], José M Sanchez-Ruiz[1], José A. Gavira[2],
1.-Departamento De Química-Física, Universidad De Granada, 2.-Laboratorio De Estudios Cristalográficos, IACT-CSIC-UGR Granada

During the last decade, different rational and high-throughput approaches have been successfully applied in the protein crystallography field to widen the||so-called "protein crystallization bottleneck" [1,2]. Despite the enormous efforts carried out by our community, the statistics presented by Structural Biology Consortiums [3] suggest that so far only the easy-to-pick fruit has been attained; thus, new approaches are necessary to further expand the crystallization limiting step to relevant targets. On the basis of previous hypothesis suggesting that the difficulties found in protein crystallization might be a result of evolutionary negative design [4], we have used two different protein engineering approaches exploiting natural sequence diversity using beta-lactamase as toolbox: i) ancestral reconstruction and ii) consensus approach [5]. Both approaches resulted in hyperstable and promiscuous ancestral derivatives. Furthermore, our initial crystallization results also suggest that both approaches increased the crystallizability of the resulting enzymes when compared to the extant TEM-1 beta-lactamase. [1] Doerr A. Widening the protein crystallization bottleneck. Nat. Methods. 2006. 3:961. [2] Warke A, Momany C. Addressing the protein crystallization bottleneck by cocrystallization. Cryst. Growth Des. 2007. 7:2219–2225. [3] Protein Structure Initiative; http://sbkb.org/ [4] Doye JP, Louis AA, Vendruscolo M. Inhibition of protein crystallization by evolutionary negative design. Phys Biol. 2004 1:P9-13. [5] Risso VA, Gavira JA, Mejia-Carmona DF, Gaucher EA, Sanchez-Ruiz JM. Hyperstability and substrate promiscuity in laboratory resurrections of precambrian β-lactamases. J Am
Chem Soc. 2013. 135:2899-2902.

PI-086 **Synthesis of selectively functionalized adiponectin**

Andreas Mattern[1], Annette Beck-Sickinger[1]
1.-University of Leipzig, Institute of Biochemistry

The adipocyte-derived hormone adiponectin has become a key player for the understanding of overweight related diseases like obesity, diabetes, atherosclerosis or the metabolic syndrome. One of its major functions are the insulin sensitizing effects, which are mediated by the activation of AMPK, p38-MAPK and PPARα (1). Furthermore adiponectin is involved into glucose regulation and fatty acid oxidation. Recently, three adiponectin receptors AdipoR1, AdipoR2 and T- cadherin have been described while an unknown fourth receptor is hypothesized (2). For only two of them (AdipoR1 and AdipoR2) the signaling transduction via adiponectin has been confirmed (3). In order to find new binding partners or co-receptors, we cloned and expressed full length adiponectin as a fusion protein with a C-terminal intein and a chitin binding domain (CBD) as well as an N-terminal His10-tag. By using the IMPACT-system, the fusion protein was cleaved to form the corresponding thioester. To separate the starting materials as well as the cleaved intein chitin binding domain, the purification was performed with chitin beads. Furthermore, the product was concentrated by Ni-NTA-affinity chromatography. Accordingly, the obtained adiponectin thioester was reacted with a TAMRA- or a biotin labeled peptide, respectively, to receive the corresponding ligation product. Finally the functionalized adiponectin was purified by size exclusion chromatography. Further studies will allow screening for interacting molecules in cell and tissue derived samples. (1) Hui et al. (2012) British Journal of Pharmacology 165, 574–590. (2) Awazawa et al. (2011) Cell Metabolism 13, 401–412. (3) Heiker et al. (2010) Biol. Chemistry 39, 1005-1018.

PI-087 **De novo catalysis in ancestral protein scaffolds**

Valeria A. Risso[1], Sergio Martinez-Rodriguez[1], Adela M. Candel[1], David Pantoja-Uceda[2], Mariano Ortega-Muñoz[3], Francisco Santoyo-Gonzalez[3], Marta Bruix[2], José A Gavira[4], Jose M. Sanchez-Ruiz[1]

1.-Departamento de Quimica Fisica, Facultad de Ciencias University of Granada, 2.-Dpto. de Quimica Fisica Biologica. Instituto de Quimica Fisica Rocasolano, 3.-Departamento de Quimica Organica, Facultad de Ciencias University of Granada, 4.-Laboratorio de Estrudios Cristalograficos, IACT-CSIC-UGR Granada

Rational design of non-natural enzyme activities has proved challenging. Here, we report the introduction of catalysis of the Kemp elimination (a model of proton abstraction from carbon) in scaffolds corresponding to Precambrian nodes in the evolution of the antibiotic resistance protein β-lactamase. We used a single-mutation, minimalist approach based on chemical intuition, and obtained catalysis levels similar to those reported in the literature for computational Kemp-eliminase designs involving multiple mutations. Remarkably, the approach was unsuccessful when performed on modern β-lactamases. We provide experimental evidence that enhanced conformational flexibility contributes to the success of the minimalist design in the ancestral scaffolds. This work has implications for the understanding of function emergence in protein evolution and demonstrates the potential of ancestral protein resurrection in enzyme engineering and design.

PI-088 **Exploring the Importance of Dimerization for DJ-1 Function through Engineered Domain Fusions**

Sierra Hansen[1], Jiusheng Lin[1], Mark Wilson[1]
1.-University of Nebraska

Parkinson's Disease is a progressive neurodegenerative disease that affects approximately 6.3 million people worldwide and is characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta. DJ-1 (PARK7) is one of several genes that are mutated in rare forms of familial parkinsonism. DJ-1 is a dimeric cytoprotective protein that defends against oxidative stress and preserves mitochondrial function. Dimerization of DJ-1 is thought to be essential for this function, as some disease-associated mutations cause poor folding and disrupt the DJ-1 dimer. However, recent reports suggest that DJ-1 may be functional as a monomer. To test this, we have engineered a non-dissociable DJ-1 dimer that is a fusion of two human DJ-1 domains. This construct cannot dissociate into monomers and thus will provide a stringent test of the importance of monomeric DJ-1. Our engineered construct is modeled on plant DJ-1 homologs, which feature naturally occurring duplicate DJ-1 domains separated by a small (19 amino acid) linker region. Using X-ray crystallography, we confirmed that this engineered non-dissociable human DJ-1 dimer has identical structure to the naturally occurring dimeric protein. We have investigated the influence of enforced dimerization of the pathogenic effects of the parkinsonian L166P and L10P mutations. CD spectroscopic analysis reveals that single and double L166P mutations in the non-dissociable DJ-1 dimer maintain a higher degree of structure than L166P mutations in the native protein. Additional characterization of the protective capacity and subcellular trafficking of this non-dissociable DJ-1 dimer is underway.

PI-089 **The purification, crystallization and preliminary characterization of SdrE from S. aureus**

Deqiang Wang[1], Ke Chen[1], Jun Zhang[2]

1.-Key Laboratory of Molecular Biology on Infectious Disease, 2.-The Department of Cell Biology and Genetics

The purification, crystallization and preliminary characterization of SdrE from S. aureus Staphylococcus aureus (S.aureus) is an important human opportunistic pathogen which colonizes about 20% of the human population persistently [1]. Surface proteins of S.aureus can excretion a kind of sortase, which represents a surface organelle responsible during the pathogenesis of bacterial infection the host circulation [2]. Sdr proteins were a component of cell wall anchored family proteins, including SdrC, SdrD and SdrE [3]. SdrE could combine with the complement regulatory protein factor H to escape the alternative pathway of complement [4]. To further investigate the functions of SdrE, we have expressed and purified the adhesive domain (residues 141-'06:15), and crystallized the recombinant protein. In addition, we also constructed the mutant S.aureus, and the cell experiments confirmed that SdrE gene participate in the bacteria invasion. Refereces: [1] Miller LS., et al., Nature Reviews 2011,8,505-518; Brien LO., et al., Molecular Microbiology, 2002, 44:1033-1044; [3] Sitkiewicz I., et al., Antonie van Leeuwenhoek, 2011, 99:409-416; [4] Sharp JA., et al., PLoS One, 2012,7(5):e38407

PI-090 **Structure based modifications of the bacterial microcompartment shell protein PduA**

David Leibly[1,2], Julien Jorda[2], Sunny Chun[3], Alan Pang[2], Michael Sawaya[2], Todd Yeates[1,2,3]
1.-Department of Chemistry and Biochemistry, University of California, 2.-UCLA-DOE Institute for Genomics and Proteomics, 3.-Molecular Biology Institute, University of California

Bacterial Microcompartments (BMCs) are proteinaceous organelles that sequester key metabolic reactions to increase enzymatic efficiency or to prevent the loss of volatile or toxic intermediates. There is an increasing desire to engineer BMCs for non-native enzymatic processes. It is thought this will increase multi-enzyme pathway efficiency and allow the expression pathways that may produce toxic or volatile intermediates in bacteria. The mechanisms of small molecule transport and retention of toxic intermediates by BMCs remain poorly understood. Better understanding of the BMCs pores critical to engineer BMCs for these non-native pathways. In order to better understand the BMC pore we have undertaken structure-guided modifications of the the hexameric PduA shell protein of the 1,2-propanediol utilization microcompartment (Pdu MCP). These modifications include pore mutations in an attempt to alter substrate specificity and permutations of PduA to allow more drastic alterations to the structure of the protein. Crystal structures of PduA pore mutants, solved to atomic resolution (2-3.3Å) provide evidence of the pore residues that confer specificity. Further, a PduA permutation (PduAp) has resulted in a closed icosahedral cage. This novel PduAp cage shows a pH and salt dependent assembly and may serve as a reaction vessel or be utilized for cargo delivery.

PI-091 **Targeted conformational transitions of large and multimeric proteins by an efficient elastic network based technique**

Yasemin Yesiltepe[1], Doga Findik[1], Arzu Uyar[1], Deniz Turgut[2], Rahmi Ozisik[2], Turkan Haliloglu[1], Pemra Doruker[1]

1.-Bogazici University and Polymer Research Center, 2.-Rensselaer Polytechnic Institute

ANM–MC is a computationally efficient, coarse-grained simulation technique that integrates anisotropic network model (ANM) with knowledge-based Monte Carlo (MC) energy minimization method (1, 2). ANM–MC is used to identify targeted transition pathways and intermediates between open and closed states of proteins. At each step of this iterative technique, the protein is deformed along the collective ANM mode showing the best overlap with the target direction and its energy is minimized via short MC run. In this work, optimization of simulation parameters (number of MC moves and their perturbation strength, ANM deformation factor in each cycle and force constant for backbone bonds) was performed in order to increase the efficiency of this technique. As a result, this technique can now be applied to much larger systems and conformational changes. The transition pathway between apo and DNA-bound conformations of the yeast RNA polymerase, which is a hetero-10-mer with more than 3500 residues, will be presented here. Moreover, the pathway intermediates for more than 10 diverse proteins were analyzed in terms of changes in local strain energy and backbone torsional angles during apo-to-complex transitions. Certain residues interacting with the ligand are detected to exhibit large changes with respect to any of these two parameters for more than half of the proteins in our dataset. References: 1. Kantarci-Carsibasi, N., T. Haliloglu, and P. Doruker. 2008. Conformational Transition Pathways Explored by Monte Carlo Simulation Integrated with Collective Modes. Biophys. J. 95:5862–5873. 2. Uyar, A., N. Kantarci-Carsibasi, T. Haliloglu, and P. Doruker. 2014. Features of Large Hinge-Bending Conformational Transitions. Prediction of Closed Structure from Open State. Biophys. J. 106:2656–2666.

PI-092 **Continuous directed evolution of receptor-selective ?-endotoxins for overcoming insecticidal resistance**

Ahmed Badran[1,2], Victor Guzov[3], Qing Huai[3], Melissa Kemp[3], Prashanth Vishwanath[3], Artem Evdokimov[3], Farhad Moshiri[3], Meiying Zheng[3], Keith Turner[3], David Liu[1,2]

1.-Department of Chemistry and Chemical Biology, Harvard University, 2.-Howard Hughes Medical Institute, Harvard University, 3.-Monsanto Company

Transgenic crops have radically reshaped the agricultural landscape. Since their introduction in the late 1990s, transgenic crops have affected economic gains greater than US$110 billion globally due to reduced production costs and increased yield gains. Crops modified to produce biological insecticides derived from the soil bacterium Bacillus thuringiensis (Bt) are among the most robust methods of pest control. Bt toxins offer many advantages over traditional insecticides, chiefly their inability to affect human biology and exquisite selectivity for defined pest species. However, the evolution of resistance to Bacillus thuringiensis ð-endotoxins (Bt toxins) in insects has been widely observed in the field, and greatly threatens the use of this mechanism of pest control in the future. We developed a Phage-Assisted Continuous Evolution (PACE) platform for the rapid generation of high-affinity protein-protein interactions and validated the system by evolving known high affinity antibody mimetics in <5 days of PACE. We applied this system to the evolution of the Bt toxin protein Cry1Ac to recognize a non-cognate cadherin-like receptor from Trichoplusia ni, a pest for which Bt toxin resistance has been observed in both the laboratory and the field. The resulting evolved Cry1Ac variants exhibits high affinity for the target receptor, and kill insect cells more potently than wild-type Cry1Ac. Our findings establish that the directed evolution of novel receptor recognition in Bt toxins can be used to target resistant pests, and has far-reaching implications for biological reagents and therapeutics.

PI-093 **Optimization of a designed protein-protein interface**

Brian Maniaci[1], Collin Lipper[2], John J. Love[1]
1.-San Diego State University, 2.-University of California

Protein-protein interactions play key roles in practically every biological process. Protein-protein interactions vary with composition, affinity, and lifetime of the complex. Studying designed protein-protein interactions will provide insight into the underlying principles of complex assembly and formation. Computational protein docking and amino acid sequence design were used previously to generate protein dimers from monomeric proteins. The normally monomeric β1 domain of Streptococcal protein-G (GB1) was computational docked to itself, followed by optimization of the interfacial side chains. Two variants, MonomerA and MonomerB, were computationally derived as a result of a designed protein-protein interface. These designed proteins were characterized using analytical ultracentrifugation and heteronuclear NMR techniques. This design resulted in a pair of protein monomers that formed a heterodimer of modest binding affinity. A tetrahedral metal-templated interface design strategy was implemented in an attempt to strengthen the MonomerA-MonomerB complex by introducing cross-monomer metal coordination. Another advantage of using the metal-templated interface is the ability to control the protein-protein interaction both temporarily and spatially. A number of newly engineered variants of Monomer A and Monomer B with metal coordination sites were designed, produced, and tested for increased affinity of the protein-protein complex. While the generation of a metal-templated MonomerA-MonomerB complex was unsuccessful, we were able to obtain MonomerA variants that form a homodimer assembly only in the presence of Zinc (II) ions. The crystal structures of metal-templated MonomerA variants in the presence of zinc provide an explanation for the observed dimer formation. The crystal structure indicates that the protein-protein interaction is not driven by the designed protein interface, but rather non-specific association via edge-strand interactions. New variants were designed with the goal of engineering a high affinity homodimer in a helix-to-helix orientation as the originally designed protein-protein interface. Current evaluation of MonomerA variants for self-association via metal coordination are being evaluated using size exclusion chromatography with a multi-angle light scattering detector for oligomerization state quantification. The results of this protein design project should lead to a greater understanding of the biophysical parameters that drive natural protein-protein interactions.

PI-094 **Continuous evolution of site-specific recombinases with highly reprogrammed dna specificities**

Jeffrey L Bessen[1,2], David B Thompson[1,2], David R. Liu[1,2]

1.-Department of Chemistry & Chemical Biology, Harvard University, 2.-Howard Hughes Medical Institute, Harvard University

The ability to precisely modify the genome of human cells has enormous potential as a novel therapy and a powerful research tool. In contrast to reprogrammable nucleases, such as TALENs or a Cas9/sgRNA pair – which specifically cleave DNA but then rely on stochastic host cells processes to effect gene insertion – site specific recombinases directly catalyze genomic integration with high efficiency. A major limitation of this approach is that recombinases, such as Cre, natively bind with high specificity to long DNA target sequences (LoxP in the case of Cre) that do not exist in the human genome. Previous attempts at evolving Cre resulted in modest changes to its specificity, or required hundreds of rounds of manual protein evolution. We developed and validated a Phage Assisted Continuous Evoluiton (PACE) selection for rapidly altering the DNA specificity of Cre recombinase towards a site present in a human genomic safe harbor locus. The PACE experiments resulted in Cre variants capable of recombining a substrate with nearly 50% of the nucleotides altered compared to LoxP. We successfully used one of these variants to integrate exogenous DNA into the genome of unmodified human cells. We are currently using sequencing methods to determine the specificity of the new recombinase clones.

PI-095 **Generation of comprehensive deletion libraries mediated by in vitro transposition**

Aleardo Morelli[1], Burckhard Seelig[1]
1.-University of Minnesota

Generation of comprehensive deletion libraries mediated by in vitro transposition Analysis of protein enzymes and ribozymes from nature, and from in vitro evolution, revealed that deletions of up to dozens of amino acids (or nucleotides) can be structurally tolerated. Furthermore, shortened variants can exhibit better stability and increased catalytic activity. In order to investigate the effects of deletions, we developed a new procedure based on in vitro transposition to build libraries of more than 10,000 deletion mutants in three to four days. We tested our procedure on DNA sequences coding for an artificial RNA ligase called ligase 10C. We used the generated library for an mRNA display selection, and isolated two active mutants containing 18 and 13 amino acids N-terminal deletions.

PI-096 **Structural characterization of PpsC, a multi-domain polyketide synthase from Mycobacterium tuberculosis using a fragment-based approach**

Alexandre Faille[1], Nawel Slama1[1], Anna Grabowska[1], David Ricard[1], Annaik Quémard[1], Lionel Mourey[1], Jean-Denis Pedelacq[1]

1.-Institut de Pharmacologie et de Biologie Structurale

Polyketide synthases are of great interest in numerous scientific fields. They are composed by multiple domains, each having a different role to play in the catalysis of sequential reactions including condensation, reduction and esterification. Their reaction products, named polyketides, represent a large variety of chemical compounds, from antibiotics to immunosuppressors or even anticancer drugs. PpsC is a 231 KDa polyketide synthase, organised into six catalytic domains (KS-AT-DH-ER-KR-ACP) with singular functions. Along with other type I polyketide synthases, PpsC is responsible for the biosynthesis of an essential polyketide for the virulence of Mycobacterium tuberculosis (Mtb) and thus is a target of choice for the design of inhibitors. To date, no structural information of any type I Polyketide synthase in its entire form has been described. Main reasons are the length of these large size enzymes and the flexibility imposed by the linkers between domains, thus making them very difficult to crystallize. Numerous questions about domain-domain interactions, spatial arrangement of this complex machinery, substrate specificity and stereochemistry are still unanswered. Addressing the structural and functional characterization of PpsC would then help answering these questions and provide valuable information for drug design. To overcome the length- and flexible- dependent problem originating from the presence of multiple domains and linkers, we decided to study domains expressed alone. For this purpose, we used our domain trapping strategy to identify soluble fragments representing a single domain from PpsC [1]. It has the advantage of not relying on the bioinformatically designed domain boundaries and can even sometimes include parts of linkers to obtain more soluble fragments. Using this strategy, we were able to identify relatively small and highly soluble fragments representing each domain of PpsC, thus facilitating the downstream structural and functional characterization. More than 20 fragments have been submitted to crystallization trials. Among these, 5 gave crystals and allowed us to determine the X-ray structure of PpsC AT, ER, in addition to the DH domain in complex with a substrate analog for which activity was confirmed in vitro. [1] J.D. Pedelacq et al. Experimental mapping of soluble protein domains using a hierarchical approach. Nucleic Acids Res. 2011 October; 39(18): e125.

PI-097 **Computational design of tighter protein-ligand interfaces**

Brittany Allison[1], Brian Bender[2], Jens Meiler[1,2]
1.-Vanderbilt University, Department of Chemistry, 2.-Vanderbilt University, Department of Pharmacology

The computational design of proteins that bind small molecules remains a difficult challenge in protein engineering. The ability to computationally design native-like interactions with high accuracy and efficiency would be an asset towards therapeutic development, enzyme design, and engineering functional proteins. We have developed a systematic approach to designing interfaces. We first identify ligands with naive binding affinity to our protein scaffold, then use RosettaLigand to computationally dock the ligand while designing the interface for a tighter interaction. This way, we are taking a 'shot in dim light' for design as opposed to a 'shot in the dark', allowing us to more thoroughly investigate the successful and not-so-successful designs, and improve the computational methods. Of ~3500 ligands screened, we identified 28 weakly-binding hits in the range of 340 – 1110 μM. Thus far, RosettaLigand has successfully designed one tighter protein-ligand interface, from 312 μM to 21 μM. In progress experiments include designing and experimentally validating more designed interfaces.

PI-098 **Structural studies of human acidic fibroblast-growth factor (FGF1) mutants with a probable anticancer activity**

Maria Cecilia Gonzalez[1], Stefano Capaldi[1], Maria Elena Carrizo[1], Laura Destefanis[1], Michele Bovi[1], Massimiliano Perduca[1], Hugo Luis Monaco[1]

1.-Biocristallography Laboratory, Department of Biotechnology, University of Verona

Lectins are carbohydrate-binding proteins ubiquitously present in nature. They play a role in biological recognition phenomena involving cells and proteins. The interaction lectin-carbohydrate is highly specific, and can be exploited for the development of nanoparticles containing on their surface lectins specifically directed to carbohydrate residues present only on malignant cells and absent on healthy ones (1). Lectins have been found to possess anticancer properties and they are proposed as therapeutic agents, binding to cancer cell membranes or their receptors, causing cytotoxicity, apoptosis and inhibition of tumor growth. Some lectins are able to prevent the proliferation of malignant tumor cells because they recognize the T-antigen (Gal β 1–3GalNAc) found specifically on the surface of tumor cells (2). The main problem is that their use as a detection agent for the T-antigen in clinical studies is not possible because the immune system can recognize them as foreign molecules and develop an immune response. Previous studies with X-ray crystallography made in our laboratory have characterized a lectin found in mushrooms called BEL β-trefoil which has antiproliferative activity on tumor cell lines, because it contains three binding sites for the T-antigen. Unlike other lectins with this property, BEL β-trefoil shows structural homology with a human protein, acidic Fibroblast Growth Factor (FGF1) (3). Superposition of their structures suggests that the human protein could be mutated to contain at least one of the binding sites for the T-antigen. Such mutations should create in FGF1 the potential capacity of recognizing tumor cells with less immunogenicity than the fungal protein. FGF1 is mitogenic and chemotactic, and mediates cellular functions by binding to transmembrane receptors, which are activated by ligand-induced dimerization requiring heparin as co-receptor. To reach our purpose, the FGF1 cDNA was cloned into a bacterial plasmid and then mutated in five different positions to eliminate its mitogenic activity and to engineer in the protein the T-antigen binding capacity. Attempts to crystalize the mutants of FGF1 were made using the hanging drop technique with the final aim to carry out their structural characterization by X-ray diffraction analysis of the crystals. . References [1] Lis H and Sharon N. Lectins as molecules and as tools. Annu Rev Biochem. 1986. 55(1): p. 35-67. [2] Ju T, Otto VI, Cummings RD. The Tn antigen-structural simplicity and biological complexity. Angew Chem Int Ed Engl. 2011. 50(8): p.1770-1791. [3] Bovi M, Cenci L, Perduca M, Capaldi S, Carrizo ME, Civiero L, Chiarelli LR, Galliano M, Monaco HL. BEL β-trefoil: a novel lectin with antineoplastic properties in king bolete (Boletus edulis) mushrooms. 2013. Glycobiology. 23(5): p. 578-592.

PI-099 **Drug-controllable protein tags for the selective visualization or selective shutoff of newly synthesized proteins of interest in mammalian cells and in vivo**

Conor Jacobs[1], Yang Geng[2], Ryan Badiee[1], Tiffany Nguyen[3], Andrew Evans[4], Hokyung Chung[1], Ying Yang[2], Mehrdad Shamloo[4], Roger Y. Tsien[5], Michael Z. Lin[2, 6]

1.-Department of Biology, Stanford University, 2.-Department of Pediatrics, Stanford University, 3.-Department of Neurology and Neurological Sciences, Stanford University, 4.-Department of Neurosurgery, Stanford University, 5.-Department of Pharmacology, UC San Diego, 6.-Department of Bioengineering, Stanford University

The de novo synthesis of proteins in response to the activation of cellular signaling pathways is a crucial element of many high-level biological processes, including the synaptic plasticity underpinning memory formation in the brain. While of fundamental biological importance, there has been a shortage of tools with which to specifically target pools of newly synthesized proteins of interest for study. Thus, we have developed TimeSTAMP and SMASh, methods for drug-dependent tagging, or destruction, respectively, of newly synthesized copies of proteins of interest. Both methods rely on protein tags that remove themselves by default via an internal Hepatitis C Virus (HCV) NS3 protease, but which are retained in the presence of cell-permeable small molecule protease inhibitors. The TimeSTAMP tag contains split YFP halves and epitope tags which are reconstituted and preserved, respectively, on proteins of interest following drug application, whereas the SMASh tag contains a strong degron which remains attached to proteins of interest following drug application, resulting in their clearance. One limitation of TimeSTAMP and SMASh is that they can only be used to independently manipulate one protein of interest at a time. Furthermore, the application of TimeSTAMP and SMASh to study endogenous protein pools in mammals has not yet been explored. Here, we report on efforts to extend these techniques by reengineering NS3 proteases which can be inhibited by two different drugs orthogonally to one another. By incorporating different drug resistance mutations into two NS3 protease variants, we engineered NS3 protease domains that are inhibitable either by asunaprevir only, or by telaprevir only. We found that these tags permit simultaneous and independent control over the newly synthesized pools of two proteins of interest within the same population of cells. We also report the development of transgenic knock-in mouse strains incorporating TimeSTAMP and SMASh tags, which allow the interrogation of newly synthesized pools of specific endogenous synaptic proteins in the context of their endogenous regulatory elements, and without relying on overexpression.

PI-100 **BRET-based protein switches for detection of Dengue serotype 1 antibodies**

Remco Arts[1], Susann Ludwig[1], Byron Martina[2], Maarten Merkx[1]
1.-Eindhoven University of Technology, 2.-Erasmus Medical Center Rotterdam

Infectious diseases are often diagnosed by the presence of specific antibodies that are produced in response to the invading pathogen. One example are antibodies that are present in patient blood after infection with the Dengue virus serotype 1 and that are directed against an epitope on the virus' non-structural protein 1 (NS-1). Traditional antibody diagnosis relies on time-consuming multi-step assays that require sophisticated equipment in a laboratory environment. A promising alternative are protein switches that are based on bioluminescence resonance energy transfer (BRET). These switches comprise a luciferase (NanoLuc) and a green fluorescent protein (mNeonGreen), which are connected via a semi-flexible linker. The linker contains two epitope sequences of NS-1 to which the antibodies bind specifically. If no antibodies are present NanoLuc and mNeonGreen are held in close proximity via two helper domains and BRET can occur; thus green light originating from mNeonGreen is visible. If antibodies are present, they bind to the specific epitopes in the linker of the switch and cause stretching of the linker and therewith break the interaction of the helper domains. As a result, NanoLuc and mNeonGreen are separated in such a way that BRET cannot occur anymore; thus only blue light originating from NanoLuc remains visible. Using this principle, monoclonal anti-NS-1 antibodies were detectable in a controlled buffer system and in spiked plasma samples. Furthermore, the developed antibody switch was applied to plasma samples of macaques after a primary infection with Dengue virus serotype 1. Signal readout was possible using a laboratory-based plate reader as well as the camera of a standard smartphone. We demonstrate that this BRET-based protein switch can quickly detect antibodies in solution in a single-step assay format using simple equipment for signal readout, such as a standard smartphone. This simplified antibody detection platform has the potential to be carried out outside of a laboratory, thus in areas with limited laboratory infrastructure and a high number of diverse infectious diseases.

PI-101 **Delivery of biologics against intracellular targets**

Paulina Kolasinska-Zwierz[1], Pawel Stocki[1], Bina Mistry[1], Sandrine Guillard[1], Alison Smith[1], Rose Marwood[1], Ben Kemp[1], Anna Czyz[1], Ronald Jackson[1], Ralph Minter[1], Tristan Vaughan[1], Herren Wu[1]

1.-ADPE Cambridge, MedImmune, Milstein Building

Proteins expressed from more than two-thirds of the human genome reside within intracellular compartments. Of these proteins many are important disease-related targets such as KRas and c-Myc which cannot be easily addressed by conventional small molecule approaches. Some of the weaknesses of small molecules can be addressed by biologic drugs, for example high target specificity and inhibition of protein-protein interactions. The challenge for biologics is how to engineer recombinant proteins to access the intracellular space. One strategy is to use systems evolved by bacteria and viruses to deliver material inside the cells. An example of such pathway is used by Pseudomonas Exotoxin A (PE). The modularity of PE allows the catalytic domain to be replaced with a biologic payload against desired intracellular target. An additional benefit of PE-based delivery is a possibility of targeting the drugs only to relevant cells in the body by modifying the cell-targeting domain of the PE. The aim of this project is to deliver functional payloads against K-Ras and c-Myc into the cell using a Pseudomonas Exotoxin A translocation domain. We used phage and ribosome display to select antibody mimetics that bind K-Ras and c-Myc. Here, we present their activity in biochemical assays and the initial results on generation of PE-based constructs.

PI-102 **Recombinant H5 antigen based on hydrolytic domain with deletion of polybasic cleavage site forms functional oligomers**

Edyta Kopera[1], Maria Pietrzak[1], Agnieszka Macioła [1], Anna Maria Protas-Klukowska[1], Konrad Zdanowski[1], Beata Gromadzka[2], Krystyna Grzelak[1], Zenon Minta[3], Krzysztof Śmietanka[3], Bogusław Szewczyk[2]

1.-Institute of Biochemistry and Biophysics, Polish Academy of Sciences, 2.-University of Gdansk and Medical University of Gdansk, 3.-National Veterinary Research Institute, Department of Poultry Diseases

Influenza virus hemagglutinin (HA) is a glycoprotein studded in the lipid bilayer of the virus (1). Hemagglutinin is synthesized as HA0 molecule assembled as noncovalently bound homotrimers on the viral surface. This precursor protein is cleaved by trypsin-like proteases to yield two subunits HA1 and HA2 linked by a single disulphide bond (2). HA0 is also post-translationally modified by N-glycosylation (3). It is well established that the virus hemagglutinin is the main antigen, inducing the neutralizing antibodies. In the attempt towards developing influenza vaccine production (the egg-based manufacturing lasts several months) that would be faster and safer the utilization of recombinant antigen alone is currently being observed. Recently we demonstrated that yeast produced influenza H5 protein although cleaved into two subunits induced strong immunological response in mice (4). In this report, we describe the biochemical and immunological characterization of the H5 antigen, based on hydrolytic domain of the H5N1 gene, with deletion of multibasic cleavage site and expressed in yeast system. The HA encoding gene from H5N1 virus with deletion of 18 nucleotides was cloned into pPICZαC vector. rHA fusion protein with His6-tag was secreted into the culture medium and was purified to homogeneity in one step using Ni-NTA agarose. The efficiency of the antigen purification was 200 mg/L. Glycosylation sites of rHA were determined using LC-MS-MS/MS. Analysis of the N-linked glycans revealed that the rHA is glycosylated at the same sites as the native HA in the vaccine strain. Next we investigated if the hemagglutinin with deletion of the cleavage site oligomerize into higher molecular forms. To determine the oligomeric forms of the recombinant antigen various approaches were applied e.g. Native-Page, Size Exclusion Chromatography or Dynamic Light Scattering. As a final experiment to measure the size of oligomers in a protein sample a combined technology SEC-MALS was conducted, using multi angle light scattering (MALS) as a detector. The immunological activity of rHA was tested in chicken and mice, where antigen elicited high immune response. The data presented here demonstrate that new influenza antigen produced in P. pastoris is highly immunogenic and might be consider as a candidate for subunit vaccine.

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PI-104 **Mining the structural universe for de novo design**

Craig Mackenzie[1], Gevorg Grigoryan[1]
1.-Dartmouth College

Structural motifs capture redundant patterns that frequently occur in proteins. Motifs associated with contiguous fragments of structure (i.e., secondary structural motifs) are well studied and have been successfully used to capture “rules” describing sequence-structure relationships in protein design and structure prediction. We have extended this concept to motifs that capture tertiary information--(i.e., tertiary structural motifs or TERMs. We have discovered that a relatively small alphabet of TERMs describes the known structural universe (all secondary, tertiary and quaternary information in the PDB) at sub-Angstrom resolution. This alphabet of universal motifs reveals the remarkable degeneracy of the protein structure space, with just a few hundred TERMs sufficient to accurately capture half of the known structural universe. We have begun to demonstrate the considerable promise this structural alphabet has for applications such as protein design, structure prediction, and docking. We have developed a novel protein design framework that selects amino acid sequences, given a desired structure, using solely information from the universal TERMs. We show that given a native backbone, this framework recovers the native sequences to a level on par with state-of-the-art atomistic protein design methods, indicating that the motifs capture the salient structural rules governing native proteins. Further, predicted sequence distributions agree closely with observed evolutionary variation. Given the apparently high degeneracy among even complex features of protein structure, methods based on mining the PDB for tertiary information should provide ample opportunities for advancement in problems of computational structural biology.

PI-106 **Sortase-mediated synthesis of protein-DNA conjugates for sensitive biosensing**

Bedabrata Saha[1], Marieke op de Beeck[1], Remco Arts[1], Maarten Merkx[1]

1.-Department of Biomedical Engineering, Eindhoven University of Technology

In recent years, semisynthetic protein-DNA conjugates have emerged as attractive biomacromolecules for different applications in bio-nanotechnology, biosensing, diagnostics and therapeutics. In protein-DNA conjugates, synthetic oligonucleotides allow the construction of desired molecular architecture with high specificity, while maintaining the original functionality of the protein molecules for desired application. However, the synthesis of site-specific and stoichiometric protein-DNA conjugates can be challenging. Due to the diversity in composition and physico-chemical properties of the proteins, few generic strategies are available for conjugation of protein molecules to a DNA scaffold. A common approach is to use thiol-based covalent conjugation, but the introduction of additional cysteines can lead to the formation of intermolecular disulfides or interfere with the formation of native disulfide bonds. As an alternative, here we have developed a site-directed protein-DNA conjugation strategy based on sortase mediated trans-peptidation reaction. The sortase recognizes a 'sorting motif' (i.e. LPXTG, X any amino acid), which is recombinantly introduced by site-directed mutagenesis at the C-terminal end of the protein molecule. The sortase cleaves the T-G peptide bond and catalyzed the formation of a new amide bond between the LPXT peptide and the N-terminal amine of any molecule bearing an N-terminal oligoglycine motif. For this purpose, a triglycine motif was introduced at the 5'-end of single-stranded DNA (ssDNA). On-column synthesis of triglycine modified ssDNA, protected on a controlled pore glass beads, simplified the purification process and enhanced the yield of triglycine-modified ssDNA (> 90%). We used this conjugation strategy in several biosensing applications. For example, we used the method to conjugate ssDNA linkers at the C-termini of a range of single-chain antibody fragments (scFv) and applied these constructs to allow oriented display of capture molecules on biosensor surfaces. ssDNA-scFv were Using an excess of triglycine modified ssDNA, we achieved ~55% conversion scFv-ssDNA conjugate, which can be further purified by in two step purification process consisting of Ni-NTA affinity column and ion-exchange chromatography. We also extended this sortase-based conjugation strategy to develop a bioluminescence based assay for sensitive target oligonucleotide detection. In this regard, the 5' and 3' end triglycine-modified ssDNA molecules were successfully conjugated with a BRET protein pairs, NanoLuc luciferase and mNeonGreen fluorescent protein. The introduction of a C-terminal sortase-His5 tag and and N-terminal Strep–tag allowed efficient purification of theseprotein-ssDNA conjugates from excess oligonucleotides and unreacted protein.

PJ-001 **Mass spectrometry based proteomics to identify the protein differences in human breast milk from breast cancer patients and controls**

Devika Channaveerappa[1], Roshanak Aslebagh[1], Kathleen F. Arcaro[2], Costel C. Darie[1]
1.-Clarkson University, 2.-University of Massachusetts

Breast cancer is the second leading cause of cancer death in women. About 12% women in the US develop breast cancer. Death rates due to breast cancer have been declined over the years due to advancements in mammography and treatment. Although, mammography helps in the early detection of breast cancer, it has few limitations. Dense breast tissue makes mammogram less accurate. Breast milk can be assessed to evaluate the risk of one getting breast cancer by comparing the proteomes of breast milk from healthy and breast cancer suffering individual. This study makes use of mass spectrometry based proteomics to identify the differences between the control and cancerous samples which would further help in identifying potential biomarkers for breast cancer. Firstly, SDS-PAGE was used to separate the proteins from the whole milk sample. The gel bands for each sample was then excised and cut into small pieces. The gel pieces were washed and trypsin digested in order to extract the peptides. Peptide mixtures in the solution were cleaned using C18 Zip-tipp and then analyzed by liquid chromatography-tandem mass spectrometry (LC- MS/MS). 60 minutes and 120 minutes gradient were used for LC-MS/MS analysis. Raw data obtained were converted to pkl files using ProteinLynx Global Served (PLGS version 2.4). Raw data were then submitted to Mascot database search for protein identification. The Mascot results were then exported as .dat files and further analyzed using Scaffold version 4.1 software. Three breast cancer milk samples were investigated against healthy control milk samples. In the SDS-PAGE gel, after Coomassie staining, the protein patterns did show minor differences. After LC-MS/MS analysis, the proteins identified by Mascot database search were imported into the Scaffold software and compared for the relative ratio between the proteins from the milk sampled from control donors and the donors with breast cancer. There were significant differences identified in the proteomes of the two sets of samples. Some of the proteins were upregulated in the breast cancer samples and some were down regulated when compared with the controls. Additional investigation of more breast milk samples is ongoing. This study focuses on identifying biomarkers directly in the milk of donors with breast cancer.

PJ-002 **Leukolike Vectors: leukocyte-inspired nanoparticles**

Claudia Corbo[1,2], Alessandro Parodi[1,2], Roberto Palomba[1,2], Roberto Molinaro[1], Michael Evangelopoulos[1], Francesco Salvatore[2,3], Ennio Tasciotti[1]

1.-The Houston Methodist Research Institute, 2.-Fondazione IRCCS SDN, 3.-CEINGE-, Biotecnologie Avanzate

Nanomedicine aims to improve drug efficiency by enhancing targeting and biocompatibility, and reducing side effects. Multiple surface modifications have been proposed to provide nanocarriers with these features, based on complex synthesis processes and very often inefficient in contemporary providing biological tolerance and targeting properties [1]. Bio-inspired approaches based on surface coatings developed from the purified cell membrane of immune cells represents a new paradigm shift for the development of carrier enable of prolong circulation and proper tumoritropic capabilities. We showed that nanoporous silicon (NPS) particles coated with leukocyte cellular membranes -Leukolike Vectors (LLVs) – possess cell-like properties [2]. LLVs can escape macrophage uptake, delay sequestration by the reticulo-endothelial system, target tumor inflamed vasculature and accumulate within the cancer parenchyma [2]. LLVs were fully characterized for their shape, size, surface charge and coating through dynamic light scattering and scanning electron microscopy. In addition we characterized the content and function of the leukocyte's proteins transferred onto the LLVs coating through high-throughput proteomic analysis and the results revealed the presence and the correct orientation of several important markers of leukocytes: CD45, CD47 and MHC-I were identified as key players in determining LLVs biocompatibility, while Leukocyte Associated Function-1 (LFA-1) and Mac-1 contributed to the LLVs targeting ability and bioactivity towards inflamed endothelium [3]. Recent investigation showed that the coating induced the formation of a singular protein corona (i.e. the protein adsorption layer) on the surface of the nanoparticles compared to negative control following in vivo injection. In addition, the proteolipid coating favored active extravasation of the LLVs in the tumor vasculature by molecular mechanisms similar to those used by tumor infiltrating leukocytes. This work shows that is possible to transfer biologically active leukocyte membrane proteins onto synthetic nanoparticles, thus creating biomimetic carriers retaining cell-like functions that are not affected by the protein corona effect that occurs in vivo. The targeting of the inflamed endothelium can be applied to a broad range of diseases and the approach used to formulate the system could open new avenues for the fabrication of the next generation of personalized treatments by using as cell membrane source the immune cells of patients. References [1] Alessandro Parodi, Claudia Corbo, Armando Cevenini, Roberto Molinaro, Roberto Palomba, Laura Pandolfi, Marco Agostini, Francesco Salvatore, Ennio Tasciotti. Enabling cytoplasmic delivery and organelle targeting by surface modification of nanocarriers. Nanomedicine UK. Accepted. [2] Parodi A. et al. Synthetic nanoparticles functionalized with biomimetic leukocyte membranes possess cell-like functions. Nature Nanotechnoly. 2013. 8(1): 61-8. [3] Corbo C, Parodi A, Evangelopoulos M, Engler DA, Matsunami RK, Engler AC, Molinaro R, Scaria S, Salvatore F, Tasciotti E. Proteomic profiling of a biomimetic drug delivery platform. Curr Drug Targets.

PJ-003 **Visualising gene regulation: a combined proteomic and genomic approach for the structural analysis of steroid hormone receptor complexes**

Andrew Holding[1]

1.-Cancer Research UK Cambridge Institute, University of Cambridge

I will present my work on the development of a novel technique for identifying key protein interactions specific to the growth of tumour cells with the potential to identify new leads for therapeutic targets. Steroid hormone receptors are intracellular receptors that initiate signal transduction in response to steroid hormones, including oestrogen and androgens. Generally, the binding of the steroid to the nuclear receptor induces the protein to form a dimer and relocate onto the chromatin, although the order of these events may vary. The location of receptor binding on the chromatin is defined by specific hormone response elements (HRE). Once located, the receptor promotes gene activation by the recruitment of other co-factors. It is this process that makes the complex of receptor protein and co-factors play a pivotal role in the regulation and activation of genes. The failure to regulate this process correctly is a key step in the development of several endocrine-driven cancers. For example: oestrogen receptor positive (ER+) breast cancer is one of the most common forms of cancer and accounts for 70% of all breast cancer cases. In ER+ tumours, the oestrogen receptor (ER) drives the tumour growth and cell proliferation. Understanding the interactions of the ER with other proteins, either directly or indirectly, can provide vital insight to the regulation of the system that drives this cancer. The progesterone receptor (PR) has also been implicated in breast cancer, and the androgen receptor (AR) is a known driver in the majority of prostate cancers. To meet the challenges of elucidating these systems, we have developed methods to purify and analyse cross-linked regulatory complexes bound to DNA by mass spectrometry (ChIP-MS). This allows for the enrichment of proteins involved in gene regulation. ChIP-MS, combined with tandem mass tags (TMT), makes it possible to realise a quantitative method to investigate the dynamic network of interactions between proteins within complexes that undertake the regulation of biological systems. ChIP-seq is a well-established method for identifying where these protein complexes are bound to the genome. This work focuses on how to combine these technologies with my previous development of cross-linking coupled mass spectrometry techniques (XCMS) to provide a strategy for visualising the dynamic organisation of the proteins on the chromatin.

PJ-004 **Global kinetic analysis of caspase protein substrates in cell lysate reveals selective roles and target specificity**

Olivier Julien[1], Min Zhuang[1], Arun Wiita[1], James Wells[1]
1.-University of California

Caspases are cysteine proteases that play important roles in development, cell differentiation and cell death. However, the limited number of known caspase substrates hinders our understanding of caspase function. Here we performed a non-biased identification and kinetic analysis of caspase-2 and caspase-6 proteolytic substrates in cell lysate, using an enzymatic N-termini enrichment approach followed by mass spectrometry. We identified 235 and 871 potential substrates for the initiator caspase-2 and putative executioner caspase-6, respectively. Our results not only confirm known substrates but also identify many more new substrates with the precise location of proteolysis. Given the emerging roles of caspases-2 and -6 in inflammation and neurodegeneration, these new substrates may provide molecular insight into the progression of related diseases. The sequence consensus logo of caspase-2 targets was very similar to a classical executioner caspase motif (DEVD), while caspase-6 revealed a VEVD motif. Using selected reaction monitoring (SRM), we quantified the kinetics of proteolysis of a large subset of these substrates by measuring the appearance of the caspase cleavage product over time. In the end, we measured 50 and 276 kcat/Km values for individual substrates cut by caspase-2 and caspase-6, respectively. By comparing these data with our previous analysis of caspase-3, -7, and -8, we found that substrates that are shared between caspases are often cleaved at rates that differ by orders of magnitude. Thus, despite having nearly identical primary sequence motifs, the caspases exhibit remarkable substrate specificity that may reflect their specialized roles within the cell.

PJ-006 **Interactomic and Enzymatic Analyses of Distinct Affinity Isolated Human Retrotransposon Intermediates**

John LaCava[1,2], Kelly Molloy[1], Martin Taylor[3], David Fenyö[2], Lixin Dai[3], Brian Chait[1], Jef Boeke[2], Michael Rout[1]

1.-The Rockefeller University, 2.-New York University School of Medicine, 3.-Johns Hopkins University School of Medicine

LINE-1 (L1) retrotransposons are catalysts of evolution and disease whose sequences comprise a significant proportion of the human genome. Despite tremendous influence on genome composition, L1 RNAs only encode two proteins. Consequently, L1 particles include a combination of permissive host factors that are essential to their lifecycle as well as repressive factors that constitute defenses against L1's mutagenic activity. We previously characterized host proteins associated with synthetic and natural human L1 retrotransposons, as expressed in cell culture, using a combination of techniques including metabolic labeling and affinity proteomics. To build on these analyses, we have implemented a series of 2D separations and post-purification treatments to produce a multi-dimensional interactomic characterization of affinity isolated L1s. These studies have revealed the presence of at least two populations of putative transposition intermediates that may exhibit distinctive intracellular localizations. We report a comprehensive, quantitative survey of the proteins partitioning within these distinct L1 populations and their associated in vitro activity. Our observations provide a basis for the classification of L1 interactors with respect to their physical and functional links, facilitating hypotheses to direct in vivo experimentation.

PJ-007 **Polyubiquitin recognition by continuous ubiquitin binding domains of Rad18 probed by modeling, small-angle X-ray scattering and mutagenesis**

Sangho Lee[1], Trung Thanh Thach[1], Namsoo Lee[1], Donghyuk Shin[1], Seungsu Han[1], Gyuhee Kim[1], Hongtae Kim[1]

1.-Department of Biological Sciences, Sungkyunkwan University

Rad18 is a key protein in double-strand break DNA damage response (DDR) pathways by recognizing K63-linked polyubiquitylated chromatin proteins through its bipartite ubiquitin binding domains UBZ and LRM with extra residues in between. Rad18 binds K63-linked polyubiquitin chains as well as K48-linked ones and mono-ubiquitin. However, the detailed molecular basis of polyubiquitin recognition by UBZ and LRM remains unclear. Here, we examined the interaction of Rad18(201-240), including UBZ and LRM, with linear polyubiquitin chains that are structurally similar to the K63-linked ones. Rad18(201-240) binds linear polyubiquitin chains (Ub2, Ub3, Ub4) with similar affinity to a K63-linked one for diubiquitin. Ab initio modeling suggests that LRM and the extra residues at the C-terminus of UBZ (residues 227-237) likely form a continuous helix, termed 'extended LR motif' (ELRM). We obtained a molecular envelope for Rad18 UBZ-ELRM:linear Ub2 by small-angle X-ray scattering and derived a structural model for the complex. The Rad18:linear Ub2 model indicates that ELRM enhances the binding of Rad18 with linear polyubiquitin by contacting the proximal ubiquitin moiety. Consistent with the structural analysis, mutational studies showed that residues in ELRM affect binding with linear Ub2, not monoubiquitin. In cell data support that ELRM is crucial in Rad18 localization to DNA damage sites. Specifically E227 seems to be the most critical in polyubiquitin binding and localization to nuclear foci. Finally, we reveal that the ubiquitin-binding domains of Rad18 bind linear Ub2 more tightly than those of RAP80, providing a quantitative basis for blockage of RAP80 at DSB sites. Taken together, our data demonstrate that Rad18(201-240) forms continuous ubiquitin binding domains, comprising UBZ and ELRM, and provides a structural framework for polyubiquitin recognition by Rad18 in the DDR pathway at a molecular level.

PJ-008 **Optimization of a protein extraction method for the proteomic study of pozol**

Cynthia Teresa Leyva-Arguelles[1], Carmen Wacher[2], Rosario Vera[3], Romina Rodríguez-Sanoja[1]
1.-Instituto de Investigaciones Biomédicas, UNAM., 2.-Facultad de Química, UNAM., 3.-Instituto de Biotecnología, UNAM

Key words: Proteomics, fermentation, pozol Pozol is a Mexican traditional no alcoholic beverage elaborated by various ethnic groups in the southeastern of Mexico. Pozol is obtained from the natural fermentation of nixtamal (heat- and alkali-treated maize) dough. The main carbohydrate in maize dough is starch (72-73%), because others such as sucrose, glucose and fructose are mostly lost during nixtamalization; so, the starch remains as the major carbohydrate available for fermentation [1]. A wide variety of microorganisms have already been isolated from the fermentation of pozol; these microorganisms include fungi, yeasts, lactic acid bacteria, and non-lactic acid bacteria [2]. However, only few bacteria are amylolytic in this fermentation and all of them are weakly amylolytic [1]. In an attempt to explain how a very low content of soluble sugars can support a diverse and abundant microbiota, a proteomic approach was designed to understand the fermentation of pozol [3]. Nevertheless, the extraction of proteins from pozol remains a limiting step in proteomic analysis mainly due to the complexity of the sample. On the basis of the aforementioned reasons, the aim of this work was to obtain a suitable extraction method of proteins for proteomic analysis. Therefore, the fermentation of pozol was continued for 48 h and samples were taken at 0, 9, 24 and 48 h. For each sample, the total sugar content was determined by the Dubois et al. method [4] and protein extraction was performed by two methods: A) Direct extraction from the dough [3] and B) Initial extraction of microorganisms and soluble proteins (this work). Comparison between the two protein methods was performed on two-dimensional gels with silver stain. Then, gels underwent to image analysis by the image master 2D Platinum software. Comparing the 2D-gels, more proteins spots were obtained with method B than that with method A, indicating a more efficient protein extraction with method B. Although, using method A higher concentration of total proteins was observed, they were mostly maize proteins, that in turn overlap and reduce the efficiently extraction of the microbial low abundant proteins. Then, method B allows a better extraction of those low abundant proteins and removes sample components that may interfere with the determination. These results could help us to find the proteins involved in carbohydrate metabolism of the microbiota and finally elucidate the dynamics of pozol fermentation. Acknowledgements. Cynthia Leyva-Argüelles is supported by a personal grant from CONACyT, Mexico. This work is supported by CONACYT grant 131'06:15 and PAPIIT grant IN218714. References: [1] Díaz-Ruiz, G. et al. (2003) Appl. Environ. Microbiol. 8: 4367-4374. [2] Ampe, F. et al. (1999). Appl. Environ. Microbiol. 12: 5464-5473. [3] Cárdenas, C. et al. (2014) J. Proteomics. 111: 139-147. [4] Dubois, M. et al. (1956) Anal. Chem. 28: 350-356.

PJ-009 **Proteomics and enology: wine yeasts study applications**

Jaime Moreno García[1], Juan Carlos Mauricio[1], Juan Moreno[2], Anna Lisa Coi[3], Marilena Budroni[3], Teresa García Martínez[1]

1.-Department of Microbiology, ceiA3, 2.-Department of Agricultural Chemistry, ceiA3, 3.-Dipartimento di Agraria

Proteomics has been applied to the enology field for numerous purposes including fermentation control, improvement of fermentation processes, ensuring wine quality, etc. According to Rodriguez et al., (2012), the information provided by wine proteomics is not only useful for these intentions, but also offers excellent prospects for innovation and diversification of winemaking processes in the near future. In this context, our group has focused research on the identification of proteins that might be important for yeast survival under typical wine elaboration conditions (standard fermentation, Sherry wine biological aging and sparkling wine second fermentation) as well as proteins that configure the content of metabolites which are ultimately responsible for wine quality. By using novel proteomic (OFFGEL fractionator and LTQ Orbitrap XL MS) and metabolomic techniques (SBSE-TD-GC-MS) we have identified a high amount of up-regulated proteins involved in processes like oxidative stress response (in biological aging) or protein biosynthesis (in second fermentation) as well as thirty-three proteins directly involved in the metabolism of glycerol, ethanol and seventeen aroma compounds excreted by the yeast under biological aging conditions. Further, in order to validate proteome data; null mutants of genes codifying proteins up-regulated in the biological aging condition were constructed. Analyses of correlated phenotypes are in progress. This technique and its combination with Metabolomics within the enology context will provide enough knowledge to design or choose yeasts or conditions that satisfy wine production and/or wine characteristics such as color/aroma/texture/flavour profile demands of wine-makers and consumers.

PJ-010 **Additional binding sites for cytochrome c on its redox membrane partners facilitate its turnover and sliding mechanisms within respiratory supercomplexes**

Blas Moreno-Beltrán[1], Antonio Díaz-Quintana[1], Katiuska González-Arzola[1], Alejandra Guerra-Castellano[1], Adrián Velázquez-Campoy[0], Miguel A. De la Rosa[1], Irene Díaz-Moreno[1]

1.-IBVF, cicCartuja, Universidad de Sevilla - CSIC, 2.-BIFI - IQFR (CSIC), Universidad de Zaragoza, 3.-Departamento de Bioquímica y Biología Molecular Celular, Universidad de Zaragoza, 4.-ARAID Foundation, Government of Aragon

Gliding mechanisms of cytochrome c (Cc) molecules have been proposed to shuttle electrons between respiratory complexes III and IV within plant and mammalian mitochondrial supercomplexes, instead of carrying electrons by random diffusion across the intermembrane bulk phase [1-2]. In this work, the binding molecular mechanisms of the plant and human Cc with mitochondrial complexes III and IV have been analyzed by Nuclear Magnetic Resonance and Isothermal Titration Calorimetry. Our data reveal that both Cc-involving adducts possess a 2:1 stoichiometry – that is, two Cc molecules per adduct –. The presence of extra binding sites for Cc at the surfaces of complexes III and IV opens new perspectives on the mitochondrial electron transport chain, where membrane respiratory complexes can be either in independent, free diffusional motion or forming macromolecular assemblies. In the latter context, such new binding sites for Cc facilitate the turnover and sliding mechanisms of Cc molecules within supercomplexes. Indeed, the accommodation of several Cc molecules between complexes III and IV in supercomplexes provide a path for Cc diffusion from complex III to IV. Such path could have physiological significance in the electron flow, which is controlled in supercomplexes to optimize the use of available substrates [3-5]. [1] Genova, G, Lenaz, A. (2013) A critical appraisal of the role of respiratory supercomplexes in mitochondria. Biol. Chem. 394, 631-639. [2] De March, M, Demitri, N, De Zorzi, R, Casini, A,

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PJ-011 **Can Bio-functionalities be deciphered from protein sequence information using computational approaches?**

Norbert Nwankwo[1]
1.-University of Port Harcourt

Background: The processes of uncovering bio-functionalities such as pharmacological activities, disease processes, physiological and structural properties by means of clinical approaches are irrational. This is because they are resource and time consuming. Sometimes, they involve sophisticated and expensive equipments, reagents and animal tissues. Contrarily, sequence information-based computerized approaches are rational and have become relevant in assessing bio-functionalities. They include geno2pheno [CORECEPTOR] [1], Position-Specific Scoring Matrix (PSSMSI/NSI and PSSMCXCR4/CCR5) [2], and Informational Spectrum Method (ISM)-based phylogenetic analysis (ISTREE) [3]. Aim: This presentation demonstrates how bio-functionalities could be deciphered from sequence information using computational approaches. Method: ISM procedure and peptides, VIPMFSALS and CAPAGFAIL are engaged. Results: Protein sequences of the peptides are converted into bio-functionality (Affinity). Affinity between the two peptides is demonstrated as significant amplitudes at the point of common interaction also referred to as Consensus Frequency, signifying remarkable affinity. Discussions: Bio-functionalities of bio-molecules are known to be expressed in one or two genes, which have been found to provide as much biological information as the bio-molecules. This indicates that biological characteristics, represented in these genes and proteins can now be extracted from their sequence information. For example, multi-drug resistances arising from a variety anti-microbial agent from several classes including alkaloids, flavonoids, etc can be retrieved from the sequence information of their encoding genes (MDR1 and MDR11). Similarly, translation of HIV infection to AIDS disease can be extracted from the protein sequence alterations in the HIV gp120. Similarly, effectiveness of anti-retroviral agent, Maraviroc on the HIV isolate H2BX2 and NDK can be deciphered from the sequence information of their V3 domain using geno2pheno [CORECEPTOR] [1]. Sequence information-based deciphering of bio-functionalities using ISM-based techniques has fetched calculation of biological functionalities, designing of biomedical device called Computer-Aided Drug Resistance Calculator, the understanding of the mechanism of HIV progression to AIDS [4], and others. They have compared the efficacies of drugs and vaccines, which formed the basis for the Innocentive Award (ID 9933477) for Assessing Vaccine Potency. Conclusions: Deciphering biological features without engaging reagents, equipments and animal tissues but biological data such as sequence information is one novel, feasible, rational and computerized research accomplishment that will revolutionize translation of therapeutic candidates into therapies.
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PJ-012 **Prediction of cleavage specificity in HCV NS3/4A serine protease and AdV2 cysteine protease systems by biased sequence search threading**

Gonca Ozdemir Isik[1], A.Nevra Ozer[1]
1.-Department of Bioengineering,Faculty of Engineering,Marmara University

Proteases are enzymes which recognize specific substrate sequences and catalyze the hydrolysis of designated peptide bonds to activate or degrade them. Due to the biological importance of proteases, it is particularly important to identify the recognition and binding mechanisms of protease-substrate complex structures in drug development studies. The assessment of substrate specificity in protease systems is crucial, where interpreting the adaptability of substrate residue positions can be useful in understanding how inhibitors might best fit within the substrate binding sites and aid in the design of potent selective inhibitors. Substrate specificity is generally determined by the amino acid profile, structural features and distinct molecular interactions. Besides experimental methods, computational tools for prediction of natural substrate cleavage sites, such as threading, have emerged as useful alternative approaches to provide valuable insights into complex enzyme-substrate interactions. In this work, the substrate variability and substrate specificity of the Hepatitis C virus (HCV) NS3/4A serine protease and the Adenovirus 2 (AdV2) cysteine protease was investigated by the biased sequence search threading (BSST) methodology. Using available crystal structures of the proteases, the template structures for the substrate-bound proteases were created in silico by performing various peptide building and docking procedures followed by energy minimization and molecular dynamics (MD) simulations. BSST was performed starting with known binding, nonbinding and some random peptide sequences that were threaded onto the template complex structures, and low energy sequences were searched using low-resolution knowledge-based potentials. Then, target sequences of yet unidentified potential substrates were predicted by statistical probability approaches applied on the low energy sequences generated. The results show that the majority of the predicted substrate positions correspond to the natural substrate sequences with conserved amino acid preferences, while some positions exhibit variability. For NS3/4A serine protease cleavage, the significant selection for Pro at P2 and Cys at P1 positions is observed at the predicted sequences. These positions are important as they surround the cleavage site in the three-dimensional structure, and are probably less tolerant to change. Moreover in previous studies, Cys at P1 position has been shown to be the dominant determinant for cleavage efficiency, while Cys, Pro and Glu at P2 position have also been shown to be correlated with increased cleavage efficiency of NS3/4A protease. For AdV2 cysteine protease, on the other hand, BSST produces similar significant results for both type 1 (XGX-G) and type 2 (XGG-X) consensus cleavage sites, where P2 and P1’ positions have Gly with highest percentage in type 1 (XGX-G) while P2 and P1 positions have Gly in type 2 (XGG-X). These indicate that the BSST seems to provide a powerful methodology for predicting the substrate specificity for the HCV NS3/4A serine protease and AdV2 cysteine protease, which are targets in drug discovery studies.

PJ-013 **Protein plasticity improves protein-protein binding description**

Chiara Pallara[1], Juan Fernández-Recio[1]
1.-Joint BSC-CRG-IRB Research Program In Computational Biology

An accurate description of protein-protein interactions at atomic level is fundamental to understand cellular processes. However the current structural coverage of protein-protein interactions (i.e. available experimental structures plus potential models based on homologous complex structures) is below 4% of the estimated number of possible complexes formed between human proteins.1,2 For these reasons, computational docking methods aim to become a complementary approach not only to solve the structural interactome but also to elucidate the basis of the protein-protein association mechanism. In spite of the advances in protein-protein binding description by docking, dealing with molecular flexibility is a major bottle-neck, as shown by the recent outcomes of the CAPRI (Critical Assessment of PRediction of Interactions) experiment.3 This data clearly confirms that the protein dynamics plays a key role in protein-protein association. The use of conformational ensembles generated from unbound protein structures in combination with computational docking simulations might represent a more realistic description of protein-protein association. Here, we present the first systematic study about the use of precomputed unbound ensembles in docking, as performed on a set of 124 cases of the Protein-Protein Docking Benchmark 3.0.4 The primary aim of our work is to understand the role of the protein conformational heterogeneity in protein-protein recognition. To do this, small conformational ensembles were automatically generated starting from the unbound docking partners, and then an extensive analysis of their binding properties was performed in the context of pyDock docking scheme.5 The results show that considering conformational heterogeneity of interacting proteins can improve docking description in cases that involve intermediate conformational changes in the unbound-to-bound transition. More interestingly, we found that protein plasticity increases chances of finding conformations with better binding energy, not necessarily related to bound geometries. The relevance for future docking methodology development and for understanding protein association mechanism will be discussed. References 1. Venkatesan K, Rual JF, Vazquez A, Stelzl U, Lemmens I, Hirozane-Kishikawa T, Hao T, Zenkner M, Xin X, Goh KI, Yildirim MA, Simonis N, Heinzmann K, Gebreab F, Sahalie JM, Cevik S, Simon C, de Smet AS, Dann E, Smolyar A, Vinayagam A, Yu H, Szeto D, Borick H, Dricot A, Klitgord N, Murray RR, Lin C, Lalowski M, Timm J, Rau K, Boone C, Braun P, Cusick ME, Roth FP, Hill DE, Tavernier J, Wanker EE, Barabasi AL, Vidal M. An empirical framework for binary interactome mapping. Nat Methods 2009;6:83-90. 2. Stumpf MP, Thorne T, de Silva E, Stewart R, An HJ, Lappe M, Wiuf C. Estimating the size of the human interactome. Proc Natl Acad Sci U S A 2008;105:6959-64. 3. Bonvin A. Coming to peace with protein complexes? 5th CAPRI evaluation meeting, April 17-19th 2013--Utrecht. Proteins, 2013; 81, 12, 2073-4. 4. Hwang H, Pierce B, Mintseris J, Janin J, Weng Z. Protein-protein docking benchmark version 3.0. Proteins, 2008; 73, 705-9. 5. Cheng TM, Blundell TL, Fernandez-Recio J. pyDock: electrostatics and desolvation for effective scoring of rigid-body protein-protein docking. Proteins 2007;68:503-15.

PJ-014 **Affimers, new affinity reagents for life science research**

Vincent Puard[1], Kit-Yee Tan[1], Kurt Baldwin[1], Enitan Carrol[2], Rebecca Patisson[3], Rob Beynon[3], Christian Tiede[4], Michael McPherson[4], Darren Tomlinson[4], Paul Ko Ferrigno[1]

1.-Avacta Life Sciences, 2.-Institute of Infection and Global Health, University of Liverpool, 3.-Centre for Proteome Research, University of Liverpool, 4.-Biomedical Health Research Centre, University of Leeds

Purpose of the research: There is increasing interest in the development of protein scaffolds that can be used to develop affinity reagents that are alternatives to antibodies. The Affimer scaffold is based on the cystatin protein fold. The Affimer scaffold is biologically inert, biophysically stable and capable of presenting a range of designed or random binding surfaces defined by peptides inserted at 2 different loops. The result is highly specific, high affinity interactions with a wide range of targets including ones that are inaccessible to antibodies. Affimers are designed to work in the same way as the very best antibodies, but with a number of key advantages. Affimers are quick to develop (typically 7 weeks) without using animals. They contain no disulphide bonds, are expressed easily in E. coli and have no batch to batch variability. Affimers are small molecules (108 aa, ~12 kDa), robust and stable (resistant to pH range, thermally stable and not sensitive to EDTA). Affimers can be a direct replacement for antibodies – no process or workflow change required – and perform identically to antibodies in assays such as ELISA, FACS, IHC, western blots, affinity purification, microarray and potentially therapeutics. We describe some applications of the technology in regards of Affimer development for custom targets on one hand and for the biomarker discovery workflow using Affimer microarrays on the other. Main results: By screening of our very large (3 x 1010) library against Yeast SUMO protein we identified Affimers with high affinity allowing their use for ELISA. Moreover, no cross-reactivity was observed when Affimers were used on western blots leading to a unique band specific to Yeast SUMO when compared to human proteins. A library of 25,000 random Affimers, expressed in E. coli, was printed on glass microscope slides and challenged with plasma from children (n=104) with sepsis and from healthy children (n=24). Unsupervised hierarchical clustering based on the 25,000 Affimers allowed differentiation between the control and patient samples. 200 Affimers were found to differentially bind proteins between the 2 groups with a > 2 fold change. The Affimer arrays identified a strong signature of sepsis and ROC curve analysis allowed confident prediction of disease (AUROC of 0.9). Affinity purification and preliminary mass spectrometry analysis identified known biomarkers of sepsis and also potentially novel biomarkers not previously associated with this disease. Major conclusions: This work demonstrates the scope of Affimer affinity reagents to develop alternative binders to antibodies, where Affimers perform identically in most assays without the disadvantages associated with antibodies. Moreover, Affimers enable a new protein microarray-based biomarker-discovery workflow and we predict that array-based validation of signatures identified using Discovery Arrays prior to affinity purification and mass spectrometry will offer a cost- and time-effective methodology compared to purely mass spec-driven workflows.

PJ-015 **NMR study of ERK-mediated hyperphosphorylation of the neuronal Tau protein**

Haoling Qi[1], François-Xavier Cantrelle[2], Amina Kamah[1], Clément Despres[1], Sudhakaran Prabakaran[2], Jeremy Gunawardena[2], Guy Lippens[1], Isabelle Landrieu[1]

1.-UMR 8576 CNRS-USTL, Lille University, 2.-Department of Systems Biology, Harvard Medical School

Tau pathologies, called 'tauopathies', are related to several neurodegenerative diseases including Alzheimer Disease (AD). In AD, Tau protein is observed hyper-phosphorylated and aggregated as Paired Helical Filament (PHF). The neuronal Tau protein is an Intrinsically Disordered Proteins (IDPs). Nuclear Magnetic Resonance spectroscopy (NMR) is here used to study the Tau protein phosphorylations and Protein-Protein Interactions (PPIs). In in vitro assays, Tau phosphorylation by rat brain extract is considered as an hyperphosphorylation model that was furthermore pointed out to enable Tau aggregation [1]. In a first step, we have identified all the phosphorylation sites of rat brain extract phosphorylated-Tau, using the analytical capacity of NMR. We showed that the protein is modified at 20 Ser/Thr sites. Among the kinases that we have characterized so far using Tau as substrate, only the extracellular signal-regulated kinase2 (ERK2) shows an ability to modify in vitro Tau protein on so many sites. We have indeed identified 14 phosphorylated Ser/Thr-Pro motifs out of 18 potential phosphorylation sites in the sequence of full length 441-residue Tau. In addition, we showed using Transmission Electron Microscope (TEM) a similar in vitro aggregation capacity of ERK-phosphorylated Tau protein compared to that of rat brain extract phosphorylated-Tau. This shows that phosphorylation by the ERK kinase generates an hyperphosphorylated Tau. Given the high efficiency of ERK towards Tau, we have next looked into the mechanism of Tau recognition. ERK kinase possesses two well-characterized docking domains: D Recruitment Sites (DRS) and F Recruitment Sites (FRS), which recruit complementary docking sites and increase the specificity and efficiency of the interaction with both its upstream regulators and downstream substrates [3]. As the interaction between Tau protein and ERK2 kinase is analyzed by NMR spectroscopy, multiple sites of interaction are observed along the Tau sequence, similar to DRS docking sites, all located in the so-called microtubule binding domain of Tau. These sites are short sequences loosely matching the reported consensus for D sites ψ1-3φxφ (ψ, φ, and x refer to positively charged, hydrophobic, or any intervening residues, respectively) [3], and also the reverse sequence φxφψ

1-3.To confirm the mapping of the interaction, two Tau recognition sites were produced as recombinant peptides of about 20 amino-acid in fusion with an N-terminal His-tag Sumo. Interaction assays using 2D [1H, 15N] HSQC spectra of the peptides confirm their binding to ERK kinase. The potential of these peptides to inhibit ERK activity with Tau as substrate is now being investigated.

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PJ-016 **How binding incorrect partners can lead to the prediction of correct interfaces: Results from a massive cross-docking study on proteins.**

Sophie Sacquin-Mora[1], Lydie Vamparys[1], Alessandra Carbone[2]
1.-Laboratoire de Biochimie Théorique, CNRS UPR9080, 2.-Génomique Analytique, Université Pierre et Marie Curie, CNRS UMR7238

While rigid-body docking has become quite successful for predicting the correct conformations of binary protein complexes, determining whether two given proteins interact remains a difficult problem. Successful docking procedures often give equally good scores for pairs of proteins for which there is no evidence of interaction. Studies investigating what we define as the 'pre-docking' problem via in silico approaches have only recently become feasible with the help of supercomputers and grid-computing systems. In a previous work, on a restricted set of protein complexes, we showed how predictions of interacting partners could be greatly improved if the location of the correct binding interface on each protein was known. Experimentally identified complexes are found to be much more likely to bring these two interfaces into contact, at the same time as yielding good interaction energies. We present data from a complete cross-docking (CC-D) study of a database of 168 proteins, including the treatment of more than 14,000 potential binary interactions. The performance of the interaction index we developed to predict binding probability compares well with other methods. By studying the interaction of all potential protein pairs within a dataset, CC-D calculations can also help to identify correct protein interaction interfaces. The present large-scale study also reveals the influence of various protein families (enzyme-inhibitor, antibody-antigen, antigen-bound antibody, etc.) on binding specificity, showing, in particular, the distinctive behavior of antigenic interfaces compared to enzymes, inhibitors or antibodies. The performance of our approach is encouraging. Although identifying interaction interfaces significantly helps in the identification of interacting proteins, further refinements will be necessary to make in silico cross-docking a viable alternative to high-throughput experimental methods.

PJ-017 **Whole-protein mass spectrometry reveals global changes to histone modification patterns in hypoxia**

Sarah Wilkins[1], Kuo-Feng Hsu[1], Christopher Schofield[1]
1.-Chemistry Research Laboratory, Oxford University

Cells respond to limiting oxygen availability (hypoxia) by altering the gene expression profile. This primarily involves changes at the level of transcription via the activity of hypoxia-responsive transcription factors, although increasing evidence suggests that changes in chromatin structure (i.e. from a condensed 'silent' state to a more open or 'active' state) are required in order for transcription to take place. In particular, post-translational modifications (PTMs) to histones have an important regulatory function in gene expression under hypoxic conditions. The N-terminal tails of histone proteins are accessible to a set of enzymes capable of 'writing' and 'erasing' PTMs including acetylation, methylation, ubiquitylation, SUMOylation and phosphorylation. To date, studies in hypoxia have employed antibody-based methods to investigate changes in histone modifications, and so have focused on individual marks in isolation. The interplay between coexisting PTMs is thought to be much more important than the effect of any single mark. Therefore, a global view of the histone modification profile is essential to gain a complete understanding of the function of histone PTMs and their roles in gene regulation. In this study, we apply whole protein mass spectrometry to investigate hypoxia-induced changes in histone marks. This 'top-down' approach provides insight into combinational modification patterns that are difficult to establish by antibody-based methods or peptide MS analysis. We investigated changes in the global PTM profiles of histones from a range of human cell-lines and tissues under severe hypoxia (<0.1% O2). We find that hypoxia causes a shift in the overall profile towards a more highly modified state, with significant changes in methylation and phosphorylation. Marked changes in histone PTMs were also observed following treatment of cells with epigenetic inhibitors and commonly used hypoxia mimetics, including several iron chelators currently in clinical trials for the treatment of anaemia. Finally, we show that this method can be used to identify the histone variant H2AX, whose phosphorylation at serine 139 is an indicator of double-stranded DNA breaks in cancer. Overall, these data provide important insights into the epigenetic changes associated with hypoxia in normal and disease contexts. We hope to further develop this method in combination with different labelling strategies to enable quantitative analysis of histone modifications in cells.

PJ-018 **Mass spectrometry-based protein biomarker discovery in neurodevelopmental disorders**

Kelly Wormwood[1], Armand Ngounou Wetie[1], Laci Charette[2], Jeanne Ryan[2], Emmalyn Dupree[1], Alisa Woods[0], Costel Darie[1]
1.-Clarkson University, 2.-SUNY Plattsburgh

Neurodevelopmental disorders are a group of disorders in which the development of the central nervous system is disturbed. These are very common with approximately 15% of children in the United States ages 3 to 17 being affected by at least one disorder. Examples include Autism Spectrum Disorder (ASD) and Smith-Lemli-Opitz Syndrome (SLOS). ASD affects approximately 1/63 children in the United States and is characterized by repetitive behaviors, communication deficits and impairments in social interactions. There is currently no biological diagnosis or known cause of ASD. SLOS is characterized by a cholesterol deficiency due to a mutation on the 7DHCR gene. Approximately 1/20,000 babies are born with SLOS. Diagnosis is achieved by measuring cholesterol and 7-dehydrocholesterol (7DHC) levels in the blood, however, there is currently no proven treatment for SLOS. Because of this, research is increasing to determine biomarkers for these disorders. Here, samples from people with ASD (sera and saliva) and SLOS (saliva), and matched controls were analyzed using a combination of gel electrophoresis (Tricine-PAGE, SDS-PAGE and Blue Native PAGE), in gel digestion or insolution digestion and nanoliquid chromatography-tandem mass spectrometry (nanolC-MS/MS) to investigate differences between the proteomes of people with these neurodevelopmental disorders and matched controls. Several alterations in protein expression were identified. These differences may lead to potential biomarkers for diagnosis, possible therapeutic targets and an altogether better understanding of the disorders.

PJ-019 **Understanding protein recognition using structural features**

Manuel A. Marin-Lopez[1], Joan Planas-Iglesias[2], Jaume Bonet[3], Daniel Poglayen[1], Javier García-García[1], Narcís Fernández-Fuentes[1], Baldo Oliva[1]

1.-Structural Bioinformatics Lab (GRIB-IMIM), Department of Experimental and Health, Universitat Pompeu Fabra, 2.-Division of Metabolic and Vascular Health, University of Warwick, 3.-Laboratory of Protein Design & Immunoengineering, School of Engineering, Ecole Polytechnique Federale De Lausanne

Protein-Protein interactions (PPIs) play a crucial role in virtually all cell processes. Thus, understanding the molecular mechanism of protein recognition is a critical challenge in molecular biology. Previous works in this field show that not only the binding region but also the rest of the protein is involved in the interaction, suggesting a funnel-like recognition model as responsible of facilitating the interacting process. Further more, we have previously shown that three-dimensional local structural features (groups of protein loops) define characteristic patterns (interaction signatures) that can be used to predict whether two proteins will interact or not. A notable trait of this prediction system is that interaction signatures can be denoted as favouring or disfavouring depending on their role on the promotion of the molecular binding. Here, we use such features in order to determine differences between the binding interface and the rest of the protein surface in known PPIs. Particularly, we study computationally three different groups of protein-protein interfaces: i) native interfaces (the actual binding patches of the interacting pairs), ii) partial interfaces (the docking between a binding patch and a non-interacting patch), and iii) back-to-back interfaces (the docking between non-interacting patches for both of the interacting proteins). Our results show that the interaction signatures in partial interfaces are much less favoured than the ones observed in native and back-to-back interfaces. We hypothesise that this phenomenon is related to the dynamics of the molecular association process. Back-to-back interfaces preserve the exposure of the real interacting patches (thus, allowing the formation of a native interface), while in a partial interface one interacting patch is sequestered and becomes unavailable to form a native interaction.

PJ-020 **Structural characterization of the cytoplasmic mRNA export platform**

Javier Fernandez-Martinez[1], Yi Shi[2], Seung Joong Kim[3], Paula Upla[4], Riccardo Pellarin[3], Daniel Zenklusen[5], David L. Stokes[4], Andrej Sali[3], Brian T. Chait[2], Michael P. Rout[1]

1.-Laboratory of Cellular and Structural Biology, The Rockefeller University., 2.-Laboratory of Mass Spectrometry and Gaseous Ion Chemistry, The Rockefeller Univ., 3.-University of California, San Francisco, 4.-The New York Structural Biology Center, 5.-Department of Biochemistry, Faculty of Medicine, University of Montreal

mRNA biogenesis is an intricate process that begins within the nucleus and culminates with the remodeling and nuclear export of the mRNP particles through the nuclear pore complex (NPC). Defects in this conserved mechanism have been shown to cause serious human diseases. The protein assembly that performs the last steps in mRNP biogenesis and export is located at the cytoplasmic face of the NPC and is formed by 14 different proteins, organized into several subcomplexes whose arrangement and molecular architecture are poorly understood. In this study we applied an integrative approach, combining cross-linking and mass spectrometry (CX-MS), electron microscopy and available high-resolution structures, to describe the molecular architecture of the endogenous NPC cytoplasmic mRNP export machinery. We generate a hybrid, close-to-atomic structure of the yeast native Nup82 complex, the core of the assembly. Our map also reveals how the Nup82 complex organizes the entire cytoplasmic mRNP export machinery, and how this in turn docks into the architectural core of the NPC. Mapping of phenotypic profiles into our structures allows us to generate a first functional map of the ensemble. We expect that our map will serve as a framework to understand the molecular mechanisms underlying this key step of mRNP biogenesis.

PJ-021 **Study of candidate proteins to pore associated with P2X7 receptor in different cell types**

Carla Oliveira[1], Anael Alberto[1], Mônica Freitas[2], Luiz Alves[1]

1.-Laboratório de Comunicação Celular – FIOCRUZ, 2.-Centro Nacional de Ressonância Magnética Nuclear – UFRJ

Aim: The P2X7R is a purinergic receptor, which differs from others subtypes due to its structural and pharmacological characteristics. When exposed for extended time or to high concentrations of its agonist (ATP), promotes an increase in membrane permeability, allowing the passage of molecules up to 900Da. There is a controversy among several authors that leave in doubt if this receptor needs a second protein for the pore formation and which protein could be. We select five pore-forming proteins: TRPV1, TRPA1, Connexins-43 (Cx-43), Pannexin-1 (Panx-1) and VDAC. We believe that different mechanisms and proteins could be associated with P2X7R, depending on the cell type and their microenvironment stimuli. In this context, our main goal is identify possible proteins that could be associated with the P2X7R pore in different cells and species. Methods and Results: We started with RT-PCR technique of cell lines: J774.G8, N2A, U373, U937, HEK-293 and primary cells from Wistar mouse and Swiss mice. We used different primers and PCR cycle for each target at different species. We observed that the P2X7, Panx-1 and Cx-43 are the most abundant and are present in all cell types except the absence of P2X7 in U373 cells and Panx-1 in mice macrophages and U373 cells (n>3). However, TRPV1 was seen at N2A and U937 cells and TRPA1 in and primary cells from mouse and mice and in J774.G8 cells (n>3). Regarding to the VDAC, it is present in mouse macrophages, J774.G8 and HEK-293 cells (n>3). The further steps, we verified if those proteins could be physically associated with the P2X7R. We co-immunoprecipitated the P2X7R of J774.G8 (with or without ATP), mice macrophages, HEK-293 and U937 cells. The samples were applied in two separated 12.5% bis/acrylamide gels: one destined to Mass Spectrometry (MS) and the other to Western Blot. At this point, we confirmed the presence of P2X7R, and observed several others proteins associated to P2X7R at different cell conditions, mainly when we exposed, J774.G8 cell, to 5 mM ATP (n=3). At this condition, we found by MS, Hsp70, 75, and 90; alpha and β tubulin; myosin Va; alpha, β and γ actin; malate and lactate dehydrogenase (n=1). Although U977 and HEK-293 had not received ATP treatment, we found several proteins associated to P2X7. The next step was to immunoprecipitated those proteins in J774.G8 (treated or not with ATP) and use it to verify if P2X7 are physically associated to them. As result we saw the P2X7 associated to Panx-1 in J774.G8 cells. Conclusion: We conclude that the P2X7R activated by extracellular ATP triggers the recruitment of variety different proteins. At this condition, we can suggest that maybe there is a conformational change, regardless of the numerous recruitment structural proteins. In addition, apparently, the pore-forming protein Pannexin-1 is associated with P2X7R, and the others pore forming proteins (VDAC, Cx-43, TRPV1, TRPA1) seems not be linked to P2X7R at J774.G8 cells

PJ-022 **CABS-dock web server for protein-peptide docking with significant conformational changes and without prior knowledge of the binding site**

Mateusz Kurcinski[1], Michal Jamroz[1], Maciej Blaszczyk[1], Andrzej Kolinski[1], Sebastian Kmiecik[1]
1.-Department of Chemistry, University of Warsaw

Protein-peptide interactions play a key role in cell functions. Their structural characterization, although very challenging, is important for discovery of new drugs. Based on our methodology for highly efficient simulation of proteins [1, 2], we developed the CABS-dock web server for protein-peptide molecular docking [3]. While other docking algorithms require pre-defined localization of the binding site, CABS-dock doesn't require such knowledge. Given a protein receptor structure and a peptide sequence (and starting from random conformations and positions of the peptide), CABS-dock performs simulation search for the binding site allowing for full flexibility of the peptide and small fluctuations of the receptor backbone [3-5]. This protocol was extensively tested over the largest dataset of non-redundant protein-peptide interactions available to date (including bound and unbound docking cases) [3]. For over 80% of the dataset cases, we obtained models with high or medium accuracy (sufficient for practical applications). CABS-dock method for coupled binding site search and protein-peptide docking can be easily complemented by other computational tools (e.g. high-resolution docking refinement protocols) or experimental data to improve the results of the docking experiment. CABS-dock web server is freely available at http://biocomp.chem.uw.edu.pl/CABSdock References [1] Jamroz M, Kolinski A, Kmiecik S. (2013) CABS-flex: Server for fast simulation of protein structure fluctuations. Nucleic Acids Res. 41, W427-31. [2] Blaszczyk M, Jamroz M, Kmiecik S, Kolinski A. (2013) CABS-fold: Server for the de novo and consensus-based prediction of protein structure. Nucleic Acids Res. 41, W406-11. [3] Kurcinski M, Jamroz M, Blaszczyk M, Kolinski A, Kmiecik S. (2015) CABS-dock web server for the flexible docking of peptides to proteins without prior knowledge of the binding site. Nucleic Acids Res. doi: 10.1093/nar/gkv456. [4] Kurcinski M, Kolinski A, Kmiecik S. (2014) Mechanism of Folding and Binding of an Intrinsically Disordered Protein As Revealed by ab Initio Simulations. Journal of Chemical Theory and Computation. 10, 2224-2231. [5] Blaszczyk M, Kurcinski M, Kouza M, Wieteska L, Debinski A, Michal J, Andrzej K, Kmiecik S. (2015) Modeling of protein-peptide interactions using the CABS-dock web server for binding site search and flexible docking. Methods (submitted), preprint at arXiv:1505.01138.

PJ-023 **Web server tools for modeling of protein structure, flexibility, aggregation properties and protein-peptide interactions**

Maciej Blaszczyk[1], Michal Jamroz[1], Mateusz Kurcinski[1], Agata Szczasiuk[1], Andrzej Kolinski[1], Sebastian Kmiecik[1]

1.-University of Warsaw, Faculty of Chemistry

Recently, we developed a series of molecular modeling tools for structure-based studies of protein functions and interactions. These tools are publicly available as web servers that are easily operated even by non-specialists: CABS-fold server for protein structure prediction [1]; CABS-flex server for modeling of protein structure flexibility [2]; Aggrescan3D server for prediction of protein aggregation propensities and rational design of protein solubility [3]; and CABS-dock server for prediction of peptide binding sites and peptide docking [4]. The web servers are freely available from the laboratory website: http://biocomp.chem.uw.edu.pl/tools

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PJ-024 **Developing a technique to detect deamidated proteins and peptides using Rig-I**

Sandy On[1], Pinghui Feng[2]

1.-University of Southern California, Keck School of Medicine, 2.-USC Norris Comprehensive Cancer Center

Developing a Technique to Detect Deamidated Proteins and Peptides Using Rig-I Sandy On, Pinghui Feng University of Southern California, Norris Comprehensive Cancer Center, Department of Microbiology, and Molecular Biology, Los Angeles CA Perhaps the most notable type of post-translational modification of proteins and peptides into a higher order structure is deamidation of asparagine and glutamine. Deamidation occurs when an amine group is removed, degrading the molecule for purpose of regulating intracellular levels. Previous studies have demonstrated that this notable post translational modification has been uncovered over time for use in DNA recombinant technology as well as use as a biological clock to facilitate the rapid turnover of biologically important components of the cell. While the effects of this non-enzymatic chemical reaction have been widely studied, the method to uncover modification sites over a large quantity of proteins remains an issue. One of the most common types of deamidation is of asparagine and glutamine residues. At this time, most researchers will depend on mass spectrometric based proteomic techniques for identification of these post-translational sites. The issue is that mass spectral analysis of deamidated proteins and peptides is complication and can lead to misassigned identification attributed by an overlapping of 13C peak of the amidated form with the deamidated monoisotopic peak; these two peaks are only separated by 19.34 mDa. While these issues can be mediated by using a mass spectrometer with a high mass measurement accuracy, and high resolving power, it is essential to establish simpler methods for identifying substrates that have undergone deamidation. If deamidation is present, different protein bands will be exhibited in the western blot, which will be compared to a triple mutant RIG-I, which resists deamidation, to observe the location of this modification on the protein. With enough testing, I will determine specific sites of digestion and use this information to make conclusions of unknown proteins. I will make results regarding whether the protein has been modified based on the digestion sites. I will use mass spectrophotometry analysis to compare the proteins on a wider scale and double check my results. I have narrowed it down to a couple of different digestion sites that indicate deamidation. Though the analysis work can be tedious, it is crucial to ensure the sites we isolate are accurate in order to establish this technique. From my research, we can apply this method for wider scale use such as in clinical settings. In areas of inflammation of Parkinson's' patients, we can review specifically the infected cells versus uninfected and isolate the proteins, usually deamidated, responsible often smaller in size and more specific. In addition, research articles have already shown that suppressing modification of certain cells such as Bcl-xl playing a major in leading the regulation of cancer cell death by apoptosis. By leading the discovery of a simpler methods to uncovering deamidation in cells, researchers will more easily and quickly be able to scan through various proteins, some of which discovered eventually may play pivotal roles in cancer research.

PJ-025 **Mass spectrometric evaluation of recombinant hemagglutinin structure conformations**

Joanna Szewczak[1], Anna Bierczyñska-Krzysik[1], Agnieszka Romanik-Chruścielewska[1], Iwona Sokołowska[1], Marcin Zieliński[1], Piotr Baran[1], Violetta Sączyńska[1], Małgorzata Kęsik-Brodacka[1], Drota Stadnik[1], Grażyna Płucienniczak[1]
1.-Institute of Biotechnology and Antibiotics

Influenza virus (IV) hemagglutinin (HA) is a homotrimeric integral membrane glycoprotein that mediates receptor-binding and membrane fusion. It constitutes the prominent viral surface antigen and a main target for neutralizing antibodies. Bacterial, recombinant HA-based vaccines indicate high potential to confer protection against highly pathogenic (HP) avian IV (AIV) H5N1 and arise as alternative for the traditional egg- or cell culture-based manufacturing. Relatively short time of bacterial HAs production can be of great importance in case of a pandemic. Escherichia coli produced protein, based on the HA sequence of A/swan/Poland/305-135V08/2006(H5N1) HPAIV*, has been successfully expressed in the form of inclusion bodies at Institute of Biotechnology and Antibiotics. Refolded and purified antigen was obtained in a soluble form, isolated by reversed phase HPLC and identified with peptide mass fingerprinting using matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI TOF/TOF MS). The performed research in a great extent allowed to confirm the amino acid sequence of the recombinant HA (rHA) assumed based on the cDNA and allowed to establish the location of a total of six disulfide bridges. However, during purification and storage of the rHA, apart from desired higher order rosette-like structures of the protein, other non-native species resulting from posttranslational modifications, misfolding, aggregation and degradation may occur what results in reduced vaccine potency. Here, besides the properly folded monomers, we indicate non-native aggregates induced by disulfide crosslinking. Moreover, several free cysteine residues and unexpected intrachain S-S were identified in rHA tryptic peptide maps. Cys 43 was found most susceptible to formation of disulfide bridges between the distinct chains of rHA. The above findings allow to assume that not all rHA particles fold to form the native structure. Reduced Cys residues exhibit tendency to undergo oxidation and uncontrolled S-S creation during storage. This may lead to activity drop and of non-native multimer formation. A covalent modification of several peptides of the rHA with a concomitant mass increase of 183 Da followed as a result of reaction with a serine protease inhibitor, 4-(2-aminoethyl) benzenesulfonyl fluoride (AEBSF).
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PJ-026 **Monoclonal-based antivenomics and biological activities revealing conserved neutralizing epitopes across elapidae family**

Carlos Correa-Netto[1,2], Ricardo Araújo[1,2], Marcelo Strauch[1], Leonora Brazil-Más[1], Marcos Machado[3], Moema Leitão-Araújo[4], Paulo Melo[3], Débora Foguel[2], Juan Calvete[5], Russolina Zingali[2]

1.-Instituto Vital Brazil, 2.-Instituto de Bioquimica Medica-UFRJ, 3.-Programa de Farmacologia e Química Medicinal-UFRJ, 4.-Fundação Zoobotânica do Rio Grande do Sul, 5.-Instituto de Biomedicina de Valencia-CSIC

Polyclonal antibodies have been used for over a century to the treatment of snakebite envenoming. New strategies and approaches to understand how antibodies recognize and neutralize snake toxins represent a challenge to improve the antivenoms. The neurotoxic activity of Micrurus venom is carried majority by two distinct proteins families, 3FTx and PLA2. The conserved structural folding of these toxins can be appreciated as model to generate inhibitors against them. In this regard, monoclonal antibodies (mAbs) can be used as tool to find hot spots for inhibit the toxins and represent the first step in order to develop recombinant neutralizing molecules. In this work our goals were analyse a set of monoclonal antibodies against the most toxic components of M. altirostris venom by proteomics approaches. The venom was fractionated; its major toxic proteins identified by in vivo tests based on murine lethal toxicity analyses (approved by the Ethical Committee for Animal Experimentation from Center of Health and Science of the Federal University of Rio de Janeiro - no. 01200.001568/2013-87). The toxic components were used to generate a panel of five monoclonal antibodies. ELISA and antivenomics results allowed us identify the specificity of all mAb and their neutralizing efficacy was measured by in vitro tests. Three mAbs showed reactivity towards 3FTx and two against PLA2. All Monoclonal antibodies against 3FTx lack a broad recognition. However, we identified a pair of monoclonal antibodies able to recognize all PLA2 molecules of M. altirostris venom and showed a synergism to inhibit the catalytic activity of them. Moreover, we challenge monoclonal antibodies against to Micrurus venom for inhibit the PLA2 activity of Naja Naja, specie taxonomically out of Micrurus cluster. Our results showed that PLA2 of M. altirostris venom share a pair of conserved antigenic regions and draw attention to use these epitopes to miming antigen to generate antibodies for antivenom production. Moreover, face to the cross reactivity and the PLA2 activity inhibition capability by mAbs towards the Naja Naja venom, our results highlight the conservation of neutralizing epitopes across the Elapidae family.

PJ-027 **A comprehensive analysis of scoring functions for protein-protein docking**

Didier Barradas[1], Juan Fernandez-Recio[1,2]

1.-Barcelona Supercomputing Center, 2.-Joint BSC-CRG-IRB Research Program in Computational Biology

Protein-protein interactions are known to play key roles in the most important cellular and biological processes such as signaling, metabolism, and trafficking. One major goal of structural biology is the structural characterization of all protein complexes in human and other organisms. These efforts can be complemented by computational approaches. In this context, computational docking attempts to predict the structure of complexes from their monomeric constituents. The docking problem presents two main challenges: the generation of structural poses or sampling, and the identification of the correct structures with a scoring function (SF). Docking methods can be successful if the interacting partners undergo small conformational changes. However, in a general situation, these algorithms generate a large number of incorrect predictions, and therefore the predictive success strongly depends on the accuracy of the SF used to evaluate the docked conformations. A variety of strategies have been developed to score putative protein-protein docked complexes. They are usually based on atomic level potentials, residue level potentials, or a combination of both. In current work, we have evaluated 73 different SF, taken from Cchappi server, on the results of 3 different rigid body docking methods, Ftdock, Zdock, and Sdock, using the docking benchmark 4.0 and a docking set built from CAPRI scorers experiment. Our results show 9 SF that showed better or similar success rate than the in-built SF. Some of these SF increase the docking success rates especially for flexible or weak-binding cases, which are the most challenging for docking. 6 of them are residue level SF robust enough to detected solutions in cases with large conformation change. In particular we found two SF that shows outstanding robustness, one designed for protein modeling and shared among docking methods, and the other is for protein docking which is also the best success rate in the top100 ranking in the CAPRI scorers set. The other 3 atomic level SF display high success rate to find a solution within weak binding proteins. The 2 most successful SF are shared between the docking methods and display high success rate in the hard cases of the benchmark 4.0 and in the CAPRI scorers set. The difference between them in the resolution level at which they work, one being atomistic the other residue-based. We found that they success rate vary according to the docking method chosen, allowing them to explode different properties of the sampling used. This way to characterize a protein complex can help to develop new combined scoring functions in protein docking or a new ranking strategy to enhance the success rate.

PJ-028 **Multi-PTK antibody: a powerful tool to detect a wide variety of protein tyrosine kinases (PTKs)**

Isamu Kameshita[1], Noriyuki Sueyoshi[1], Yasunori Sugiyama[1]
1.-Kagawa University

The eukaryotic protein kinases consist of large families of homologous proteins and play pivotal roles in various cellular functions. These enzymes are classified into two major groups; protein serine/threonine kinases and protein tyrosine kinases (PTKs). PTKs are believed to be involved in various cellular events such as cell cycle, proliferation, differentiation, apoptosis, and cell adhesion in multicellular eukaryotes. As many as 90 PTK genes have been identified in the human genome and many of these PTKs are known to be closely correlated with various diseases such as cancer. Therefore, it is important to elucidate the expression profiles of the entire PTK family in cells and tissues. To investigate the expression profiles of the cellular PTKs, we produced an antibody that detects a wide variety of PTKs. For production of the antibody, antigenic peptides corresponding to amino acid sequences of a highly conserved region (subdomain VIB) of PTKs were synthesized and immunized to BALB/c mice. Among various antigens, a peptide with 11 amino acids, CYVHRDLRAAN, efficiently produced a polyclonal antibody with a broad reactivity to PTKs. We established a hybridoma cell line producing a monoclonal antibody, YK34, which appeared to cross-react with various PTKs. At least 68 PTKs could be detected by YK34 antibody, as evidenced by its reactivity with the recombinant Src tyrosine kinases whose subdomain VIB had been replaced by those of the other PTKs. When differentiated HL-60 cells were analyzed by Western blotting after two-dimensional electrophoresis with YK34 antibody, we observed significant changes in the immunoreactive spots in HL-60 cell extracts along with the changes in the morphology of the cells. These results suggest that the Multi-PTK antibody, YK34, will be a powerful tool for the analysis of a variety of cellular PTKs.

PJ-030 **Analysis of the Siglec-9 and hVAP-1 interactions**

Leonor Carvalho[1], Vimal Parkash[1], Heli Elovaara[2], Sirpa Jalkanen[2], Xiang-Guo Li[4], Tiina Salminen[1]

1.-Structural Bionformatics Laboratory, Department of Biosciences, 2.-MediCity Research Laboratory, 3.-Department of Pharmacology, Drug Development and Therapeutics, 4.-Turku PET Center

Sialic acid-binding immunoglobulin (Ig)-like lectins (Siglec) are type I transmembrane proteins. Siglec-9 has an N-terminal V-set domain followed by two C2-set domains in the extracellular region. It contains an immunoreceptor tyrosinebased inhibitory motif (ITIMs) in its cytoplasmic tail and can function as an inhibitory receptor by dampening the tyrosine kinase-driven signaling pathways. These proteins are expressed primarily on leukocyte subsets and, thus, are thought to be involved in regulation of leukocyte functions during inflammatory and immune responses. Recently, phage display screening experiments identified Siglec-9 as leukocyte surface ligand for human vascular adhesion protein -1 (hVAP-1; AOC3 gene product) and their interaction was confirmed by cell adhesion and enzymatic assays (Kivi et al., 2009; Aalto et al., 2011). Based on our preliminary data, hVAP-1 sugar units with sialic acid (SA) might mediate interactions with the V-set domain in Siglec-9. Furthermore, it is known that the Siglec peptides binding to hVAP-1 are located in the CE loop of the second C22- set of domain (Siglec-9_C22). Based on current hypothesis an arginine in Siglec-9_C22 interacts with the TPQ residue in the active site of hVAP-1. The CE loop of Siglec-9_C22 has two arginines (R284 and R290) and, therefore, the interacting arginine is unclear. We will now study the interaction mode of hVAP-1 and Siglec-9 in silico to predict the role of the arginines in the C22 domain and the role of SA-binding using the 3D model of the full-length ectodomain of Siglec-9 and the hVAP-1 crystal structure. The in silico analysis will be conducted in parallel with experimental site-specific mutational studies and the result will be combined to elucidate the mechanism of hVAP-1- Siglec-9 interaction.

PJ-031 **Molecular basis of polyubiquitin chain formation by Ube2K**

Adam Middleton[1], Catherine Day[1]
1.-Department of Biochemistry, University of Otago

Attachment of ubiquitin to substrate proteins regulates almost all cellular processes, including protein degradation and cell division. Ubiquitylation involves a cascade of three families of proteins: ubiquitin activating (E1), ubiquitin conjugating (E2) and ubiquitin ligase (E3) enzymes. The 8.5 kDa protein can be attached as a monomeric moiety or as a polyubiquitin chain, and the type of modification spells out the 'ubiquitin code' that directs the fate of the substrate. Polyubiquitin chains can be formed via eight different linkage types, and the arrangement of chain formation is typically directed by the E2 enzymes. Forming a polyubiquitin chain involves binding of two molecules to the E2: the donor (UbD) and acceptor (UbA) ubiquitin. UbD is linked to the E2 via a thioester bond between its C-terminal Gly and the active site Cys of the E2, and when primed for catalysis it interacts with a particular face of the E2. In contrast, coordination of UbA by E2s is transient and cannot be easily measured; however, UbA binding defines the linkage type of polyubiquitin chains. The E2, Ube2K, directs Lys48 chain synthesis, which results in modified proteins being degraded by the proteasome. We generated a stable form of the Ube2K~Ub conjugate and crystallized it, and showed that both Ube2K and its ubiquitin conjugate are monomeric. Using molecular docking, we modelled the position of both UbD and UbA and investigated the interfaces with site-directed mutagenesis. These experiments led to a molecular model that revealed how Ube2K can synthesise Lys48-linked ubiquitin chains. This molecular explanation provides a foundation for understanding how other E2s generate Lys48-linked polyubiquitin chains.

PJ-032 **The two chromophorylated linkers of R-Phycoerythrin in Gracilaria chilensis**

Marta Bunster, Francisco Lobos-González, José Aleikar Vásquez, Carola Bruna, José Martínez-Oyanedl

1.-Fac de Cs Biol. , Universidad de Concepción

The two chromophorylated linkers of R-Phycoerythrin in Gracilaria chilensis. Francisco Lobos-González, José Aleikar Vásquez, Carola Bruna, José Martínez-Oyanedel, Marta Bunster. Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción. Phycoerythrin is a phycobiliprotein present in phycobilisomes in Gracilaria chilensis as a complex with chromophorylated linker proteins. Our interest is to discover the role of these linkers in the function of phycobilisomes. Phycobilisomes(PBP) are auxiliary light harvesting protein complexes in charge of channeling energy towards photosystem II in alga, cyanobacteria and cryptophyta. This is possible thanks to fluorescent proteins called phycobiliproteins (PBP) and the chromophores (phycobilins, open-chain tetrapyrrols) attached to specific cysteines. Phycobiliproteins share a common general structure; they are organized as (alfaβ) heterodimers which themselves assemble as trimers(alfaβ)3 or hexamers (alfaβ)6; this complexes are organized in high order structures to form the core and the rods. Besides PBPs, PBS have linker proteins in charge of the assembly and stabilization of the complex , and also it has been proposed that they collaborate in the fine tuning of the energy transfer steps between chromophores. These linkers are located within the rods, the rod-core interface, the core and the core-membrane interface. Although most linker proteins are colorless, chromophore bearing linkers have been described, which suggest its participation in the energy transfer process. Two of them, γ 31 and γ 33 are associated to R-phycoerythrin in Gracilaria chilensis, nevertheless the information available on these linkers in eukaryots is still limited. To understand how these linkers collaborate with the function of the phycobilisome, we need structural information, especially the coordinates of all the chromophores present in the complex; we have sequenced both linkers from the genomic dna, performed sequence analysis and also we have purified the linkers by anion exchange, molecular sieve and HPL Chromatography. The characterization was performed by denaturant electrophoresis, absorption and emission spectroscopy and by mass spectrometry. The results show that they have molecular masses as predicted, with a peptide signal for chloroplasts, an internal sequence repeat; residues 67 - 170 with residues 179 – 273 for γ 31 and residues 107-200 with residues 219-315 for γ 33 , and the presence of conserved cysteine residues putative sites of chromophorylation. The spectroscopy shows that they have different composition of phycobilins and a very short t1/2. A preliminary model for both linkers shows that they belong to αα structural class and that they share a common fold (HEAT like motifs) frequently involved in protein-protein interactions. Acknowledgement: FONDECYT 113.0267

PJ-033 **Post-docking analysis by physicochemical properties of protein-protein interactions generated from rigid-body docking processes**

Nobuyuki Uchikoga[1], Masahito Ohue[2], Yuri Matsuzaki[3], Yutaka Akiyama[3]

1.-Dept. of Phys., Chuo Univ., 2.-Grad. Sch. of Inform. Sci. and Eng., Tokyo Tech, 3.-ACLS, Tokyo Tech

Rigid-body docking algorithms are useful for predicting tertiary structures of near-native protein complexes. However, this algorithms generate many protein complex poses including false positives. Then, near-native poses are searched in a post-docking process. There are many computational softwares with rigid-body docking algorithms, for example, ZDOCK. We developed a high-performance protein-protein interaction prediction software, MEGADOCK, which is basically used on supercomputing environments for a large scale and network level problems in system biology [Matsuzaki, et al. (2013) Source code Biol. Med. 8:18]. When some large scale network docking analysis, we can use MEGADOCK by every pairs of proteins for selecting native protein-protein pairs rather than for prediction of interaction surfaces of native poses. On the other hand, we developed a profile method for searching near-native protein complexes from docking poses, which are composed of residues patterns of protein-protein interaction [Uchikoga & Hirokawa (2010) BMC Bioinform. 11:236]. This profile method is used for exploring more docking space by clustering interaction surfaces of protein complex poses and iterative docking processes, which is called ‘re-docking’ [Uchikoga et al. (2013) PLoS ONE 8:e69365]. In this work, we then tried to use these docking softwares and the profile method for understanding mechanisms of protein-protein interactions. We focused on some physicochemical properties, electrostatic and hydrophobicity, of a set of protein complex poses generated by a rigid-body docking process. From these poses, we obtained sets of possible interacting amino acid pairs. A set of interaction profiles has some information of docking spaces. From the view of a network prediction, the docking spaces of a set of protein complex poses are one of the properties for discriminating native protein-protein pairs from non-native pairs. In this work, ensemble docking process is performed by MEGADOCK ver. 4.0 and ZDOCK ver. 3.0.1. Cluster analysis is used with profiles of physicochemical properties. We used a dataset composed of typical 44 monomer-monomer protein pairs and will discuss mainly differences between native and non-native protein pairs.

PJ-034 **The structural studies of the two thermostable laccases from the white-rot fungi Pycnoporus sanguineus**

Marta Orlikowska[1], Grzegorz Bujacz[1]
1.-Institute of Technical Biochemistry, Lodz University of Technology, Poland

Laccases (EC 1.10.3.2, benzenodiol oxygen oxidoreductases) are enzymes that have the ability to catalyze the oxidation a wide spectrum of phenolic compounds with the four-electron reduction of molecular oxygen to water [1]. It has been found that the active site is well conserved in between laccases from different organisms. It contains four copper atoms: one paramagnetic type 1 cooper (T1) that is responsible for their characteristic blue color and where the oxidation of the reducing substrate occurs, one type 2 cooper (T2) and two type 3 coopers (T3) that conform a trinuclear cluster in which molecular oxygen is reduced to two molecules of water [2]. Laccases are present in many different species and they have been isolated from plants, fungi, prokaryotes, and arthropods In most cases laccases are monomeric glycoproteins of around 500 amino acids with molecular weights in the range of 60-85 kDa. The various functions carried out by those enzymes include the antagonistic ones such as their involvement in lignin biosynthesis (in plants), lignin degradation, pigment production, fruiting body formation, pathogenesis (in fungi) and spore protection against UV light (in bacteria) [1, 3]. The diversified functions of laccases make them an interesting enzyme for study from the point of view of their structure, function and application. Laccases of white-rot fungi (WRF) are of special interest because one of its role is to degrade lignin and most of them are extracellular enzymes helping purification procedures [1]. During the last two decades, there has been an increasing interest in the genus Pycnoporus for its ability to overproduce high redox potential laccases as the ligninolytic enzymes. We present the crystal structures of two thermostable lacasses produced by strain Pycnoporus sanguineus CS43 (LacI and LacII). The molecular weights of LacI and LacII, determined by SDS-electrophoresis, is 68 and 66 kDa, respectively [3]. Both isoforms shows high amino acids sequence similarity (91%) between them and high thermal stability, at 50°C and 60°C. They remained active at high concentration of organic solvent (acetonitrile, ethanol or acetone). The unique properties make them promising candidates for industrial applications in wasterwater treatment. LacI exerted a higher thermal and pH stability, tolerance against inhibitors and was a more efficient catalyst for ABTS and DMP (laccases substrate) then LacII [3]. Based on the structures we would like to understand the isoforms differences that confers LacI a markedly better performance than LacII in pH and thermal stability as well as better resistance to inhibitors. [1] Baldrian F., FEMS Microbiol. Rev., 30, 215–242 (2006) [2] Mot A. & Silaghi-Dumitrescu R., Biochemistry, 77, 1395-497 (2012) [3] Rivera-Hoyos E. et al., Fungal Biol. Rev., 27, 67-82 (2013) [4] Ramirez-Cavazos L. et al., J. Mol. Catal. B Enzym., 108, 32-42 (2014)

PJ-035 **Analysis of liver proteome in cystathionine ß-synthase deficient mice using 2D IEF/SDS-PAGE gel electrophoresis, MALDI–TOF mass spectrometry, and label-free based relative quantitative proteomics**

Izabela Bielińska[1], Łukasz Marczak[1], Hieronim Jakubowski[1,2]
1.-Institute of Bioorganic Chemistry, Polish Academy of Sciences, 2.-Rutgers University, New Jersey Medical School

Homocysteine (Hcy) arises from the metabolism of the essential dietary protein amino acid methionine. Levels of Hcy are regulated by remethylation to Met and transsulfuration to Cys. Cystathionine β-synthase (CBS) catalyzes the conversion of homocysteine to cystathionine (first step of transsulfuration reaction). Human CBS deficiency is a recessive inborn error of homocysteine metabolism that casues severe hyperhomocysteinemia (HHcy) and diverse clinical manifestations, including fatty liver disease [1]. Although the causes of fatty liver disease in CBS deficiency have been studied the underlying mechanism is not understood. We hypothesize that CBS deficiency induces changes in gene expression that could impair liver homeostasis. To test this hypothesis and gain insight into hepatic functions of Cbs we analyzed the liver proteome of Cbs -/- and Cbs +/+ mice [2,3] Using 2D IEF/SDS-PAGE gel electrophoresis and MALDI-TOF mass spectrometry (n=14) we identified twelve liver proteins whose expression was significantly altered as a result of the Cbs gene inactivation. Expression of three proteins was up-regulated and of nine down-regulated by the Cbs-/- genotype. Two up-regulated liver proteins are involved in iron metabolism (Ftl and Fth). Those proteins are associated with oxidation stress and inflammation. Third up-regulated liver protein (Cbr3) is related to oxidation-reduction process. The down-regulated protein are involved in the hydrolysis of N-acylated or N-acetylated amino acids (Acy1), regulation of endopeptidase activity (A1at4), cholesterol biosynthetic process (Fpps), amino acid degradation (Huth), cellular calcium ion homeostasis and L-ascorbic acid biosynthetic process (Rgn). Using label-free based relative quantitative proteomics (n=8) we identified fourteen liver proteins whose expression was significantly altered as a result of the Cbs gene inactivation. Expression of four proteins was up-regulated and of ten proteins was down-regulated. The down-regulated liver proteins are linked with regulation of bone mineralization and inflammatory response (Ahsg) or regulation of mRNA splicing (Roa2). The up-regulated liver proteins are involved in tricarboxylic acid cycle (Suca), oxidation-reduction process (Cy250), cholesterol metabolic process, iron ion homeostasis (Fech), fatty acid metabolic process (Ssdh; Eci1) and response to oxidative stress (Lonm). Our findings suggests that Cbs interacts with diverse cellular processes, including lipid metabolism, that are essential for normal liver homeostasis. Deregulation of genes involved in lipid metabolism provides a possible explanation for fatty liver disease associated with CBS deficiency. [1] Mudd SH, Levy HL, Kraus JP. Disorders of transsulfuration. In Scriver C. R., Beaudet A. L., Sly W. S., Valle D., Childs B., Kinzler K. W., Vogelstein B., editors. McGraw-Hill; New York: The Metabolic and Molecular Bases of Inherited Disease. 2001;2:2007–2056 [2] Gupta S et al Mouse models of cystathionine β-synthase deficiency reveal significant threshold effects of hyperhomocysteinemia. FASEB J 2009;23:883–893 [3] Jakubowski H et al. Genetic or nutritional disorders in homocysteine or folate metabolism increase protein N-homocysteinylation in mice. FASEB J 2009;23:1721–1727 Supported by the NCN grant 2013/09/B/NZ5/02794, 2013/11/B/NZ1/00091

PJ-037 **Investigating protein-protein interactions of the language-related transcription factor FOXP2 in live cells with bioluminescence resonance energy transfer**

Sara B. Estruch[1], Sarah A. Graham[1], Pelagia Deriziotis[1], Swathi Mookonda Chinnappa[1], Simon E. Fisher[1,2]

1.-Max Planck Institute for Psycholinguistics, Language and Genetics Department, 2.-Donders Institute for Brain, Cognition and Behaviour, Radboud University

Transcription factors play central roles in coordinating developmental processes, as evidenced by the increasing number of transcription factor-related developmental disorders being uncovered by next-generation sequencing and genome-wide studies of copy number variation. The action of a transcription factor in regulating gene expression depends on interactions with other transcription factors, co-activators/co-repressors and chromatin modifying and remodeling complexes. Transcription factors are commonly regulated by post-translational modifications. However the study of protein-protein interactions and post-translational modifications of transcription factors by common techniques such as co-immunoprecipitation and mass spectrometry is hampered by the difficulty in preserving interactions and modifications through cell lysis. To circumvent this issue, we developed a Bioluminescence Resonance Energy Transfer (BRET) assay, which allows protein-protein interactions to be observed in live cells. In this assay, a protein of interest is expressed as a fusion with luciferase from Renilla reniformis, and its putative interaction partner as a fusion with Yellow Fluorescent Protein (YFP). Upon addition of a cell-permeable substrate, the distance-dependent non-radiative transfer of energy from luciferase to YFP is quantified by measurement of light emission at two wavelengths to assess the interaction between the two fusion proteins. To validate the utility of this assay for investigating transcription factor interactions, we confirmed homodimerization of the FOXP2 transcription factor, haploinsufficiency of which causes a rare and severe speech and language disorder, as well as interaction of FOXP2 with other members of the FOXP family. We also confirmed the interaction between FOXP2 and multiple candidate interactors identified through yeast two-hybrid assays, including the autism-related transcription factor TBR1, the co-repressors CtBP1 and CtBP2, and post-translational modification enzymes of the PIAS family. The role of PIAS enzymes in sumoylation – the covalent modification of proteins with Small Ubiquitin-like Modifier (SUMO) proteins – led us to further explore this process, which is notably difficult to investigate because of the dynamic and labile nature of the modification, which is also typically present on only a minor fraction of molecules of a given protein. Combining the BRET assay with gel-shift techniques we demonstrated that FOXP2 is sumoylated. Finally, we used the BRET assay to examine the effects of etiological FOXP2 variants in speech and language disorder on protein-protein interactions and post-translational modification. In summary, the BRET assay is a sensitive, reliable and potentially high-throughput technique for exploring protein biology in the context of live cells. We have demonstrated applications of the assay in validating putative protein-protein interactions, assessing post-translational modifications, and investigating functional effects of protein variants identified in patient cohorts. These investigations have provided novel insights into the function of the FOXP2 transcription factor in neurodevelopment and into the etiology of FOXP2-related speech and language disorder.

PJ-038 **The directly interaction between PreS1 of human virus B and human heat shock protein 70 (HSP70)**

Deqiang Wang[1], Chen Ke[1], Jun Zhang[2]

1.-Key Laboratory of Molecular Biology on Infectious Disease, 2.-The Department of Cell Biology and Genetics

The directly interaction between PreS1 of Human virus B and Human Heat Shock protein 70 (HSP70). Hepatitis B virus (HBV) has infected 2 billion people worldwide, and 350 million of them are chronically infected. The chronic virus infection, a major public health problem worldwide, leads to bout two-thirds of hepatocellular carcinoma (HCC). The HBV envelope consists of the large (L), middle (M) and small (S) envelope proteins, which contain preS1-preS2-S, preS2-S, and S domain alone, respectively [2]. The preS1 domain is believed to mediate virus attachment to the high-affinity receptor. Yan et al employed a novel technique to propose sodium taurocholate co-transporting polypeptide (NTCP) as the candidate HBV receptor, and consequently, NTCP is a target for a new family of anti-HBV agents [3]. Whereas, it remains a query to clarify that NTCP is the only or major HBV receptor in vivo. To illuminate if other host proteins cooperatively participate the HBV infection, we detect the interaction between PreS1 and many candidate host proteins. Fortunately, we have found that the human heat shocking protein 70 (HSP70) could directly interact with the PreS1 domain of the HBV virus protein. Both the pull down and the size exclusion chromatography experiments verify that the GRP78 have the ability binding to PreS1. Whereas, whether the interaction between HSP70 and PreS1 relates to the HBV infection need further experiments to clarify. Reference: [1] Lavanchy. J. Viral Hepat. 2004, 11: 97–107. [2] Schulze et al. Hepatology 2007, 46: 1759–1768. [3] Yan et al., Elife 2012, 1: e00049

PJ-039 **A new hydrophobicity scale of amino acids based on IEF-MST calculated log P and log D**

William J. Zamora[1], Josep M. Campanera[1], F. Javier Luque[1], Jody McGinness[1]

1.-Departament de Fisicoquímica and Institut de Biomedicina (IBUB), 2.-Departament de Fisicoquímica and Institut de Biomedicina (IBUB), 3.-Departament de Fisicoquímica and Institut de Biomedicina (IBUB), 4.-The member sponsorship

In the vast world of naturally occurring peptides, where more than 7000 peptides are known and approximately 140 peptide therapeutics are currently being evaluated in clinical trials (Fosgerau & Hoffmann, 2015), the rapid and accurate determination of their physicochemical properties is key in peptide drug discovery. Among these properties, hydrophobicity is crucial for understanding molecular recognition and biomolecular aggregation. Hence, there is a great interest in determining hydrophobicity scales for amino acid structures. In this work, octanol/water partition (log P) and octanol/water distribution (log DpH, Fig. 1) of N-acetyl-L-amino-acid methyl amides were determined by means of quantum mechanical IEF-MST solvation calculations taking into account the intrinsic conformational preferences of each amino acid according to Dunbrack`s libraries (Dunbrack & Karplus, 1993;1994). The results reveal log D7.4 differences for α-helical and β-sheet conformations in Arg, Lys, Hid, Asn, Gln, Met, Cys, Leu and Ile. Furthermore, by decomposing the octanol/water transfer free energy into electrostatic and non-electrostatic components, we estimated that the non- electrostatic cost of transferring the amino acid side chain amounts to 23.9 ± 3.0 cal/mol.Å2, in agreement with previous estimates reported in the literature. Comparison of our scale with other theoretical and experimental hydrophobicity scales yields satisfactory results, leading to correlation coefficients ranging from 0.61 to 0.94. Additionally, the MST-derived hydrophobicity scale led to significant correlations with the RP-HPLC retention factors measured for eight decapeptides (r = 0.97) and for 195 influenza virus hemagglutinin 13-mer (Ac-YPYDVPDYASLRS-Amide) peptides (r = 0.80). Finally, the hydrophobicity scale was able to reproduce the experimental log P for 118 random neutral peptides (r = 0.92) and log D7.4 for '01:15 random charged peptides (r = 0.95), Fig. 2. Future studies will address the application of this methodology to nonproteogenic amino acids, the prediction of peptide hydrophobicity at global and atomic level in peptides, and the scoring of peptide-protein interactions.

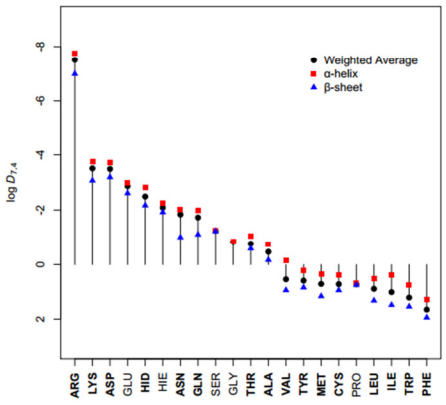

Figure 1. Representation of log $D_{7.4}$ values for twenty-one amino acid species. Black circle, red box and blue triangle represent the log $D_{7.4}$ value in total, alpha-helix and beta-sheet conformers respectively. Residues in bold show different values between alpha-helix and beta-sheet conformers

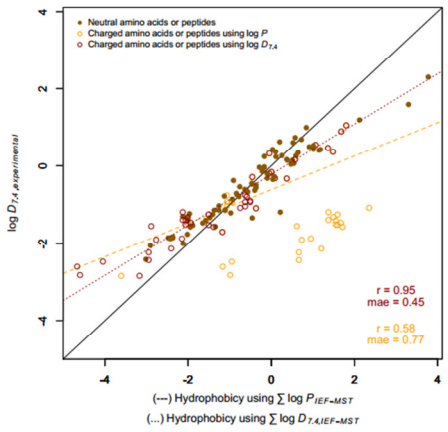

Figure 2. Representation of experimental log $D_{7.4}$ for 115 random peptides versus a) computed hydrophobicity using our log P values (correlation in yellow) b) computed hydrophobicity using our log $D_{7.4}$ values (correlation in red).

PJ-040 **Docking-based tools for discovery of protein-protein modulators**

Mireia Rosell Oliveras[1], Juan Fernández Recio[2]
1.-Barcelona Supercomputing Center, 2.-Barcelona Supercomputing Center

Docking-based tools for discovery of protein-protein modulators. Protein-protein interactions (PPIs) play an essential role in many biological processes, including disease conditions. Strategies to modulate PPIs with small molecules have therefore attracted increasing interest over the last few years. Although protein-protein interfaces (PPIfs) are considered difficult to target with small molecules given its lack of well defined cavities. Successful PPI inhibitors have been reported into transient cavities from previously flat PPIfs. Recent studies emphasize on hotspots (those residues contribute for most of the energy of binding) as promising targets for the modulation of PPI. PyDock algorithm is one of the few computational methods that use energy of solvation to predict protein-protein interfaces and hotspots residues. We present an approach aimed at identifying hotspots and transient pockets from predicted protein-protein interfaces in order to find potential small molecules capable of modulating PPIs. The method uses pyDock to identify PPIfs and hotspots and molecular dynamics (MD) techniques to propose putative transient cavities. We benchmarked the protocol in a small set of protein-protein complexes for which both structural data and PPI inhibitors are known. The method applies to the unbound proteins of the complexes the fast Fourier transform algorithm, followed by the energy-based scoring from pyDock to calculate the normalized interface propensity (NIP) values derived from rigid-body protein docking simulations to predict the PPIfs and hotspots residues without any prior structural knowledge of the complex. Then we used MD to describe the possible fluctuations of the interacting proteins in order to suggest transient pockets that could be useful as targets of small molecules for the modulation of PPIs. Finally, we evaluated by ligand docking, the validity of predicted hotspots and pockets for in silico drug design. We found that the NIP-based method from pyDock protein-protein docking identifies hotspots residues that are located within the binding site of known inhibitors of PPIs. Predicting PPIfs from a three dimensional structure is a key task for the modulation of PPIs. The use of the NIP-based hotspots prediction method improve the identification of transient cavities from MD simulation when compared to known binding cavities. This approach can be extremely useful in a realistic scenario of drug discovery targeting PPIfs, when there is no information at all about the protein-protein complex structure.

PJ-041 **Identification of transient protein complexes by using intrinsic disorder and network topology**

Inhae Kim[1], Sangjin Han[1], Jihye Hwang[2], Sanguk Kim[1]

1.-Department of Life Sciences, Pohang University of Science and Technology, 2.-Division of IT Convergence Engineering, Pohang University of Science and Technol

Protein complexes are the fundamental molecular organizations that assemble multiple proteins to achieve various biological processes. Identification of protein complex membership should provide a genotype-phenotype map to elucidate human gene-disease associations. It has been routinely assumed that network clusters with dense connections inside and sparse connections outside would form functional protein complexes. Therefore, searching highly modular subgraphs in protein-protein interaction networks was explicitly or implicitly implemented in the algorithms to find protein complexes. However, to our surprise, we found a large portion of complexes with a medium-to-low modularity from the analysis of 719 experimentally confirmed protein complexes. We also discovered that these complexes have cellular functions enriched in highly time- and space-dependent expression, such as signal transduction or subcellular localization. We further developed an algorithm to find such complexes by weighing network connections to capture transient interactions with intrinsically disordered regions. We confirmed that our method improved the identification of biologically relevant members of protein complexes and covered more complexes with a medium-to-low modularity. Furthermore, newly discovered subunits in protein complexes could explain more disease-gene associations, indicating its utility to expand current genotype-phenotype map of human diseases.

PJ-042 **Expanding template-based protein-protein complex prediction using ab-initio docking**

Sergio Mares-Sámano[1], Luis Ángel Rodríguez-Lumbreras[1], Juan Fernández-Recio[1]
1.-Joint BSC-CRG-IRB Research Program in Computational Biology

Structural characterization of protein-protein interaction (PPI) networks is crucial for understanding the underlying molecular mechanisms whereby life processes and disease arise. However, due to inherent limitations of experimental techniques, such characterization only covers an extremely reduced fraction of the human PPI network (interactome). Recent studies have shown that although available structural templates may suffice to model a significant proportion of the interactome, model accuracy and binding specificity remain unsolved problems. Consequently, improving the ability to predict PPIs structurally will help to provide a better 3D profile of the known interactome, which may ultimately lead to the development of new therapeutic applications. Here we show a novel approach that combines template-based modeling with protein-protein computational docking to the structure-based prediction of PPIs. Our approach samples different protein-protein structural models derived from docking simulations. Models are subsequently ranked using a function that incorporates an energy-based scoring term and a structural template similarity score. The energy-based scoring function includes electrostatics, van de Waals and desolvation calculations, whilst the template similarity score accounts for the degree of structural similarity of models against a high-resolution and diverse dataset of structural templates. Our approach highly improved the predictive success rate over individual ab-initio docking and template-based techniques across a large benchmark dataset, including 176 protein-protein complexes. When compared to the performance of the ab-initio docking algorithm, we found that the approach increased consistently the success rate, by approximately 30%, for the top 1, top 5 and top 10 solutions. The success rate improvement was even more notorious when the comparison was performed against the predictions from the traditional template-based docking. Though incorporating ab-initio docking expands considerably the scope of the template-based docking method, challenges remain for interacting proteins in which high conformational changes occur upon binding and also the size and diversity of the repertoire of structural templates needs to be increased.

PJ-043 **A Common Role for Cytochrome c in Programmed Cell Death in Humans and Plants**

Katiuska González-Arzola[1], Blas Moreno-Beltrán[1], Jonathan Martínez-Fábregas[1], Carlos A. Elena-Real[1], Antonio Díaz-Quintana[1], Irene Díaz-Moreno[1], Miguel Á. De la Rosa[1]

1.- Instituto de Bioquímica

A Common Role for Cytochrome c in Programmed Cell Death in Humans and Plants

González-Arzola, Katiuska; Moreno-Beltrán, Blas; Martínez-Fábregas, Jonathan; Elena-Real, Carlos A.; Díaz-Quintana, Antonio; Díaz-Moreno, Irene; De la Rosa, Miguel Á.

Instituto de Bioquímica Vegetal y Fotosíntesis, cicCartuja
Universidad de Sevilla – CSIC, Sevilla, Spain
Email: katiuska.gonzalez@ibvf.csic.es

Programmed Cell Death (PCD) is essential for the development of multicellular organisms. In mammalian cells, early events in PCD involve the release of cytochrome c (Cc) from mitochondria to the cytoplasm, so letting Cc play a key role in assembling the apoptosome and triggering apoptosis. In plants, PCD is part of a general process – the so-called hypersensitive response – in which mitochondrial Cc is likewise released into the cytosol but its further role and cytoplasmic partners remain veiled. Such a coincidence in Cc release made us think of a common link for PCD in such evolutionarily distant species along evolution.

To go deeper in understanding the PCD-dependent role of Cc, a proteomic approach based on affinity chromatography with Cc as bait was run using human and plant cell extracts. Upon combining this approach with Bimolecular Fluorescence Complementation (BIFC), a total of eight and nine unknown proteins interacting with Cc under PCD conditions were identified in human and plant cells, respectively [1,2]. Such novel Cc-partners – which are located in the cytoplasm and even in the nucleus – are involved in protein folding, translational regulation, oxidative stress, DNA damage, energetic and mRNA metabolism [3]. Strikingly, some of the novel human Cc-partners are closely related to those for plant Cc, so indicating that the evolutionarily well-conserved event of Cc release from mitochondria could involve a common signalosome consisting of a wide range of common targets [3].

To also understand such a promiscuity of Cc from a structural point of view, the Cc surface residues involved in complex formation with each one of its counterparts were mapped by using NMR spectroscopy. The resulting data shows that the heme crevice of Cc is at the Cc-partner interface in most of the complexes, which is in agreement with the vast majority of known redox adducts of Cc. In contrast, however, to the high turnover number of the redox Cc adducts inside the mitochondria, the complexes formed by Cc under PCD conditions lead to the formation of rather stable nucleo-cytoplasmic ensembles.

Altogether, these findings suggest that extra-mitochondrial Cc interacts with nuclear and/or cytoplasmic pro-survival, anti-apoptotic proteins in both humans and plants so as to lead living cells to dye.

Keywords: Cytochrome c, Programmed Cell Death, Signalosome.

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PJ-044 **Phosphorylation of cytochrome c at positions 28 and 47 could affect its double role in the cell**

Alejandra Guerra-Castellano[1], Katiuska González-Arzola[1], Francisco Rivero-Rodríguez[1], Adrián Velázquez-Campoy[2], Miguel Ángel De la Rosa[1], Irene Díaz-Moreno[1], Antonio Díaz-Quintana[1]
1.-IBVF - cicCartuja, University of Seville - CSIC, 2.-BIFI-IQFR, University of Saragossa – CSIC

Post-translational phosphorylation often modulates the function of proteins. In particular, they affect the role that cytochrome c (Cc) plays in cell life and death [1]. Cc is phosphorylated in vivo in Tyr48 and Tyr97 residues [2, 3], but recently, two new phosphorylation sites have been described at positions 28 and 47 [4]. Hence, we aim at understanding the structural and functional changes induced by Thr28 and Ser47 phosphorylation Cc. For this purpose, we designed two phosphomimetic mutants of Cc by replacing either Thr28 or Ser47 by the canonical amino acid aspartic acid (T28D and S47D). As control, two other mutants at the same two positions (T28A and S47A) were analyzed so as to differentiate the effects due to the presence of a negatively charged residue. Remarkably, the S47A mutant is significantly less stable than the wild-type species. We found that phosphorylation at position Thr28 diminishes the redox potential and oxygen consumption. In addition, T28D mutation affects the ability of Cc to bind the distal site pCc1, thereby suggesting that phosphorylation at this position affects the electron carrier capacity of Cc. Work supported by JAE Program (JaePre_2011_01248), ESF 2007-2013, Andalusian Government (CVI-BIO198), Ministry of Economy and Competitiveness (BFU2012-31670). 1García-Heredia, JM et al. J. Biol. Inorg. Chem. (2011) 16, '01:155-1168 2Lee, I et al. Biochemistry (2006) 45, 9121-9128 3Yu, H et al. Biochim. Biophys. Acta (2008) 1777, 1066-1071 4Zhao, X et al. Mol. Cell. Proteomics. (2011) 10, 1-14

PJ-046 **msBiodata Analysis Tool, a web tool to extract relevant information from proteomics experiments**

Pau Marc Muñoz Torres[1], Robert Baluzic[1], Ivana Grebesa[1], Oliver Vugrek[1]
1.-Translational Medicine Group

Mass spectrometry (MS) is widely used techniques to gain knowledge about biomolecules [1, 2]. It produces a high amount of data which is often presented as a list containing thousands of proteins. That list usually contains few hits interesting for our research. The pocess to select those proteins may include integrating experimental with annotation data. It requires spending some time in both, performing calculus and searching in databases. In this poster we present msBiodata Analysis Tool, a web service thought to deal with this tedious work. With this tool, researchers can set rules to select the most interesting hits in his lists using both, experimental data and Gene Ontology [3] annotation. The data can be upload to the web using an excel spreadsheet or a flat files in a mztab format, and rules are easily constructed by means logical sentences. Those sentences are composed by one or more terms linked by logic operators (and and or). Each term in the logical sentence indicates to our program the conditions 1 that selected hits must meet. Once the alysis is finished, the results are delivered by e-mail. msBiodat analysis tool do not requires any programming knowledge to be used and is freely available at: http://msbiodata.innomol.eu Keywords Bioinformatics / Data analysis / proteomics / Data mining / Mass spectrometry. References [1] Kondethimmanahalli Chandramouli and Pei-Yuan Qian. Proteomics: challenges, techniques and possibilities to overcome biological sample complexity. Hum Genomics Proteomics, 2009, 2009. [2] Bruno Domon and Ruedi Aebersold. Mass spectrometry and protein analysis. Science, 312(5771):212–217, Apr 2006. [3] M. Ashburner, C.A. Ball, J.A. Blake, D. Botstein, H. Butler, J. M. Cherry, A. P. Davis, K. Dolinski, S. S. Dwight, J. T. Eppig, M. A. Harris, D. P. Hill, L. Issel-Tarver, A. Kasarskis, S. Lewis, J. C. Matese, J. E. Richardson, M. Ringwald, G. M. Rubin, and G. Sherlock. Gene ontology: tool for the nification of biology. the gene ontology consortium. Nat Genet, 25(1):25–29, May 2000.

PK-001 **Effect of three aporphine alkaloids on bacillus subtilis 168**

Fatma Gizem Avci[1], Berna Sariyar Akbulut[1]
1.-Marmara University, Department of Bioengineering

Extensive and misuse of antimicrobial drugs have generated selective pressure on bacteria which resulted in the development of various resistance mechanisms rendering the drugs ineffective. Increased resistance is a matter of serious public health concern worldwide. Discovery and development of new antimicrobials are crucial steps to overcome bacterial resistance. In this sense, plant-derived substances have drawn attention as novel and promising sources of antimicrobial agents. Alkaloids are among the secondary metabolites of plants with different activities. So far, isoquinoline alkaloids have proven to possess therapeutic potential. In this work, the effect of three aporphine alkaloids, boldine, bulbocapnine and roemerine, on the microbial viability has been investigated using a systems approach. The cells were allowed to grow in the presence of the alkaloids for determining MIC values. Cytoscape was then used to analyze protein interaction networks. Despite their structural similarities, whereas -(-)roemerine had a high antimicrobial activity against Bacillus subtilis 168, boldine and bulbocapnine had no significant effect. The network constructed using cytoscape enabled us to evaluate the protein interactions comparatively under the influence of the three different alkaloids. -(-)Roemerine is a potential antibacterial agent that could be valuable in the fight with rising resistance to available drugs. Acknowledgements: This work was supported by TUBITAK-MAG project 113M052.

PK-002 **Protein degradation systems in the control of salmonid fish growth**

Liudmila Lysenko[1], Nadezda Kantserova[1], Marina Krupnova[1], Nina Nemova[1]
1.-The Institute of Biology, Karelian Research Centre of Russian Academy of Science

Beside the rate of protein synthesis, the regulation of protein degradation plays a crucial role in the white muscle protein accumulation and overall fish growth. Intracellular proteolysis in salmonid species, such as Atlantic salmon, Salmo salar L. and rainbow trout, Oncorhynchus mykiss Walb., was studied to evaluate the basic mechanisms of protein degradation that could possess a potential target to regulate the body mass accumulation in farmed fish. A number of white muscle proteases such as cathepsins B, L, and D, proteasomes, and calcium-dependent proteases (μ- and m-calpains), was studied in the juvenile specimens of different size- and age-groups both wild and farmed salmonids. The correlations between the protease activity and expression levels and morphometric characteristics of fish were found. The size- and age-related differences in intracellular protease activity revealed in fish muscles indicate both general role of proteolysis regulation in salmonid growth and the specific role of the individual proteolytic enzymes as well. The data on negative correlation of cathepsin D and calpain activity in muscles and the rate of weight increase in juvenile salmonids were obtained. A revealed positive correlation of cathepsin B activity and morphometric parameters in fish young presumably indicates its primary contribution to non-myofibrillar protein turnover. Ubiquitin-proteasome system seems to contribute to background protein turnover as the proteasome activity was not corresponded with growth rate. Summarizing the data obtained the autophagy-lysosomal and calpain-related protein degradation pathways were recognized to be directly involved in body growth and muscle protein retention in salmonid fish. The work was carried out using technical facilities of IB KarRC RAS Equipment Centre and financially supported by the Russian Science Foundation, grant No. 14-24-00102 “Salmonids of the North-West Russia: ecological and biochemical mechanisms of early development”.

PK-003 **Solving the proteomic organization of fitness-related genes in Uropathogenic Escherichia coli**

Marc Torrent Burgas[1,2]

1.-Microbiology Department, Vall d'Hebron Institut de Recerca, 2.-Biochemistry Department, Universitat Autònoma de Barcelona

Certain isolates of Escherichia coli are associated to a wide range of diseases and affect both humans and animals worldwide. Uropathogenic E. coli (UPEC) belongs to a subset of the extraintestinal pathogenic E. coli (ExPEC) pathovar and is the primary etiological agent of urinary tract infections (UTI). Patients with UTI can develop pyelonephritis and are at risk for developing bacteremia that may result in life threatening sepsis. Nowadays, complete genomes for almost all major bacterial pathogens are available, helping researchers to identify virulence factors. However we still ignore how these genes are organized at the proteome level and how this association influences bacteria pathogenicity. We integrated available databases on UPEC E. coli (strain CFT073) to investigate the genomic and proteomic organization of genes related to UPEC fitness in the host. Intriguingly, we found that most fitness-related genes have orthologs not only in other pathogenic strains but also in non-pathogenic bacteria such as E. coli K-12. These genes are organized in clusters and operons with similar structure. By integrating protein-protein interaction data we observed that genes with high impact on fitness also display a highly clustered organization when compared to other genes. Overall, our results show that protein-protein interaction clusters associated to UPEC fitness in the host represent a promising target for the design of new antibiotics.

PK-004 **Elucidating the molecular mechanisms by which the HNH endonuclease gp74 activates the terminases in bacteriophage HK97**

Sasha Weiditch[1], Karen Maxwell[4], Voula Kanelis[1,3]

1.-Cell & Systems Biology, University of Toronto, 2.-Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, 3.-Chemical & Physical Sciences, University of Toronto, 4.-Department of Molecular Genetics, University of Toronto

Elucidating the molecular mechanisms by which the HNH endonuclease gp74 activates the terminases in bacteriophage HK97. Bacteriophages are the most abundant entities in the biosphere (1). The last gene in the genome of the bacteriophage HK97 codes for gp74, an HNH endonuclease(2). HNH endonucleases are characterized by two highly conserved His residues and an Asn residue(3). Gp74 is essential for phage head morphogenesis, likely because gp74 enhances the activity of the HK97 terminase enzymes toward the cos site(4). Notably, enhancement of the terminase-mediated cleavage of the phage cos site requires the presence of an intact HNH motif in gp74. Mutation of the canonical metal binding His in the HNH motif abrogates gp74 mediated-terminase activity. Although phages are widely studied, there is no definitive structural or mechanistic evidence as to how the HNH endonuclease within gp74 functionally interacts with the adjacent terminase enzymes to facilitate phage morphogenesis. Previous work on HNH-containing bacteriophage proteins does not address explicitly how the requirement for divalent metal binding at the HNH endonuclease site induces interaction with the terminase enzymes that are so crucial for phage DNA packaging during morphogenesis (4, 5). In addition, gp74 possesses no sequence similarity to HNH proteins for which the structure has been determined (3), making structural studies of gp74 necessary. Toward these ends, we use nuclear magnetic resonance (NMR) spectroscopy to probe metal and terminase binding of gp74 in the wild type state and bearing metal binding mutations. We also report backbone resonance assignment of gp74. Our NMR studies have elucidated residues within gp74 required for metal binding and terminase activity. These data are being used to assess the role of specific gp74 residues in phage morphogenesis. Together, this work will identify the enigmatic role describing how metal binding in HNH endonucleases is crucial in the replication and morphogenesis of phages. 1.Campbell, A. (1994) Comparative molecular biology of lambdoid phages, Annu Rev Microbiol 48, 193-222. 2. Juhala, R. J., Ford, M. E., Duda, R. L., Youlton, A., Hatfull, G. F., and Hendrix, R. W. (2000) Genomic sequences of bacteriophages HK97 and HK022: pervasive genetic mosaicism in the lambdoid bacteriophages, J Mol Biol 299, 27-51. 3.Stoddard, B. L. (2005) Homing endonuclease structure and function, Q Rev Biophys 38, 49-95. 4.Kala, S., Cumby, N., Sadowski, P. D., Hyder, B. Z., Kanelis, V., Davidson, A. R., and Maxwell, K. L. (2014) HNH proteins are a widespread component of phage DNA packaging machines, Proc Natl Acad Sci U S A 111, 6022-6027. 5.Quiles-Puchalt, N., Carpena, N., Alonso, J. C., Novick, R. P., Marina, A., and Penades, J. R. (2014) Staphylococcal pathogenicity island DNA packaging system involving cos-site packaging and phage-encoded HNH endonucleases, Proc Natl Acad Sci USA 111, 6016-6021.

PK-005 **Analysis of the binding of mycotoxins to proteins involved in ASD with a combined computational/experimental approach**

Bernardina Scafuri[1], Antonio Varriale[2], Angelo Facchiano[2], Sabato D'Auria[2], Maria Elisabetta Raggi[3], Anna Marabotti[1]

1.-Dept. Chemistry and Biology, University of Salerno Bernardina Scafuri, 2.-Institute of Food Science, CNR Antonio Varriale, 3.-IRCCS "E. Medea" Ass. "La Nostra Famiglia" Maria Elena Raggi, 4.-2 Sabato D'auria, 5.-2 Angelo Facchiano, 6.-2 Anna Marabotti, 7.-1 Anna Marabotti

Autism spectrum disorder (ASD) is a group of neurodevelopmental disabilities characterized by persistent deficits that manifestwith impaired social communication and social interaction, restricted and repetitive patterns of behavior, interests or activities [1]. The etiology of ASDis unknown, but it is believed that it involves genetic and environmental components. The purpose of this work is to assess the possible involvement of food contaminants, such as mycotoxins, in the etiology of ASD. The hypothesis is that the mycotoxins ingested with the diet could bind to proteins and expose the entire organism,including CNS, to the negative effects of xenobiotics, in genetically predisposed patients. In this study some possible protein targets for the mycotoxinswere identified to evaluate if the bond between any protein target and the mycotoxin in exam could play a role in ASD. Twelve mycotoxins were selected (ochratoxin A, gliotoxin, aflatoxin B1, aflatoxin B2, aflatoxin M1, aflatoxin M2, aflatoxicol, a-zearalanol, b-zeralanol, zearalenone, deoxynivalenol, patulin),which are contaminants of milk and cereals.For each of these molecules,possible protein targets were searched by a reverse docking approach using the idTargetserver[2].From the results given by idTarget, human protein targets expressed in the brain or involved in brain diseaseswere selected. Subsequently, a direct docking was made using AutoDock 4.2 [3], in orderto verify the strength of the interaction between selected proteins and each mycotoxin, and to identify the mycotoxins' binding site on each of the selected protein. Finally, the bond of some mycotoxins to selected protein targets has been experimentally tested. For each mycotoxin, idTarget returned thousands of possible protein targets,and only those with the best binding energy were selected and evaluated. Among them, human protein targets that are expressed in the brain or that are involved in cerebral diseases,have been selected; moreover the protein targets that were not human but that idTargetselected for five or more mycotoxins, were replaced with their human counterparts. At the end of the procedure, nineteen protein targets have been identified for the following direct docking approach. From the docking results, eight proteins have been selected for experimental tests, having a predicted binding energy lower than -7 kcal/mol. Finally, the interactions between Acetylcholinesterase (AChE), β-secretase (BACE1) and Neuroligin-4, X-linked (NLG4X) with Aflatoxin B1, Aflatoxin B2, Gliotoxin, Ochratoxin A and Deoxynivalenol, were evaluatedusing fluorescence spectroscopy and microscale thermophoresis. These experiments confirmed the presence of an interaction between BACE1 and Aflatoxin B1; NLG4X and Aflatoxin B1,Gliotoxin and Ochratoxin A; and Deoxynivalenol,AChE and Aflatoxin B1. These results suggest that the interaction between mycotoxins and proteins involved in neuronal plasticity is possible also in vivo, supporting the hypothesis of a putative role of mycotoxins in the etiology of ASD. References [1] Singh K, Zimmermann AW;Sleep in Autism Spectrum Disorder and Attention Deficit Hyperactivity Disorder; SeminPediatrNeurol 22,113-125 (2015) [2]Chu PY, Chen CM, Lin JH;idTarget: a web server for identifying protein targets of small chemical molecules with robust scoring functions and a divide-and-conquer docking approach, Nucleic Acids Res 34, W219-W224 (2006) [3]Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ;AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility, J ComputChem 30, 2785-2791 (2009) Acknowledgements This work was made in the frameshift of the project GR-2009-1570296: "The relationshipamongfood, mycotoxins, gastrointestinaldisorders and autism: a multidisciplinaryapproach for the molecularinvestigation" funded by the Ministry of Health (program "Ricerca Finalizzata e Giovani Ricercatori 2009"). AF acknowledges the

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The Authors would also acknowledge the support of NanoTemper Technologies GmbH, and in particular of dr. Francois-Xavier Ogi, for the microscale thermophoresis experiments

PK-006 **Developing of microbial consortia for enzymatic valuable conversion of keratin-rich slaughter-house waste**

Roall Espersen[1], Milena Gonzalo[3], Samuel Jacquiod[3], Waleed Abu-Alsud[3], Søren J. Sørensen[3], Jakob R. Winther[4], Per Hägglund[2], Birte Svensson[1]

1.-Enzyme and Protein Chemistry, Department of Systems Biology, Technical Universit, 2.-Protein and Immune Systems Biology, Department of Systems Biology, Technical Uni, 3.-Section of Microbiology, Department of Biology, University of Copenhagen, 4.-Section for Biomolecular Sciences, Department of Biology, University of Copenhag

Meat production from pigs for human consumption is a resource heavy process, indeed every part of the animal that is not used constitutes a protein food-chain loss, which is neither economically nor environmentally viable. The goal of this project is to better harness slaughterhouse waste such as the keratin rich pig bristles and nails through microbial conversion. Instead of using identified single microorganisms, it is the goal to define microbial consortia where microorganisms synergistically show the ability of efficient keratin degradation/conversion. Candidate consortia have been obtained by selecting for microorganisms growing on enriched media that contains milled pig bristles as sole carbon and nitrogen source. By using mass spectrometry and various biochemical analyses to investigate keratinolytic enzymes, methods will be established for identifying and characterizing suitable consortia. Protein families likely to be involved are keratinases, which are specialized proteases including serine, cysteine and metallo proteases, as well as systems capable of reducing or otherwise breaking disulfide bonds which are highly abundant in hair and nails. Furthermore, interactions and symbiosis of microorganisms in a consortium will be investigated at the meta-proteomics level. The project will lead to development of biotechnological degradation of keratin rich fibers, and provide new insights into functional dynamics and efficacy of microbial consortia.

PK-007 **A comprehensive protein domain analysis to map cancer-type-specific somatic mutations**

Jihye Hwang[1], Sangjin Han[2], Inhae Kim[2], Sanguk Kim[2]

1.-Department of IT Convergence and Engineering, POSTECH, 2.-Department of Life Science, POSTECH

Recent development of whole genome sequencing technology has provided a large amount of genetic variants of cancers. This data may be informative to find cancer markers for diagnosis and stratification of cancer patients into different risk groups. However, the identification of type-specific markers for various cancers is still elusive because each genetic variant often accounts for only small portion of patients. Thus, it is urgent to develop analytic frameworks to infer the genotype-phenotype relationship in cancers from such amount of genetic variants. In this study, we mapped protein domain information to genetic variants from whole-genome/exome sequencing data of 3,527 specimens across 12 cancer types from The Cancer Genome Atlas project (TCGA project). We found that protein domain instances are often enriched with cancer-type-specific somatic mutations, which enabled us to define cancer-type-specific protein domains. Based on the cancer-type-specific protein domains, we could stratify cancers with distinct phenotypes better than gene-based approaches. Thus, we expect that protein domain analysis offers a new opportunity to decipher functional relationships between cancers and would helpful to prioritize cancer-type-specific mutations for discovering marker genes and classification of cancer patient groups.

PK-008 **Identification of cancer-type-specific modules comprised of cancer-type-specific variants through phenotype similarity between cancer types**

Sangjin Han[1], Jihye Hwang[2], Inhae Kim[1], Sanguk Kim[1,2]

1.-Department of Life Science, POSTECH, 2.-Department of IT Convergence and Engineering, POSTECH

Interpretation of the genome-wide association studies (GWAS) of cancer patients to find cancer-type-specific biomarker is challenging due to the mutational heterogeneity of cancer types. Network approaches to find cancer-type-specific variants and biological pathways are increasing since genes tend to act together to display phenotypic or disease outcomes. Phenotype similarity has proven to reflect the relationship of functionally related genes. We applied phenotype similarities between various diseases for expanding molecular connections of cancer-type-specific variants to discover cancer-type-specific modules. Specifically, cancer-type-specific variants of 7 cancer types from The Cancer Genome Atlas (TCGA) were analyzed to find phenotype-inferred relationships among the variants. We find that cancer variants that cause the similar disease phenotypes tend to be linked as a cluster of biological pathways or functions. Moreover, cancer-type-specific modules could explain the underlying pathogenicity of specific symptoms which manifest in particular cancer types. Cancer-type-specific modules and pathways found from phenotype similarity/dissimilarity based on cancer symptoms improved the discrimination performance to sort cancer-type-specific variants to accurately predict patient groups. Our method will be further developed to find genetic biomarkers for the diagnosis or prognosis of specific cancer types

PK-009 **Engineering a stable, symmetric membrane protein scaffold**

Amanda Duran[1], Jens Meiler[1]
1.-Vanderbilt University-Department of Chemistry

Computational protein engineering has the potential to contribute to various fields including drug design, protein therapeutics, and materials science. Protein-ligand interface design and the construction of large, stable proteins rely on stable scaffolds. Symmetry is a great tool for protein stability both in protein engineering and nature. Several membrane protein structures exhibit pseudo-symmetry and are proposed to be the result of gene duplication, fusion and diversification events originating from a monomeric gene. Aquaporins (AQP) are a class of membrane proteins that exhibits a two-fold inverted pseudo-symmetry. The Escherichia coli AQP Glycerol facilitator protein (GlpF) was originally computationally engineered to be perfectly symmetric in sequence and presumably in structure. The symmetric gene was assembled, cloned, and expressed. However, after facing many challenges experimentally, the computational study has been expanded to 13 AQPs of known structure for a more extensive symmetric backbone search. MAMMOTH structural alignment was used to align the structures to their inverted counterparts. Cutpoints were calculated based on α-Carbon distance. Finally, the Rosetta Protein Modeling Software Suite was used to refine and energetically minimize the symmetric backbones. From over 1500 generated symmetric backbones, 20 candidates were chosen for experimental verification. These studies are ongoing.Currently, the symmetric backbone models have scored to be more stable than the wild-type proteins. Experimental verification of these symmetric backbones will provide valuable information for the current state of membrane protein modeling and design using computational methods.

PL-001 **Intrinsically disordered proteins drive heritable transformations of biological traits**

Daniel Jarosz[1], James Byers[1], Sohini Chakrabortee[2], Sandra Jones[3], Amelia Chang[2], David Garcia[1]

1.-Stanford University, 2.-Whitehead Institute for Biomedical Research, 3.-Rockefeller University

The transmission of information from one generation to the next generally occurs via nucleic acids. The only known protein-based molecular memories are prions, which drive heritable biological traits based upon self-templating changes in protein conformation. These protein-based genetic elements have previously been identified systematically, but at least three do not share the sequence biases or structural characteristics that have informed such studies. Here we employed a comprehensive library of yeast proteins to examine the breadth of protein-based inheritance. Transient overexpression of more than forty proteins created new traits that were heritable and beneficial. Some shared properties of known prions, but most employed distinct genetic and biochemical mechanisms to act as elements of inheritance. Traits with these characteristics were common in wild yeast strains and could also be elicited using orthologous mammalian proteins. The inducing proteins were strikingly enriched in intrinsically disordered sequences that have been widely conserved across evolution. Intrinsically disordered proteins are associated with human disease and with dosage sensitivity in yeast, flies and worms. Our results suggest another widespread role for such intrinsically disordered sequences: induction of heritable epigenetic switches that transform phenotypic landscapes and drive adaptation to stressful environments.

PL-002 **Prediction of binding affinity in protein complexes: contacts do matters**

Anna Vangone[1], Alexandre MJJ Bonvin [1]

1.-Computational Structural Biology group, Bijvoet Center for Biomolecular Research

Almost all critical functions in cells rely on specific protein-protein interactions. Understanding these is therefore crucial in the investigation of biological systems. Despite all past efforts, we still lack a thorough understanding of the energetics of association of proteins. Here, we introduce a new and simple approach to predict binding affinity based on functional and structural features of the biological system, namely the network of interfacial contacts. We assess its performance against a protein-protein binding affinity benchmark and show that both experimental methods used for affinity measurements and conformational changes have a strong impact on prediction accuracy. Using a subset of complexes with reliable experimental binding affinities and combining our contacts- and contact types-based model with recent observations on the role of the non-interacting surface in protein-protein interactions, we reach a high prediction accuracy for such a diverse dataset outperforming all other tested methods.

PL-003 **Free radical oxidation – a new method for obtaining stable protein coatings on magnetic nanoparticles**

Anna Bychkova[1], Alexandra Vladimirova[1], Mariya Nezhivaya[1], Tatiana Danilova[1], Pavel Pronkin[1], Maria Gorobets[1], Alexander Tatikolov[1], Vyacheslav Misin[1], Mark Rosenfeld[1]
1.-N. M. Emanuel Institute of Biochemical Physics, Russian Academy of Sciences

Magnetically targeted nanosystems (MTNSs) are now considered to be applicable in different areas of biology and medicine such as hyperthermia, magnetic resonance imaging, immunoassay, cell and molecular separation, a smart delivery of drugs to target cells. Proteins are promising materials for creation of coatings on magnetic nanoparticles (MNPs) due to their biocompatibility, an ability to protect magnetic cores from influence of biological liquids and prevent agglomeration of MTNSs in dispersion, their possible functional activity as therapeutic products and biovectors. The creation of stable protein coatings with retention of native properties of molecules is still an important biomedical problem because of disadvantages of the commonly used methods such as formation of a polydisperse ensemble of particles, nonselective linking of proteins leading to cross-linking of macromolecules in solution, and desorption of coatings.

A novel method in obtaining stable single-layer coatings assembled from protein molecules on the surface of magnetite nanoparticles has been developed. It is based on protein liability to free radical modification, leading to the formation of intermolecular covalent cross links. Free radicals are locally generated on the surface of nanoparticles via the Fenton reaction thereby proteins adsorbed on the surface are subjected to the cross-linking. O-phenylenediamine was used for detection of free radical generation initiated by nanoparticles. The proteins drastically differing in their structure and properties, namely, serum albumin, thrombin and immunoglobulin G were selected for creating the protein coatings. The properties of the obtained coatings and their stability have been studied with the help of dynamic light scattering (DLS), UV/Vis spectrophotometry, antibody-antigen test and the method of spectral-fluorescent probes. Albumin molecules in MNPs coatings have been shown to retain their capability of binding with a dye and be conformationally stable. The dye 3,3`-di-(γ-sulfopropyl)-5,5`–diphenyl-9-ethiloxacarbocyanine-betaine interacting with albumin with a growth of fluorescence and with partial cis–trans conversion of the dye has been used.

It has been proven that coatings composed of protein macromolecules are 1) stable, 2) formed around individual nanoparticles and 3) have several nanometers in thickness. The free radical linking of thrombin and immunoglobulin G on the surface of nanoparticles has been shown to almost completely keep native properties of the protein molecules.

The free radical linking method reveals new possibilities for design of single-layer multiprotein polyfunctional coatings on the surfaces of all the nano-, micro- and macroobjects containing metals of variable valence (for example, Fe, Cu, Cr).

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PL-004 **Regulation of neuronal SNAREs by accessory proteins**

Shrutee Jakhanwal[1], Reinhard Jahn[1]
1.-Department of Neurobiology, Max Planck Institute of Biophysical Chemistry

Regulation of Neuronal SNAREs by accessory proteins 1Shrutee Jakhanwal and 1Reinhard Jahn 1Department of Neurobiology, Max Planck Institute of Biophysical Chemistry, Fassberg, Goettingen, Germany-37075. Synaptic vesicle exocytosis lies at the heart of the process of neurotransmitter release. And, the family of proteins that is central to the process of synaptic vesicle exocytosis is the family of SNARE proteins. There are three kind of neuronal SNARE proteins namely Syntaxin, SNAP25 and Synaptobrevin. These three SNARE proteins interact through their SNARE-motifs to form a highly stable four-helix bundle, which in turn, pulls two membranes together to mediate fusion. Years of work in this field have established that the four-helix bundle is critical for the membrane fusion to occur. However, the process of regulation of SNARE-mediated fusion remains very poorly understood. The major regulatory proteins involved in the process are Munc 18, Munc 13, Synaptotagmin and Complexin. The major aim of my project is to obtain a closer look at the regulation process of SNARE-mediated fusion by focusing on the interaction between the SNARE proteins and the regulatory proteins. To achieve this objective, I express and purify the different proteins involved in the process of SNARE-mediated fusion and thereafter subject them to appropriate biochemical characterization. In order to assess the role of the purified proteins in the process of fusion, I reconstitute them into liposomes and perform in-vitro lipid-mixing assays. These assays are based on Fȯrster Resonance Energy Transfer (FRET). Based on the discretion of assessing the protein-protein or protein-lipid interactions, either the proteins or the lipids can be fluorescently labeled. Also, the lipid compositions can be varied in order to assess the effect of lipid on the function of the respective protein. Fluorescence-based anisotropy measurements can also provide information about the degree of freedom of a protein, indirectly providing information about the kinetics of a reaction. Employing these techniques, I observe that Munc 18-1 leads to displacement of Syntaxin from a complex of Syntaxin and SNAP25. Also, a complex of Syntaxin and Munc 18 is resistant to the action of the AAA-ATPase, NSF and its co-factor αSNAP, implicating this complex as a strong candidate for acting as the starting point for the process of neurotransmitter release. Munc 18 also appears to enhance lipid-mixing by interacting with the SNARE-complex. Further investigations on the same lines can provide very useful insights into the process and can help us unravel the secrets that underlie the beauty of the exquisitely regulated process of neurotransmitter release.

PL-005 **Binding of thymidine nucleotides to a viral thymidine monophosphate kinase**

Aldo A. Arvizu-Flores[1], Eduardo Guevara-Hernández[2], Enrique F. Velazquez-Contreras[1], Francisco J. Castillo-Yañez[1], Luis G. Brieba[3], Rogerio R. Sotelo-Mundo[2]

1.-Universidad de Sonora, Departamento de Ciencias Químico Biológicas, 2.-Centro de Investigación en Alimentación y Desarrollo, A.C., 3.-Laboratorio Nacional de Genómica para la Biodiversidad

Nucleotide phosphorylation is a key step towards DNA replication during viral infections since suitable levels of nucleotide triphosphates pool are required for DNA synthesis. Deoxythymidine triphosphate (dTTP) is produced either by de novo or salvage pathways, being the enzyme thymidine monophosphate kinase TMK) at the junction of both pathways. The TMK from the white spot syndrome virus (WSSV) represents a major target for drug design directed to shrimp quaculture applications in many countries. This enzyme catalyzes the ATP-dependent phosphorylation of deoxythymidine monophosphate (dTMP) to yield deoxythymidine diphosphate (dTDP). In this work, we used isothermal titration calorimetry and molecular docking to assess the binding thermodynamics for hymidine nucleotides, such as dT, dTMP, dTDP and the nucleosidic analog of phosphorylated stavudine (d4TMP), to the TMK from WSSV. Dissociation onstants (Kd) for dTMP and dTPDP were 3.2 and 6.2 µM, respectively. Both ligands showed similar binding energetics, with a subtle difference of 1.5 kcal/mol in the enthalpic component. Interestingly, the Kd for d4TMP was 3.5 µM, whereas for dT was 11.6 µM. For all thymidine nucleotides, the Kd values are almost dentical to that of dTMP, despite of being different in one hydroxyl or phosphate group. These results suggest that nucleoside analogues like d4TMP, could be considered as a feasible treatment strategy for the WSSV disease in farmed shrimp.

PL-006 **A cold-adapted trypsin in sardine fish (Sardinops sagax caerulea): molecular modeling and recombinant expression**

Aldo A. Arvizu-Flores[1], Manuel I. Carretas-Valdez[2], Francisco J. Castillo-Yañez[1], Karina D. Garcia-Orozco[3], Carmen A. Contreras-Vergara[3], Rogerio R. Sotelo-Mundo[3], Maria A. Islas-Osuna[1,3]1.-Departamento de Ciencias Químico Biológicas, Universidad de Sonora, 2.-Departamento de Investigación y Posgrado en Alimentos, Universidad de Sonora, 3.-Centro de Investigación en Alimentación y Desarrollo

THEME: Biochemistry There is great interest in the evolution and activities of fish trypsins, since they appear to have evolved into different families. The cDNA for trypsin III from the Monterey sardine (Sardinops sagax caerula) was obtained and its deduced amino acid sequence matched its identity with a purified protease from the fish by mass spectrometry analysis. Molecular modeling of sardine trypsin III compared to other homologs showed a typical trypsin fold with all the cognate components for catalysis, and specific amino acid distribution that are possible factors that explain the cold adaptation. From phylogenetic analysis, sardine trypsin III belongs to the novel Y family, which is proposed to have evolved for cold adaptation. The obtained recombinant trypsin III showed a low catalytic efficiency, but it remained active at cold temperatures, similar to other cold-adapted trypsins. The cold-adaptation of sardine trypsin III opens a wide range of biotechnological applications for this protease and is also interesting from the serine protease structure-function relationship point of view.

PL-007 **Fungicidal mechanism of scolopendin 2, a cationic antimicrobial peptide from centipede**

Heejeong Lee[1], Dong Gun Lee[1]
1.-Kyungpook National University

Scolopendin 2, AGLQFPVGRIGRLLRK, is a 16-mer peptide derived from the centipede Scolopendra subspinipes mutilans. To investigate its property against fungal and bacterial pathogens, antimicrobial tests were performed. We observed that this peptide exhibited antimicrobial activity in a salt-dependent manner and showed no hemolysis. The circular dichroism (CD) analysis observed that α-helical structure properties. We determined the mechanism(s) of action using flow cytometry and investigated the release of potassium. The results showed that the microbial membrane in Escherichia coli O157 and Candida albicans was permeabilized with loss of potassium ions. Additionally, the bis-(1,3-dibutylbarbituric acid) trimethine oxonol [DiBAC4(3)] and 3,3'-dipropylthiacarbocyanine iodide [DiSC3(5)] assay showed membrane depolarization. Using calcein-encapsulating giant unilamellar vesicles (GUVs) and FITC-dextran containing large unilamellar vesicles (LUVs), scolopendin 2 disrupted the cell membrane and the damage size is between 4.8 to 5.0 nm against composition of microbial plasma membrane of E. coli and C. albicans. Thus, we demonstrated that a cationic antimicrobial peptide, scolopendin 2, possesses broad-spectrum antimicrobial effects that formed pore on the cell membrane.

PL-008 **Structural and functional investigation of the far C-terminal domain (CTD) of the bifunctional enzyme traI using NMR Spectroscopy**

Krishna Chaitanya Bhattiprolu, Evelyne Schrank, Klaus Zangger

Protein Structural Biology Structural and functional investigation of the far C-terminal domain (CTD) of the bifunctional enzyme TraI using NMR Spectroscopy B.Krishna Chaitanya, Evelyne Schrank and Klaus Zangger Institute of Chemistry / Organic and Bioorganic Chemistry University of Graz, Austria Corresponding author email ID: krishna.bhattiprolu@uni-graz.at Bacterial conjugation is a complex process for the horizontal transfer of single stranded DNA from one cell to another. This mechanism also leads, for example, to the spread of antibiotic resistance genes and virulence factors among bacterial species. Multi-protein complexes formed at the origin of transfer (oriT) region of DNA and at the cytoplasmic membrane of the bacterial cell, initiate this process. Inside the membrane, the relaxosome identifies the single strand for transfer in a plasmid DNA, relaxes and unwinds it, whereas the transferosome is involved in pilus formation (Type IV secretion system) and transferring the gene through the cytoplasmic membrane. These events take place in the donor bacterial cell along with several other auxiliary proteins [1] The bifunctional enzyme TraI of plasmid R1 plays a crucial role in the relaxosome activity, as it contains both a relaxase and helicase domain. To exert its functions on DNA, TraI works in close co-ordination with other relaxosome proteins like TraY, TraM and the integration host factor. TraI is a 1756 residual protein and contains 3 major domains: N-terminal relaxase domain, a central helicase domain and a C terminal domain (CTD). The structure of the C-terminal domain until residue 1629 has been solved by crystallography, while the structure and function of the remaining ~130 residues remained undetermined [2]. There are SAXS models and crystallographic structures for different parts of TraI and also for the full length protein. All of them miss the information about this particular domain. This region is of interest, particularly because it is an intrinsically disordered (ID) region and ID regions in most of the proteins are involved in regulatory functions. Previous mating pair experiments have strong evidences which show that the translocation frequency of the conjugative DNA decreases drastically (from 6.5 x 10-3 to 2 x 10-3 colonies) upon deletion of this ~130 residues domain from the full length TraI. We are investigating the structure and function of this very C-terminal end of TraI using NMR spectroscopy. For the backbone assignment we used slice-selectively homonuclear broadband decoupled spectra along with standard experiments. Three-bond scalar coupling constants were obtained through real-time J-upscaling experiments. With the backbone assignments, we have the first hand evidence which shows that his domain is for the most part intrinsically disordered, but contains short α-helical regions. Structural development, interaction studies to find the binding partner and transition of disorder to order orientation of this domain will be further investigated in this project. References: [1] Redzej, Adam et al. “Structure of a Translocation Signal Domain Mediating Conjugative Transfer by Type IV Secretion Systems.” Mol. Microbiol.89 (2) (2013): 324–333. [2] Guogas, Laura M. et al. “A Novel Fold in the Trai Relaxase-Helicase C-Terminal Domain Is Essential for Conjugative DNA Transfer.” J. Mol. Biol. 386(2) (2009): 554–568.

PL-009 **Sodium chloride induced aggregation of monoclonal antibodies at low ph: prevention by additives**

Fabian Bickel[1, 2], Hans Kiefer[1]

1.-Institute of Applied Biotechnology, Biberach University of Applied Sciences, 2.-International Graduate School in Molecular Medicine Ulm, Ulm University

Protein Aggregation Aggregation is a known phenomenon in downstream processing of monoclonal antibodies (mAbs) in connection with certain stress factors like shear forces, low pH values and high salt concentrations. Here we investigated a model system where mAb aggregation is induced by increasing the ionic strength (NaCl) at low pH. The aggregation depends both on protein and sodium chloride concentration. With Nanoparticle Tracking Analysis (NTA) and Micro Flow Imaging (MFI) the aggregation formation was further characterized. Aggregation can be partially reverted by lowering the ionic strength as determined by soluble monomer concentration measurement using SE-HPLC: Parts of insoluble aggregates could be solubilized as soluble aggregates, dimers or even monomers. A quasi equilibrium is formed in between the subtypes. The whole aggregation process was examined by FTIR and CD-Spectroscopy to identify structural changes of the mAb. Screen of protective additives: The effect of osmolyte additives on aggregation kinetics and final aggregate concentration is investigated, revealing protective effects in both cases. In a screen with more than 200 compounds not only the aggregation propensity was studied but also structural changes. The Aggregation Index (quantity for colloidal stability) and the melting point (quantity for conformational stability) measured by differential scanning fluorimetry were determined. The used MTP format screen has potential for buffer optimization and formulation development.

PL-011 **Conformational flexibility of CD81 cellular receptor head-subdomain – implications on Hepatitis C binding modes**

Eva S. Cunha[1], Pedro Sfriso[2], Adriana Rojas[1], Adam Hospital[2], Modesto Orozco[2], Nicola Abrescia[1]

1.-Structural Biology Unit, CIC bioGUNE, 2.-Institute for Research in Biomedicine (IRB Barcelona)

Structural Biology and Protein Dynamics Tetraspanin CD81 has a broad range of cellular functions, such as integrin association forming tetraspanin-enriched domains, synapse formation between B and T cells, cell adhesion, motility, invasion and signalling. Furthermore, CD81 is one of the four receptors involved in the cell entry of Hepatitis C virus (HCV) and therefore infection onset, one of the major causes for chronic liver disease resulting in cirrhosis and hepatocarcinoma. Human CD81 Large-Extracellular-Loop (hCD81LEL) is composed of a “stalk” and a “head” subdomain; with the latter interacting with HCV-E2 glycoprotein. We present four novel hCD81LEL crystal forms. Analysis of the fourteen independent observed hCD81LEL high-resolution X-ray structures suggests that the dynamism of the hCD81LEL head-subdomain is an inherent molecular property, an observation supported also by Molecular Dynamics (MD) studies. We classify the conformations in three distinct clusters (closed, intermediate and open), which are seen both in the crystal structures and in the molecular dynamics simulations. The MD simulations also show that conformational variability is modulated by pH changes, with distinct probability for each cluster at acidic and neutral pH. Furthermore, in silico docking of the recent E2core structure with three of the major types of hCD81LEL head-subdomain clusters highlights hydrophobic interactions as the major forces in the E2core: hCD81LEL recognition mechanism. We propose that the flexibility of the hCD81LEL is exploited by HCV at different stages of cell entry from virus attachment to internalization and fusion with the endosomal membrane. Our results provide important insights on the basic mechanism governing HCV binding to hCD81, and can help structure-based drug design of entry-inhibitors of HCV.

PL-012 **Allophycocyanin of gracilaria chilensis: from gene to function**

Jorge Dagnino-Leone[1], José Martinez-Oyanedel[1], Marta Bunster-Balocchi[1]
1.-Universidad de Concepción

THEME: Structure-Function relationship of proteins

The phycobilisomes (PBS) are auxiliary photosynthetic complexes that allow cyanobacteria and red algae to enhance the energy uptake in the range of 490-680 nm. In Gracilaria chilensis, an eukaryotic red algae, PBS is composed of Phycoerythrin (PE), Phycocyanin (PC) and Allophycocyanin (APC); these proteins possess chromophores which capture energy and then transfers it to photosytems. PBPs are oligomers of a αβ heterodimer; it oligomerizes into a trimer (αβ)3, this trimer has discoidal shape and it is associated in hexamers (αβ)6, several of this hexamers forms cylinder-like structures. PBS has 2 components: antennas and core. The antennas are composed of PE and PC, whose function is to capture energy between 490-570 and 590-625 nm respectively and transfer it to the core. The core is formed by APC, which can absorb energy in the 620-650 nm range. APC emission allows transferring energy to the photosystems with high efficiency. PBS is also composed by linker proteins which allow the correct assembly of PBS and possibly regulate the energy transfer. The main goal in our group is to build an atomic model of the Gracilaria chilensis phycobilisome. We have solved the crystal structure of PE and PC and created an antenna model. At present we are working in APC and the chromophorilated linker proteins. The objective of the present work is to create a model of the core of Gracilaria chilensis; to achieve these we have used molecular biology, biochemistry and bioinformatics techniques. We designed oligonucleotides primers for the four allophycocyanin subunits genes and for the globular domain of the apcE linker. These primers were used in PCR experiments to obtain the genes sequences. The sequences were translated to a aminoacid sequences and used to build a 3D model for APC subunits and trimers using the software Modeller. On the other hand we purified and analyzed the spectroscopic properties of APC from Gracilaria chilensis using absorption and fluorescence spectroscopy. We also determined APC oligomerization state using Gel filtration. Molecular docking using the CLUSPRO server was performed to obtain a hexamer and APC cylinder models. Based on electron micrographs obtained by our lab a tri-cylindric core model was built. All the models were submitted to a molecular dynamics using GROMACS software. Finally we determine possible energy transfer pathways in the core model applying the extended Forster equation, spectroscopic data from literature and the transition dipole moments of each of the chromophores present in the core. As conclusion of this work we built the first atomic model of Gracilaria chilensis phycobilisome core and propose energy transfers pathways inside the core in the context of a phycobilisome.

PL-013 **Novel practical strategies to access artificial metalloenzymes**

Marco Filice[1], Jose Miguel Palomo[1]
1.-Departamento de Biocatálisis, Instituto de Catálisis, CSIC

Protein Chemistry and Engineering Since the first report, the design of artificial metalloenzymes has rapidly been converted into an important topic in biological and inorganic chemistry due to their potential applications in synthetic chemistry, nanoscience and biotechnology. The combination of a catalytically active organometallic moiety with a macromolecular host has permitted the creation of biohybrids, a new kind of heterogeneous catalytic entities combining the attractive features of both homogeneous and enzymatic systems. Presenting our most recent achievements in this research area, here we describe two novel powerful and promising approaches focusing the practical synthesis and large scale production of heterogeneous artificial metalloenzymes showing chimeric activity. The first strategy is based on the in situ synthesis of noble metal nanoparticles and their supramolecular assembly with a microbial lipase from Candida antarctica (fraction B) finally creating an ultra-active organometallic-enzyme heterogeneous nanobiohybrid. In the second approach, combining different protein engineering protocols (molecular biology, orienting immobilization, solid-phase bioorganic modification and bioinformatic tools), an orthogonal solid-phase strategy creating novel unnatural catalytic sites was designed and optimized. The application of such a strategy onto the structure of the lipase from Geobacillus thermocatelunatus permitted the generation of a heterogeneous artificial metallolipase with chimeric activity. As proof-of-concept, the combinatorial library of generated artificial metalloenzymes obtained by both strategies was successfully assessed in a set of different synthetic reactions (selective C-C bond formation as Suzuki, Heck or Diels-Alder reactions) and also combining both activities (metallic and enzymatic) in cascade processes such as dynamic kinetic resolution of amines or production of arylamines. The obtained results were excellent in all cases. Extending this strategy to other enzymes, proteins and catalytic metals, we envisage the creation of a combinatorial library of programmable artificial enzymes useful for a wide set of applications (i.e. fine organic and medicinal chemistry, bioremediation or biomedicine).

PL-014 **Proteomic examination of the yeast nuclear pore complex dynamics**

Zhanna Hakhverdyan[1], Kelly Molloy[2], Brian Chait[2], Michael Rout[1]

1.-Laboratory of Cellular and Structural Biology, 2.-Laboratory of Mass Spectrometry and Gaseous Ion Chemistry

Protein turnover and exchange Nuclear pore complexes (NPCs) are proteinaceous assemblies situated in nuclear envelopes of eukaryotic cells. The main function of the NPC is the selective transport of macromolecules. NPCs also partake in other functions, such as nuclear organization and gene regulation. The core scaffold of the NPC is thought to be a stable structure, while the peripheral components exchange at various rates. However, these phenomena have not been elucidated in detail. The recent findings that yeast daughter cells get a higher proportion of the old NPCs and the core scaffold hardly turns over raise the possibility that the exchange of the peripheral nucleoporins can be a repair mechanism. Yeast provides a useful organism for the interrogation of nucleoporin exchange, as it performs closed mitosis; hence the only mixing of NPC constituents is due to exchange. We have developed a panel of genetic tools providing for conditional induction and repression of nucleoporins. By combining these switches with stable isotope metabolic labeling and affinity capture, cross linking coupled to mass spectrometry, we are able to distinguish between pre-existing and newly synthesized proteins and quantify their relative amounts in the NPC. Our preliminary findings are in agreement with results obtained in other organisms: the core scaffold of the NPC (inner ring, outer ring) appears to be stable, however does exchange slowly over time, while peripheral components exchange faster. By looking at the exchange rates of yeast nucleoporins we hope to gain insight into the NPC biology of actively dividing eukaryotic cells.

PL-015 **Active site clustering identifies functional families of the peroxiredoxin superfamily**

Angela Harper[1], Janelle Leuthaeuser[2], Patricia Babbitt[2], Jacquelyn Fetrow[3]
1.-Department of Physics, Wake Forest University, 2.-Department of Molecular Genetics and Genomics,Wake Forest University, 3.-Departments of Physics and Computer Science, Wake Forest University

Bioinformatics Understanding the relationships between proteins is vital to increasing our knowledge of the protein universe. While there are large databases of sequence information, the massive data influx over the past decade has prevented adequate classification of proteins at the molecular function level. However, it has been previously suggested that a protein's active site information may correlate with these known molecular functional differences; thus, active site profiling was developed to use residues around the active site of a protein to relate proteins. Subsequently the Deacon Active Site Profiler (DASP) was developed to create these active site profiles and search them in a database, such as GenBank, in order to find proteins with similar active site environments. By using DASP to computationally cluster proteins based on the similarity of their active site profiles, the Peroxiredoxin (Prx) superfamily was analyzed through active site similarity methods. The residues from the active site of each Prx structure were extracted and clustered, and these profiles were iteratively searched in GenBank through a Multi-level Iterative Sequence Searching Technique (MISST). The Prx superfamily has been studied by experts, allowing the results of these searches to be compared to a well-annotated group of proteins. While previous sequence based evolutionary methods have been unable to identify functional differences between some subgroups of the Prxs, notably the AhpC-Prx1 and Prx6 subgroups, MISST discretely separates these subgroups. Classifying Prx proteins into functionally relevant groups using computational active site similarity methods lays the foundation for an automated process for identifying protein functional groups beyond the Prx superfamily.

PL-016 **Synthesis and conformational studies of glycoprotein N homolog of bovine herpesvirus 1 (BHV-1) by using CD, NMR and molecular modelling**

Natalia Karska[1], Andrea D. Lipińska [2], Małgorzata Graul[2], Franciszek Kasprzykowski[1], Emilia Sikorska[1], Igor Zhukov[3], Magdalena J. Ślusarz[1], Sylwia Rodziewicz-Motowidło[1]

1.-Faculty of Chemistry, University of Gdansk, 2.-Intercollegiate Faculty of Biotechnology, University of Gdansk, 3.-Nano Bio Medical Centre University of Poznan

UL49.5 protein (gN homolog) is a key player in the immune evasion strategies of several varicelloviruses, including bovine herpesvirus-1. During viral infection UL49.5 exerts dual activity: it serves as a chaperone for viral glycoprotein M and, in its gM-unbound form, acts as an inhibitor constraining the transporter associated with antigen processing (TAP). The UL49.5/gM complex formation is required for the maturation and proper trafficking of both viral proteins. In the absence of gM, UL49.5 blocks transport of antigenic peptides by TAP and their MHC I-restricted presentation. The molecular mechanism of UL49.5 activity still remains elusive. In order to investigate the structural requirements for biological function UL49.5 study was conducted using CD, NMR and Molecular Dynamics methods. The data obtained with the use of high purity synthetic peptides encompassing UL49.5 confirmed the presence of an alpha-helix structure, formed preferentially in the presence of dodecylphosphocholine (DPC) micelles as a membrane-like environment.

In order to determine the three-dimensional structure of UL49.5 protein in the present work its NMR solution structure in the presence of membrane-like environment was performed. The NMR data were used as a set of restraints for a simulated annealing protocol that generated 3Dstructures of the peptides in DPC micelles [3]. In the next step, the calculation of spatial structure and “assembling” of the whole protein from the obtained peptide structures were performed by using molecular dynamics of the protein in the fully hydrated 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine (POPC) [4]. The obtained structural model may contribute to identification of UL49.5 active sites and elucidation of its mode of action.

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PL-017 **Functional and mechanistic studies of dysferlin, an essential protein in cell membrane repair**

Colin Johnson[1], Sara Codding[1]
1.-Oregon State University

Membrane proteins Resealing of tears in the sarcolemma of myofibers is a necessary step in the repair of muscle tissue. Defects in this repair process are responsible for muscular dystrophy and cardiomyopathy. The repair pathway is triggered by the influx of calcium through lesions in the membrane, which result in membrane fusion and patching of the wound. Recently dysferlin has been identified as a calcium binding protein essential for sarcolemma repair, as well as other SNARE mediated exocytotic events including cytokine and acid sphingomyelinase secretion. In this presentation we demonstrate a direct interaction between dysferlin and the SNARE proteins syntaxin 4 and SNAP-23. In addition, FRET and in vitro reconstituted lipid mixing assays indicate that dysferlin accelerates SNARE heterodimer formation and SNARE mediated lipid mixing in a calcium sensitive manner. Our results suggest a model whereby dysferlin acts as a calcium sensing SNARE effector for exocytosis and membrane fusion.

PL-018 **Exploring the therapeutic potential of a peptide derived from a poxviral immune evasion protein: NMR determination of the solution structure of VIPER and its inactive mutant**

Jiyoon Kim[1], Dylan Lawless[1], Manuel Ruether[2], Andrew Bowie[1], Kenneth H. Mok[1,3],
1.-Trinity College Dublin, Trinity Biomedical Sciences Institute (TBSI), 2.-Trinity College Dublin, School of Chemistry, 3.-Trinity College Dublin, Centre for Research on Adaptive Nanostructure/devices

Toll-like receptors (TLRs) have a role in viral detection leading to cytokine and IFN induction, and as such they are targeted by viruses for immune evasion. The poxviral protein A46 has been identified to inhibit TLR signaling by interacting with TIR domain-containing proteins of the receptor complex to collectively inhibit all TLR adaptor proteins that positively regulate transcription-factor activation (1). One 11 aa peptide (KYSFKLILAEY) termed VIPER (Viral Inhibitory Peptide of TLR4) was reported to retain the inhibitory properties of full length A46 against TLR4 signaling. A 9R homopolymer delivery sequence at the C-terminus provided delivery of the peptide into cells. Structural comparisons are presented between 9R-VIPER, which is active in preventing TLR4-dependent cytokine induction in cell culture, and a mutant that exhibited loss of function (9R-VIPER L6A,E10A), through solution NMR spectroscopy. We find that despite a relatively minor sequence difference, the loss of hydrophobicity as well as negative electrostatic interactions result in subtle but potentially significant differences in the region of the peptide proposed to interface with TLR4. Reference: (1) Stack J, Bowie AG, "Poxviral protein A46 antagonizes Toll-like receptor 4 signaling by targeting BB loop motifs in Toll-IL-1 receptor adaptor proteins to disrupt receptor:adaptor interactions" J Biol Chem 287: 22672-82, 2012.

PL-019 **Active site profile-based protein clustering is an efficient, accurate method to define protein functional groups**

Janelle Leuthaeuser[1], Angela Harper[2], Gabrielle Shea[2], Patricia Babbitt[3], Jacquelyn Fetrow[1,2]
1.-Wake Forest University, 2.-Wake Forest University, 3.-University of California San Francisco

Protein Function Prediction The elucidation of protein molecular function lags far behind the rate of high-throughput sequencing technology; thus, it is essential to develop accurate and efficient computational methods to define functional relationships. Protein clustering based on sequence similarity has emerged as a simple, high-throughput method for defining protein relationships, but sequence-based techniques often inaccurately define molecular function details. Active site profiling (ASP) was previously developed to identify and compare molecular details of protein functional sites. Protein similarity networks were created using both active site similarity and sequence similarity for four manually curated superfamilies, and results demonstrate that ASP-based clustering identifies detailed functional relationships more accurately than sequence-based clustering. Building on this, two iterative pipelines were developed using active site profiling and profile-based searches to cluster protein superfamilies into functional groups. First, the Two Level Iterative clustering Process (TuLIP) utilizes active site profiling and iterative PDB searches to divisively cluster protein structures into groups that share functional site features. Across eight superfamilies, TuLIP clusters exhibit high correlation with expert functional annotations. Subsequently, the Multi-level Iterative Sequence Searching Technique (MISST) utilizes iterative profile-based GenBank searches to identify protein sequences that belong in each TuLIP group. The results indicate that these ASP-based methods accurately and efficiently identify functionally relevant groups through a process that can be applied systematically and on a large-scale. Moreover, the approach can be applied more quickly than detailed manual curation, suggesting its value in guiding annotation efforts.

PL-020 **Insertion of the hydrophobic C-terminal domain of apoptotic BH3-only proteins into biological membranes**

Ismael Mingarro[1], Vicente Andreu-Fernández[2], Manuel Bañó-Polo[1], Maria J. García-Murria[1], Mar Orzáez[2]

1.-Dept. Biochemistry and Molecular Biology. University of Valencia, 2.-Lab of Peptide and Protein Chemistry. Centro de Investigación Príncipe Felipe

Membrane Proteins Changes in the equilibrium between pro-survival and pro-apoptotic members of the B-cell lymphoma-2 (Bcl-2) protein family at the mitochondrial outer membrane (MOM) induce structural changes that committed cells to apoptosis. Bcl-2 homology-3 (BH3)-only proteins participate in this process activating pro-apoptotic effectors and promoting permeabilization of the MOM. The membrane association of BH3-only proteins is a controversial issue due to the lack of a canonical carboxyl-terminal (C-terminal) transmembrane (TM) domain. We used an in vitro transcription/translation system to study the insertion capacity of these hydrophobic C-terminal regions of the BH3-members Bik, Bim, Noxa, Puma and Bmf into microsomal membranes, and an Escherichia coli complementation assay to validate our results in bacterial cells. Furthermore, we have fused these hydrophobic regions to GFP to investigate the subcellular sorting. These results will allow further refinement in the elaboration of the Bcl-2 protein-protein and protein-membrane interactome network.

PL-021 **A computational investigation of tight junctions**

Alexis Peña[1], Flaviyan Jerome Irudayanathan[1], Shikha Nangia[1]
1.-Syracuse University, Dept. of Biomedical and Chemical Engineering

Computational Modeling, Biostatistics, Biomedical and Chemical Engineering Tight junctions (TJ) are vital intracellular barriers that are responsible for regulating paracellular transport. Claudins, a family of small transmembrane proteins with approximately 27 members, are an integral part of the TJ strands. Tight junctions provide molecular-level protection and prevent infection and toxins from entering the body; in the same sense TJs allow nutrients and vital solutes to pass through. Claudins are associated with various diseases including metastatic cancer as well as an entry point for many viruses. Despite their importance and abundance in all cell membranes and their ubiquitous nature, the exact 3-D structure of Claudins has remained elusive to traditional X-ray crystallographic and NMR studies. In this investigation, a computational approach was used to determine the Claudin structure of claudin 1-10. Homology modeling, molecular dynamic simulations, and reverse mapping were employed to predict the protein structures with relative accuracy. Understanding structure of claudin proteins and its interaction at the molecular level can lead to effective drug delivery technology.

PL-022 **Determination of optimal conditions for an isothermal titration calorimetry essay to obtain kinetic parameters of trypsin i from pyloric caeca of monterey sardine (Sardinops sagax caerulea)**

Idania Emedith Quintero Reyes[1], Francisco Javier Castillo Yáñez[1], Enrique fernando Velázquez Contreras[1], Rocío Sugich Miranda[1], David Octavio Corona Martínez[1], Aldo Alejandro Arvizu Flores[1], Ivet Cervantes Domínguez[1]

1.-Universidad de Sonora

Protein Kinetics Determination of Optimal Conditions for an Isothermal Titration Calorimetry Essay to Obtain Kinetic Parameters of Trypsin I from Pyloric Caeca of Monterey Sardine (Sardinops sagax caerulea) Trypsin is the most studied alkaline protease and it´s very common to found isoforms from this protein as the case for Monterey sardine (Sardinops sagax caerulea); as it shows an expression of trypsin I and trypsin III according to the cDNA characterization. Trypsin I was determine to be a cold adapted enzyme as it shows a higher catalytic efficiency (kcat/KM) than the mesophilic counterparts. The kinetic parameters were obtained by spectrophotometric essays, which are not fallible for all the enzymes because native, recombinant or mutant enzyme activity could be below the detection limit of the assay, opaque or turbid solutions interfere with spectrophotometric detection, etc. Alternative tools as the isothermal titration calorimetry (ITC) can measure enzyme kinetics using thermal power generated by the enzymatic conversion of substrate to product; were the rate of reaction is directly proportional to thermal power. The objective of this study was to stablish the optimum conditions to obtain kinetic parameters of Trypsin I from pyloric caeca of Monterey sardine using ITC. To reach the objective Trypsin I was purified from viscera of Monterey sardine using molecular exclusion and affinity chromatography obtaining a yield of 1.1 mg/mL. At 20 °C kcat and KM of Tryipsin I form Monterey sardine were 14.6 s-1 and 1.4 µM respectively. At 15°C were 13.6 s-1 and 4 µM (kcat and KM) and at 4°C kcat was 0.454 s-1 and KM 0.52 µM. The kinetic parameters obtained by spectrophotometric assay at 25°C were kcat and KM 436 s-1 and 1.8 µM respectively. At 20°C the kcat was 409.7 s-1 and KM 1.8 µM and at 15°C kcat 288 s-1 and KM 3µM. Comparing the values obtained for kcat with the spectrophotometric essay were higher 29 fold than those obtained by ITC and the values in KM were similar by both methods. Even though the differences in kcat, we can reassert the psychrophilic behavior of trypsin I as the catalytic efficiency is higher by both methodologies. In the understanding that the kinetic behavior of enzymes is important to not only understanding biochemical pathways and catalytic mechanisms but is again a fruitful area for drug discovery and development; so the ITC provides a universal approach to determining the kinetic behavior of enzymes and can yield in a single experiment a complete set of kinetic parameters for an enzyme-catalyzed reaction that can be applied for the different alkaline proteases from pyloric caeca of Monterey sardine (Sardinops sagax caerulea).

PL-023 **Mysterious world of stress-responding sigma factors in Bacillus subtilis**

Olga Ramaniuk[1]
1.-Institute of Microbiology, Academy of Sciences of The Czech Republic

Protein-DNA interaction Bacterial transcription is mediated by the RNA polymerase holoenzyme containing sigma factors - essential proteins for the initial step of transcription that recognize and bind to promoter DNA. The primary sigma factor is essential in exponential phase of growth while alternative sigma factors are active during transcription under stress conditions. This project has three main aims. The first aim is to explore the binding properties of B. subtilis alternative sigma factors; specifically, whether sigma factors lacking the autoinhibitory domain 1.1 can bind to promoter DNA in the absence of RNAP. The second aim explores whether RNAP associated with alternative sigma factors is regulated by the concentration of the initiation nucleoside triphosphate. The third aim is to define the regulon of Sigma I. In order to achieve our aims, 7 out of 17 alternative sigma factors were successfully purified using affinity chromatography and ion exchange chromatography. We set up in vitro transcription system with selected sigma factors and initiated experiments with Sigma I regulon determination. Results named above and our future findings will help to better understand gene expression regulation on the level of transcription initiation. This work was supported by grant No. P305-12-G034 from the Czech Science Foundation.

PL-024 **Assessing the costs and benefits of protein aggregation**

Natalia Sanchez de Groot[1], Marc Torrent Burgas[2], Charles N. J. Ravarani[1], Salvador Ventura[3], M. Madan Babu[1]

1.-MRC Laboratory of Molecular Biology, 2.-Vall d'Hebrón Research Institute, UAB, 3.-Inst. de Biotec. i Biomed. and Dprt. de Bioq. i Biol. Mol., UAB

Protein aggregation and cell fitness Protein aggregation has been associated with numerous diseases but also with important cellular functions such as epigenetic inheritance. Here we present a population genetics approach to infer the costs and benefits of protein aggregation on cell fitness. This information is crucial to understand how cellular systems tolerate the formation of protein deposits and which factors modulate this event. Using our experimental system, we measured different protein aggregation effects (deleterious, neutral or beneficial) within the same genomic background. Single cell analyses, within the same population, showed stochastic variability in the aggregate's size and in its effect on cell fitness. Our data indicates that, in certain conditions, protein aggregation can enhance population variability and survival expectancy. Overall, these results suggest that the presence and formation of protein aggregates could be almost harmless whereas the associated gain and loss of function are critical for the cell.

PL-025 **Revealing the key role of negatively charged residues of heme sensor proteins involved in Geobacter sulfurreducens' signal transduction pathways**

Marta A. Silva[1], Telma C. Santos[1], Teresa Catarino[2], Carlos A. Salgueiro[1]
1.-UCIBIO-Requimte, Departamento de Química, FCT-UNL., 2.-Instituto de Tecnologia Química e Biológica, UNL

Signal transduction proteins Bacterial chemotaxis systems sense and regulate the microbe mobility in response to environmental conditions. Such mechanisms constitute a striking example of cell motility to gain advantages for cell survival and permit the bacteria to fill important niches in a diversity of anaerobic environments [1]. Geobacter sulfurreducens (Gs) is an anaerobic bacterium with a considerable respiratory versatility whose genome encodes for an unusual family of methyl-accepting chemotaxis proteins (MCP), each containing at least one heme c-binding motif [2]. These sensor proteins, GSU0582 and GSU0935, are involved in signal transduction pathways mediated by chemotaxis-like systems [3]. The thermodynamic and kinetic characterization of the sensors GSU0582 and GSU0935 by visible spectroscopy and stopped-flow techniques, at several pH and ionic strength values revealed that sensor GSU0935 midpoint reduction potentials are lower than those of GSU0582 at all pH and ionic strength values and the same were observed for the reduction rate constants [4]. The origin of the different functional properties of these closely related sensor domains are rationalized in the structural terms showing that GSU0935 has two extra negatively charged residues in the vicinity of the heme group, which have no counterpart in GSU0582: Glu89 and Asp57. Residue Asp57 is less exposed compared to Glu89 and it was suggested that its carboxylic group might have a role in the modulation of the heme reduction potential of GSU0935. To investigate this, both residues were replaced by a positively charged amino acid (lysine) and by a neutral one (asparagine or glutamine). For the mutants with enough expression, a functional characterization was carry out, using several spectroscopic techniques, including UV-visible and CD, together with kinetics and potentiometric measurements. Significant changes on the reduction potential values are observed when a negative charge is replaced by a positive one at position 57 or 89. Therefore, the decrease of the reduction potential in Asp57 and Glu89 mutants reinforces the hypothesis that the higher reduction potential observed for heme sensor domain GSU0582 is related with the less negative electrostatic surface around the heme. This work provides, for the first time, evidence for the co-existence of two similar methyl-accepting chemotaxis proteins functioning in different working potential ranges. These proteins are responsible to allow Geobacter sulfurreducens triggering an adequate cellular response in different anoxic subsurface environments. Acknowledgments: This work was supported by project grant PTDC/BBB-BEP/0753/2012 and UID/Multi/04378/2013 from Fundação para a Ciência e a Tecnologia (FCT), Portugal. TCS is recipient of grant PD/BD/106037/2015 from FCT, respectively. References 1. MK Chan. Curr Opin Chem Biol, 5 (2001) 216-222. 2. DR Lovley, JD Coates. Curr Opin Microbiol, 3 (2000) 252-256. 3. PR Pokkuluri, M Pessanha, YY Londer, SJ Wood, NE Duke, R Wilton, T Catarino, CA Salgueiro, M. Schiffer. J Mol Biol, 377 (2008) 1498-1517. 4. MA Silva, RC Valente, PR Pokkuluri, DL Turner, CA Salgueiro, T Catarino. Biochim Biophys Acta, 1837 (2014) 920-928.

PL-026 **Appearance of stabilizing interactions in the evolution of a dimeric TIM barrel**

Mariana Schulte-Sasse[1], Nancy O. Pulido Mayoral[1], Miguel Costas-Basín[2], Enrique García-Hernández[3], Adela Rodríguez-Romero[3], D. Alejandro Fernández-Velasco[1]

1.-National Autonomous University of Mexico, Faculty of Medicine, 2.-National Autonomous University of Mexico, Faculty of Chemistry, 3.-National Autonomous University of Mexico, Institute of Chemistry

Molecular Evolution The glycolytic enzyme triosephosphate isomerase (TIM) is an oligomeric (β/alpha)8 barrel that catalyses the interconversion of D-Glyceraldehyde 3-phosphate and dihydroxyacetone phosphate in a diffusion-limited reaction. Although each subunit has its own active site, naturally occurring monomeric TIMs have not been reported; in fact, monomer association is very tight. TIM topology is well conserved among the three domains of life. Nevertheless, their folding mechanism and inhibition properties vary across species. Comparative studies of proteins have proved to be very useful in understanding the relationship between sequence and physicochemical properties, however, they lack the capacity to give a more integrative and evolutive correlation. In order to elucidate how the catalytic properties, the oligomerization state and the stability of extant TIMs arose, in this work we examined the molecular history of eukaryotic TIM through ancestral protein reconstruction methods (Maximum Likelihood) and the subsequent physicochemical characterization of the resurrected enzymes. We first characterized in detail the protein corresponding to the last common ancestor of animals and fungi (TIM63). The CD and fluorescence spectra of TIM63 are similar to those of extant TIMs. Secondary structure is lost in a cooperative transition with Tm = 68.7 °C. The enzyme loses activity upon dilution suggesting that only the dimer is active. Dilution experiments followed by isothermal titration calorimetry indicate that dissociation enthalpy is small; moreover the heat capacity change observed is three times higher than the one predicted for a rigid body dissociation process, suggesting partial unfolding of the monomers. When compared with extant TIMs, the catalytic efficiency of TIM63 is reduced 10-fold, whereas binding of PGH, a transition-state analogue, shows a similar thermodynamic signature. These data indicate that although monomer association may have been less tight in ancestral TIMs, catalysis has been always linked to oligomerization. Analysis of the crystal structure of TIM63, obtained at 1.9 Å resolution, suggests that the lack of four salt bridges observed in the interface of extant TIMs is responsible for the low dimer stability. In order to test this hypothesis we also studied the stability of four younger reconstructed ancestors that acquired the salt bridges in two different phylogenetic lineages. We found a correlation between the appearance of stabilizing interactions in the interface, dimer stability and catalysis; suggesting that these salt bridges are partially responsible for extant dimer stability and shed light on the dimeric nature of extant TIMs.

PL-027 **Receptor protein-tyrosine phosphatases: dimerization, receptor kinase interaction and allosteric modulation**

Elizabeth Dembicer[1], Damien Thevenin[1]
1.-Department of Chemistry, Lehigh University

THEME: receptor tyrosine kinase and receptor protein phosphatase signaling Many cell-signaling events are regulated through reversible tyrosine phosphorylation of proteins, which is controlled by the counterbalanced actions of two key enzyme families: Protein tyrosine kinases and protein tyrosine phosphatases. Interestingly, both families include transmembrane receptor-like enzymes, namely the receptor tyrosine kinases (RTKs) and the receptor-like PTPs (RPTPs). While the regulation and actions of many RTKs are well characterized, the mechanisms controlling the enzymatic activity of RPTPs and how they interact with their substrates remain to be fully explained. Thus, understanding how these receptors function and interact will give fundamental insights into how tyrosine phosphorylation is finely tuned in cells, and how it can be modulated. Increasing evidence indicates that RPTPs, like RTKs, are regulated by homodimerization. However, it appears that homodimerization inhibits the activity of most RPTPs. Even though the transmembrane (TM) and the juxtamembrane domains have been proposed to be involved in this process, there is no clear structure-based proposal for the role of these regions. Moreover, several RPTPs have been identified as candidate regulators of RTKs. In particular, the Receptor-type tyrosine-protein phosphatase eta (PTPRJ; also known as DEP1 or CD148) is capable of attenuating EGFR tyrosine phosphorylation. Physical interactions of EGFR with PTPRJ at the cell surface have been documented, but the basis for these interactions is unknown. Here, using a dominant-negative transcriptional activator-based assay (DN-AraTM), and mutagenesis analysis, we show that: (1) PTPRJ has a strong tendency to homodimerize, (2) PTPRJ heterodimerizes with EGFR through TM-TM interactions, (3) these interactions are mediated by specific residues, and can be modulated by the delivery of peptide binders. This work represents the first structure-function study of RPTP-RTK interaction, and may not only result in significant progress towards a better understanding of the basic biology of RPTPs in cancer cells, but also offer new possibilities for targeting protein tyrosine phosphatases for therapeutic modulation of EGFR in oncology.

PL-028 **Inhibiting EGFR dimerization and signaling through targeted delivery of juxtamembrane domain peptide mimics using pHLIP**

Anastasia Thevenin[1], Kelly Burns[1], Janessa Guerre-Chaley[1], Damien Thevenin[1]
1.-Department of Chemistry, Lehigh University

Regulating Receptor Tyrosine Kinase Signaling The elevated phosphorylation of key regulatory tyrosines on oncogenic signaling proteins that result from aberrant protein tyrosine kinases activity plays well-established roles in promoting tumorigenesis and in the high frequency with which resistance arises to existing therapeutic treatment. For instance, this is the case for the epidermal growth factor receptor (EGFR). Thus, there is a clear need for novel specific targeting methods to inhibit the activity of receptor protein tyrosine kinases, such as EGFR, in cancer. EGFR becomes activated upon ligand binding to the extracellular domain, leading to receptor dimerization. The juxtamembrane (JM) domain of EGFR is critical for intrinsic tyrosine kinase activity and receptor dimerization by stabilizing the active conformation of EGRR through the formation of a antiparallel helical dimer. Therefore, peptides mimicking the JM domain – if specifically delivered to cancer cells – have the potential to prevent EGFR dimerization, receptor activation, downstream signaling, and thus to attenuate aberrant EGFR activity in cancer cells. Here, pHLIP (pH Low Insertion Peptide), a peptide that can selectively target cancer cells and tumors based solely on their extracellular acidity, is used to selectively translocate the JM domain of EGFR in cancer cells to prevent EGFR dimerization. At pH above 7, pHLIP is soluble and unstructured, however, when exposed to lower pH such as observed in tumors, pHLIP inserts as a transmembrane (TM) alpha-helix, allowing the direct translocation of cargo molecules into the cytoplasm. Using the dominant negative AraC-based transcriptional reported assay (DN-AraTM), which assesses JM and TM domain interactions in cells membranes of E. coli, we show that pHLIP-JM is able to disrupt EGFR dimer by 50%. Current work is focused on testing the ability of such pHLIP-JM peptide conjugate to perturb EGFR homodimerization and decrease downstream signaling through soluble kinases, such as Akt and ERK, in cancer cells.

PL-029 **The thumb subdomain of yeast mitochondrial RNA polymerase is involved in processivity, transcript fidelity and mitochondrial transcription factor binding**

Gilberto Velazquez[1], Luis Brieba[2], Rui Sousa[3]

1.-Universidad de Guadalajara, 2.-Langebio Cinvestav, 3.-University of Texas HealthSscience Center at San Antonio

DNA protein interaction ABSTRACT Single subunit RNA polymerases have evolved two mechanisms to synthesize long transcripts without falling off a DNA template: binding of nascent RNA and interactions with an RNA:DNA hybrid. Mitochondrial RNA polymerases share a common ancestor with T-odd bacteriophage single subunit RNA polymerases. Herein we characterized the role of the thumb subdomain of the yeast mtRNA polymerase gene (RPO41) in complex stability, processivity, and fidelity. We found that deletion and point mutants of the thumb subdomain of yeast mtRNA polymerase increase the synthesis of abortive transcripts and the probability that the polymerase will disengage from the template during the formation of the late initial transcription and elongation complexes. Mutations in the thumb subdomain increase the amount of slippage products from a homopolymeric template and, unexpectedly, thumb subdomain deletions decrease the binding affinity for mitochondrial transcription factor (Mtf1). The latter suggests that the thumb subdomain is part of an extended binding surface area involved in binding Mtf1.

PL-030 **Design principles of membrane protein structures**

Vladimir Yarov-Yarovoy[1], Diane Nguyen[1]
1.-University of California Davis

Membrane Protein Structure Membrane proteins play key role in cellular signaling and ion transport. Statistical analysis of expanding database of high-resolution membrane protein structures in Protein Data Bank (PDB) provides useful information about membrane protein structure and function. We used RosettaMembrane software (Yarov-Yarovoy V et al (2006) Proteins) to analyze ~300 unique alpha helical membrane protein structures in PDB and derive knowledge based energy function for membrane protein structure prediction, membrane protein-protein docking, and membrane protein design. The RosettaMembrane residue environment energy term is based on amino acid propensities in hydrophobic, interface, and water layers of the membrane and depends on the residue burial state – from being completely buried within a protein environment to being completely exposed either to the lipid or water environments. Residue buried state is determined from the number of residue neighbors within 6 and 10 Å spheres. The RosettaMembrane residue-residue interaction term is based on the propensities of amino acid pairs to be in close proximity to each other within hydrophobic, interface, and water layers. Results of our statistical analysis reveal fine details of favorable and unfavorable environments for all amino acids types in all membrane layers and residue burial states. We find that large hydrophobic amino acids are favorable facing the hydrophobic core of the lipid bilayer. Small amino acids are favorable facing the protein core within the hydrophobic layer of the membrane. Aromatic or positively charged amino acids and favorable facing the lipid head groups. Residue-residue interactions are often favored between polar and charged amino acids and also between some of small and large hydrophobic amino acids inside of the protein core within the hydrophobic layer of the membrane. These data will be useful for rational design of novel membrane protein structures and functions.

PL-031 **Coordinated gripping of substrate by subunits of a AAA+ proteolytic machine**

Ohad Yosefson[1], Andrew Nager[1], Tania Baker[1], Robert Sauer[1]
1.-Department of Biology, Massachusetts Institute of Technology

Protein quality control' or 'Protein degradation' Hexameric AAA+ protein-remodeling machines use conserved loops that line the axial pore to apply force to substrates during the mechanical processes of protein unfolding and translocation. An open question in the AAA+ field is whether pore loops from different subunits of the hexameric ring grip the substrate coordinately (all six subunits involved), independently (one subunit at a time involved), or partially coordinated (two or three subunits at a time). To answer this question, we studied covalently linked hexamers of the E. coli ClpX unfoldase bearing different numbers and configurations of wild-type and mutant pore loops and challenged these variants with protein substrates with a broad range of stabilities. We find that successful unfolding of increasingly resistant substrates requires the coordinated action of a greater number of wild-type pore loops. Our results support a mechanism in which a power stroke initiated in one subunit of the ClpX hexamer results in the simultaneous movement of all six pore loops, which coordinately grip and apply force to the substrate.

PL-032 **Structure and function of the Toc159 M-domain, and its role in targeting the preprotein receptor to the chloroplast outer envelope membrane**

Matthew Smith[1], Shiu-Cheung Lung[2], Prem Nichani[1], Nicholas Grimberg[1], J. Kyle Weston[1], Shane Szalai[1], Simon Chuong[2]

1.-Deartment of Biology, Wilfrid Laurier University, 2.-Department of Biology, University of Waterloo

Chloroplast biogenesis and function rely on the import of thousands of nucleus-encoded preproteins from the cytosol. Preprotein import is supported by the Toc and Tic (Translocon at the outer and inner envelope membranes of chloroplasts) complexes, which work cooperatively to translocate preproteins across the double-membrane envelope to the chloroplast interior. Toc159 is one of the preprotein receptors of the Toc complex, is also encoded in the nucleus and post-translationally targeted to the chloroplast, and is comprised of 3 distinct domains: 1) the intrinsically disordered N-terminal Acidic (A-) domain; 2) the central GTPase (G-) domain; and 3) the C-terminal Membrane (M-) domain that anchors the protein to the chloroplast outer membrane (COM) through an unknown mechanism. The M-domain has no known homologues and does not contain a predicted trans-membrane domain, but does contain intrinsic chloroplast targeting information at the extreme C-terminus. The M-domain also contains a predicted β-helix motif, which may be important for anchoring the protein to the COM. We are interested in characterizing the structure of the M-domain and determining the nature of its association with the COM, as part of our larger goal of understanding the role Toc159 plays in protein import into chloroplasts. We are also interested in defining the precise nature of the targeting information contained within the extreme C-terminus of Toc159, elucidating the targeting pathway that is used, and whether other COM proteins use this pathway. We will present our most recent data on the structure, function and targeting of the Toc159 M-domain.

PL-033 **Structural investigation of NlpC/P60 protein acquired by Trichomonas vaginalis through a lateral gene transfer event**

Jully Pinheiro[1,2], Augusto Simoes-Barbosa[1], David Goldstone[2]

1.-Microbiology , School of Biological Sciences, University of Auckland, 2.-Structural Biology , School of Biological Sciences, University of Auckland

Trichomonas vaginalis is an extracellular flagellated protozoan parasite that causes the most common non-viral sexually transmitted disease, with approximately 200 million cases worldwide annually. Nevertheless, the biochemical processes behind T. vaginalis infection and its interaction with the vaginal microbiota are still not well defined. In 2007 the draft genome sequence of Trichomonas vaginalis strain G3 was described, identifying 60,000 protein-coding genes. Of these, nine genes encode NlpC/P60-like members. This superfamily is widely represented in the different kingdoms of life and has diverse enzymatic functions, such as amidases, endopeptidases and acetyltransferases. Previous studies have shown that members of this superfamily hydrolyze specific peptide linkages in bacterial cell walls affecting germination, vegetative growth, sporulation and division or cell lysis/invasion. As a typical eukaryote, the protozoan parasite T. vaginalis does not have a cell wall itself. Previous studies suggest that the T. vaginalis NlpC/P60 genes were acquired via lateral gene transfer from bacteria and must have an important function, possibly controlling the vaginal microbiota and aiding parasite invasion and infection. To investigate the function of the NlpC/P60 family of proteins in T. vaginalis we have expressed, purified and crystallized a member TVAG_119910 and report its three-dimensional structure, determined at 1.5 Å resolution, by X-ray diffraction. The structure of the protein reveals a typical papain-like fold resembling peptidoglycan hydrolases from the NlpC/P60 family with a conserved cysteine and histidine; forming the catalytic residues. The protein contains two bacterial SH3 domains at the N-terminus. This domain acts as a general binding domain and is likely to aid the interaction of the NlpC/P60 domain with substrate components. Combined with biochemical and enzymatic characterization, the structure of this NlpC/P60 protein will help to elucidate the molecular origin of its hydrolase activity and to decipher their putative role in the parasite infection.

PL-034 **Novel DNA polymerases from Red Sea brine-pools: new potential polymerases for PCR application**

Masateru Takahashi[1], Etsuko Kimura[1], Mohamed Salem[1], Ulrich Stingl[1], Samir Hamdan[1]
1.-King Abdullah University of Science and Technology

Protein Biotechnology The polymerase chain reaction (PCR) is a key tool in medical and biological research. The most common PCR reaction relies on the thermal cycling method that consists of repeated cycles of heating and cooling steps for DNA melting and extension by the DNA polymerase, respectively. The introduction of new DNA polymerases to the market is a major area of development that tremendously helped in improving the performance and quality of PCR. Nonetheless, PCR still requires optimization of salt and metal ion concentrations leaving a room in the market for introducing new DNA polymerases that are robuster in their salt and metal ion concentration dependence. In this study, we will present the characterization of a novel archaeal DNA polymerase from the Red Sea brine-pool (termed BR3) and demonstrate how its enzymatic activity reflects on every aspects of the environment of the brine-pool – high tolerance to concentrations and types of salts and metal ions including utilization of Zn2+ ions in its active site. These results suggest that the brine-pool microorganisms are likely to contain novel chemical pathways to deal with its exterior harsh conditions. We will further show the mechanism of BR3 polymerase how it was adjusted to be active in harsh condition.

PL-035 **Structural basis for the identification of the n-terminal domain of coronavirus nucleocapsid protein as an antiviral target**

Ming-Hon Hou[1], Shing-Yen Lin[1], Chia-Ling Liu[1], Yu-Ming Chang[2], Jincun Zhao[3], Stanley Perlman[3]
1.-Institute of Genomics and Bioinformatics, National Chung Hsing University., 2.-Institute of Biological Chemistry, Academia Sinica., 3.-Department of Microbiology, The University of Iowa

Drug Discovery Coronaviruses (CoVs) cause numerous diseases, including Middle East respiratory syndrome and severe acute respiratory syndrome, generating significant health-related and economic consequences. CoVs encode the nucleocapsid (N) protein, a major structural protein that plays multiple roles in the virus replication cycle and forms a ribonucleoprotein complex with the viral RNA through the N protein's N-terminal domain (N-NTD). Using human CoV-OC43 (HCoV-OC43) as a model for CoV, we present the 3D structure of HCoV-OC43 N-NTD complexed with ribonucleoside 5'-monophosphates to identify a distinct ribonucleotide-binding pocket. By targeting this pocket, we identified and developed a new coronavirus N protein inhibitor, N-(6-oxo-5,6-dihydrophenanthridin-2-yl)(N,N-dimethylamino)acetamide hydrochloride (PJ34), using virtual screening; this inhibitor reduced the N protein's RNA-binding affinity and hindered viral replication. We also determined the crystal structure of the N-NTD-PJ34 complex. On the basis of these findings, we propose guidelines for developing new N protein-based antiviral agents that target CoVs.

PL-036 **Thermal and structural stability of ß-Glucosidases GH1**

Maira Artischeff Frutuoso[1]

1.-Departamento de Bioquímica do Instituto de Química da Universidade de São Paulo

Enzymology We compared the stability of thermophilic β-glucosidases GH1 to mesophilic ones in the presence of denaturants as urea and high temperature by following the transitions between the native and unfolded states by tryptophan fluorescence, enzymatic activity and differential scanning fluorimetry (DSF). The bacterial β-glucosidases (bglA) and (bglB) of the mesophile Paenibacillus polimyxa and β-glucosidase (bglThm) of the thermophile Thermotoga maritima were expressed as recombinant proteins in NovaBlue (DE3) and purified by affinity chromatography (Ni-NTA resin). These recombinant enzymes have very similar folding type structure (β/α)8 barrel, as shown in crystal structures and exhibited a characteristic peak between 330 and 340 nm in the tryptophan fluorescence spectra, indicating that those proteins are folded. Circular dichroism analysis in the far-UV region (190 nm to 240 nm) also showed typical spectra of folded proteins with secondary structure composition of 47% of α-helix and 13% of β-sheets for bglA, 61% of α-helix and 2.5% of β-sheets for bglB and 30% of α-helix and 20% of β-sheets for bglThm. The average degree of accessibility to the exposed tryptophan residues in the native enzyme to increasing concentrations of the acrylamide suppressor (Stern-Volmer constant - KSV) is greater to bglA (9.49), but similar to bglB (3.17) and bglThm (3.84). The thermal stability determined by DSF was higher for bglB (Tm 43.8ºC) than for bglA (Tm 35.2ºC). The bglThm was stable at 47°C and remained stable for up to 4 h at 80°C. In addition the thermal inactivation kinetics at 47°C evaluated by the relative remaining activity showed that bglA denaturation (kinactivation of 1.9 s-1) is faster than bglB (kinactivation of 31.3 s-1). On the other site, bglThm inactivation at 95°C was a two-step process, which exhibited an initial fast step (kinactivation of 2.9 s -1) followed by a slow step (kinactivation of 0.2 s-1). The chemical denaturation by urea followed using tryptophan fluorescence showed a transition (c50) at 7.9 ± 0.2 mol•L-1 for bglA and 7.1 ± 0.2 mol•L-1 for bglB, the bglThm was stable at 9M of urea (showing 95% of native state). Moreover the 'm' parameter, which represents the denaturant effect on the protein stability, is 669 cal•mol-1 for bglA and 860 cal•mol-1 for bglB. In conclusion, bglB showed intermediate stability between bglA and bglThm. Supported by FAPESP and CAPES.

PL-037 **Computational modeling of INI1/SMARCB1 and novel insights into its interaction with HIV-1 Integrase**

Savita Bhutoria[1], Sheeba Mathew[2], Menachem Spira[2], Xuhong Wu[2], Kalpana Ganjam[2], Seetharama Acharya[1]

1.-Department of Hematology , Albert Einstein College of Medicine, 2.-Department of Genetics, Albert Einstein College of Medicine

Protein structure modeling, protein- protein interaction Computational modeling of INI1/SMARCB1 and novel insights into its interaction with HIV-1 Integrase Savita Bhutoria1, Sheeba Mathew2, Menachem Spira2, Xuhong Wu2, Ganjam V Kalpana2* and Seetharama A Acharya1* Departments of 1Hematology and 2Genetics, Albert Einstein College of Medicine, Bronx, New York. *Equal corresponding authors The INI1/SMARCB1 gene encodes a component of the SWI/SNF ATP-dependent, chromatin-remodeling complex. INI1/SMARCB1 is present in all mammalian SWI/SNF complexes. It was isolated as an interacting protein for HIV-1 Integrase (IN) and subsequently demonstrated to be associated with cMYC, the carboxyl-terminal SET domains of ALL-1 and EBNA (Epsteain Bar Virus, nuclear antigen)1. INI1/SMARCB1 has no known structural homologues, and its amino-acid sequence yields little insight into its function. A detailed understanding of structure-function relationships is hampered by the lack of structural information for INI1. Computational methods that model protein/peptide structures with sufficient accuracy to facilitate functional studies have had notable successes. We carried out combination of sequence analysis ab initio structure modeling and dynamics studies of Integrase Binding Domain of INI1 and found it to be similar to that of Phospholipase A2 Activating Protein, PLAA. Structural similarity with this distant protein suggests divergent evolution of the two proteins. The modeled structure sheds light on various protein-protein interactions of INI1. By integrating the experimental studies about the binding, we have shown through docking, how a fragment of INI1 binds to the HIV-1 IN. Molecular docking and experimental studies indicated that two proteins bind tightly through charged/polar residues surrounding a hydrophobic cleft. These studies provide first modeled structure of INI1/SMARCB1 or any component of the SWI/SNF complex, and provide structural basis for IN-INI1 interactions. This molecular interpretation of the intermolecular interactions is expected to facilitate design of inhibitors as novel class of anti-HIV-1 therapeutic agents. References Morozov A, Yung E, Kalpana GV (1998) Structure-function analysis of Integrase Interactor 1/hSNF5L1 reveals differential properties of two repeat motifs present in the highly conserved region. ProcNatlAcadSciUSA 95: 1120–1125. Maillot B, Lévy N, Eiler S, Crucifix C, Granger F, et al. (2013) Structural and Functional Role of INI1 and LEDGF in the HIV-1 Preintegration Complex. PLoS ONE 8(4): e60734.

PL-038 **Structural determinants for human RNase 6 antimicrobial mechanism of action**

Javier Arranz Trullén[1], Guillem Prats-Ejarque[1], Jose Antonio Blanco[1], Marcel Albacar[1], Diego Velazquez[1], David Púlido[2], Mohammed Moussaoui[1], Ester Boix[1]

1.-Department of Biochemistry and Molecular Biology, Biosciences Faculty, UAB, 2.-Department of Life Sciences, Imperial College

Host Defence & Immunity Structural determinants for human RNase 6 antimicrobial mechanism of action. The RNase A superfamily is a vertebrate specific family that includes eight functional members in humans. Together with their catalytic activity towards RNA substrates, other biological properties have been reported and evolution studies suggest an ancestral host-defence function in vertebrates. Indeed, genetic studies confirmed a rapid molecular evolution within the family, a distinctive trait for host defence proteins exposed to a changing pathogen environment. Previous studies from our laboratory characterized the wide spectra antimicrobial activity of two highly cationic human RNases: the eosinophil RNase 3 and the skin derived RNase 7 (Boix et al., 2012). However, the family photo still remained incomplete. In the present study we have explored the structural determinants required for human RNase 6 mechanism of action. RNase 6 is a secretion protein expressed in innate cell types. Its induced secretion at the urinary tract during infection suggests a physiological protective role (Becknell et al., 2014)(Pulido et al., 2013). We present here the characterization of the RNase catalytic activity together with its membrane binding mode and bactericidal properties. Our results show that the protein displays a high antimicrobial activity against both Gram negative and Gram positive species together with a cell agglutinating ability. By applied site-directed mutagenesis we have spotted the protein residues contributing to the protein distinctive features. Becknell, B., Eichler, T. E., Beceiro, S., Li, B., Easterling, R. S., Carpenter, A. R., … Spencer, J. D. (2014). Ribonucleases 6 and 7 have antimicrobial function in the human and murine urinary tract. Kidney International, 87, 151–161. Boix, E., Salazar, V. a., Torrent, M., Pulido, D., Nogués, M. V., & Moussaoui, M. (2012). Structural determinants of the eosinophil cationic protein antimicrobial activity. Biological Chemistry, 393(August), 801–815. Pulido, D., Torrent, M., Andreu, D., Nogues, M. V., & Boix, E. (2013). Two human host defense ribonucleases against mycobacteria, the eosinophil cationic protein (RNase 3) and RNase 7. Antimicrobial Agents and Chemotherapy, 57(RNase 3), 3797–3805.

PL-039 **Covalent structure of single-stranded fibrinogen and fibrin oligomers cross-linked by fxiiia. The influence of free radical oxidation**

Anna Bychkova[1], Vera Leonova[1], Alexander Shchegolikhin[1], Marina Biryukova[1], Elizaveta Kostanova[1], Mark Rosenfeld[1]

1.-N. M. Emanuel Institute Of Biochemical Physics, Russian Academy Of Sciences

Protein structure and function Native fibrinogen is a key blood plasma protein whose main function is to maintain hemostasis by virtue of producing the cross-linked fibrin clots under the effect of thrombin and fibrin-stabilizing factor (FXIIIa). FXIIIa-mediated isopeptide γ–γ bonds are known to be produced between γ polypeptide chains of adjacent fibrinogen or fibrin molecules. But there are apparently conflicting ideas regarding the orientation of γ–γ bonds. In this study several peculiarities of self-assembly of fibrin(ogen) and induced oxidation of the proteins have been studied with the aid of elastic and dynamic light scattering, UV-, FTIR- and Raman spectroscopy methods. In the presence of FXIIIa both the non-oxidized and oxidized fibrinogen molecules has been shown to bind to each other in the “end-to-end” fashion to form the flexible covalently cross-linked fibrinogen homopolymers. To identify the orientation of γ–γ bonds in fibrin protofibrils a novel approach based on self-assembly of soluble cross-linked fibrin protofibrils and their dissociation in the urea solution of moderate concentrations has been applied. The results of elastic and dynamic light scattering coupled with analytical ultracentrifugation indicated the protofibrils to exhibit an ability to dissociate under increasing urea concentration to yield single-stranded structures entirely brought about by γ–γ bonds. The results of this study provide an evidence to support the model of the longitudinal γ–γ bonds that form between the γ chains end-to-end within the same strand of a protofibril. Since fibrinogen is known to be sensitive to ROS the mechanisms of fibrinogen and fibrin self-assembly under induced oxidation have been investigated. In both cases the polypeptide chains of the oxidized fibrin(ogen) proved to be involved in the enzymatic cross-linking more readily than those of unaffected molecules. The enhancing role of the D:D interaction under oxidation could be considered as an compensatory mechanism in the assembly of fibrin when the D:E interaction is impaired. The experimental data on fibrinogen and fibrin oxidation acquired in the present study, being combined with our earlier findings, make it reasonable to suppose that the spatial structure of fibrinogen could be evolutionarily adapted to some ROS actions detrimental to the protein function. The study was supported by the RFBR, Research Projects 14-04-31897mol_a and 15-04-08188a.

PL-040 **Structural and thermodynamic analysis of co-stimulation receptor CD28 phosphopeptide interactions with Grb2, Gads, and PI3-kinese SH2 domains**

Satomi Inaba[1], Nobutaka Numoto[2], Hisayuki Morii[3], Teikichi Ikura[2], Ryo Abe[4], Nobutoshi Ito[2], Masayuki Oda[1]

1.-Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, 2.-Medical Research Institute, Tokyo Medical and Dental University (TMDU), 3.-National Institute of Advanced Industrial Science and Technology, 4.-Research Institute for Biomedical Sciences, Tokyo University of Science

In addition to the signaling produced by the binding of antigen-major histocompatibility complex to T-cell receptors, co-stimulatory signals from other receptor-ligand interactions are required for full activation of T-cells. The CD28 receptor on the T-cell surface has been well characterized, and the binding of ligand to CD28 is critical for producing co-stimulatory signals. CD28 has no enzymatic activity and its cytoplasmic region consists of 41 amino acids that contain the sequence YMNM, in which the tyrosine residue is phosphorylated by kinase. The phosphorylated sequence, pYMNM, is recognized by Src homology 2 (SH2) adaptor proteins, such as growth factor receptor binding protein 2 (Grb2), Grb2-related adaptor downstream (Gads), and the phosphatidylinositol 3-kinase (PI3-kinase) regulatory subunit, p85. The consensus sequence for the binding of Grb2 SH2 and Gads SH2 is pYXNX, and that of p85 N-terminus SH2 (nSH2) and C-terminus SH2 (cSH2) is pYXXM. We reported the high-resolution crystal structure of Grb2 SH2 in complex with the CD28 phosphopeptide [Higo et al., PLOS ONE 8, e74482, 2013], and recently determined those of Gads SH2, p85 nSH2, and p85 cSH2. These data along with the results of binding thermodynamics analyzed using isothermal titration calorimetry, helped to elucidate the molecular recognition mechanisms of CD28 by adaptor proteins. The SH2 proteins were over-expressed in Escherichia coli, and were purified using affinity and gel-filtration chromatography. The CD28 phosphopeptides, 8-residue (OctP) and 12-residue (DdcP02), were synthesized using the solid-phase supported technique, and were purified using reversed-phase chromatography. The crystals were obtained by the hanging-drop vapor diffusion method. X-ray diffraction data were collected at synchrotron radiation facilities, and the structures were determined by the molecular replacement method. The models of Grb2 SH2, Gads SH2, p85 nSH2, and p85 cSH2 in complex with OctP were refined at 1.35, 1.2, 1.0, and 1.1 Å resolutions, respectively. The crystal structures showed that the phosphotyrosine phosphate moiety directly interacted with the side-chain of arginine in SH2, which is common in all complex structures. In the Grb2 SH2 and Gads SH2 complexes, the side-chain of asparagine at the pY+2 position forms a pair of hydrogen bonds with the main-chain amide and carbonyl groups of lysine in SH2. Alternatively, in the p85 nSH2 and cSH2 complexes, the side-chain of methionine at the pY+3 position is located in hydrophobic pockets of nSH2 and cSH2, in which the hydrophobic interactions of cSH2 would be stronger than those of nSH2. This idea is supported by the observed binding thermodynamics. The binding affinity of cSH2 to DdcP02, because of a favorable enthalpy change, is about 10-fold higher than that of nSH2. The binding affinity of Grb2 SH2 to DdcP02 is similar to that of Gads SH2 to DdcP02, and is about 10-fold lower than that of nSH2 to DdcP02. These results indicate that the contribution of hydrophobic interactions of nSH2 and cSH2 at the pY+3 position are stronger than those of hydrogen bonds of Grb2 SH2 and Gads SH2 at the pY+2 position.

PL-041 **Novel kinetochore protein complex from silkworm holocentric chromosomes**

Takahiro Kusakabe[1], Hiroaki Mon[1], JaeMan Lee[1]
1.-Kyushu University Graduate School

The kinetochore, which consists of centromere DNA and a multilayered protein complex, plays important roles in chromosome organization and segregation. Interactions between chromosomes and spindle microtubules allow chromosomes to congress to the middle of the cell, and to segregate the sister chromatids into daughter cells in mitosis, which is followed cytokinesis. In contrast to monocentric chromosomes, in which the centromere is normally present at a single region on each chromosome, the holocentric chromosomes have centromeric activity along the entire length of the chromosome. It has been known that the silkworm, Bombyx mori, has holocentric chromosomes since 1970s, none of silkworm kinetochore proteins, however, have been identified so far. Here we report the identification of a novel set of genes for outer kinetochore proteins in silkworm by using bioinformatics and RNA interference-based screening. Under the hypothesis that depletion of essential kinetochore genes causes cell cycle arrest in mitosis, we performed RNAi in the silkworm cell line, BmN4-SID1, targeting a set of candidate genes. Knockdown of five genes caused significant cell cycle arrest at the G2/M phase. We also found that these five proteins make a complex, and that all of them are localized along the chromosome arms, indicating that the silkworm kinetochore extends along the chromosome.

PL-042 **Inactivation of βine aldehyde dehydrogenase from spinach by its physiological substrate βine aldehyde**

Rosario A. Muñoz-Clares[1], Andrés Zárate-Romero[1], Dario S. Murillo-Melo[1], Carlos Mújica-Jiménez[1], Carmina Montiel[1]

1.-Facultad de Química, Universidad Nacional Autónoma de México

To contend with osmotic stress caused by drought, salinity, or low temperatures some plants synthesize the osmoprotectant glycine βine (GB) from βine aldehyde (BAL). The last step—the irreversible NAD+-dependent oxidation of BAL—is catalyzed by ALDH10 enzymes that exhibit βine aldehyde dehydrogenase (BADH) activity. We here report that the Spinacia oleracea BADH (SoBADH) is reversibly inactivated by BAL in the absence of NAD+ in a time- and concentration-dependent mode to approximately 50% of the original activity. Inactivation kinetics are consistent with a partial reversible, two-steps mechanism that involves the formation of an active non-covalent enzyme•BAL complex before formation the inactive enzyme-BAL complex. Crystallographic evidence indicates that in the enzyme previously inactivated by BAL the aldehyde forms a thiohemiacetal with the nonessential Cys450 (SoBADH numbering) located at the aldehyde-entrance tunnel, thus totally blocking the access to the catalytic cysteine. Accordingly, BAL does not inactivate the C450S SoBADH mutant. Two crystal structures of the inactivating enzyme-BAL complex showed that the trimethylammonium group of BAL is inside the active-site aromatic box, as in the productive way of binding. This explains why the inactivation of the A441I mutant—where the binding of the trimethylammonium group is hindered—requires non-physiologically high BAL concentrations, while the A441C mutant—where the binding is allowed—is inactivated similarly to the wild-type enzyme. Cys-450 is conserved in most plant ALDH10 enzymes of known sequence, and in all of them with proven or predicted BADH activity. Inactivation by BAL appears therefore to be a common feature of plants BADHs. This short-term regulation may be of great physiological importance since the irreversibility of the BADH-catalyzed reaction would unbalance the NAD+/NADH ratio if the aldehyde concentrations are high, the NAD+ concentrations low and the reaction is not slowed down. Plants BADHs are prone to this situation since they work under osmotic stress conditions, when high BAL concentrations are required for the synthesis of high levels of the osmoprotectant GB. The partial nature of the regulatory mechanism that we are reporting will contribute to prevent both NAD+ exhaustion and accumulation of the toxic BAL. To the best of our knowledge, this is the first report of a novel reversible covalent modification of an ALDH enzyme involving its own substrate.

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PL-044 **Study of denaturation of proteins by surfactant using the taylor dispersion analysis and dynamic light scattering**

Anna Lewandrowska[1], Aldona Jelińska[1], Agnieszka Wiśniewska[1], Robert Hołyst[1]
1.-Institute of Physical Chemistry Polish Academy of Sciences

The protein-surfactant systems are commonly used in biological, pharmaceutical and cosmetic applications. Ionic surfactants are said to cause unfolding of proteins and in consequence losing their biological function. Therefore, knowledge of the structure of protein-surfactant complexes is important to understand the mechanism of denaturation. In these studies we employed the Taylor dispersion analysis and dynamic light scattering to study denaturation process of a few proteins under the influence of sodium dodecyl sulfate (SDS). We carried out series of measurements at constant protein concentration and varying SDS concentration. The structural changes were analyzed based on the diffusion coefficients of the complexes which were formed at different surfactant concentration. We observed that diffusion coefficient for these proteins was decreasing with increasing concentration of the surfactant at concentrations below the critical micelle concentration (CMC). The results obtained using the Taylor dispersion analysis are well correlated with those obtained using dynamic light scattering. Acknowledgement: This research was supported by the National Science Center Grant Opus 4 (UMO-2012/07/B/ST4/01400).

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PL-046 **Paraoxonase 1 (Pon1) regulates water homeostasis by controlling the expression of Fxr and Aqp2 proteins in mice**

Marianna Wieloch[1,2], Hieronim Jakubowski[1,2,3]

1.-Institute of Bioorganic Chemistry, 2.-Department of Biochemistry and Biotechnology, University of Life Sciences, 3.-Dep.of Microbiology Biochemistry & Molecular Genetics,Rutgers-New Jersey Medical

Paraoxonase 1 (Pon1) regulates water homeostasis by controlling the expression of Fxr and Aqp2 proteins in mice The kidney is responsible for maintenance of water and sodium homeostasis, which is controlled by a complex balance of water intake, renal perfusion, glomerular filtration and tubular reabsorption of solutes, and reabsorption of water from the renal collecting ducts. Urine volume depends on aquaporin (Aqp) water channels located in epithelial cells of renal tubules. Aqp2 expression is regulated by the bile acid receptor Fxr, a transcription factor also known to regulate lipid and glucose metabolism (Zhang X et al. Farnesoid X receptor (FXR) gene deficiency impairs urine concentration in mice. Proc Natl Acad Sci U S A 2014;111:2277-82). Inactivation of the Fxr gene reduces Aqp2 expression and impairs urine concentrating ability, which leads to a polyuria or urine dilution phenotype. We have previously found that Pon1-/- mice exhibit a polyuria phenotype and produce twice as much 24-h urine as their wild type Pon1+/+ littermates (Borowczyk K et al. Metabolism and neurotoxicity of homocysteine thiolactone in mice: evidence for a protective role of paraoxonase 1. J Alzheimer's Dis 2012;30:225-31). Pon1 is expressed in many organs, including the kidney, circulates in the blood attached to high-density lipoprotein (HDL), participates in homocysteine (Hcy) metabolism by hydrolyzing Hcy-thiolactone, and contributes to atheroprotective function of HDL by reducing oxidative stress and protein damage by N-homocysteinylation (Perła-Kaján J, Jakubowski H. Paraoxonase 1 and homocysteine metabolism. Amino Acids 2012;43:1405-1417). The purpose of the present work was to test a hypothesis that Pon1 maintains water homeostasis and prevents polyuria by controlling the expression of Fxr and Aqp2 proteins. Towards this end we quantified Fxr and Aqp2 proteins in kidneys of Pon1-/- and Pon1+/+ mice by Western blotting using anti-Fxr and anti-Aqp2 antibodies. We found that expression of both FXR and AQP2 was significantly reduced (2-fold) in kidneys of Pon1-/- mice (n=4) relative to Pon1+/+ animals (n=4). In conclusion, these findings demonstrate that Pon1/HDL play a critical role in controlling water homeostasis by controlling the expression of Fxr and its target gene Aqp2. Supported in part by NCN grants 2011/02/A/NZ1/00010, 2012/07/B/NZ7/01178, 2013/09/B/NZ5/02794, 2013/11/B/NZ1/00091.

PL-047 **Development and application of novel non-Ewald methods for calculating electrostatic interactions in molecular simulations**

Ikuo Fukuda[1], Narutoshi Kamiya[1], Han Wang[2], Kota Kasahara[1], Haruki Nakamura[1]
1.-Institute for Protein Research, Osaka University, 2.-Freie Universitaet Berlin

To understand the structure, function, and dynamics of protein systems in a microscopic description, molecular simulation is an important tool. The most time-consuming part of molecular simulation is the calculation of long-range interactions of the particles. In particular, appropriate treatment of the electrostatic interaction is critical, since the simple truncation cannot be used due to the slow decay of the Coulombic function. Thus, it is highly demanded to calculate the electrostatic interactions with high accuracy and low computational cost. For this purpose we have developed the Zero-multipole (ZM) summation method [1]. In this method the artificial periodic boundary conditions are not necessary and the Fourier part evaluations are not needed, in contrast to the conventional Ewald-based methods. Instead, a pairwise function that is suitably redefined from the Coulombic function is used with a cutoff scheme. The underling physical idea is simple: (a) in a biological system, a particle conformation for which the electrostatic interactions are well cancelled is more stable than other conformations [2]; (b) since such well-cancelled conformations are essentially physical, we should clip a subset of such a conformation out of the conformation within an ad-hoc given cutoff sphere and calculate the interactions only from this subset. This idea is realized by a rigid mathematical consideration that leads to the deformation of the Coulombic function. The efficiency of the ZM method has been validated in applications to fundamental systems, such as ionic systems [3] and bulk water [4], and heterogeneous bimolecular systems, including DNA [5], membrane protein [6], motor protein [7], and DNA-protein complex [8]. In the presentation, we will provide the theory and the numerical results of our method and show its efficiencies in detail. We will also discuss how the treatment of the electrostatic calculations seriously affects the simulation results of protein systems. [1] I. Fukuda, J. Chem. Phys. 139, 174107 (2013); Fukuda et al., ibid. 140,194307 (2014). [2] I. Fukuda and H. Nakamura, Biophys. Rev. 4, 161 (2012). [3] I. Fukuda, et al. J. Chem. Phys. 134, 164107 (2011). [4] I. Fukuda, et al. J. Chem. Phys. 137, 054314 (2012). [5] T. Arakawa et al., PLoS One 8, e76606 (2013). [6] N. Kamiya, et al., Chem. Phys. Lett. 568-569, 26 (2013); T. Mashimo et al., J. Chem. Theory Comput. 9, 5599 (2013). [7] Y. Nishikawa, et al., J. Mol. Biol. 426, 3232 (2014). [8] K. Kasahara, et al., PLoS ONE 9, e112419 (2014).

PL-048 **Isolation and characterisation of the zearalenone degrading hydrolase ZenA**

Sebastian Fruhauf[1], Michaela Thamhesl[1], Patricia Fajtl[1], Verena Klingenbrunner[1], Elisavet Kunz-Vekiru[2], Gerhard Adam[3], Gerd Schatzmayr[1], Wulf-Dieter Moll[1]

1.-Biomin Research Center, 2.-Christian Doppler Laboratory for Mycotoxin Metabolism (IFA-Tulln), 3.-IAGZ, University of Natural Resources and Life Sciences

Zearalenone is a mycotoxin produced by Fusarium graminearum and related Fusarium species. F. graminearum is a powerful plant pathogen and infects major crop plants around the world. Acute toxicity of zearalenone is low, but due to its structural similarity to β-estradiol it has binding affinity to the estrogen receptor, which results in interference with hormonal balance. Typical effects seen in animals include symptoms like hyperestrogenism and reproductive disorders (reduced fertility, reduced litter size or swelling of uterus and vulva). To reduce the risk for human and animal health posed by the ingestion of contaminated food or feed different decontamination strategies have been studied, including biotransformation. Today many microorganisms are known to degrade zearalenone, but for most of them the degradation pathway and formed metabolites remained unknown, hence it is unknown if this degradation also means detoxification. Only for the fungal strains Trichosporon mycotoxinivorans and Gliocladium roseum ZEN degradation has been studied in detail and loss of estrogenicity of reaction products has been confirmed. We screened for, and isolated zearalenone degrading bacteria from soil samples. The most promising new bacterial isolate was taxonomically assigned to the species Rhodococcus erythropolis and designated PFA D8-1. The zearalenone catabolism pathway of PFA D8-1 was found to be identical as known from G. roseum. The primary reaction product, hydrolysed zearalenone, has so far only been postulated in G. roseum. We prepared hydrolysed zearalenone by preparative HPLC and showed loss of estrogenicity in assays with the breast cancer cell line MCF7 and the estrogen reporter yeast strain YZHB817. A genomic library was prepared and screened in zearalenone degradation deficient R. erythropolis PR4. The gene encoding zearalenone hydrolase was found and named zenA. The hydrolase was identified as member of the α/β-hydrolase family and named ZenA. It was cloned, recombinantly expressed in E. coli and purified by 6 x His-tag mediated immobilised metal affinity chromatography. Activity of His-tagged and untagged enzyme ZenA was compared in cleared lysate and ZenA was purified for enzyme characterisation. The influence of pH and temperature on enzyme activity and stability was evaluated and kinetic parameters were determined.

PL-049 **A new biding site for snake venom C-type lectins?**

Maria Cristina Nonato Costa[1], Ricardo Augusto Pereira de Pádua[1], Marco Aurelio Sartim[1], Suely Vilela Sampaio[1]

1.-University of São Paulo, FCFRP

C-type lectins are proteins that bind different glycan molecules by interactions with a calcium atom present in a carbohydrate recognition domain (CRD). Many organisms (plants, bacteria, virus and animals) use these proteins in various biological events like lymphocyte adhesion, erythrocyte agglutination and extracellular matrix organization. The C-type lectin fold is plastic and possible for about 1013 different sequences, what promoted its adaptation to diverse functions, similarly to the observed for the immunoglobulin fold (1014-1016 sequences). It is comprised of about 110-130 amino acid residues that folds in two four-stranded β sheets sandwiched by two alpha helices. Interestingly, C-type lectins present in snake venoms are possible anti-cancer agents since they are toxic to cancer cells and inhibit the adhesion and proliferation of various cancer cell lines. Therefore, we have purified a lactose binding C-type lectin from the venom of Bothrops jararacussu (BJcuL) to study its structure and binding properties to different sugars. BJcuL crystals were obtained by vapor diffusion and the structure solved by X-ray crystallography to 2.9 Å resolution. BJcuL structure is a decamer formed by a pseudo fivefold axis rotation of a dimer hold by a disulfide bond. Each monomer binds a calcium atom and possibly another metal at a second and opposed binding site. The decamer possesses a donut shaped structure with 10 calcium ions on the surface available for interactions with carbohydrate molecules. Binding specificity was evaluated for 20 carbohydrates using differential scanning fluorimetry (DSF) that showed BJcuL interacts with galactose and lactose but less with glucose and sacarose. Surprisingly, high levels of thermostabilization of BJcuL was achieved with the antibiotic aminoglycosides geneticin (G418) and gentamicin in a calcium concentration dependent manner, but not kanamycin. Intriguingly, while lactose and galactose inhibited erythrocyte agglutination by BJcuL, G418 and gentamicin did not affect hemagglutination implying a second site of binding. DSF analysis also suggested the presence of a second binding site for the antibiotics and crystallization of the complexes are in progress in order to understand fully this new binding mechanism of C-type lectin with antibiotics.

PL-050 **Ab initio modelling of structurally uncharacterised antimicrobial peptides**

Mara Kozic[1]

1.-Institute of Integrative Biology, University of Liverpool

Ab initio modelling of structurally uncharacterised antimicrobial peptides Mara Kozic 1* 1 Institute of Integrative Biology, Biosciences Building, University of Liverpool, Crown Street, Liverpool L69 7ZB, United Kingdom * Mara.Kozic@liverpool.ac.uk Antimicrobial resistance within a wide range of infectious agents is a severe and growing public health threat. Antimicrobial peptides (AMPs) are among the leading alternatives to current antibiotics, exhibiting broad spectrum activity. An understanding of the structure of a protein can lead us to a much improved picture of its molecular function. Furthermore, an improved understanding of structure-function relationships facilitates protein design efforts to enhance their activity. Currently, the 3D structures of many known AMPs are unknown. To improve our understanding of the AMP structural universe we have carried out large scale ab initio 3D modelling of structurally uncharacterised AMPs. Such ab initio modelling is facilitated by the typical small size of AMPs as well as their tendency to contain disulphide bonds, these providing valuable additional information to simulations. Preliminary results reveal unexpected similarities between the predicted folds of the modelled sequences and structures of well-characterised AMPs. For example, Lacticin Q was revealed to contain a helical bundle fold that bears a striking resemblance to Enterocin 7A. We also found a remarkable similarity between the predicted structure of Silkworm 001 peptide and β-hairpin AMPs such as Tachyplesin I. Our results improve the understanding of the structure-function relationship of AMPs.

PL-051 **Surface aggregation-propensity as a constraint on globular proteins evolution**

Susanna Navarro[1], Marta Diaz[2], Pablo Gallego[2], David Reverter[2], Salvador Ventura[1]
1.-Institut de Biotecnologia i Biomedicina and Departament de Bioquimica i Biologia, 2.-Institut de Biotecnologia i Biomedicina, Universitat Autònoma de Barcelona

In living cells, functional protein–protein interactions compete with a much larger number of nonfunctional interactions. Theoretical studies suggest that the three-dimensional structures of present proteins have evolved under selective pressure to avoid the presence of aggregation-prone patches at the surface that may drive the establishment of anomalous protein contacts. However, no experimental evidence for this hypothesis exists so far. The α-spectrin SH3 domain (SPC-SH3) has been used as a protein model to decipher the sequential aggregation determinants of proteins. Here we use it to address the structural determinants of protein aggregation and their link to protein evolution. To this aim we exploit Aggrescan3D (A3D), a novel algorithm developed by our group, which takes into account both protein structure and experimental data to project aggregation propensities on protein surfaces. We used A3D to design a series of SPC-SH3 variants with progressively stronger aggregation-prone surfaces and characterized their thermodynamic, structural and functional properties. Our data support evolution acting to constraint the aggregation propensities of globular protein surfaces in order to decrease their potential cytotoxicity and the protein quality control machinery acting to buffer this negative selective pressure.

PL-052 **Utilizing 3D structure for the annotation of structural motifs in the Conserved Domain Database**

Narmada Thanki-Cunningham[1], Noreen Gonzales[1], Gabriele Marchler[1], Myra Derbyshire[1], James Song[1], Roxanne Yamashita[1], Christina Zheng[1], Stephen Bryant[1], Aron Marchler-Bauer[1], Farideh Chitsaz1[1]

1.-Conserved Domain Database, Structure Group CBB/NCBI/NLM/NIH

The Conserved Domain Database (CDD) is a protein classification and annotation resource comprised of multiple sequence alignments representing ancient conserved domains. CDD protein domain models are curated by NCBI and use 3D protein structure explicitly to define domain extent and the location of conserved core structures, and to provide accurate alignments between diverse family members via structure superposition. CDD also imports external collections such as Pfam and TIGRFAM. Recently, a novel class of annotation labeled as “structural motifs” has been introduced to supplement current capabilities. These annotations define compositionally-biased and/or short repetitive regions in proteins, which are difficult to model as functional domains conserved in molecular evolution. Structural motifs include transmembrane regions, coiled coils, and short repeats with variable copy numbers. For many types of short tandem repeats, a few position-specific score matrices (PSSMs) suffice to annotate more than 90% of the known instances of that structural motif. Unfortunately, a lack of sequence similarity within coiled-coil regions prohibits the development of only a few generic models; therefore, models for coiled-coil regions in the context of specific families have been developed using the Spiricoil Database as a reference. Increased coverage of coiled-coil regions in CDD, specific site annotations of these structural motifs as well as their representation on the webpages will be discussed.

PL-053 **Biophysical characterization of the Sema3A C-terminal basic domain interaction with glycosaminoglycans**

Roman Bonet[1], Miriam Corredor[1], Cecilia Domingo[1], Jordi Bujons[1], Yolanda Perez[1], Ignacio Alfonso[1], Angel Messeguer[1]

1.-Department of Chemical Biology and Molecular Modelling, IQAC-CSIC

Semaphorin 3A (Sema 3A) is a protein originally described as an axonal chemorepellent cue involved in many physiological processes ranging from embryonic development to bone homeostasis or immune responses [1]. Sema3A signal transduction requires the formation of a heteromeric complex with Neuropilin-1 (Nrp1) and PlexinA [2]. In addition, Sema3A interaction with Nrp1 is modulated by the furin protease cleavage at its C-terminal basic domain [3]. This C-terminal basic domain has also been suggested to mediate the binding to glycosaminoglycans (GAGs), an association that locates Sema3A to perineuronal nets and enhances its function in restricting neuronal plasticity and inhibiting axonal regeneration in the central nervous system (CNS) [4, 5]. In this work, we used a combination of biophysical techniques to gain insight into the interaction of the Sema3A C-terminal domain with GAGs. Two peptides corresponding to the highly positively charged regions on the domain were shown to bind to immobilized heparin by surface plasmon resonance (SPR) and the affinity dramatically increased when the complete domain was assayed. The binding was confirmed by nuclear magnetic resonance (NMR) and Circular Dichroism (CD), which also revealed that the Sema3A C-terminus is mainly unstructured in solution with a short helix in its N-terminus, as previously described for Sema3F [6]. The conserved cysteine within this motif, necessary for the dimerization of Sema3A [7], is also critical for the helix formation. In addition, fluorescence spectroscopy studies showed that the N-terminal region also has a contribution in the binding to GAGs. We acknowledge the financial support from the European Union Seventh Framework Programme (FP7/2007-2013) under the Project VISION, Grant Agreement n° 304884. 1.Xu, R., Semaphorin 3A: A new player in bone remodeling. Cell Adh Migr, 2014. 8(1): p. 5-10. 2.Janssen, B.J., et al., Neuropilins lock secreted semaphorins onto plexins in a ternary signaling complex. Nat Struct Mol Biol, 2012. 19(12): p. 1293-9. 3.Parker, M.W., et al., Furin processing of semaphorin 3F determines its anti-angiogenic activity by regulating direct binding and competition for neuropilin. Biochemistry, 2010. 49(19): p. 4068-75. 4. De Wit, J., et al., Semaphorin 3A displays a punctate distribution on the surface of neuronal cells and interacts with proteoglycans in the extracellular matrix. Mol Cell Neurosci, 2005. 29(1): p. 40-55. 5.Dick, G., et al., Semaphorin 3A binds to the perineuronal nets via chondroitin sulfate type E motifs in rodent brains. J Biol Chem, 2013. 288(38): p. 27384-95. 6.Guo, H.F., et al., Mechanistic basis for the potent anti-angiogenic activity of semaphorin 3F. Biochemistry, 2013. 52(43): p. 7551-8. 7.Koppel, A.M. and J.A. Raper, Collapsin-1 covalently dimerizes, and dimerization is necessary for collapsing activity. J Biol Chem, 1998. 273(25): p. 15708-13.

PL-054 **Functional clustering of the crotonase superfamily**

Julia Hayden[1], Janelle Leuthaeuser[2], Patricia Babbit[3], Jacquelyn Fetrow[4]

1.-Dickinson College, Molecular Biology and Chemistry Department, 2.-Department of Molecular Genetics, Wake Forest University,, 3.-Department of Pharm. Chem., University of California, San Francisco, CA, 4.-Department of Chemistry, University of Richmond, Richmond, VA

As the number of sequenced proteins have grown, the reliance on computational annotation has likewise grown, leading to rampant misannotation and difficulties grouping proteins functionally. Prior attempts to create functionally relevant groupings of proteins in the Crotonase superfamily suggest that this superfamily is difficult to cluster functionally due in part to the functionally diverse nature of the protein superfamily. We have developed two novel procedures to combat this difficulty: TuLIP (Two-Level Iterative clustering Process), a process that utilizes structural information from active sites to cluster protein structures into hypothesized functional groupings, and MISST (Multi-level Iterative Sequence Searching Technique), a process that uses the protein groupings created in TuLIP as a starting point for iterative GenBank searches and further clustering after each search. Through these two methods, the total coverage of the Crotonase superfamily has increased, and the generated groups contain proteins from subgroups and families that did not have a structural representative. Novel hypothesized functional protein groupings have been created, most notably for a large number of proteins that lack annotation data at the subgroup or family level, and for proteins of the enoyl-CoA hydratase family. Our results demonstrate the novel processes TuLIP and MISST are able to cluster proteins of the Crotonase superfamily into hypothesized functional groupings.

PL-055 **Peptidic probes for intravascular molecular imaging of inflammation using clinically translatable polymeric microbubbles**

Olga Iranzo[1,2], Ana C. Fernandes[1], Teresa Sorbo[2], Ivan Duka[2], Lia Christina Appold[3], Marianne Ilbert[4], Fabian Kiessling[3], Ricardo J. F. Branco[5]

1.-Instituto de Tecnologia Química e Biológica António Xavier, UNL, 2.-Aix Marseille Université, Centrale Marseille, CNRS, iSm2 UMR 7313, 3.-ExMI, Helmholtz Institute for Biomedical Engineering, RWTH-Aachen University, 4.-BIP, IMM, Aix Marseille Université, CNRS, UMR 7281, 5.-UCiBio-REQUIMTE, Faculdade de Ciências e Tecnologia, UNL

E-selectin is a cell-adhesion molecule induced on the surface of endothelial cells in response to cytokines. Its upregulation has been reported in many disorders, including inflammatory and cardiovascular diseases, tumor angiogenesis and metastasis [1]. This profile suggests E-selectin as a promising target to develop molecular imaging probes for the detection of these diseases. Recently, we have reported the specific in vivo ultrasound imaging of E-selectin expression in tumors using a microbubble contrast agent covalently attached to the peptide ligand IELLQAR, known to bind to E-selectin [2]. However, it was observed that this probe has a limitation in the imaging of cardiovascular diseases where higher shear stresses prevent microbubbles from remaining attached to the target. Therefore, peptides with higher E-selectin affinity are needed to design probes capable of imaging these diseases. In this context, automated docking and molecular dynamics methodologies were combined and applied to different E-selectin binding peptides. These studies predicted the energetically more favorable binding mode as well as the key interactions between the peptide ligands and the E-selectin receptor. Some of these peptides were prepared by solid-phase peptide synthesis and their interactions with E-selectin analyzed by surface plasmon resonance technique. The results showed that these peptides have different affinities for E-selectin. These data were correlated with the computational studies and evaluated to obtain crucial information of the key recognition elements needed for higher E-selectin affinity. These recent results will be presented. [1] E. Jubeli, L. Moine, J. Vergnaud-Gauduchon, and G. Barratt, J. Control. Release 2012, 158, 194-206. [2] S. Fokong, A. Fragoso, A. Rix, A. Curaj, Z. Wu, W. Lederle, O. Iranzo, J. Gätjens, F. Kiessling, and M. Palmowski, Invest. Radiol. 2013, 48, 843-850.

PL-056 **A search for anti-melioidosis drug candidates targeted to sedoheptulose-7-phosphate isomerase from Burkholderia pseudomallei**

Jimin Park[1], Daeun Lee[1], Sang A Yeo[1], Mi-Sun Kim[1], Dong Hae Shin[1]
1.-College of Pharmacy, Global Top5 Research Program, Ewha Womans University

Burkholderia pseudomallei is the causative agent of melioidosis, a serious invasive disease of animals and humans in tropical and subtropical areas. Sedoheptulose-7-phosphate isomerase from B. pseudomallei (BpGmhA) is the antibiotics adjuvant target for melioidosis. In general, BpGmhA converts d-sedoheptulose-7-phosphate to d-glycero-α-d-manno-heptopyranose-7-phosphate (M7P). This is the first step of the biosynthesis pathway of NDP-heptose responsible for a pleiotropic phenotype. Therefore, this biosynthesis pathway is the target for searching novel antibiotics increasing the membrane permeability of Gram-negative pathogens or adjuvants synergistically working with known antibiotics. The crystal of this enzyme has been solved at 1.9 Å resolution. There is an active site pocket where a putative metal binding site is located. To find out inhibitors of BpGmhA, in-silico virtual screening with ZINC, a free database of commercially-available compounds, has been performed. Tens of thousands of chemical compounds were docked into the active site of BpGmhA. A number of putative BpGmhA binding compounds better than M7P were found using Surflex-Dock included in the SYBYL software package. Characteristics of these compounds were surveyed and classified to identify common binding properties with BpGmhA.

PL-057 **Mapping the structure of laminin using cross-linking and mass spectrometry**

Gad Armony[1], Toot Moran[1], Yishai Levin[2], Deborah Fass[1]

1.-Weizmann Institute of Science, Department of Structural Biology, 2.-Weizmann Institute of Science, Israel Center for Personalized Medicine

Laminin, a ~800 kDa heterotrimer, is a major element in the extracellular matrix (ECM). Within the ECM, laminin contributes to the adhesion and migration of cells, both in health and disease. The laminin trimer was observed by rotary shadowing electron microscopy to be cross shaped: the three short arms of the cross are formed by the amino-terminal halves of the three subunits, whereas the long arm of the cross holds the three chains together in a long coiled coil. The narrow and flexible arms of the laminin cross complicate studying its structure to high resolution by crystallography or electron microscopy single particle reconstruction. To advance our understanding of this remarkable quaternary structural assembly, we have used cross-linking and mass spectrometry to analyze the organization of the laminin trimer. This technique was validated by known crystal structures of isolated laminin domains. In all cases the crystal structure distances agree with the cross-linker length. The identified cross-links were particularly helpful in assigning the register and the subunit order of the long coiled coil due to the high content of cross-linkable residues in this region. Using known X-ray crystal structures, homology modeling, and distance restraints provided by two cross-linker chemistries, a clearer picture of the laminin quaternary structure is obtained.

PL-058 **Non-sequential protein structure alignment program MICAN and its applications**

Shintaro Minami[1], George Chikenji[2], Motonori Ota[1]
1.-Dept. of Info. Sci., Nagoya Univ., 2.-Dept. of Comp. Schi. & Eng., Nagoya Univ.

In some proteins, secondary structure elements are arranged spatially in the same manner, but they are connected in the alternative ways. Analysis on such non-sequential structural similarity in proteins is important because it provides a deeper understanding of the structural geometry of protein. This can be also observed even in the homologous proteins, indicating the non-sequential structural similarity is significant in the protein evolution. However, the non-sequential structural similarity in proteins is less investigated. We developed a novel non-sequential structural alignment program MICAN, which can handle Multiple chains, Inverse direction of chains, Cα models, Alternative alignments, and Non-sequential alignments. We performed comprehensive non-sequential structural comparison among homologous proteins in the same SCOP superfamily by using the MICAN program. Based on the result, we found that approximately 8% of superfamilies include at least one protein pairs showing non-sequential structural similarity. 85% non-sequential structurally similar pairs are aligned in a simple way, e.g. circular permutation, $\β$ strand flip/swap, but 15% are complicated. Interestingly, most of such complicated non-sequential similarities can be explicable by combination of 2-4 simple non-sequential relationships. This result indicates that accumulation of simple structural changes in the course of protein evolution produces completely different fold homologs.

PL-059 **Effects of cell-like infrastructures on transient protein interactions**

Ciara Kyne[1], Peter Crowley[1]
1.-School of Chemistry, National University of Ireland Galway

As early as 1919, Ritter surmised that the cell's molecules cooperate to form a "special apparatus and an organised laboratory".1 Despite supporting evidence from Srere, McConkey and others, efforts to understand molecular organisation in vivo are still in their infancy. However, important aspects of the cell interior have already been revealed. For example, weak molecular interactions structure the cytoplasm into time-evolving, functional zones.2 Weak interactions are difficult to capture and can preclude protein detection in cells by many biophysical techniques, including NMR spectroscopy.3, 4 We explored the effects of cell-like milieus on the cytochrome c (cyt c)-flavodoxin (fld) interaction. These oppositely charged proteins interact weakly with a number of cognate partners. Neither cyt c4 nor fld is detectable by NMR in Escherichia coli confirming their "sticky" nature (Figure 1A). The cyt c-fld interaction was assessed in buffer, 8 % polyacrylamide gels and in solutions containing 100 g/L of macromolecular crowders (Figure 1B). 1H, 15N HSQC NMR revealed that the interaction was transient in buffer, proceeding via the known binding site for both proteins. Substantial line broadening was effected in crowded and confined solutions suggesting that the cyt c-fld complex is stabilised under native-like conditions. The stabilising effect of macromolecular crowders was also observed by native gel electrophoresis and crystallization. These findings coincide with Spitzer and Poolman's model for cytoplasmic structuring, emphasising the role of charge-charge interactions and crowding in the formation of macromolecular "clusters". 5 The implications for cytoplasmic structuring will be discussed alongside related investigations of cationic protein interactions in E. coli extracts.3, 4

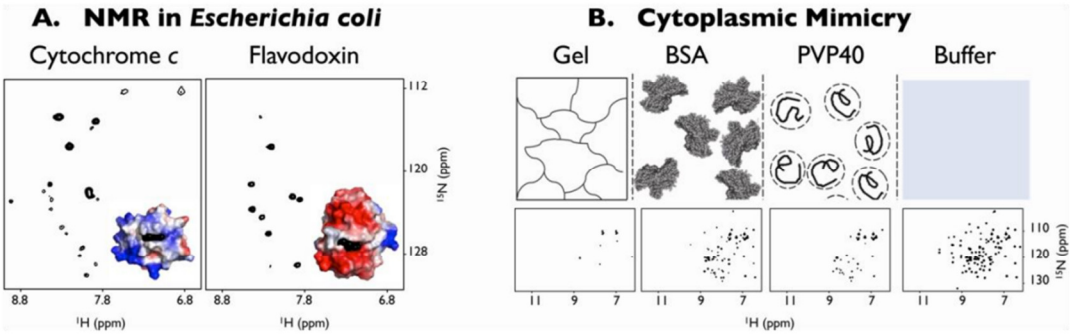

Figure 1. 1H, 15N HSQC spectra of: A. E. coli cell suspensions containing over-expressed cyt c and fld. B. cyt c with 1 equivalent of fld in media mimicking the cytoplasm. These include 8 % polyacrylamide gel, 100 g/L bovine serum albumin or polyvinylpyrrolidone 40, and buffer alone for comparison. Electrostatic surface representations of the proteins are shown with their in-cell spectrum.

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PL-060 **A search for anti-melioidosis drug candidates targeted to D-glycero-D-manno-heptose-1,7-bisphosphate phosphatase from Burkholderia pseudomallei**

Jimin Park[1], Sang A Yeo[1], Daeun Lee[1], Mi-Sun Kim[1], Dong Hae Shin[1]
1.-College of Pharmacy, Global Top5 Research Program, Ewha Womans University

Burkholderia pseudomallei is the causative agent of melioidosis, a serious invasive disease of animals and humans in tropical and subtropical areas. D-glycero-D-manno-heptose-1,7-bisphosphate phosphatase from B. pseudomallei (BpGmhB) is the antibiotics adjuvant target for melioidosis. In general, BpGmhB converts D-glycero-D-manno-heptose-1β,7-bisphosphate to D-glycero-D-manno-heptose-1β-phosphate. This is the third step of the biosynthesis pathway of NDP-heptose responsible for a pleiotropic phenotype. Therefore, this biosynthesis pathway is the target for inhibitors increasing the membrane permeability of Gram-negative pathogens or adjuvants synergistically working with known antibiotics. To find inhibitors of BpGmhB, we performed homology modeling of BpGmhB and in-silico virtual screening with ZINC, a free database of commercially-available compounds. Tens of thousands of chemical compounds were docked into the active site of BpGmhB. A number of putative BpGmhB binding compounds better than D-glycero-D-manno-heptose-1β,7-bisphosphate were found using Surflex-Dock included in the SYBYL software package. Characteristics of these compounds were surveyed and classified to identify common binding properties with BpGmhB.

PL-061 **Crystal structure of dimeric D-glycero-D-manno-heptose-1,7-bisphosphate phosphatase from Burkholderia thailandensis**

Jimin Park[1], Mi-Sun Kim[1], Daeun Lee[1], Keehyung Joo[2], Gil-Ja Jhon[3], Jooyoung Lee[2], Dong Hae Shin[1]

1.-Collegy of Pharmacy, Global Top5 Research Program, Ewha Womans University, 2.-School of Computational Sciences, Korea Institute for Advanced Study, 3.-Department of Chemistry and Nano Science, Ewha Womans University

We have solved the crystal structures of D-glycero-D-manno-heptose-1,7-bisphosphate phosphatase from Burkholderia thailandensis (BtGmhB) catalyzing the removal of the phosphate at the 7 position of D-glycero-D-manno-heptose-1,7-bisphosphate. It belongs to the haloacid dehalogenase (HAD) superfamily with an α/β Rossman fold composed of six parallel β-strands sandwiched between two sets of three α-helices. In solution, BtGmhB presents both as monomer and dimer. The crystal structure of BtGmhB revealed a dimeric form without catalytically important metal ions. This metal-free BtGmhB shows the importance of zinc and magnesium ions for both structural and functional reasons. It also contains one disulfide bond mediated by Cys95. A biochemical study shows that this disulfide bond may be not essential for dimerization but responsible for enzyme inactivation due to the absence of catalytic metal ions. A further biochemical study is going on to identify a biorelevance of active and inactive dimeric forms coupled to oxidation.

PL-062 **Refined crystal structure of predicted fructose-specific enzyme IIB(fruc) from E. coli**

Jimin Park[1], Daeun Lee[1], Mi-Sun Kim[1], Keehyung Joo[2], Gil-Ja Jhon[3], Jooyoung Lee[2], Dong Hae Shin[1]

1.-Collegy of Pharmacy, Global Top5 Research Program, Ewha Womans University, 2.-School of Computational Sciences, Korea Institute for Advanced Study, 3.-Department of Chemistry and Nano Science, Ewha Womans University

We have solved the crystal structures of predicted fructose-specific enzyme IIB(fruc) from Escherichia coli (EcEIIB(fruc)). EcEIIB(fruc) belongs to a sequence family with more than 5,000 sequence homologues with 25~99 % amino-acid sequence identity. It reveals a conventional Rossman-like α-β-α sandwich fold with a novel β-sheet topology. Its C-terminus is longer than its closest relatives and forms an additional β-strand whereas the shorter C-terminus is random coils in the relatives. Interestingly, its core structure is similar to that of enzyme IIB(cellobiose) from E. coli (EcIIB(cel)) transferring a phosphate moiety. In the active site of the closest EcEIIB(fruc) homologues, a unique motif CXXGXAHT comprising a P-loop like architecture including a histidine residue is found. The conserved cysteine on this loop may be thiolated to act as a nucleophile similar to that of EcIIB(cel). The conserved histidine residue is presumed to accommodate negatively charged phosphate during enzymatic catalysis. Therefore, we propose that the catalytic mechanism of EcEIIB(fruc) is similar to that of EcIIB(cel) transferring phosphoryl moiety to a specific carbohydrate.

PL-063 **NMR studies of the insertase BamA in three different membrane mimetics**

Leonor Morgado[1], Kornelius Zeth[1,2,3], Björn M. Burmann[1], Timm Maier[1], Sebastian Hiller[1]
1.-Biozentrum, University of Basel, 2.-Department of Biochemistry, University of the Basque Country, 3.-Ikerbasque - Basque Foundation for Research

β-barrel membrane proteins are ubiquitous in the outer membrane of Gram-negative bacteria, mitochondria and chloroplasts, and are essential for transport, protein biogenesis, cellular adhesion, and other cellular processes. In bacteria, outer membrane proteins are inserted into the outer membrane by the β-barrel assembly machinery (Bam) (1). This complex is composed of five proteins: the central unit BamA and the lipoproteins BamB, BamC, BamD and BamE. BamA is a β-barrel membrane protein with five periplasmic N-terminal polypeptide transport associated (POTRA) domains. The BamA structure has been determined recently by X-ray crystallography (2,3), however its functional mechanism is not well understood. This mechanism comprises the insertion of substrates from a dynamic, chaperone-bound state into the bacterial outer membrane, and NMR spectroscopy is thus a method of choice for its elucidation. In this work, BamA was reconstituted and characterized in three membrane mimetic systems: LDAO micelles, DMPC:DiC7PC bicelles and MSP1D1:DMPC nanodiscs. The impact of biochemical parameters on the NMR spectral quality was investigated, including the total protein concentration and the detergent:protein ratio. The transmembrane β-barrel of BamA is folded in either micelles, bicelles or nanodiscs, however an N-terminally attached single POTRA5 domain is flexibly unfolded, due to the absence of stabilizing contacts with other protein domains. Measurements of backbone dynamics show distinct time scales of dynamic behavior for BamA β-barrel and parts of its extracellular loop L6, revealing high local flexibility within the the lid loop. This work presents the first high-resolution 2D solution NMR spectra of the BamA barrel and establishes improved biochemical preparation schemes, which will serve as a platform for structural and functional studies of BamA and its role within the Bam complex. References 1. Rigel, NW, Silhavy, TJ (2012) Curr Opin Microbiol, 15, 189. 2. Noinaj, N, et al. (2013) Nature, 501, 385. 3. Ni, D, et al. (2014) FASEB J, 28, 2677.

PL-064 **Biochemical characterization of the substrate specificity of two unique members of the mammalian protein arginine methyltransferase family, PRMT7 and PRMT9**

Andrea Hadjikyriacou[1], You Feng[1], Yanzhong Yang[2], Mark Bedford[3], Steven Clarke[1,4]

1.-Department of Chemistry and Biochemistry, University of California Los Angeles, 2.-Department of Radiation Biology, Beckman Research Institute, City of Hope, 3.-Department of Epigenetics and Molecular Carcinogenesis UT MD Anderson Cancer, 4.-Molecular Biology Institute, University of California Los Angeles

Protein arginine methylation is a widespread and important posttranslational modification in eukaryotic cells, shown to be involved in the activation or repression of transcription, modification of the splicing machinery, signaling, and DNA repair. Mammalian protein arginine methyltransferases include a family of nine sequence-related enzymes that transfer one or two methyl groups onto the terminal guanidino groups on arginine residues, producing monomethylarginine only (MMA, type III), symmetric dimethylarginine (SDMA) and MMA (Type II), or asymmetric dimethylarginine (ADMA) and MMA (Type I). While PRMT1, 2, 3, 4, 6, and 8 have been characterized as type I enzymes, and PRMT5 as a type II enzyme, the role and activity types of the two final members of this family of enzymes, PRMT7 and PRMT9, had been unclear due to conflicting results in the literature, and the substrates for these enzymes had been elusive. Both PRMT7 and PRMT9 are distinct members of the family with two methyltransferase or methyltransferase-like domains and containing acidic residues in otherwise well-conserved substrate double E binding motif, features not seen in the other PRMT enzymes. Recent work in our laboratory confirmed PRMT7 as the only type III MMA-forming enzyme in the group, with a unusual low temperature optimum for activity, and a heretofore not seen preference for a basic stretch of residues in an R-X-R sequence for methylation. Mutations of the acidic residues in the substrate-binding motif results in a loss of the specific R-X-R activity and the appearance of a G-R-G specificity typical of many of the other PRMTs. The physiological substrate of PRMT7 has yet to be confirmed, although histone H2B is an effective in vitro substrate. PRMT9, on the other hand, had no reported activity, until immunoprecipitation from HeLa cells showed it pulled down two splicing factors, SF3B2 and SF3B4, in a complex. Amino acid analysis showed that PRMT9 methylates SF3B2 to produce both MMA and SDMA, thus making it the second type II enzyme in mammals. PRMT9 knockdown results in modulation of alternative splicing events. This enzyme appears to be relatively specific for the SF3B2 protein; a peptide containing the methylatable arginine residue was not found to be a substrate, and typical substrates of other PRMTs are not recognized by PRMT9. We found that the position of the methylated arginine residue in SF3B2 is important, and the acidic residues in the substrate-binding motif also play an important role in substrate recognition. Thus, PRMT7 and PRMT9 represent unique members of the mammalian PRMT family.

PL-065 **Ornithine decarboxylase participates in autophagy by ultraviolet Binduced cell injury**

Guang-Yaw Liu[1], Yen-Hung Lin[2], Hui-Chih Hung[2]

1.-Institute of Microbiology & Immunology, Chung Shan Medical University, and Divi, 2.-Department of Life Sciences, National Chung Hsing University (NCHU)

Ornithine decarboxylase (ODC) plays an essential role in various biological functions, including cell proliferation, differentiation and cell death. In this study, we revealed that overexpression of ODC in HeLa and MCF-7 cells decreased cellular ROS (Reactive oxygen species) after low dose of ultraviolet B radiation (UVB), leading autophagy inhibited, and it was restored by knocking down ODC (shODC) in ODC overexpressing HeLa and MCF-7 cells. Furthermore, the results demonstrated that AMPK was increased after high dose of UVB radiation in ODC ovexpressing HeLa and MCF-7 cells, leading autophagy induced and apoptosis inhibited. We demonstrated that knocked down autophagy by shRNA (shAtg5, shBECN1, and shAtg12) and chloroquine (CQ) could enhance high dose of UVB induced cell death in ODC overexpressing HeLa and MCF-7 cells. Here, we also observed that knocked down ODC in ODC overexpressing HeLa and MCF-7 cells inhibited autophagy and enhanced high dose of UVB radiation. Because of Atg12 can regulate cell apoptosis and utophagy. Site directed mutagenesis was used to mutant the amino acid which can regulate cell apoptosis and autophagy on Atg12, respectively in these two ODC overexpressing cells. According to the results, mutated the amino acid which can regulate apoptosis on Atg12 leading the cells more survival. Relatively, mutated the amino acid which can regulate autophagy on Atg12 leading the cells died. Therefore, inhibition of ODC and autophagy could be a promising strategy for adjuvant chemotherapy in human breast and cervical cancers. Keywords: Ornithine decarboxylase, Autophagy, Apoptosis, Ultraviolet B

PL-066 **Fish ß-parvalbumin acquires allergenic properties by amyloid assembly**

Javier Martínez[1], Rosa Sánchez[1], Milagros Castellanos[2], Ana M. Fernández-Escamilla[3], Sonia Vázquez-Cortés[4], Montserrat Fernández-Rivas[4], María Gasset[1]

1.-Instituto Química-Física 'Rocasolano', CSIC, 2.-Centro Nacional de Biotecnología, CSIC, 3.-Estación Experimental del Zaidín, CSIC, 4.-Allergy Department, Hospital Clínco San Carlos, IdISSC

Principles: Amyloids are highly cross-β-sheet-rich aggregated states that confer protease resistance, membrane activity and multivalence properties to proteins, all essential features for the undesired preservation of food proteins transiting the gastrointestinal tract and causing type I allergy. Methods: Amyloid propensity of β-parvalbumin, the major fish allergen, was theoretically analyzed and assayed under gastrointestinal relevant conditions using the binding of thioflavin T, the formation of SDS-resistant aggregates, circular dichroism spectroscopy and atomic force microscopy fibril imaging. Impact of amyloid aggregates on allergenicity was assessed by dot blot. Results: Sequences of β-parvalbumin of species with commercial value contain several adhesive hexapeptides capable of driving amyloid formation. Using Atlantic cod β-parvalbumin (rGad m 1) displaying high IgE crossreactivity, we have found that formation of amyloid fibers under simulated gastrointestinal conditions accounts for the resistance to acid and neutral proteases, for the presence of membrane active species at gastrointestinal relevant conditions and for the IgE-recognition in allergic patient sera. Incorporation of the anti-amyloid compound epigallocathequin gallate prevents rGad m1 fibrillation, facilitates its protease digestion and impairs its recognition by IgE. Conclusions: rGad m 1 amyloid formation explains its degradation resistance, its facilitated passage across the intestinal epithelial barrier and the epitope architecture as allergen. Financial Support: This work was partially supported by Ministerio de Economía y Competitividad (BFU2009-07971 and SAF2014-52661 to MG, BIO2011-28092 and CSD2009-00088 to MC), Fundación CIEN (MG) and Raman Health (MG)

PL-067 **Peptidylarginine Deiminase 2 Assigns Activated T Cell Autonomous Death**

Guang-Yaw Liu[1], Wen-Hao Lin[2], Hui-Chih Hung[2]

1.-Institute of Microbiology & Immunology, Chung Shan Medical University, and Divis, 2.-Department of Life Sciences, National Chung Hsing University (NCHU)

Peptidylarginine deiminase type 2 (PADI2) is a protein post-translational modification enzyme that catalyzes arginine residues into the citrulline residues. Previous studies have been shown that PADI2 promotes protein citrullinations in lymphocytes and it could plays an important role in inflammation. We found that overexpression of PADI2 promotes apoptosis in activated T cells previously. Whether PADI2 participate in the pathway of activated T cell autonomous death (ACAD) is still curious. In the delicate PADI2-mediated ACAD, we found that overexpression of PADI2 displayed the higher levels of citrullinated protein which induced the ER stress significantly. The high levels of citrullinated protein results unfolding protein response (UPR) of ER stress and increases the loading of protein degradation. Autophagy could degrade the citrullinated and unfolding protein. Herein, PADI2 could enhance autophagy in Jurkat T cells and lead to a degradation of p62 and the accumulation of LC3-II. Autophagy and apoptosis are two critical mechanisms which participate against cellular stress, cell activation, survival and homeostasis. PAD2-overexpressed Jurkat T cells caused the activation of Th17 cells to increase mRNA expression of cytokines, such as IL-17, IL-21, IL-22 and TNF. Cytokines provoked caspase expression and led to caspase-mediated cleavage of Beclin-1 which was an important factor of apoptotic signaling. Knockdown of BCEN1 rescued cell survival due to the increase of Bcl-xL and the decrease of caspase-3. We suggested that PADI2 participated in the activated T cell-induced autonomous death through triggering ER stress pathway, stimulating the expression of cytokines and promoting autophagy by PADI2-citrullinated protein.

Key words：PADI2, ER stress, Autophagy, Cytokine, Activated T cell autonomous death (ACAD).

PL-068 **Studies on secondary metabolites production and proteins and enzymes of in vitro cultivated Artemisia alba Turra and relations with some endogenous phytohormones**

Yuliana Raynova[1], Krassimira Idakieva[1], Vaclav Motyka[2], Petre Dobrev[2], Yuliana Markovska[3], Milka Todorova[1], Antoaneta Trendafilova[1], Ljuba Evstatieva[4], Evelyn Wolfram[5], Kaliva Danova[1]
1.-Institute of Organic Chemistry with Centre of Phytochemistry, BAS, 1113 Sofia, B, 2.-Institute of Experimental Botany, CAS, Prague, Czech Republic, 3.-Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia 1164, Bulgari, 4.-Institute of Biodiversity and Ecosystem Research, BAS, 1113 Sofia, Bulgaria, 5.-Zurich University of Applied Sciences, Institute of Biotechnology, Phytopharmacy

Studies on secondary metabolites production and proteins and enzymes of in vitro cultivated Artemisia alba Turra and relations with some endogenous phytohormones Yuliana Raynova1, Krassimira Idakieva1, Vaclav Motyka2, Petre Dobrev2, Yuliana Markovska3, Milka Todorova1, Antoaneta Trendafilova1, Ljuba Evstatieva4, Evelyn Wolfram5, Kalina Danova1 1 Institute of Organic Chemistry with Centre of Phytochemistry, BAS, 1113 Sofia, Bulgaria 2 Institute of Experimental Botany, CAS, Prague, Czech Republic 3 Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia 1164, Bulgaria 4 Institute of Biodiversity and Ecosystem Research, BAS, 1113 Sofia, Bulgaria 5 Zurich University of Applied Sciences, Institute of Biotechnology, Phytopharmacy, Wädenswil, Switzerland Aim: Artemisia alba Turra is an essential oil bearing shrub, characterized with great variability of the essential oil profile of wild grown plants, related to genetic, geographic and environmental factors. It was previously established that inhibition of rooting in vitro caused by cytokinin/auxin treatment affected the essential oil profile of the plant and these changes were also related to bioactive endogenous cytokinin levels in vitro (1, 2). The aim of the present work was to perform a complex study on the relations between soluble protein levels, enzyme activities (studied spectrophotometrically and by SDS-PAGE and zymography); malondialdehyde and hydrogen peroxide levels, endogenous hormones (cytokinins, salycilic acid, as well as jasmonic acid and its conjugates), polyphenolics and terpenoids in a model system of A. alba in vitro with inhibition of rootng and stimulation of callusogenesis by means of individual and combined cytokinin and cytokinin/auxin treatments. Results: It was established that inhibition of rooting and stimulation of callusogenesis caused by benzyl adenine (BA) or combinations of BA and indole-3-butiric acid (IBA) in vitro were related to elevation of sesquiterpenoids in the essential oils, as well as polyphenolics content, accompanied by a drop of stress hormones, bioactive cytokinins and preservation of oxidative stress and lipid peroxidation levels, as compared with non-treated control. Individual treatments with either IBA or BA, also increased the sesquiterpenoid content in the essential oil of the plant, in a concentration related manner, this effect being more profound after BA treatment. In addition, BA treated plants exhibited a drop of protein levels of the aerial samples, as well as profound differences of enzymatic activity in the callus tissues, as compared with callus of plants treated with different combinations of BA and IBA. Conclusion: The results of the present work indicate that alterations of endogenous phytohormonal levels, caused by exogenous plant growth regulators treatment, might be the mediator between primary and secondary metabolism by means of affecting protein levels and activity of key enzymes in vitro. Acknowledgements: PhytoBalk, SNF No. IZEBZ0_142989 and SD-MEYS No. DO2-'01:153; MEYS CR, project No. LD14120; bilateral cooperation project between BAS and CAS, Reg. No. 29. References 1. Danova K, Todorova M, Trendafilova A, Evstatieva L (2012) Cytokinin and auxin effect on the terpenoid profile of the essential oil and morphological characteristics of shoot cultures of Artemisia alba. Natural Product Communications 7: 1-2. 2. Krumova S, Motyka V, Dobrev P, Todorova M, Trendafilova A, Evstatieva L, Danova K (2013) Terpenoid profile of Artemisia alba is related to endogenous cytokinins in vitro. Bulgarian Journal of Agricultural Science, 19: 26–30.

PL-069 **Evaluation of human salivary ?-defensins by LC-ESI-MS**

Nadia Ashrafi[1], Cris Lapthorn[1], Fernando Naclerio[2], Frank Pullen[1], Birthe Nielsen[1], Yue Fu[2], Jack Miller[2], Christian Watkinson[2], Marcos Seijo[2]

1.-University of Greenwich (Faculty of Engineering and Science), 2.-University of Greenwich (Centre for Sport Science and Human Performance)

Human neutrophil α-defensins (HNP 1-4) are small cationic, structurally homologous peptides which play a central role not only in infection and inflammation but also have direct antimicrobial activity against various bacteria, viruses, fungi, and parasites. Human neutrophils are granulocytes that are predominantly found in the blood and may account for as much as 70% of the total circulating leukocyte population. Several study has demonstrated an increased concentration of HNPs in biological fluid (plasma, blood, saliva, serum) of patients with lung diseases, crohn's disease, uncreative colitis, oral disease, gastric, type 1 diabetes and colorectal cancer. Salivary HNP 1-3 are conventionally measured using an enzyme-linked immunosorbent assay (ELISA) which does not discriminate between individual HNPs due to their structural similarities. Considering the biological importance of salivary human neutrophil α-defensin (HNPs), there is therefore, a need to develop an analytical method that will discriminate between the defensins. An LC-MS method has been established for the separation and detection of HNP 1-4. The method has been optimised, validated and applied to examine the relative level of HNP 1-4 in participants undertaking a circuit resistance training workout. To date, no studies have systematically investigated the effect of acute (min to hours) and chronic (days to weeks) change in salivary α-defensins family before and after exercise by LC-ESI-MS. Twelve resistance trained athletes participated in the study. Participants consumed a placebo or a multi-nutrient supplement during exercise and the HNP 1-4 response was investigated pre, post 30 and 60 minutes of the workout. The data reveals that the difference between HNP 1-4 levels pre and post exercise were significant; $p < 0.03$ for participants consuming either the placebo or the multi-nutrient drinks. The increased HNP levels may be a part of the normal stress response. Supplementing with the multi-nutrient drink resulted in decreased level of HNPs compared to placebo data which indicates that the supplementing reduce exercise-induced airway inflammation. The present work demonstrated that, HNP 1-4 could be used as potential stress biomarkers.

PL-070 **Characterising interactions between alginates of different sizes and ß-lactoglobulin**

Emil G. P. Stender[1], Sanaullah Khan[2], Outi E. Mäkinen[3], Kristoffer Almdal[2], Peter Westh[4], Richard Ibsen[2], Maher Abou Hachem[1], Birte Svensson[1]

1.-Technical University of Denmark – DTU – Department of Systems Biology, 2.-2Technical University of Denmark – DTU – Department of Micro- and Nanotechnology, 3.-University of Copenhagen – Department of Food Science, 4.-Roskilde University – RUC – Department of Science, Systems and Models

Alginate is a polysaccharide from brown algae consisting of (1→4)-β-D-mannuronic acid and α-L-guluronic acid (1). Here interaction is characterised between β-lactoglobulin the most abundant whey protein and two alginates of different average molecular weight. At pH below the pI of β-lactoglobulin large particles are formed whose hydrodynamic diameter depends on pH as deduced from dynamic light scattering data (2). The binding strength and stoichiometry as assessed at pH 3 and pH 4 by aid of isothermal titration calorimetry showed no difference in dissociation constants at these pH values, while the binding stoichiometry is increased 2.5 fold. Furthermore, the binding stoichiometry varied 7 fold among the two alginates corresponding to their difference in average molecular weight and in addition 20 fold higher binding affinity was found with the high as compared to the low molecular weight alginate. In conclusion, the binding stoichiometry of β-lactoglobulin with alginate increases by a factor that correlates to the average molecular weight of the alginate and also a much higher affinity was found for the high molecular weight alginate.

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PL-071 **Validation of a LC-MS method for the detection of human salivary ?-defensins**

Nadia Ashrafi[1], Cris Lapthorn[1], Birthe Nielsen[1], Fernando Naclerio[2], Frank Pullen[1], Patricia Wright[1]

1.-University of Greenwich (Faculty of Engineering and Science), 2.-University of Greenwich (Centre for Sport Science and Human Performance)

A LC-MS method for the detection of human salivary alpha-defensins (HNP1-4) in stimulated whole saliva has been developed and validated which extend to sample preparation. HNP 1-3 have almost identical amino acid sequences. HNP 1-4 differs in their N-terminal amino acid residues. The presence of mucins and other high molecular weight glycoproteins in saliva makes the direct analysis of defensins difficult. The LC-MS method was linear for concentrations of HNP-2 between 0.05 and 1 ng/mL (R2 = 0.99) with a LOD of 0.05 ng/mL. Inter and intra assay precision was 0.94 – 15%, respectively. Saliva sample were clean up by solid phase extraction (SPE) and without-solid phase extraction (WSPE) method. Mobile phase composition was delivered at a flow rate of 0.6 mL/min for 3 mm internal diameter column and 0.3 mL/min for 2.10 mm internal diameter column in relation to the method transfer . During LC-MS optimisation, two different mobile phases compositions (MeOH: H2O; MeCN: H2O) with three different additives ((0.1% (v/v); formic acid, acetic acid, ammonium format with formic acid) have been investigated in response to ion intensity of ESI-MS for individual HNP 1-4 in saliva. Kinetex® column separation efficiency was evaluated using two different column dimensions (50 x 2.1 mm and 50 x 3 mm.) and two different stationary phases (C18 and C8). Kinetex® column (homogenous porous shell) performance was also compared to new ultra ACE® (encapsulated bonded phase) column. Sample optimisation revealed that the SPE method removes interference from salivary glycoproteins and consequently yields larger peak area (30-90%) for all HNPs. HNPs were extracted by SPE with a recovery of 80-91%. The MeOH: H2O: acetic acid (0.1%) provided enhanced (P>0.05) HNP1-3 ion intensities. The Kinetex® C8 (50 x 3.0 mm, 2.6 µm) column facilitated a better separation efficiency of the four HNPs as compared to the Ultra Core Super C18 ACE® (50 x 3.0 mm, 25 µm) column, the Kinetex® C18 (50 x 3.0 mm, 2.6 µm) and the Kinetex® C18 (50 x 3.0 mm, 5 µm) column.. The relative levels of the HNPs were determined in healthy volunteers before and after a rigorous exercise regime: It is possible that prolonged strenuous exercise will affect oral innate immunity and therefore also the level of salivary defensins. HNP1-3 are traditionally detected in an enzyme-linked immunosorbent assay (ELISA) which does not discriminate between the different HNPs due to their structural similarities. There has therefore been a need to develop a mass spectrometry method that will discriminate between the defensins. As part of the method validation, the HNP1-3 level was determined by ELISA and the data was compared with the LC-MS data. Here we present this cross-validation; the data revealed no significance difference between the two methods (R2= 0.96) which confirms that the developed LC-MS method is and equal sensitive method for the detection of these potential antimicrobial markers. This method can easily be adopted for similar molecular weight of peptides as HNPs and also for any other biological matrix.

PL-072 **Moonlighting proteins: relevance for biotechnology and biomedicine**

Luis Franco Serrano[1], Sergio Hernández[1], Alejandra Calvo[2], Gabriela Ferragut[2], Isaac Amela[1], Juan Cedano[2], Enrique Querol[1]

1.-Institut de Biotecnologia i Biomedicina. Universitat Autònoma de Barcelona, 2.-Laboratorio de Inmunología, Universidad de la República Regional Norte-Salto

Multitasking or moonlighting is the capability of some proteins to execute two or more biochemical functions. The identification of moonlighting proteins could be useful for researchers in the functional annotation of new genomes. Moreover, the interpretation of knockout experiments, in which the result of a gene knocking does not produce the expected results, might be enhanced. The action of a drug can also be facilitated because it might have an off-target or side effect with somewhat hidden phenotypic traits. It would be helpful that Bioinformatics could predict this multifunctionality. In the present work, we analyse and describe several approaches that use protein sequences, structures, interactomics and current bioinformatics algorithms and programs to try to overcome this problem. Among these approaches there are: a) remote homology searches using Psi-Blast, b) detection of functional motifs and domains, c) analysis of data obtained of protein-protein interaction databases (PPIs), d) matches of the sequence of the query protein to 3D databases (i.e., algorithms like PISITE), e) mutation correlation analysis between amino acids using algorithms like MISTIC. Remote homology searches using Psi-Blast combined with data obtained from interactomics databases (PPIs) have the best performance. Structural information and mutation correlation analysis can help us to map the functional sites. Mutation correlation analysis can only be used in very specific situations because it requires the existence of a multialigned family of protein sequences, but it can suggest how the evolutionary process of second function acquisition took place. We have designed a database of moonlighting proteins, MultitaskProtDB (http://wallace.uab.es/multitask/). From this database we determine the frequencies of canonical and moonlighting coupled functions (being an enzyme and a transcription factor the highest), the percentage of moonlighting proteins involved in human diseases (65% of the human moonlighting proteins in the database) and the percentage of moonlighting proteins acting as a pathogen virulence factor (20% of the moonlighting proteins in the database).

PL-073 **Correlation between potential human neutrophil antimicrobial peptides (HNP 1-3) and stress hormones in human saliva**

Nadia Ashrafi[1], Frank Pullen[1], Birthe Nielse[1], Cris Lapthorn[1], Fernando Naclario[2]

1.-University of Greenwich (Faculty of Engineering and Sciene), 2.-University of Greenwich (Centre of Sports Science and Human Performance)

Numerous studies have investigated the effect of exercise on mucosal immunity but the focus has mainly been on salivary immunoglobulins lysozymes and hormones (cortisol, testosterone). This is not surprising given that IgA and IgG are the predominant immunoglobulins in saliva and there is a relationship between mucosal immunity and upper respiratory illness. It is well known that physical and mental stress provoke the release of cortisol from hypothalamic pituitary adrenal axis, by which stress can modulate various immune responses. In general, cortisol and growth hormones helps to induce the activation of neutrophils. To date, this study represents the first study that investigated the correlation between human neutrophil alpha defensins family against cortisol (stress hormone) and testosterone (growth hormone) in human saliva before and after exercise or training. Twelve resistance trained athletes volunteered to participate in the study. Participants consumed supplements during exercise and the HNP 1-3, cortisol and testosterone response was investigated pre, post 30 and 60 minutes of the workout. The correlation between salivary antimicrobial peptide (HNP 1-3) and stress hormone (cortisol and testosterone) has been investigated using ELISA. Cortisol showed no significant ($p = 0.818$) difference for (pre to 30 min post) between CHO and PL (CHO: 483.07 ± 912.77 ng/mL; PL= 583.82 ± 1134.33 ng/mL) conditions but a strong trend ($p = 0.074$) was observed for (pre to 60 min post) post (CHO: 1023.19 ± 1500.40 ng/mL; PL= 1480.33 ± 2214.80 ng/mL) condition. Testosterone showed no significant ($p = 0.167$; $p = 0.156$) difference for (pre to 30 min post) between CHO and PL (CHO: 23.32 ± 44.11 ng/mL; PL= 10.40 ± 14.19 ng/mL) and for (pre to 60 min post) post (CHO: 26.42 ± 19.11 ng/mL; PL= 23.72 ± 17.91 ng/mL) condition. HNP 1-3 showed no significant ($p = 0.348$) difference for (pre to 30 min post) between CHO and PL (CHO: 72.26 ± 148.82; PL= 125.20 ± 70.00) conditions but significant difference ($p = 0.026$) was observed for (pre to 60 min post) between CHO and PL (CHO: 35.18 ± 182.69; PL= 228.74 ± 151.63) condition. The present findings suggested that there is no correlation between salivary HNP 1-3 and cortisol for (PL: $R2 = 0.02$ and CHO: $R2 = 0.01$); HNP 1-3 and testosterone (PL: $R2 = 0.20$ and CHO: $R2 = 0.10$). A worth note from previous study which suggested that using murine skin model (an increase in endogenous glucorticoids (cortisol) by physiological stress reduced mRNA levels of antimicrobial peptide (cathelicidin). It is not clear that the correlation between hormones and antimicrobial peptide has been affected by the time interval of the exercise. Both cortisol and antimicrobial peptide demonstrated a transient increase after exercise but it is surprising that they are not correlate to each other. One of the hypothesis from the present finding could be cortisol responses slow and it will be interesting to do further research with longer interval. The second hypothesis demands a further investigation to determine the synergism between substances.

PL-074 **GWIDD: Genome-Wide Docking Database**

Madhurima Das, Varsha D. Badal, Petras J. Kundrotas, Ilya A. Vakser
1.-Center for Computational Biology, The University of Kansas

GWIDD: GENOME-WIDE DOCKING DATABASE Madhurima Das, Varsha D. Badal, Petras J. Kundrotas and Ilya A. Vakser Center for Computational Biology, The University of Kansas, Lawrence, Kansas, USA Structural characterization of protein-protein interactions (PPI) is essential for understanding molecular mechanisms in living systems. Genome-Wide Docking Database (GWIDD) provides the most extensive data repository of structures and models of PPI on a genomic scale. Currently, we are expanding the GWIDD dataset to 800,365 PPI in 1,652 organisms, up from 128,818 PPI in 771 organisms in the previous release. The PPI data were imported from INTACT and BIOGRID databases and were subjected to in-house modeling pipeline. GWIDD current implementation contains 11,073 experimentally determined complexes, and 12,426 sequence homology and 28,811 structure homology models of complexes. The user-friendly interface offers flexible organism-specific search with advanced functions for a refined search for one or both proteins. The new GWIDD version includes also a new interactive visualization screen that allows to view search results in different residue representations with the emphasis on the PPI interface. GWIDD is available at http://gwidd.compbio.ku.edu.

PL-075 **Refolding and activation of recombinant trypsin i from sardine fish (sardinops sagax caerulea)**

Manuel Carretas-Valdez[1], Francisco Cinco-Moroyoqui[1], Marina Ezquerra-Brauer[1], Enrique Marquez-Rios[1], Rogerio Sotelo-Mundo[2], Idania Quintero-Reyes[3], Aldo Arvizu-Flores[3],
1.-Universidad de Sonora, Departamento de Investigación en Alimentos, 2.-Centro de Investigación en Alimentación y Desarrollo, A.C., 3.-Universidad de Sonora, Departamento de Ciencias Químico Biológicas

Trypsin (EC 3.4.21.4) is the principal member of the serine protease family, and catalyzes the hydrolysis of proteins and peptides specifically at the carboxyl group of lysine and arginine residues. The trypsin I studied from Monterey sardine (Sardinops sagax caerulea) is described as a cold-adapted enzyme. Previous to evaluate structure-function relationships by site-directed mutagenesis, we first established the experimental conditions to perform the recombinant expression, purification, refolding and activation for wild-type trypsin I from Monterey sardine. Trypsin I was overexpressed in E. coli BL21 as a fusion protein of trypsinogen and thioredoxin, which was obtained in an insoluble form. Inclusion bodies were extracted and disolved in urea 4 M and DTT 30 mM. Refolding was achieved with buffer Tris-HCl, 55 mM, pH 8.8, NaCl 264 mM, KCl 11 mM, polyethylenglycol 0.055 %, GSSG 1 mM and GSH 5 mM. The recovery of the refolded recombinant trypsinogen I was 29 mg per liter of LB medium. Before activation, the 45 kDa trypsinogen-thiorredoxin did not show trypsin-like activity against BApNA. Activation was achieved by the addition of 0.01 U/mL of native trypsin I purified from sardine pyloric caeca (non-recombinant). The activated recombinant trypsin showed up to four times more activity than the non-recombinant trypsin. The described protocol allowed us to obtain sufficient amounts of protein for further biochemical and biophysical characterization. Therefore, the recombinant trypsin I from Monterey sardine is feasible as a model for structure-function studies for cold-adapted proteins.

PL-076 **WapA and SMU_63c are Amyloidogenic Proteins of Streptococcus mutans**

Richard Besingi[1], L. Jeannine Brady[1]

1.-Department of Oral Biology, University of Florida

We showed previously that adhesin P1 (Ag I/II) of the acidogenic bacterium Streptococcus mutans, a causative agent of human tooth decay, is capable of amyloid fibrillization. Known inhibitors of amyloid fibril formation inhibit biofilm formation by amyloidogenic microbes, including S. mutans, thus suggesting a potential mechanism for therapeutic intervention. Amyloid is detectable in human dental plaque and is produced by both clinical and laboratory strains of S. mutans, further supporting a functional role. S. mutans lacking P1 demonstrates residual amyloid forming properties, however, a mutant lacking sortase, the transpeptidase which covalently links P1 and several other proteins to the peptidoglycan cell wall, is defective in cell-associated amyloid-like properties. The objectives of this study were to identify additional amyloid forming proteins of S. mutans and to evaluate the effects of buffering conditions and pH on the ability of the identified proteins to form amyloids. A P1-deficient mutant strain was grown to stationary-phase in defined minimal media, and secreted proteins from spent culture supernatants were fractionated by ion exchange chromatography. Partially purified protein fractions were tested for binding of the amyloidophilic dyes Congo Red (CR) and Thioflavin T (ThT), and for characteristic birefringent properties following staining with CR and visualization under crossed polarizing filters. Proteins from fractions that tested positive for amyloid-like material were separated by SDS PAGE, and identified by LC/MS. These included WapA, GbpA, GbpB, SMU_2147c and SMU_63c. Recombinant proteins were expressed in Escherichia coli, and purified for confirmation and characterization of individual amyloidogenic properties in vitro. Recombinant WapA and SMU_63c displayed all the biophysical characteristics of amyloid, including visualization of fibrillar aggregates when viewed by transmission electron microscopy. In contrast, GbpA and SMU_2147c produced amorphous aggregates. WapA and SMU_63c form amyloid at different pH, SMU_63c under acidic conditions and WapA under neutral to basic conditions. This suggests that the prevailing environmental pH may represent different in vivo triggers for amyloid fibrillization of different S. mutans proteins. Genes encoding P1, WapA, and SMU_63c have been deleted from the S. mutans genome individually and in combination with each other. This will facilitate subsequent assessment of their individual susceptibilities to amyloid inhibitors, their contributions to amyloid formation under varying environmental conditions, and their respective roles in biofilm formation.

PL-077 **Characterization of the membrane-localized interaction network between the GTP-ase Rheb and the FKBP12-like protein FKBP38 by NMR**

Maristella De Cicco[1], Sonja A. Dames[1]
1.-The Affiliation Is Technische Universität München

Rheb is a homolog of Ras GTPase. Like other small GTPases, the activity of Rheb is dictated by its guanine nucleotide binding states: it is active in its guanosine 5'-triphosphate (GTP) bound form and inactive in the guanosine diphosphate (GDP)–bound form. Rheb proteins play critical roles in regulating growth and cell cycle, and this effect is due to its role in regulating the insulin/TOR/S6K signaling pathway [1-3]. Rheb binds directly to a region N-terminal of the kinase domain in mTOR and activates it in a GTP-dependent manner. C-terminal farnesylation allows Rheb to associate with the endomembranes. Conditions preventing Rheb endomembrane localization impair its ability to interact with the components of the mTOR complex 1 (mTORC1) to activate downstream targets [4]. The heterodimeric Rag GTPases localize mTORC1 to lysosomes by their amino-acid-dependent interaction with the lysosomal Ragulator complex. Rheb is also thought to reside on lysosomes to activate mTORC1 [5]. Based on coimmunoprecipitation and an in vitro binding assays Rheb regulates mTOR through FKBP38, a member of the FK506-binding protein (FKBP) family that is structurally related to FKBP12 [6]. FKBP38 binds to mTOR and inhibits its activity. Rheb interacts directly with FKBP38 and prevents its association with mTOR in a GTP–dependent manner. Moreover, FKBP38 bound to GTP-γ-S, a nonhydrolyzable GTP analogon, has a much higher binding affinity for Rheb than the GDP-bound form [6]. However, two other publications re-evaluated the results and came to different conclusions. The first study confirmed the interaction between Rheb and FKBP38 but disagreed, that the bound nucleotide has an effect on the interaction [7]. The second study contradicted both studies, since they could not detect any interaction between Rheb and FKBP38 [8]. To clarify whether there is an interaction and if it is nucleotide dependent, NMR monitored interaction studies were performed employing a C-terminal truncated construct of human Rheb (1-170 = RhebΔCT) that cannot be farnesylated and the biochemically defined binding region on FKPB38 (FKBP12-like = FKBP38-BD). Based on our data RhebΔCT –GDP does not significantly interact with FKBP38-BP. 15N-FKBP38-BD titrated with a 9-mer peptide corresponding to the Rheb switch 1 did also not result spectral changes. Thus the proposed importance of the switch 1 region for the interaction with FKBP38 maybe indirect by influencing the nucleotide binding. However, we observed a weak interaction between RhebΔCT bound to a GTPanalogon (GppNHp) and FKBP38-BD. Mapping of the observed spectral changes on the structure of Rheb-GTP suggests that FKBP38 targets the switch 2 region, loop ~109-112 and the neighboring b-sheet region. We further analyzed the backbone dynamics of RhebΔCT –GDP and –GppNHp using 15N relaxtion data (T1, T2 and heteronuclear NOE) .Based on these data the phosphorylation loop, the switch regions and the loop around residues ~109-112 show increased backbone dynamics that modulated by the nucleotide binding. These increased dynamics may allow Rheb to interact with several different interaction partners such as FKBP38.

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PL-078 **Unraveling the nature of TDP-43 aggregates from its putative aggregation domain**

Miguel Mompeán[1], Rubén Hervás[2], Yunyao Xu[3], Timothy H. Tran[4], Emanuele Buratti[5], Francisco Baralle[5], Liang Tong[4], Mariano Carrión-Vázquez[2], Ann E. McDermott[3], Douglas V Laurents[1]
1.-Instituto Química Física Rocasolano, 2.-Instituto Cajal, IC-CSIC, 3.-Department of Chemistry, Columbia University, 4.-Department of Biological Sciences, Columbia University, 5.-International Centre for Genetic Engineering and Biotechnology

TDP-43 is an RNA processing protein that can form inclusions of debatable nature implicated in neurodegenerative diseases. Within the putative aggregation domain, repeats of residues 341-366 can recruit endogenous TDP-43 into aggregates inside cells1. Recently, we showed that a coil to β-hairpin transition in a short peptide corresponding to TDP-43 residues 341-357 enables oligomerization2. We have used a broad battery of biophysical experiments, including chromophore and antibody binding, electron microscopy (EM), circular dichroism (CD), solution and solid-state NMR, and X-ray to shed light on the nature of these aggregates. Based on these findings, structural models for TDP-43(341-357) oligomers have been constructed, refined, verified, and analyzed using computational methods, ranging from Docking and Molecular Dynamics simulations to Semiempirical Quantum Mechanics calculations. Interestingly, TDP-43(341-357) β-hairpins assemble into a novel parallel β-turn configuration showing cross-β spine, cooperative H-bonding and tight side chain packing3. These results expand the amyloid foldome and could guide rational drug design to prevent this process.

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PL-079 **The role of the structural NADP+ binding site in human glucose 6-phosphate dehydrogenase**

Mona Alonazi[1,2], Paul Engel[1]

1.-School of Biomolecular and Biomedical Science, Conway Institute, UCD., 2.-King Saud University, Sciences, Biochemistry department.

The crystal structure of a human glucose 6-phosphate dehydrogenase (G6PD) shows that each subunit has two NADP+ sites; in addition to a catalytic site there is a “structural” site which is distant from the catalytic coenzyme site. Mutations causing severe deficiency tend to cluster round and close to the dimer interface and the structural NADP+, indicating that the integrity of these areas is important for enzyme stability and therefore for maintenance of activity. In order to understand the molecular basis of G6PD deficiency, and to have a clearer indication about the role of some features of the three-dimensional structure, a fuller study of the second, “structural” NADP+ binding site is needed. Human G6PD controls the first committed step in the pentose phosphate pathway. It catalyses the oxidation of glucose 6-phosphate to gluconolactone 6-phosphate, generating NADPH which is essential, amongst other things, for protection against oxidative stress. The human enzyme can be active in dimer or tetramer forms. Human G6PD of “structural” NADP+ per subunit of enzyme. This tightly-bound NADP+ can be reduced by G6P, probably following migration to the catalytic site. The importance of NADP+ for stability is explained by the structural NADP+ site, which is not conserved in prokaryotes. After removing the tightly bound “structural” NADP+ the enzyme is still active but not stable. The effects of different NADP+ fragments on the stability of human recombinant G6PD have been investigated. NADP+ is crucial for the long term stability of human G6PD, and only one of NADP+ analogues which is adenosine diphosphate ribose - 2'-phosphate was able to slightly promote the stability of enzyme. .

PL-080 **Molecular characterization of specific positively selected sites in mammalian visual pigment evolution**

Miguel A. Fernández-Sampedro[1], Eva Ramon[1], Brandon M. Invergo[2], Jaume Bertranpetit[2], Pere Garriga[1]

1.-Grup de Biotecnologia Molecular i Industrial., 2.-IBE – Institute of Evolutionary Biology

Visual rhodopsin is a member of the G-protein coupled receptors superfamily. This membrane protein consists of a 11-cis-retinal cromophore bound to a seven transmembrane protein, opsin, by means of a protonated Schiff base linkage. It has an important role as a dim light photoreceptor in the retina of the eye. By statistical models, where episodic selection in rhodopsin is tested on one branch of the phylogeny against a background of neutral or purifying selection on the rest of the tree, we have found some significant evidence of specific positively selected sites in early mammalian divergence. We have chosen the three amino acid sites identified with the highest posterior probability of having been targets of positive selection to perform experimental studies, i.e. 13 (positively selected from M to F), 225 (positively selected from R to Q) and 346 (positively selected from S to A). We have constructed, expressed, immunopurified and functionally characterized the proposed candidates, F13M, Q225R and A346S rhodopsin mutants located at the N-terminus, the transmembrane domain and the C-terminus region of the protein respectively. From the analysis of the molecular features of the F13M mutant, we conclude that position 13 is very important for protein folding and also for proper protein glycosylation, since we only could observe cromophore regeneration after its rescue in the double cysteine (N2C/D282C) mutant background that stabilizes the N-terminal extracellular domain of the protein. Our results also show that mutants Q225R and A346S alter the G-protein activation rate, and hydroxylamine susceptibility in the dark-adapted state. In the case of Q225R, disrupting critical interactions with the neighbouring Y136 of the conserved D/ERY motif, critical in Gt activation, could cause the lower Gt activation ability. The mutant A346S would create a potential additional phosphorylation site in the protein which could affect rhodopsin phosphorylation after photoactivation and, in turn, could affect the binding affinity of arrestin, a regulator of rhodopsin deactivation. This extra phosphorylation site could provide an evolutionary explanation for the enhanced response observed in the case of Gt activation. In conclusion, these results highlight the importance of molecular investigations of positive selected sites in rhodopsin evolution and the relevance of structural and functional analysis of these sites in unravelling the molecular basis of visual pigment evolution.

PL-081 **Natural evolution sheds light on modern drug resistance in protein kinases**

Marc Hoemberger[1], Christopher Wilson[1], Roman Agafonov[1], Dorothee Kern[1]
1.-HHMI & Department of Biochemistry, Brandeis University

The anti-cancer drug imatinib exhibits highly specific binding to the human kinase and oncogene Abl with a three thousand fold weaker affinity for the structurally and functionally very similar kinase Src. It has been shown recently that the major difference in binding of imatinib to Abl and Src stems from an induced fit after binding of the drug. To further understand the mechanism of imatinib binding to its target we used ancestral sequence reconstruction (ASR) and resurrected enzymes along the node from the common ancestor of Abl and Src up to the extant kinases. We show that imatinib affinity is gained towards the evolution of extant Abl while it is lost towards evolving Src. The combination of ASR and crystallographic data of the ancestors in addition to kinetics data allowed us to identify a subset of residues involved in imatinib specificity sufficient to switch from an intermediate binder to a tight binder. Preliminary data shows that a network of hydrogen bonds and packing interactions stabilize the kinked p-loop conformation for tight binders thus allowing for more interactions between the kinase and the drug. Strikingly, many of these residues were identified in human cancer patients as “hot spots” for the development of resistance mutations. Further investigation into the identified subset of residues in combination with these commonly found imatinib resistance mutations will allow us to understand emerging drug resistances better.

PL-082 **An evolutionary view of the cold adapted catalysis of enzymes**

Vy Nguyen[1], Christopher Wilson[1], Dorothee Kern[1]
1.-HHMI & Department of Biochemistry, Brandeis University

The diversity in protein function that we see today arose as a result of life adapting to a cooling earth. How did enzymes, the catalysts of many crucial cellular processes, achieve this cold adaptation? This is a challenging question to answer because ancient sequences of proteins that existed billions of years ago are not available. To address this question we used ancestral sequence reconstruction to create adenylate kinase (Adk) enzymes from the divergence of Anaerobic and Aerobic Firmicutes towards modern day thermophilic, mesophilic and psychrophilic organisms. Adk is a phosphotransferase that catalyzes the conversion of two ADP molecules into ATP and AMP. We make the following observations. First, all ancestral enzymes are active with optimal catalytic rates linearly corresponding to the temperature of the environments where these proteins would have been found. Most strikingly, the catalytic rate of our oldest Adk ancestor exhibits a higher enthalpy of activation at low temperatures as compared to the modern thermophilic Adk. This suggests a large enthalpic penalty had to be paid for reactions to occur at cold temperatures in an ancestor that existed in a hot environment. Second, several high resolution crystal structures of extant proteins that we solved (1.2Å – 1.6Å), show that the oldest ancestors were more rigid than the modern Adks due to an intricate salt-bridge network. This work, thus shows for the first time, the molecular and thermodynamic determinants of cold adaptation in an enzyme over a time period that spans billions of years.

PL-083 **Induced oxidative modification of plasma and cellular fibrin-stabilizing factor**

Anna Bychkova[1], Tatiana Danilova[1], Alexander Shchegolikhin[1], Vera Leonova[1], Marina Biryukova[1], Elizaveta Kostanova[1], Alexey Kononikhin[0], Anna Bugrova[1], Evgeny Nikolaev[0], Mark Rosenfeld[1]

1.-N. M. Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, 2.-Institute for Energy Problems of Chemical Physics, Russian Academy of Sciences

The main function of plasma fibrin-stabilizing factor pFXIII is to catalyze the formation of the intermolecular covalent cross-links between both γand αfibrin polypeptide chains. The crosslinking crucially affects mechanical strength of fibrin and its resistance against fibrinolysis. The precise role of cellular fibrin-stabilizing factor cFXIII remains poorly understood. pFXIII is a heterotetramer (FXIII-A2B2) consisting of two single-stranded catalytic A subunits (FXIII-A2), and two identical single-stranded inhibitory/carrier B subunits (FXIII-B2). The subunits are held together by weak non-covalent bonds. Contrary to plasma FXIII, cFXIII is a dimer (FXIII-A2) devoid of B subunits. As well as many other proteins circulating in the bloodstream, pFXIII is known to be a target for reactive oxygen species (ROS) causing processes of protein oxidative modification. Since the conversion of pFXIII to the active form of the enzyme (FXIIIa) is a multistage process, ozone-induced oxidation of pFXIII has been investigated at different stages of its enzyme activation. The biochemical results point to an inhibition of enzymatic FXIIIa activity depending largely on the stage of the pFXIII conversion into FXIIIa at which oxidation was carried out. UV-, FTIR- and Raman spectroscopy demonstrated that chemical transformation of cyclic, NH, SH and S–S groups mainly determines the oxidation of amino acid residues of pFXIII polypeptide chains. Conversion of pFXIII to FXIIIa proved to increase protein susceptibility to oxidation in the order: pFXIII < pFXIII activated by thrombin < pFXIII in the presence of calcium ions < FXIIIa. With the aid of mass-spectrometry it has been demonstrated that oxidation leads to decreasing FXIII-A and FXIII-B coverage both in the forms of zymogen and in the presence of calcium ions. A group of amino acid residues involved in oxidation modification of pFXIII is identified in this study. The oxidation of either cFXIII or cFXIIIa has revealed an almost complete loss of enzyme activity caused by dramatic changes in the primary and secondary structure of the proteins detected by the FTIR data. Taking into account these new findings, it seems reasonable to assume that the inhibitory/carrier FXIII-B subunits can serve as scavengers of ROS. Hypothetically, this mechanism could help to protect the key amino acid residues of the FXIII-A subunits responsible for the enzymatic function of FXIIIa. The study was supported by RFBR, research project No. 15-15-04-08188a. Mass spectrometry study was supported by the Russian Scientific Foundation grant No. 14-24-00114.

PL-084 **Performance and quality. making microcalorimetry simple with microcal peaq-itc**

Natalia Markova[1], Ronan O'Brien[1], Mark Arsenault[1]
1.-MicroCal, Malvern Instruments Ltd.

Dynamic interactions involving biomolecules drive and regulate all biological processes. Studies of biomolecular interactions are fundamentally important in all areas of life sciences. Data provided by Isothermal Titration Calorimetry (ITC) enables scientists in academia and industry to directly and quantitatively characterize these interactions in solution. MicroCal PEAQ-ITC, the latest generation of MicroCal ITC instrumentation, offers a whole range of solutions for addressing current bottlenecks associated with interaction analysis. Among the most recognized challenges are the needs to adequately address a broad range of binding affinities and to reliably interpret binding data complicated by the presence of inactive protein fraction or inherent uncertainty in the concentration of a ligand. Consistently high performance of MicroCal PEAQ-ITC enables increased confidence and data resolution when measuring low heats at low or uncertain sample concentrations and complex binding modes. The new MicroCal PEAQ-ITC analysis software allows for utomated data analysis, minimizing analysis time and user subjectivity in assessing data quality. Data quality is determined and advanced fitting performed in a few seconds per experiment allowing for analysis of large data sets of 50 or more experiments in a matter of seconds.

PL-085 **Glutamine-rich activation domain of transcription factor Sp1 - biochemical activity and structure**

Jun Kuwahara[1], Chisana Uwatoko[1], Emi Hibino[2], Katsumi Matsuzaki[2], Masaru Hoshino[2]
1.-Faculty of Pharmaceutical Sciences, Doshisha Women's University, 2.-Graduate School of Pharmaceutical Sciences, Kyoto University

Transcription factor Sp1 is ubiquitously expressed in a mammalian cell, activates reasonably large subset of mammalian genes, and is involved in the early development of an organism. The protein comprises two glutamine-rich (Q-rich) regions (A and B domains) located in its N-terminal half, while three tandem repeats of C2H2 zinc finger motif at its C-terminus binds directly to a GC-rich element (GC box) of DNA. In general, Q-rich domain is one of the typical motifs found in trans-activation domain of transcription factors together with acidic and proline-rich domains. Transcriptional signal of Sp1 are transmitted via interaction between Q-rich domains of Sp1 and different classes of nuclear proteins, such as TATA-binding protein (TBP) associated factors (TAFs) in components of basic transcription factor complexes (TFII). In addition, self-association of Sp1 via Q-rich domains is also important for its regulation of transcriptional activity. It has been considered that an Sp1! molecule bound to a 'distal' GC-box synergistically interacts with another Sp1 molecule at a 'proximal' binding site. Although formation of multimers via Q-rich domains seems functionally important for Sp1, little is known about relevance between biological activity and structural nature of Q-rich domains. We analyzed nature of glutamine-rich domains of Sp1 by biochemical and physicochemical methods. We found that Q-rich domains do not have clear secondary structure whereas they can indicate biochemical activity. Detailed analysis of NMR spectra indicated interaction between the domains. The Q-rich domains of Sp1 might be one of the intrinsically disordered proteins (IDP).

PL-086 **CHIPping away at the yeast proteome: redesigning an E3 ubiquitin ligase for targeted protein degradation**

Michael Hinrichsen[1], Lynne Regan[1]
1.-Yale University

One of the central goals of synthetic biology is to exploit biological systems in order to produce compounds of therapeutic or industrial value1. Often, these efforts are complicated by the many natural biochemical pathways in cells that can compete for the same small molecule precursors. Currently, the most common solution is to simply delete the genes coding for the competing enzymes2. While such an approach has been successful, it is only applicable to nonessential genes and can produce unintended off-target effects such as decreased cell viability2. An alternative strategy is to instead target proteins directly for degradation. Using this strategy, scientists would first grow cultures of engineered cells to high densities under permissive conditions (i.e. targeted proteins are stably expressed). Then, once sufficient cell density has been reached, enzymes of competing pathways would be rapidly degraded, resulting in the rapid production of high concentrations of the compound of interest. We propose to create such a tool by reengineering the C-terminus of Hsp70 interacting protein (CHIP), an E3 ubiquitin ligase. CHIP recognizes substrate proteins through a short C-terminal peptide tag on target proteins3. We have shown that fusing this tag to non-native substrates is sufficient for ubiquitination in vitro (data not published). Cellular assays have also been performed in S. Cerevisiae, a model organism commonly used in metabolic engineering applications1. As a number of native yeast proteins possess C-termini similar to that of CHIP's native substrates (data not published), it was necessary to develop an orthogonal CHIP-peptide pair. This was achieved by replacing CHIP's natural TPR ligand-binding domain with a ligand-binding domain engineered previously in the Regan Lab4. The altered CHIP construct has been shown to be active both in vitro and in vivo, and produces an altered growth phenotype when targeted against an enzyme involved in uracil biosynthesis. Future work will focus on further kinetic characterization of the engineered enzyme, increasing its activity, and introducing the system into a proof of concept synthetic biology application.

PM-001 **Functional characterization of proteins by domain architecture**

Roxanne Yamashita[1], Lewis Geer[1], Lianyi Han[1], Lanczycki Christopher[1], Shennan Lu[1], Jane He[1], Josie Wang[1], CDD Curation Team[1], Aron Marchler-Bauer[1]

1.-National Institutes of Health/National Center for Biotechnology Information

Advances in modern sequencing techniques have resulted in an explosion of genomic data. Correctly classifying this new wealth of information can be daunting not only because of the sheer volume of sequence data, but also because the propagation of erroneous and less-than-ideal names and functional characterizations in the current databases gets in the way of functional classification by mere sequence similarity. We are investigating the extent to which protein domain architecture can be utilized to define groups of proteins with similarities in molecular function, and whether we can derive corresponding functional “labels”, starting with some of the most common domain architectures found in bacteria. To this end, we have developed an in-house procedure called SPARCLE ('SPecific ARChitecture Labeling Engine') that lets us track and examine specific or sub-family domain architectures, resulting from annotating protein sequences with domain footprints provided by the Conserved Domain Database (CDD), which includes hierarchical classifications for many common domain families. We will discuss how the proteins are grouped into specific architectures, our successes in assigning functional labels, and the major limitations we have encountered to date. While we will be able to assign functional labels to a large fraction of protein models derived from genome sequences, this effort has the added benefit of pointing out insufficient coverage and resolution of the current protein domain model collections that constitute CDD. We will also discuss alternative procedures that utilize pre-computed domain annotation for clustering protein sequences at a level that is well suited for functional labeling. We hope that this preliminary study will help to identify approaches that facilitate rapid and accurate annotation of genomes with a minimum of manual intervention.

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