BOOK OF ABSTRACTS

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APTAMERS in Bordeaux

ORAL PRESENTATIONS

POSTERS

01

EMERGENCE OF COOPERATIVITY DURING *IN VITRO* SELECTION OF A SELF-SYNTHESISING RIBOZYME

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In vitro evolution can reveal surprising capabilities of individual RNA molecules. Recently, during such an experiment to develop ribozymes that assemble other RNAs, we isolated the first RNA molecule able to build a copy of itself from small building blocks, assembling itself in stages from RNA trinucleotide 'triplets'. Unusually, this species emerged as a cooperative RNA heterodimer from the in vitro selection pool, an underexplored possibility in SELEX experiments. I will describe its synthetic capabilities, the behaviour of the complex RNA pools that led to its emergence, and the implications for the inception of RNA self-replication (both modern and primordial).

02

FULL-LENGTH ANALYSIS OF CAENORHABDITIS ELEGANS TRANSCRIPTOME USING NANOPORE SEQUENCING TECHNOLOGY

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A recent meta-analysis of alternative exon usage in Caenorhabditis elegans based on publicly available RNA-seq dataset (Tourasse et al., Genome Research, 2017) refined our comprehension of C. elegans transcriptome, especially regarding the splicing quantitative aspects of alternative splicing in messenger RNAs. However, Next-Generation Sequencing technologies (NGS) like Illumina technology are proving to be limited to fully characterize one's transcriptome. PCR-based sequencing methods are known to introduce amplification bias affecting the overall distribution of mRNAs detected in one experiment and short-reads are not suited to accurately predict the frequency of isoforms derived from multiple alternative splicing events. In this study, we are exploiting the new possibilities offered by Oxford Nanopore Technology (ONT) to overcome those limitations. Nanopore-based sequencing allow to directly sequence nucleic acids without any prior amplification step and generates long-reads covering up to the full-length of the molecule. Hence, we are aiming to further characterize C. elegans transcriptome by providing a more accurate measure of isoforms ratios, a better comprehension of exons associations during alternative splicing and by characterizing differentially trans spliced mRNAs. To do so, we analyzed two different populations of mRNAs: a library of poly(A) mRNAs representing the whole-animal transcriptome and a library of SL1-enriched mRNAs. Those libraries were sequenced using an ONT MinION device and analyzed using a combination of tools recommended for long-reads analysis and in-house python scripts. We assessed the efficiency of three different sequencing kits commercialized by ONT that are recommended for transcriptomics. Our results suggest that direct cDNA sequencing is most suited for transcriptome analysis in C. elegans, in regard to the quantity of data generated while preserving the quality of the dataset. The two libraries were compared together at the level of both genes and isoforms. We are reporting a set of non-SL1 genes that are found highly expressed in poly (A) libraries but not detected in SL1-enriched libraries. Additionally, we are also showing that alternatives promoters can lead to populations of isoforms exhibiting different trans-splicing status.

O3

STRUCTURE GUIDED FLUORESCENCE LABELING REVEALS A TWO-STEP BINDING MECHANISM OF NEOMYCIN TO ITS RNA APTAMER

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The understanding of RNA dynamics on a molecular level relies on suitable spectroscopic reporter groups. A small and very rigid cytidine analogue was introduced as DNA (Ç) or RNA label ($Ç_m$), either as spin label or in the reduced version as fluorophore ($Ç_{(m)}^f$).[1-3] This label class stands out, because its rigid incorporation supresses conformational ambiguity and allows a direct comparison between EPR and fluorescence methods.

In an initial study, we compared the photophysical properties of the fluorescent, methoxy group protected, RNA-label $Ç_m^f$ to the well-established non-fluorescent spin-label $Ç_m$ and clarified the quenching mechanism.[4] Quantum chemical calculations support the experimental finding that the $Ç_m$ emission is quenched via a fast (≤ 1 ps) internal conversion into a non-fluorescent, spectroscopically dark, doublet state. This dark state is located on the nitroxide moiety of the label. The quenching process also could be interpreted as a fast internal Dexter energy transfer. The full spectroscopic characterization of $Ç_m$ and $Ç_m^f$ provides an expanded view on the photochemistry of fluorophore-nitroxide-compounds in general.

As a next step, $Ç_m^f$ was incorporated into RNA model sequences for hybridization studies.[5] It turned out, that the $Ç_m^f$ emission sensitively reports local changes of its microenvironment. Fluorescence lifetime or quantum yield measurements could discriminate between labelled single and double strands. Furthermore, both observables are affected by the neighboring bases of the $Ç_m^f$ label. Thus, a discrimination of pyrimidine and purine neighbors is possible. Fluorescence anisotropy indicates a very rigid incorporation of $Ç_m^f$ into the RNA strands. Overall, it was shown that $Ç_m^f$ is ideally suited for kinetic studies of hybridization or ligand binding.

Consequently, the neomycin binding aptamer (N1) was labelled with the fluorophore $Ç_m^f$ at four different positions adjacent to the binding pocket.[5] Steady state emission experiments confirm the conformational selection mechanism previously proposed in NMR studies.[6,7] Furthermore, fluorescence stopped flow measurements demonstrate a very fast ligand binding to the aptamer, which is best described by a two-step model, consisting of an unspecific ligand binding to the preformed aptamer as a first step and a subsequent step accompanied by the formation of specific H-bonds and minor conformational adjustments.

Currently, we test RNA-systems with $Ç_m^f$ as a FRET-donor in combination with tC_{nitro} as acceptor.[8,9] The rigidity of the $Ç_m^f$ should be useful to gain distance and orientation information within RNA structures. Currently this is tested on RNA model sequences, where different singly labelled strands were combined to gain a variety of donor-acceptor distances within the RNA duplexes. A clear distance dependence is observed while an orientation dependence on the other hand seems to be averaged out due to the flexibility of the RNA. Subsequently, FRET and PELDOR measurements on identically labelled N1 aptamers are planned to gain more information on ligand binding and conformational dynamics of this aptamer.

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04

FINE-TUNING THE BINDING AFFINITY OF A STRUCTURE-SWITCHING APTAMER USING DANGLING NUCLEOTIDES

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Altering the binding affinity of biomolecules in a designed manner is an important, but difficult to achieve goal. We have used a structure-switching or ligand-induced folding construct of the cocaine-binding aptamer to increase the binding affinity of the aptamer by introducing dangling nucleotides into the sequence. Depending on the identity of the terminal base pair and the identity of a 5' or 3' dangling nucleotide the introduction of a dangling nucleotide can stabilize (or destabilize) the structure with a known ΔG value. For structure-switching aptamers, the unbound state is unfolded, or loosely folded, and the aptamer folds with ligand binding. The incorporation of the dangling nucleotide reduces the needed free energy supplied by ligand binding to fold the aptamer, resulting in a tighter observed binding affinity. We obtain a linear relationship between the predicted ΔG of stabilization by the dangling nucleotide and the resulting increase in binding affinity. We believe this method of introducing dangling nucleotides is a general method for increasing the affinity of structure-switching aptamers.

05

APTAMER DELIVERY OF SIRNAS TO KNOCKDOWN CANCER

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Immune checkpoint blocking antibodies have revolutionized cancer therapy. They can restore immune surveillance and durably control some cancers. However, for most cancers only a minority of patients responds. An important obstacle to immune therapy is that many tumors are not recognized as "foreign" by the immune system. Tumor cells have many ways to evade immune control. Here we describe a way to induce gene silencing in vivo in epithelial cancers that uses RNA aptamers that bind with high affinity to a cell surface receptor to deliver covalently linked siRNAs into cells that express the recognized receptor. EpCAM, the first described tumor antigen, is highly expressed on epithelial cancers and their especially malignant subpopulation of cancer stem cells. EpCAM aptamer-siRNA chimeras (AsiCs) that use a high affinity EpCAM aptamer that recognizes both mouse and human EpCAM selectively bind to and knock down gene expression in EpCAM+ breast tumor grafts, but not normal tissue, suppress tumor initiation and inhibit tumor growth in vivo. Recently we have used EpCAM-targeted gene knockdown in the tumor to make immunologically "cold" aggressive Her2+ and triple negative breast tumors visible to the immune system and to counteract tumor strategies of immune evasion and immune suppression. In particular EpCAM-AsiCs were used to induce tumor neoantigen expression to render immunologically ignored tumors visible and to activate anti-tumor functional immunity by knocking down immune evasion genes expressed by the tumor. AsiC cocktails targeting multiple genes enhanced immune responses to the tumor and increased tumor regression. By targeting the tumor, rather than activating immune cells nonspecifically, tumor-targeted immune therapy should have few side effects.

06

GENETICALLY ENCODED LIGHT-UP APTAMERS FOR LIVE-CELL SUPER-RESOLUTION RNA IMAGING

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In light of numerous diverse roles of RNA in processes such as transcription, translation, catalysis and gene regulation at the cellular level, there is an urgent need to establish a versatile, easy-to-use technology for specific, genetically encoded fluorescence labeling of RNA, especially for live-cell imaging applications. To this end, we developed two orthogonal light-up aptamers which bind to small, cell-permeable, bright and photostable fluorophores with distinct spectral colors for super-resolution RNA imaging in live cells using STORM (stochastic optical reconstruction microscopy), SIM (structured illumination microscopy) and STED (stimulated emission depletion) microscopy.

The first aptamer, named SiRA, binds to silicon rhodamines (SiRs) which are photostable, NIR-emitting fluorogenic dyes. 50-nucleotide SiRA aptamer was generated via SELEX and binds to SiR with nanomolar affinity (K_D ≈ 400 nM). SiRs change their open-closed equilibrium between the non-colored spirolactone and the fluorescent zwitterion in response to their environment. SiRA aptamer preferentially binds to the open-form resulting in a significant fluorescence increase upon interaction. Remarkably, SiRA is resistant to photobleaching and constitutes the brightest far-red light-up aptamer system known. SiRA allowed us to visualize the expression of RNAs in bacteria in no-wash live-cell imaging experiments. We also reported the first super-resolution STED microscopy images of aptamer-based, fluorescently labeled mRNA in live cells.[1]

The second aptamer, named SRB-2, binds to sulforhodamine B (SR) fluorophore with high specificity and affinity. To convert this aptamer/fluorophore pair into a fluorescence light-up system, SR fluorophore was conjugated to dinitroaniline (DN) which diminishes the fluorescence of SR via contact quenching. SR-DN is essentially non-fluorescent in solution; however, upon binding to SRB-2 (K_D ≈1.4 µM), the fluorescence intensity increases >100-fold. We used this method to image abundant RNAs in live bacteria.[2] Then, we determined the binding constants of various structurally different rhodamine-based dyes to SRB-2. The obtained structure-activity relationships allowed us to rationally design of a novel, bright, orange fluorescent turn-on probe (TMR-DN) with low background fluorescence and high affinity to SRB-2 (K_D ≈ 35 nM). The utility of SRB-2/TMR-DN was demonstrated by imaging mRNAs in bacteria and mammalian cells.[3] To further improve the properties of the SRB-2/TMR-DN complex, we created a library of SRB-2 mutants and selected aptamers that bind to TMR-decorated beads. Using SELEX, we discovered a mutant (SRB-3) that binds to TMR-DN with higher affinity (K_D ≈ 20 nM) and is 50% brighter than SRB-2. After rationally improving the folding features of SRB-3, the resulting SRB-4 allowed imaging low copy number mRNAs in bacteria and high copy number mRNAs in mammalian cells using SIM and STORM with very high resolution.

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07

LIGHT-UP APTASENSORS- A TOOL FOR THE DETECTION OF RNA HAIRPINS AND SMALL MOLECULES

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Cells have to adapt to their ever-changing environment. To do so, they evolved sophisticated regulatory networks involving several types of regulators such as non-coding RNAs. However, it is known that gene expression can significantly vary from one cell to another even within isogenic populations [1]. Therefore, proper characterization of regulatory networks requires being able to monitor target RNAs over the time, on the same population and with a single-cell resolution. These two conditions can be fulfilled by using fluorescence microscopy imaging and genetically-encoded fluorogenic molecules.

Our group is specialized in the development of fluorogenic modules made of a light-up RNA aptamer able to specifically interact with a pro-fluorescent dye (fluorogen) and activate its fluorescence [2]. The small size of these RNA aptamers strongly contrasts with approaches like MS2-GFP that require the use of tandem repeats of bulky protein-binding sites. The development efficiency of these RNAs is dramatically improved by using ultrahigh-throughput functional screening technologies like droplet-based microfluidics [3,4]. Indeed, this technology makes possible to screen, in a single experiment, millions of mutants individualized and in vitro expressed within picoliter water-in-oil droplets with an exquisite control over droplet dispersity and composition.

In this talk, I will present how we used this innovative evolutionary technology to develop a new generation of bright and photostable orange emitting fluorogenic module. This new tool will allow exploring RNA-mediated gene expression regulation in cells both in a dynamic way and with a single-cell resolution.

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08

EVOLUTION OF EFFICIENT RNA-BASED FLUORESCENT PROBES USING ULTRAHIGH-THROUGHPUT FUNCTIONAL SCREENING

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Cells have to adapt to their ever-changing environment. To do so, they evolved sophisticated regulatory networks involving several types of regulators such as non-coding RNAs. However, it is known that gene expression can significantly vary from one cell to another even within isogenic populations [1]. Therefore, proper characterization of regulatory networks requires being able to monitor target RNAs over the time, on the same population and with a single-cell resolution. These two conditions can be fulfilled by using fluorescence microscopy imaging and genetically-encoded fluorogenic molecules.

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In this talk, I will present how we used this innovative evolutionary technology to develop a new generation of bright and photostable orange emitting fluorogenic module. This new tool will allow exploring RNA-mediated gene expression regulation in cells both in a dynamic way and with a single-cell resolution.

09

HIGH-THROUGHPUT SCREENING OF FLUORESCENT RNA APTAMERS

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The discovery of Green Fluorescent Protein few decades ago has revolutionized the way we do molecular biology research. Today we are witnessing a similar process in the RNA world with the development and utilization of various RNA mimics of the GFP such as Spinach and Broccoli, which bind different chemical fluorophores with high affinity and induce their fluorescence. They can be fused to RNAs expressed in the cell and used for investigation of their localisation and stability in vivo. Here we show how we repurposed commercially available gene expression microarray for high-throughput screening of fluorescent RNAs. We designed a library of all possible single and double mutants of the Broccoli RNA aptamer and a fraction of mutants of Spinach RNA, fused to a different sequence complementary to a specific probe on the microarray. We used microscope imagining to measure fluorescence of each mutant from library in various conditions: ranges of magnesium, potassium or fluorophore (DFHBI) concentrations, pH and temperature. Collected data allowed us to recapitulate 2D structure of Broccoli - different from prediction by free energy minimization, identify crucial positions involved in forming of G-quadruplexes containing structure (responsible for fluorophore binding) and mutants with shifted emission spectrum. We also noticed interesting lack of correlation between affinity to DFHBI and brightness of the mutants. Among other possibilities such approach will potentially provide a set of well characterized aptamers which can serve as fluorescent intracellular sensors of pH or ions concentration.

O10

SINGLE-STRANDED DNA BINDING PROTEIN-ASSISTED FLUORESCENCE POLARIZATION APTAMER ASSAY: GENERALIZATION TO AN UNSTRUCTURED APTAMER

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We herein describe a new method generalizing the fluorescence polarization aptamer assay (FPAA) strategy based on the use of the single-stranded DNA binding (SSB) protein from Escherichia coli as a strong FP signal enhancer tool for the specific detection of small molecules.1
Our team developed the SSB assisted FP assay in 2012 using pre-structured aptamers (anti adenosine and anti argininamide aptamers): SSB was successfully used as a signal enhancer as it preferentially bound to the free aptamer than to the aptamer-target complex resulting in a much higher anisotropy variation than in the absence of SSB. In the absence of the target, the binding of the labeled aptamers to SSB governed a very high fluorescence anisotropy increase (in the 0.130-0.200 range) as the consequence of (i) the large global diffusion difference between the free and SSB-bound tracers and (ii) the restricted movement of the dye in the SSB-bound state. When the analyte was introduced into the reaction system, the formation of the folded tertiary structure of the target-aptamer complex triggered the release of the labeled nucleic acids from the protein, leading to a strong decrease in the fluorescence anisotropy.
The originality of the new method lies in showing that the SSB assisted FP assay can be extended to aptamers that are not pre-structured in their free state. To demonstrate the feasibility of our sensor, the anti- tyrosinamide aptamer was used as a model. The 49mer usually used sequence of this aptamer was truncated to a 23 mer oligonucleotide and dyes of different photochemical properties (fluorescein and texas red) were tested either on the 3' or on the 5' position of the aptamer. The strategy was to take advantage of the higher affinity of SSB for unstructured DNA sequences rather than for complexed DNA. Unlike our initial method, the L-tyrosinamide target was first mixed to its aptamer to generate a stable complex. SSB was secondly added to the solution and bound preferentially to the free aptamer. As a consequence, the more the target, the lower the anisotropy of the solution.
Experimental conditions were optimized by adjusting the amount of SSB, the reaction time, the buffer concentration as well as the nature and position of the fluorescent dye on the aptamer to reach a limit of detection of about 20 nM at RT. The temperature also had a significant influence as we could show that decreasing the solution temperature to 4°C improved the limit of detection (2nM). FP assay selectivity was demonstrated against the closely related compounds: L-tyrosine and L-phenylalanine. In addition, the method allowed the enantioselective sensing of tyrosinamide: it was found that 10 nM of target L enantiomer could be detected in the presence of 100 µM of non-target D enantiomer (at RT).
As a conclusion, our SSB assisted FP aptamer assay dedicated to small molecule detection could be generalized to aptamers that are not pre-structured in their free state by using a smart strategy that consisted in firstly generating the target-aptamer complex. The FA method appeared to be sensitive, selective and widely applicable.

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011

A TETR-BINDING APTAMER AS VERSATILE REGULATORY ELEMENT

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One of the most exciting areas of Synthetic Biology is the control of cellular behaviour using engineered genetic circuits. Genes with selected features combined in a building block manner and transferred to organisms of interest, achieve desired biological functions. However, the expression level of the corresponding genes have to be regulated and fine-tuned to avoid unbalanced gene expression and the accumulation of toxic intermediates.

Synthetic RNA-based systems have increasingly been used for the regulation of gene expression. Due to their structural properties, riboregulators provide a convenient basis for the development of ligand-dependent controllable systems. Here, we demonstrate reversible conditional control of eukaryotic gene expression with an TetR aptamer domain as a sensing unit. Our designed devices are capable of robust and reversible control of miRNA processing, mRNA splicing and mRNA localization. Thus we offer novel investigational tools to study the complex life of mRNA. In addition, we will present the structure of the TetR-aptamer complex that allows interesting insights into the regulatory systems.

012

HOW TO REDIRECT MOLECULAR EVOLUTION WHEN IT GOES IN THE WRONG DIRECTION

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In vitro selection of aptamers is a molecular evolution process that selects and enriches a population of oligonucleotides into sequences with a high affinity for a target. However, it is well known that this method can sometimes evolve into sequences with an affinity for undesirable targets such as other compounds used for the selection. Counter-selection steps are often used to avoid this problem, for example by retrieving sequences that do not bind to the selection material before the selection. But, this method may be imperfect and could lead some undesirable aptamers to survive. This is particularly true for Cell-SELEX when the goal is to direct the selection of aptamers to a specific membrane protein while cells express many other proteins on their surface.

We experienced such difficulties during the selection of aptamers against the vascular endothelial growth factor receptor 2 (VEGFR2). At every round of selection, we incubated the library on HEK-293 cells that don't express VEGFR2 and unbound sequences were recovered for the selection on the same cells modified to over-express the target. No aptamers were identified to bind to VEGFR2 after 15 rounds of selection. In contrast, several aptamers were identified to bind to other targets demonstrating that counter-selection steps were inefficient. In order to remove these unwanted aptamers from the library, we used antisense oligonucleotides during 3 more rounds selection. Almost all antisense led to a decrease in their aptamer targets and, consequently, to the enrichment of new sequences, one of which was identified as an aptamer against VEGFR2.

This redirection of molecular evolution was analyzed by High-throughput sequencing of every round and compared to classical cloning and sequencing method.

013

FIRST DNA-APTAMERS SPECIFIC TO NON-PHYSIOLOGICAL MEDIA- A SELEX TOWARDS ATP IN IONIC LIQUID

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Functional nucleic acids are very versatile molecules. Thanks to their specific hybridization they enable creating nanoscale structures, they may specifically recognize a wide variety of molecules, or even serve as catalysts. These unique and intrinsic properties of nucleic acids have been made use of in manifold sensing, separation or catalytic applications. However, applications commonly are limited by the need for physiological conditions, i.e., aqueous environments, as the use of molecular organic solvents widely annihilates nucleic acid function. During the last decade, a possible alternative to molecular organic solvents has emerged, namely ionic liquids (ILs). These solvents are electrolytes but due to weak electrostatic interactions remain liquid over a very wide temperature range. Furthermore, due to the multiple combinations possible between anions and cations, they can be tuned which is why they have also been denoted "designer-solvents".

Previously, we have systematically studied the function and stability of the well-known ATP DNA-aptamer in ionic liquid (IL)/buffer media. We observed that while DNA would be stable in different ILs, the sensitivity towards the target (ATP) suffered a significant decrease. This was not at all surprising considering that the aptamer had been originally selected under physiological conditions. We therefore conducted a SELEX for an ATP-aptamer including an ionic liquid (choline lactate) from the beginning. The aim of this selection was to explore whether it would be possible to identify new sequences that could recognize the target more efficiently in presence of ionic liquid than in buffer. If so, the concept that nucleic acids may exert their function also beyond the physiological context would be proven, paving in this way the path to a whole new range of applications.

To prove the concept, we conducted two SELEX for the ATP DNA-aptamer in parallel, one in presence of choline lactate as the ionic liquid and another with the original buffer conditions (Huizenga and Szostak 1995). In buffer, enrichment was achieved after eight rounds of selection and Huizenga and Szostak, validating our selection procedure, in line with those reported the sequences found. In presence of choline lactate, enrichment was observed after twelve rounds and while some sequences comprised the original binding motifs, four entirely different sequences were detected with different binding motifs in the random region. Radioactive assays confirmed that in presence of choline lactate, these new sequences were able to recognize ATP with higher efficiency than those selected under buffer conditions. Even more, one new sequence had a binding percentage of ATP in choline lactate similar to the one found by classic motifs in buffer. Most interestingly, two of the new sequences found did not recognize ATP in absence of choline lactate, so their function was clearly medium-specific.

The results obtained suggest that we can use a versatile functional molecule such as DNA beyond physiological conditions, and in tunable solvents, which will enable entirely new opportunities in bio nanotechnology.

014

ONE-POT SELEX: SIMULTANEOUS SELECTION OF SPECIFIC APTAMERS AGAINST DIVERSE STEROID TARGETS

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Aptamers are well-established biorecognition molecules used in a wide variety of applications for the detection of their respective targets. However, individual SELEX processes typically performed for the identification of aptamers for each target can be quite time-consuming, labour-intensive and costly. An alternative strategy is proposed herein for the simultaneous identification of different aptamers binding distinct but structurally similar targets in one single selection. This one-pot selection, using the steroids estradiol, progesterone and testosterone as model targets, was achieved by combining the benefits of counter-SELEX with next generation sequencing. Successive incubation steps of a single stranded DNA library with each of the target molecules were performed to achieve selective enrichment for each target. Using next generation sequencing, the composition of each pool was compared to identify sequences preferentially abundant in only one of the pools corresponding to one steroid target. Binding studies demonstrated the high affinity of each selected aptamer for its respective target, and low nanomolar range dissociation constants calculated were similar to those previously reported for steroid-binding aptamers selected using traditional SELEX approaches. Finally, the selected aptamers were exploited in microtitre plate assays, achieving nanomolar limits of detection, whilst the specificity of these aptamers was also demonstrated. Overall, the one-pot SELEX strategy led to the discovery of aptamers for three different steroid targets in one single selection without compromising their affinity or specificity, demonstrating the power of this approach of aptamer discovery for the simultaneous selection of aptamers against multiple targets.

015

DEVELOPMENT AND CHARACTERIZATION OF A CHEMICALLY MODIFIED DNA APTAMER AGAINST A T-CELL EXHAUSTION MARKER USING THE PUREAPTA™ PLATFORM

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Several lines of evidence indicate that certain T-cell-specific receptors play a crucial role in T-cell exhaustion, thus enabling cancer progression. The current cancer immunotherapy includes several monoclonal antibodies, which block certain receptors such as PD-1 to restore T-cell function. There is still a significant need for new therapeutic agents to explore other immunomodulatory pathways. One of the promising solutions is the use of nucleic acid-based aptamers – they are highly specific, easily manipulated to serve a specific purpose, lack batch to batch variations and have low immunogenicity and low cost of production. In this work, we describe the identification of a new chemically modified DNA aptamer specifically directed towards an undisclosed T-cell receptor. The aptamer was selected with a slightly modified SELEX procedure using our modular platform termed PureApta™, which enables facile introduction of different chemical groups into the aptamer library, increasing or enhancing the possible target interactions. Here, we present a selected aptamer with high specificity and affinity (KD ~7 nM) to the target molecule. The features of this aptamer are very encouraging and represent an attractive potential alternative to the monoclonal antibodies currently being developed. Further research is planned to probe the ability of the aptamer to promote T-cell function and tumour growth inhibition and if its therapeutic effects are comparably to known antibody agents.

016

INTEGRATING LIGAND RECEPTOR INTERACTIONS AND IN VITRO EVOLUTION FOR STREAMLINED DISCOVERY OF ARTIFICIAL NUCLEIC ACID LIGANDS AGAINST T-CELL RECEPTOR-CD3 COMPLEX IN HUMAN T-CELLS

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Empirical evidence generated over five decades has demonstrated that cells undergo structural changes at the molecular level in response to environmental cues. As membrane receptor proteins are most attuned to such cues, they remain the most attractive therapeutic targets. In order to discover specific DNA ligands against cell-surface receptors, our group recently introduced a unique variant of SELEX, termed Ligand-guided Selection (LIGS). LIGS exploits the inherent evolutionary step of competition between weak and strong binders in a SELEX library. The iterative process in conventional SELEX is designed to outcompete low-affinity binders through a competitive process whereby high affinity binders move on through the selection process. By introducing a naturally occurring stronger, highly specific binder, for example, an antibody (Ab) interacting with its cognate epitope, LIGS out-competes specific aptamers from a partially enriched SELEX pool against cells expressing the same epitope. Here, we show that LIGS can be expanded to evolve functional nucleic acid (NA) ligands against the multi-component cell-surface receptor T-cell receptor-CD3□ complex expressed by cultured and primary cells using two monoclonal Abs, OKT3 and UCHT1, as competing ligands. Bioinformatics analysis of the sequences obtained from Illumina sequencing, a total of five aptamer candidates were identified. These aptamers show affinities from 3.06±0.485 nM to 325±62.7 nM towards TCR-CD3□. The aptamer family was validated utilizing multiple strategies, including competitive binding analysis with respective antibodies used in LIGS, and a double-knockout Jurkat cell line generated by CRISPR technology. Collectively this report shows that, by exploiting the inherent competition between weak and strong binders in a combinatorial library with existing ligand receptor interactions, multiple NA ligands against a multi-component receptor protein can be identified without changing the cell-surface landscape.

017

DE-NOVO DISCOVERY OF TUMORICIDAL APTAMERS IN A DNA SEQUENCING CHIP

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We describe a technique for the rapid de-novo discovery of target-tailored tumoricidal DNA aptamers inside an Illumina sequencing chip. By sequencing oligonucleotide pools we generate a physical microfluidic map of hundreds of millions of potential aptamer clusters, in which every cluster is mapped to a specific set of spatial coordinates. Tumor cells, pre-loaded with a fluorogenic reporter of apoptosis, are then injected into the chip and monitored over time. Apoptotic tumor cells are identified and analyzed across the entire map, automatically revealing the coordinates of aptamers that induced this effect. We demonstrate this method by identifying, within just a few hours, new aptamers capable of directly and selectively inducing apoptosis in primary human tumor cells. Our platform could be perfected towards a miniaturized device that receives patient-derived, primary tumor cells, and rapidly identifies de-novo, selective, and effective aptamers tailored to this target. Such a capability could lead to a new paradigm of personalized cancer therapy.

While our main focus in this work is the rapid discovery of aptamers inducing apoptosis in tumor cells, the platform we developed can be used for rapid discovery of functional or binding aptamers for numerous targets. Any target that is either visible in brightfield microscopy – cells, bacteria and proteins or small molecules mounted on solid phase beads – or that is fluorescently labelled, can be assayed for binding to up to hundreds of millions of unique aptamers. The ability to carry out a fast, direct and cheap binding assay for a very large number of aptamers could be a breakthrough for aptamers discovery, where direct binding characterization is generally limited to only a few aptamers.

This work has been submitted to Nature Methods and is available on:
https://www.biorxiv.org/content/10.1101/630830v1.full

018

OPTORIBOGENETICS TO CONTROL CELLULAR PROCESSES

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Optogenetic makes use of light to control cellular behaviour in a spatial and temporal manner. Several processes, such as channel conformation, enzymatic activity or DNA binding have been shown to be compatible with light regulation. Photoreceptor proteins, e.g. LOV (light, oxygen, voltage)-domain containing proteins play an important role in developing those processes since they are able to reversibly change their conformation upon light absorption and therefore convert the incoming signal into a specific output.
However, until now it is difficult to apply optogenetic control to RNA biochemistry, e.g. RNA localization or function. We applied a light dependent selection strategy to develop aptamers specific for binding a LOV photoreceptor protein. This strategy yielded two RNA aptamers selectively interacting with the LOV photoreceptor in its light conformation. By conjoining these RNA elements with naïve RNA molecules, light dependent control of RNA mediated biological processes was obtained.

019

INTERLOCKED DNA NANOSTRUCTURES FOR MOLECULAR ENGINEERING

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Mechanically interlocked DNA nanostructures are useful as flexible entities for operating DNA-based nanomachines. Interlocked structures made of double-stranded (ds) DNA components can be constructed by irreversibly threading them through one another so that they are mechanically linked. The interlocked components thus remain bound to one another while still permitting large-amplitude motion about the mechanical bond. The construction of interlocked dsDNA architectures is challenging because it usually involves the synthesis and modification of small dsDNA nanocircles of various sizes, depending on intrinsically curved B-DNA. This lecture describes the design, generation, and characterization of interlocked dsDNA structures such as catenanes, rotaxanes, and daisy chain rotaxanes. Their construction requires precise control of threading and hybridization of the interlocking components at each step during the assembly process. Characterization of these nanostructures usually involves gel electrophoresis and atomic force microscopy (AFM), including high-resolution AFM images. Additional functionality can be implemented into the DNA architectures by incorporating proteins, aptamers, molecular switches such as photo-switchable azobenzene derivatives, or fluorophores for studying the mechanical behavior by fluorescence quenching or FRET experiments. These modified interlocked DNA architectures provide access to more complex mechanical devices and nanomachines that can perform a variety of desired functions and operations.

O20

A DNAZYME-BASED COLORIMETRIC PAPER SENSOR FOR HELICOBACTER PYLORI

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Infections by pathogenic bacteria are a major threat to public health as they cause many costly outbreaks around the world each year. Among bacterial pathogens, Helicobacter pylori (HP) is particularly important, as more than 50% of the world population is infected by this pathogen. HP is strongly related to gastric carcinoma and is responsible for ~80% of gastric ulcers and ~95% of duodenal ulcers. Moreover, this pathogen has been considered as an emerging superbug due to increased reports of drug resistant strains of HP. Therefore, accurate diagnosis of HP is critical to managing gastrointestinal health.

In this presentation, we describe the in vitro selection of an RNA cleaving DNAzyme that is activated by a protein present in HP, and the subsequent development of a simple, paper-based colorimetric device capable of providing specific and sensitive detection of HP in stool samples. The selection of the DNAzyme is based on the use of the crude extracellular mixture (termed CEM for simplicity) of the HP bacterium as the target in the positive selection step and the CEM from a mixture of unintended bacteria as the target in the counter selection step. As such, it does not require a pre-validated biomarker of a bacterium of interest to initiate the DNAzyme selection.

Following selection and optimization, the RNA-cleaving DNAzyme was designed to release a DNA strand upon RNA cleavage that carries a urease enzyme. The release urease undergoes lateral flow to a readout area containing colorimetric reagents for the urease reaction, producing a color change. The colorimetric paper sensor, designed on the basis of the RNA-cleaving property of the DNAzyme, is capable of delivering sensitive detection of H. pylori in human stool samples with minimal sample processing, and provides results in minutes. It remains fully functional under storage at ambient temperature for at least 130 days. This work lays a foundation for developing DNAzyme-enabled paper devices as point-of-care diagnostics for monitoring pathogens in complex samples.

021

DEVELOPMENT OF A NOVEL APTAMER-BASED BIOSENSOR TO DETECT INVASIVE MAMMAL PESTS

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Invasive mammal pests (e.g. rat, mouse and possum) are a danger to New Zealand's flora and fauna, threatening its biodiversity and endemic species. Current pest management approaches predominantly rely on traps and baits that need to be physically checked frequently by humans in order to monitor the pest population in an area of bush, a costly process in both money and time. There is a need for the development of new technologies that can enable the remote detection of pests, for example camera based technologies to identify pests.1 We are investigating an alternative approach based on the detection of species-specific biomarker molecules using an aptamer-based biosensor (aptasensor) device. We have identified a rat-specific biomarker molecule and used this as the target molecule for the selection of DNA aptamers following the Flu-Mag SELEX method2. This yielded thirty-five aptamer sequences, which could be grouped into three families. Four aptamer candidates were chosen to take forward for further characterization using a dot blot assay approach. Once binding ability has been confirmed for each aptamer, Electrochemical Impedance Spectroscopy will be performed to determine their affinity constants and limit of detections. The best candidate will be then be coupled to a remote sensing device.

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022

EVOLUTION, CRYSTAL STRUCTURE, AND APPLICATION OF CUBANE-MODIFIED APTAMERS – "CUBAMERS" - FOR MALARIA DIAGNOSIS

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Non-natural chemistries within evolutionary selection systems have the potential to radically expand the possible applications of nucleic acid aptamers. We have been developing aptamers to discriminate between biomarker targets important for the diagnosis of malaria. In previous work we had solved the crystal structure of a simple DNA aptamer in complex with malaria biomarker Plasmodium falciparum lactate dehydrogenase (PfLDH) but had encountered challenges for the development of Plasmodium vivax specific aptamers when using unmodified natural DNA. Here, we demonstrate how using cubane base-modified uridines in the aptamer evolutionary process enabled evolution of discriminatory PvLDH cubane-modified aptamers – 'cubamers'. Cubanes are unusual Platonic box-shaped benzene bioisosteres with intriguing bioactivities. We solved the crystal structure of the cubamer in complex with PvLDH, and observed unusual hydrophobic clustering effects at the cubamer-protein binding interface as a unique mechanism of nucleic acid-protein interaction. The cubamer was specific for binding to PvLDH as demonstrated by surface plasmon resonance, electrophoretic mobility shift assay and by microscale thermophoresis. Evolutionary experiments that integrate non-natural chemistries allow potentially limitless possibilities for aptamer functionality and translational application.

023

RNA APTAMERS TARGETING INTEGRIN α5β1 AS PROBES FOR CYTO- AND HISTO-FLUORESCENCE IN GLIOBLASTOMA

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Nucleic acid aptamers are often referred as chemical antibodies. As they possess several advantages like their smaller size, temperature stability, ease of chemical modifications, lack of immunogenicity/ toxicity and lower cost of production, aptamers are promising tools for clinical applications. Aptamers against cell-surface protein biomarkers are of particular interest for cancer diagnosis and targeted therapy.

In this study, we identified and characterized RNA aptamers targeting cells expressing integrin α5β1. This αβ heterodimeric cell-surface receptor is implicated in tumor angiogenesis and solid tumor aggressiveness. In glioblastoma, integrin α5β1 expression is associated with an aggressive phenotype and a decrease in patient survival. We used a complex and original hybrid SELEX (selective evolution of ligands by exponential enrichment) strategy combining protein-SELEX cycles on the recombinant α5β1 protein, surrounded by cell-SELEX cycles using two different cell lines.

We identified an aptamer named H02. Directly coupled to the cyanine 5 fluorophore, aptamer H02 was able to discriminate between ten GBM cell lines expressing high and low levels of integrin α5. Aptamer H02 is internalized at 37°C. As a proof-of-concept, we also demonstrated that aptamer H02 is very efficient in apta-fluorescence assays to characterize GBM tumor tissues from patient-derived tumor xenografts expressing high levels of α5 from GBM tumor tissues expressing low levels of α5.

Internalized, an aptamer targeting integrin α5β1 might open roads for α5β1-specific therapeutic payloads delivery.

024

TRACELESS APTAMER-MEDIATED ISOLATION OF CD8+ T CELLS FOR CAR T CELL THERAPY

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The increasing number of CAR T cell therapy clinical trials utilizing defined product compositions underscores the need for continued development of robust and cost-efficient methods for selection of specific T cell subsets. Using a modified method of cell-SELEX we identified new DNA aptamers, that preferentially bind human cytotoxic T cell marker CD8. We applied one of those aptamers to a traceless cell isolation strategy with a complementary oligonucleotide reversal agent that undergoes toehold-mediated strand displacement with the aptamer and thereby disrupts its secondary structure for label-free elution of captured cells. We show that this approach gives high yields of CD8+ T cells and that CAR T cells manufactured from these cells are comparable to antibody-isolated CAR T cells in proliferation, phenotype, effector function, and anti-tumor activity in vivo. These findings represent an important technology advance towards a fully synthetic system that will enable multiple cell selections from a single apparatus by employing multiple aptamers and reversal agents.

025

TOWARDS CONTROLLING VASCULARIZATION WITHIN ENGINEERED TISSUES VIA PROGRAMMABLE HYDROGELS

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Growth factors are of vital importance for controlling and coordinating blood vessels formation, organization and maturation. Among other important factors, spatiotemporally regulated bioavailability of growth factors is of critical need for controlling vascularization within an engineered tissue. However, conventional approaches for growth factor localization mainly focus on their physical entrapment or chemical coupling within the engineered matrices (hydrogel) via metalloproteinase-sensitive linker. Even though these approaches allow passive release rate and growth factor delivery on demand, they fail to provide spatial or temporal control on growth factor's release rates which is essential for mimicking native tissue/organ developmental environment. To address this issue, nucleic acid aptamers have emerged as a class of affinity ligands that could be selected from DNA/RNA libraries for binding to growth factors with high affinity and specificity.1,2 This approach provides an alternative system that could allow sequential loading of different growth factors and provide temporal control on their release rate.

To this end, in the present study, aptamer conjugated gelatin methacrylate (GelMA) hydrogels have been developed for programmable release of vascular endothelial growth factor (VEGF). Furthermore, the effect of programmable VEGF release on angiogenic properties of human umbilical vein derived endothelial cells (HUVECs) co-cultured with mesenchymal stem cells (MSCs) have been studied. The results obtained from VEGF ELISA experiments revealed that acrydite functionalized aptamers could sustain a controlled VEGF release up to 10 days, if no complementary sequence (CSs) for these VEGF specific aptamers were provided. However, immediately after adding the CSs, triggered VEGF release was observed. In co-culture experiments, the developed programmable hydrogels supported HUVECs and MSCs in terms of cell viability and vascular network formation over a span of 7 days within the hydrogels (triggered VEGF release was observed on Day 5). Therefore, the results of this study demonstrated a successful programmable VEGF release within a hydrogel and its positive effect on vascular formation in HUVECs/MSCs co-culture.

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026

APTAMER-STAT3 RNA BIO-DRUG AS TOOL TO ERADICATE GLIOBLASTOMA

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An important drawback in glioblastoma (GBM) treatment is that conventional therapies poorly affect a small population of stem-like cancer cells (glioblastoma stem cells, GSCs) that remain capable of repopulating the tumour. Thus, the development of strategies able to target GSCs represents an important challenge in oncology in order to effectively render the tumours unable to maintain themselves or grow. The signal transducer and activator of transcription-3 (STAT3) has been reported as key regulator of the highly aggressive mesenchymal GBM subtype and of survival and propagation of GSCs. Using an aptamer that binds to and antagonizes the oncogenic receptor tyrosine kinase PDGFRβ (Gint4.T), we recently designed a novel aptamer-siRNA chimera (AsiC, Gint4.T-STAT3) able of efficient delivery and silencing of STAT3 in PDGFRβ expressing GBM cells. Because of the pivotal role of STAT3 in maintaining the tumor initiating capacity of GSC population and tumor relapse, here we explored the potential of Gint4.T-STAT3 to inhibit the STAT3-dependent gene expression impairing the stem-like GSCs phenotype. We showed that the aptamer conjugate is able to effectively and specifically prevent patient-derived GSC function and expansion. Results highlighted the potential of Gint4.T-STAT3 as a high promising inhibitor of adaptative resistance to conventional therapeutics and GBM tumor dissemination.

027

APTOLL, FILLING THE GAP OF THERAPEUTIC MOLECULES IN ISCHEMIC STROKE TREATMENT

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Ischemic stroke is a devastating and debilitating medical condition, being the second cause of death, the second cause of dementia (after Alzheimer disease) and the leading cause of adult disability worldwide. One of the main processes involved in the pathophysiology of stroke is the inflammatory response. In this context, the toll-like receptor type 4 (TLR4) plays a crucial role inducing the overexpression of several proinflammatory mediators which activate the innate immune response and attract proinflammatory cells to the ischemic tissue, exacerbating the brain damage (Caso et al., Circulation 2007). Unfortunately, at this moment, no effective therapies to reduce this inflammatory response and minimize brain damage after stroke have been developed.

Currently, aptamers are emerging as a powerful therapeutic tool in several diseases. They have certain advantages over other molecules like antibodies and small molecules. Their production is easily scalable and cheap, and due to their small size, they present an optimal body diffusion reaching the target organ better than others. We aim to use this innovative technology to fill the gap in therapies for ischemic stroke. To this end, from a library of unmodified oligonucleotides, we have selected two aptamers with high affinity to the TLR4 receptor by cell-SELEX methodology. The size of these selected sequences was optimized removing the primer sequences flanked the molecule. Among four sequences, ApTOLL was the strongest candidate showing the best antagonistic effect and high affinity and specificity in both, in vitro and in vivo assays. In the in vivo studies, ApTOLL has been tested in experimental ischemic models in rats and mice under permanent (28.98 ± 5.08% mid protection) and transient (45.23 ± 17.2% mid protection) middle cerebral artery occlusion and displayed protective effect even administered 6h after the experimental stroke (Fernández et al, Mol Ther 2018). Due to its nature, based on unmodified nucleotides, ApTOLL exhibits short half-life (2.8h rats and 1.4h monkeys) fitting perfectly for the acute treatment of the disease. Additionally, ApTOLL showed neither drug-to-drug interactions nor off-target effects in vitro and, in regulatory studies conducted in rats and monkeys, did not show any toxicological alteration in biochemistry and pathophysiology.

All these data together demonstrate the strong protective effect of ApTOLL in experimental ischemic models in rodents with a very promising pharmacokinetic and safety profiles which have prompted us to initiate, this year, the clinical phase I trial (first-in-human) in healthy volunteers.

028

APTAMERS AS REVERSIBLE LIGANDS FOR THERAPEUTIC, IMAGING AND CLEAN CELL PURIFICATION APPLICATIONS

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We have been exploring the concept of utilizing aptamer-antidote pairs as reversible ligands now for over a decade. Our studies demonstrate that aptamer-binding can be rapidly reversed in vitro, in vivo and in patients using short antidote oligonucleotides. We will describe three different applications of this powerful approach to generated switchable ligands. First our recent results evaluating this approach to control a therapeutic antiplatelet aptamer in the setting of ischemic stroke will be described. As stroke treatment is limited by serious bleeding, we believe that the development of a rapidly reversible antiplatelet agent for this clinical setting will significantly improve the treatment options of the millions of patients that experience stroke around the world each year. Next we will describe how a rapidly reversible aptamer can be utilized to image an active thrombus in vivo in real time. As current methods of detecting thrombi are indirect, we believe that the development of this approach will greatly facilitate rapid and definitive clinical diagnoses and more accurately direct clinical care of patients experiencing acute thrombotic events. Finally we discuss our studies demonstrating that aptamer-antidote pairs can be utilized to purify cells in their native states, which will greatly improve their function following isolation. As antibodies are difficult to remove from cells and aptamers are not through the use of our antidote-reversal approach, we believe that aptamer-antidote based cell purification methods represent a novel and more useful strategy to isolate cells in their "native state" for both research and clinical applications. In summary our discovery that aptamer-antidote pairs represent rapidly reversible ligands has many potential applications particularly in settings where reversible binding is important and valuable.

029

APTAMERS AS TOOLS FOR THE STUDY AND TREATMENT OF PARKINSON'S DISEASE

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The central nervous system is a particularly challenging domain for aptamer applications due to a number of physical constraints, in particular the blood brain barrier. Nevertheless, given the limited treatment strategies for CNS disorders and the growing burden of neurological and neurodegenerative diseases, developing aptamer systems for diagnostics and treatments continues to be of interest. In this presentation, the selection of DNA aptamers for alpha synuclein, a protein implicated in Parkinson's Disease, will be described. A modification to the traditional SELEX method facilitated the selection of aptamer candidates that interfered with alpha-synuclein aggregation. Aptamers were identified that had a marked effect on the aggregation of alpha-synuclein monomer in vitro. Building on our recent work delivering the dopamine aptamer into the brain using a modified liposome system1, successful delivery of the aptamer to the brain in a mouse model system was confirmed and a reduction in alpha synuclein was noted. Applications for the mechanistic study of Parkinson's disease will be discussed, as well as its possible use as a therapeutic.

1) Erin M. McConnell, Katelyn Ventura, Zach Dwyer, Vernon Hunt, Anna Koudrina, Matthew R. Holahan, and Maria C. DeRosa. In Vivo Use of a Multi-DNA Aptamer-Based Payload/Targeting System To Study Dopamine Dysregulation in the Central Nervous System. ACS Chem. Neuro. 2018, 10, 371-383.

O30

ICOS CO-STIMULATION AT THE TUMOR SITE IN COMBINATION WITH CTLA-4 BLOCKADE THERAPY ELICITS ANTITUMOR IMMUNE RESPONSES

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CTLA-4 blockade therapy is able to induce long-lasting antitumor responses in a fraction of cancer patients. Nonetheless, there is still room for improvement in the quest for new therapeutic combinations. ICOS costimulation has been underscored as a possible target to include with CTLA-4 blocking treatment. Herein we describe an ICOS agonistic aptamer that potentiates T-cell activation, and induces stronger antitumor responses when locally injected at the tumor site in combination with anti-CTLA-4 antibody in two different models of melanoma and hepatocarcinoma. Furthermore, ICOS agonistic aptamer was engineered as a bi-specific tumor targeting aptamer in order to reach any disseminated tumor lesions after systemic injection. Treatment with the bi-specific aptamer showed enhancement of CTLA-4-mediated antitumor immunity under conditions where CTLA-4 treatment alone did not display any significant therapeutic benefit. Thus, this work provides strong support for the development of combinatorial therapies involving anti-CTLA-4 blockade and ICOS agonist tumor-targeting agents.

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INHALATION OF AN RNA BIO-DRUG TARGETING EXTRACELLULAR HISTONES PROTECTS FROM ACUTE LUNG INJURY

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Acute lung injury (ALI) can result from sepsis, bacterial pneumonia, aspiration of gastric contents, inhalation of toxic substances, and epidemic viruses. When severe, ALI leads to significant morbidity and mortality. Despite extensive research into its pathogenesis and clinical trials of new therapeutics, there remains no effective pharmacotherapy to interrupt the progression of ALI. Patients with severe ALI often develop acute respiratory distress syndrome (ARDS) which is accompanied by a high incidence of morbidity and mortality. The early exudative phase of ALI is characterized by increased alveolar-capillary permeability with clinical manifestation of this barrier disruption manifested by pulmonary edema, impairment of lung compliance and hypoxemia. Damage to lung tissue causes release of damage-associated molecular patterns (DAMPs), which act as endogenous danger signals to promote and exacerbate the inflammatory response. One of these DAMPs are the histone proteins. Extracellular histones are implicated in the propagation of ALI and serum levels of histones in critically ill patients predicts mortality. Histones normally reside in the nucleus where they partner with DNA, but when released from damaged cells, histones cause additional tissue injury via activation of Toll-like receptors, calcium influx, platelet aggregation, and cell death. The goal of this study was to develop a therapeutic to inactivate circulating histones for administration in field situations to prevent the morbidity and mortality associated with ALI/ARDS. Because histones (cationic proteins) normally associate with DNA in the nucleosome, we hypothesized that oligonucleotides such as RNA aptamers (anionic molecules) will have extraordinarily high affinity and specificity for histones, making them the preferred reagent for binding and neutralizing circulating histones. Using in vitro-SELEX, we previously identified chemically stabilized RNA aptamers that bind with high affinity and specificity to the histone subunits mostly commonly implicated in tissue damage (H3 and H4) but do not bind to other serum proteins. Direct exposure of cultured human endothelial cells to calf thymus-derived histones (CTH, 50 µg/ml) for 24 hours caused cell apoptosis (25%) as compared to the vehicle group (<10%). Administration of a histone-targeted RNA aptamer (KU7) to cells 30 minutes after CTH exposure reduced cell apoptosis (17%, $p<0.05$ vs CTH). Similarly, CTH exposure caused concentration-dependent death of cultured human epithelial cells, but KU7 given to cells 1 hour after CTH protected from this histone toxicity. In vivo CTH administration by oropharyngeal aspiration induced ALI in C57BL/6 mice as evident by an increase in broncheoalveolar lavage fluid (BALF) neutrophils, albumin and cytokines and histologic evidence of alveolar edema, inflammatory infiltrate, and alveolar disruption. We next determined the retention and distribution of the RNA aptamer in lung following oropharyngeal aspiration of fluorescent-labeled KU7 (1.7 nmol in 50uL PBS). The aptamer was distributed throughout all segments of the lung and remained present in BALF at 24 hours without causing an increase in inflammatory cells or injury. Next, mice received CTH (300 µg in 50 µL PBS) to initiate ALI. Treatment with KU7 (4:1 Molar ratio CTH:KU7) 30 minutes prior to, or 30 minutes after CTH, attenuated BALF albumin. In summary, intratracheal delivery of RNA aptamers protected from histone-mediated lung toxicity and may have therapeutic potential in ALI.

032

SOMAMERS AND SOMASCAN: MOLECULAR SNUGGLING, BINDING SPECIFICITY, AND PROTEOMICS

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SomaLogic scientists have now performed around 10,000 successful SELEX experiments over 20 years. Over time we migrated from RNA and DNA aptamers to DNA-based SOMAmers (Slow Off-Rate Modified Aptamers) which are derived from starting libraries augmented with molecular features natural nucleic acids lack. The structures from a handful of protein target: SOMAmer co-crystals have revealed the profound role these non-natural chemical entities play in facilitating both SOMAmer folding and protein recognition. We are struck by the snuggling capabilities of nucleotides (reflected in their ability to create exquisitely precise complementary surfaces), even when compared to amino acids, the traditional binding units of antibodies.

SOMAmers are the binding molecules used in the SOMAscan assay, the means by which we have realized our long-standing goal of simultaneously measuring many proteins in biological samples. In this assay, we were able to achieve specificity comparable to high quality ELISAs using only a single binding reagent by taking advantage of several intrinsic features of nucleic acid ligands in combination, such as low Kd's, polyanionic competitors to minimize non-specific binding, and kinetics, to achieve that specificity. The proteomic data from SOMAscan contain more biomarkers for medical and lifestyle conditions than one might have thought available. Some, but not all biomarkers, make sense to molecular biologists and other scientists who understand biology as we currently know it. Interestingly, correlation analyses from SOMAscan data lead us toward a non-standard view of biology from which sense may yet emerge.

P1

CHEMILUMINESCENT LABEL-FREE ASSAYS BASED ON USE OF HEMIN APTAMER

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Peroxidase-mimicking DNAzyme (PMDNAzyme) is a noncovalent complex of hemin and its aptamer in G-quadruplex conformation. In the present work, aptamer EAD2 (CTGGGAGGGAGGGAGGGA) was used. This peroxidase mimetic, PMDNAzyme is able to catalyze luminol oxidation with a formation of light. An extension of the aptamer by attaching additional DNA sequence prevents a formation of G-quadruplex of EAD2 or strongly deformed it, if the additional sequence is able to specifically react with the aptamer sequence. In turn, these structural changes diminish the catalytic activity of PMDNAzyme. In the same time, in the presence of targets PMDNAzyme reduces its activity. Based on this fact, we modeled and synthesized some probes with additional sequences which were complementary both to targets (fragment of HBV DNA and miRNA-141) and to EAD2. Such probes were used in the development of chemiluminescent assays for detection of HIV DNA and miRNA-141. The detection limit for both targets was 100 pM. The linear range values for HIV DNA and miRNA-141were 0.1-50 nM and 0.1–10 nM, respectively. The values of coefficient of variation measured within the working range varied within 2-4%, which indicates a high precision of the proposed assays.

The effect of allosteric activation of PMDNAzyme was also applied in the development of a method to measure exonuclease III activity. For this, we synthesized similar probes (hairpins) with blunt 3'-ends. The catalytic activity of such probes was low. In the presence of exonuclease III (exo III) one of stands of a stem was digested with a production of aptamer EAD2, which after reaction with hemin shows the high activity. This effect allowed developing simple and versatile assay for estimation of exo III activity with a detection limit of 0.01 U/μL.

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P2

NEW APTAMER-BASED BIOSENSORS FOR THE DETECTION OF CHAGAS DISEASE

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Chagas is an infectious disease caused by Trypanosoma cruzi that generates severe morbidity and affects approximately eight million humans worldwide. Although the clinical symptoms of Chagas disease are well characterized, direct detection of the parasite that causes it remains a challenge. Here, bioinformatic and in vitro evolution techniques were employed to identify potential novel biomarkers and to develop aptamers as novel biosensors, respectively. Using ribosome profiling databases, ten highly expressed proteins of Trypanosoma cruzi were identified as potential biomarkers and the protein encoded by the TcCLB.510323.60 gene was selected as a suitable candidate for aptamer development. This protein was the second most abundant in the metacyclic trypomastigote and was highly conserved among T. cruzi strains. Systematic evolution of ligands by exponential enrichment (SELEX) and Next-Generation Sequencing (NGS) allowed to identify suitable aptamer candidates and the Multiple Em for Motif Elicitation (MEME) software identified a common guanine-rich motif in all the sequences. An ELISA-like assay was used to probe the candidates binding capacity in simple and complex matrixes. These showed specific binding for a short segment of the protein biomarker (peptide TC1), three evaluated aptamers (TA1, TA2 and TA11) were able to bind the peptide differentially ($p < 0.05$). Additionally, the aptamers recognized the complete protein in crude T. cruzi lysates, showing reactivities 2 to 3 times greater than the cut-off established in lysates (0.071196 for total soluble protein and 0.082576 for membrane protein). Comparisons between total protein and total membrane protein lysates suggest that the biomarker encoded by the TcCLB.510323.60 gene is membrane associated. A Stopped-flow assay showed that aptamers incubated with fluorescent probes in the 5´and 3´ ends had a differential fluorescent and FRET signal while binding the peptide, suggesting that they could work as a molecular beacon. For these reasons, the identified aptamers isolated in this work could be used to develop a direct detection assay for T. cruzi in biological samples. Further research should focus on structural and binding site evaluation through bioinformatic modelling and experimental truncations.

Aptamers; Biomarker; Chagas, Molecular Beacon

P3

DEVELOPMENT OF A RAPID DETECTION STRATEGY FOR CARDIAC TROPONIN T BASED ON AN ENZYME-LINKED OLIGONUCLEOTIDE ASSAY (ELONA)

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Nowadays, many efforts are devoted to the design and synthesis of innovative and effective biomimetic receptors, e.g. aptamers, affibodies, molecularly imprinted polymers (MIPs), as promising alternative to antibodies used in clinical immunoassays [1-2]. Herein, we purpose a rapid approach involving simple analytical platforms coupled with a new pair of aptamers for the detection of cardiac Troponin T (cTnT). Cardiac troponins T and I are the gold standard biomarkers for the diagnosis of acute myocardial infarction (AMI), due to their enhanced sensitivity and specificity, almost replacing the previous biochemical markers, e.g. aspartate aminotransferase, total lactate dehydrogenase, myoglobin and creatine kinase [3-5]. The aptamers were first characterized by using Surface Plasmon Resonance (SPR), and then we have applied a direct Enzyme-Linked-OligoNucleotide Assay (ELONA) for cTnT detection, offering many advantages over other methods: expensive equipment is not required; different reporter systems can be used to obtain a quantifiable output and different samples can be analyzed simultaneously in the same platform (polystyrene microplate). We have successfully reproduced, on polystyrene microplate, a single-aptamer ELISA-like experiment to quantify cTnT and, as proof of concept, spiked human serum solutions has been investigated to evaluate matrix effects and possible interferents in cTnT-Aptamer recognition reaction, giving encouraging results. Moreover, we are studying a dual-aptamers strategy to improve the sensitivity of the assay.
Our approach represents a first example of aptamer-based bioassay for the quantification of cTnT, which could be further implemented to be integrated with the emerging smartphone-based point-of-care (POC) technology for the development of rapid colorimetric assay.

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P4

POLYMERASE SYNTHESES OF SEQUENCE-SPECIFIC MONODISPERSED DNA POLYMERS DECORATED BY FOUR DIFFERENT BASE-MODIFIED DNTPS

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The novel base-modified dNTPs were synthesized and tested as building blocks for various DNA polymerases in primer extension (PEX) to decorate DNA scaffold by set of 4 different functional groups.

In order to point them out to the major groove of DNA, all the modifications are attached at position 5 of pyrimidines and at position 7 of 7-deazapurines. Having specific hydrophobic aromatic or aliphatic groups, they mimic amino acid side-chains. The modifications are either linked through rigid an alkyne or flexible alkyl linkers and have been prepared by the aqueous Sonogashira cross-coupling reactions. We could reach the alkyl linker by catalytic hydrogenation of the alkyne linker serie performed either on nucleoside or nucleotide. There is no paper reporting simultaneous use of four dNRTPs with hydrophobic modifications in polymerase synthesis of such hypermodified DNA.

In order to synthesize single-stranded partially or fully modified oligonucleotides (ssONs), we employed either PEX with biotinylated template followed by magnetoseparation on streptavidine beads or PEX with 5´-phosphorylated template followed by digestion of template by □ exonuclease, as well as nicking enzyme amplification reaction (NEAR) producing short ssONs offering also great diversity. Combination of all four dNRTPs were tested in PCR amplification incorporating them into both strands with high fidelity. This methodology will enable us to synthesize variety of sequence-specific monodispersed DNA polymers and to study effect of novel modifications on hybridization, duplex stability or folding by such base-modified analogues of DNA.

Combination of mentioned approaches will be later applied for construction of libraries with modified sequences, SELEX or other methods for in vitro selection of aptamers to target undruggable proteins of relevant choice to cancer or viral diseases etc.

P5

PROSPECTS OF EGFR APTAMEROTHERANOSTICS FOR GLIOBLASTOMA

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Theranostics is a contemporary trend in treating diseases. A large part of that is immunotheranostics, which applies monoclonal antibodies as molecular recognizing elements (MoRE) to specifically block functioning of therapeutically important target. MoRE made of nucleic acids, instead of polypeptides, are aptamers. Aptamers are more robust to produce, handle and apply, not mentioning a lack to provoke immune response. For glioblastoma (GBM), some aptamers were made, which are able to interact specifically with glioma cells gaining to block proliferation; they are described in about 65 publications (comparing with 6,440 for antibody to glioma). Among them are the following examples: for EphB2/3 receptors, for PDGFRβ, for U87-EGFRvIII cells, GBI-10 to tenascin-C, AS1411 to nucleolin, DNA aptamers from whole-cell SELEX. Some applications of aptamers have been developed: surface-immobilized aptamers for cancer cell isolation and cytology, quantum dot (QD) labeled aptamer nanoprobes, aptamer guided gadolinium-loaded liposomes, aptamer-guided liposomes with siRNA and QDs, aptamer functionalization of nanosystems to get through the blood-brain barrier, microRNA-based targeting to eradicate GBM stem-like cells.

In this research, EGFR has been chosen as specific target to explore interactions with aptamers in more detail gaining to halt proliferation of EGFR positive clones of GBM. The topic is already covered in a dozen publications, not mentioning initial classical research of A. Ellington et al. The results are rather diverse and could not be easily compared.

We made inventory of published EGFR-binding aptamers, both RNA and DNA, and analyzed them for possible secondary structures, gaining to find putative scaffold and recognizing parts of aptamers. All promising candidates were tested for affinity to the immobilized extracellular domain of human recombinant EGFR by surface plasmon resonance and compared with commercial monoclonal antibodies (H11 and 225). Derivatives of active aptamers were made to reveal putative scaffold and recognizing domain, which were also tested for affinity. Studying of interactions of several aptamers with EGFR positive cell lines, including standard A431, as well as with tissue cultures, and primary cultures from surgical samples of GBM, were made by flow cytometry. EGFR expression in cells was tested by qRT-PCR for mRNA and by western blot for the protein. Signal transduction from EGFR was studied by western blot for phosphokinase. Competitive experiments for aptamers with either EGF or antibody were made. Some particular aptamers have very promising structural and functional properties. They show distinctive putative structures, which easy to modify, and they exhibit rather high affinity.

Conclusions. Despite a hundred papers for the EGFR binding aptamers had been described till now (comparing with about 18,000 for antibody to EGFR), most of the aptamers do not exhibit properties for further translational study. Therefore, there is a need for more success stories of selection, and methodology for developing and translating aptamers into theranostic drugs. The study was funded by RFBR Projects № 17-00-00160, 17-00-00157 and 17-00-00162 (K).

P6

BI-HD1 (BI-TBA) REDUCES PROLIFERATION OF LINEAR LUNG CANCER CELLS AND HUMAN GLIOBLASTOMA

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G-quadruplex DNAs possess antiproliferative activity. Interestingly, the antithrombin aptamer, TBA, which also has the structure of a G-quadruplex, also showed an anti-proliferative effect. The covalent dimer HD1, bi-HD1, has an anticoagulant effect and its structure presumably consists of two G-quadruplexes. Therefore, it would be interesting if it retains antiproliferative properties. And if so, on which type of cancer it acts most effectively.For the study, a panel of cell lines of malignant tumors of various etiologies was used, consisting of breast cancer, glioblastoma, neuroblastoma, prostate cancer, rectal carcinoma. The effect of bi-HD1 on cell lines was assessed by the MTT method as an indicator of a decrease in proliferative potential. bi-HD1 reduces proliferative activity in a number of cell lines of different tissue specificity: human embryonic fibroblasts cells (hEF), glioblastoma cell line (U87), epithelioid cervix carcinoma cells (HeLa), colon cells (HCT116), prostate cancer cells (PC3), breast adenocarcinoma cells (MCF7), lung cancer cells (RL-67) and melanoma cells (mS). The antiproliferative effect was cytostatic. The best anti-proliferative effect of bi-HD1 was shown on lung cancer line RL-67 and human glioblastoma U87 line. The efficiency of aptamer penetration into the cell was analyzed by flow cytofluorimetry and secondary ion mass spectrometry (ToF-SIMS). For this bi-HD1, labeled TAMRA or J2 was used. Addition of ExGen DNA carrier to the cell culture medium was found to enhance antiproliferative action.
Conclusions.The bi-HD1 exhibits dose-dependent antiproliferative activity toward several cancer cell lines, with the highest activity toward lung cancer cell line RL-67, and ‘glioblastoma’ cell line U87. The bi-HD1 turned out to be a unique two-modular structure with high antiproliferative activity that illustrates possibility to build multi-functional nano-constructs based on the GQ.
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P7

NEXT GENERATION PROTEOMICS PROFILING OF CANCER PATIENTS BY PRE-ENRICHED APTAMER LIBRARIES AND HIGH-RESOLUTION MASS-SPECTROMETRY

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Despite significant improvements in sensitivity of mass-spectrometry, the proteomics profiling of clinical samples remains challenging due to the significant fraction of low abundant proteins and their wide dynamic range, limitations to analyze protein complexes and low throughput.

Our Poly-Ligand Profiling (PLP) platform generates and employs aptamer libraries trained in the unbiased recognition of molecular complexes from patients with clinically relevant differences in health or drug response status. We have demonstrated PLP applications in both a plasma exosome-based breast cancer diagnostic (AUC 0.73) as well as a tissue-based classifier for breast cancer patient outcomes to trastuzumab (300 days survival increase). To uncover the molecular background of the PLP profiles, we utilized the trained libraries to affinity purify proteins from patient-derived blood plasma or FFPE tissue and subjected them to high-resolution mass-spectrometry (MS). MS identified proteins known to be involved in cancer related biological processes and drug response as well as novel targets not previously associated with cancer biology. Currently, we are developing a Next-Generation Profiling (NGP) platform which capitalizes on dramatically improved proteomics resolution enabled by PLP on a per patient basis and machine-learning algorithms to assemble complex molecular signatures that underlie the nature, natural course and predicted response to therapy of individual cancers. To ensure NGP does not exceed the typical turnaround time for a clinical assay, we developed a high-throughput one round protocol for the training of PLP-libraries on FFPE tissue. Starting with library, containing >1 copy/oligo and utilizing stringent post-binding washes/dilutions, the training can be completed in one day and scaled to process 100's of patient samples. Resulting libraries have significantly reduced complexity and ability to bind FFPE tissue as shown by NGS and chromogenic staining, respectively. Enrichment of proteins for MS from FFPE tissue lysates via affinity purification was performed on pancreatic, breast and colon cancer, utilizing the following criteria: 99% protein threshold; minimum 2 peptides per protein with 95% threshold; at least 2-fold change for precursor ion intensity between trained and untrained starting library, "No library" and total lysate controls; coefficient of variation between binding replicates less than 25%. Up to 70% of the identified cancer related targets were previously described based on cell line analyses, while PLP enriched them from patient samples. Up to 40% of the identified proteins had no previously reported association with cancer; many of these were verified by IHC with corresponding antibodies and represent biomarker-candidates.

In summary, our data indicate that NGP using trained aptamer libraries has the potential to overcome some of the current challenges in proteomics, like wide dynamic range, low sensitivity and low throughput. NGP can provide insight into the mechanisms of cancer and drug response, and may deliver new biomarkers for personalized medicine.

P8

LIGHT-UP APTAMERS- A TOOL FOR THE DETECTION OF SMALL MOLECULES AND BIOLOGICAL LIGANDS

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The pair comprising a conditionally non-fluorescent dye and its cognate nucleic acid aptamer that activates the dye's fluorescence upon binding emerged as a promising tool for fluorescence based biosensing and molecular imaging. A number of RNA light-up aptamers have been developed already against some fluorogenic dyes (MG= Malachite green, DFHBI=Z-4-(3,5-difluoro-4-hydroxybenzylidene)-2-methyl-1-(2,2,2-trifluoroethyl)-1H-imidazol-5(4H)-one, TO= Thiazole orange). In this study we have converted a MG specific light-up aptamer into a structure-switching aptasensor malaswitch. We destabilized the stem-loop structure of the native aptamer and we introduced an apical loop which can form kissing interaction with an RNA hairpin, exclusively in presence of small molecule. The ternary MG-malaswitch-RNA hairpin complex is much more stable than the binary MG-malaswitch one. The malaswitch-MG combination was used for the detection of microRNA precursors. On the basis of this malaswitch, we developed light-up aptamers responding to the presence of small molecules like adenosine and theophylline. We also selected a DNA light-up aptamer against DFHBI from a pool of candidates with a randomized 40 nucleotide window. This multi modality approach of 'light up aptasensor' holds an immense potential for small molecule detection and sensing other biomolecules causing various diseases and disorders.

Keywords: light-up aptamer, Kissing complex, DNA/RNA aptasensor

P9

APTAMERS AS A TOOL FOR SELECTIVE TARGETING OF MUSCLE STEM CELLS IN DUCHENNE MUSCULAR DYSTROPHY

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Duchenne Muscular dystrophy (DMD) is a X-linked muscle disorder caused by mutations in the dystrophin gene, which has immense adverse effects on males. It occurs with muscle weakness associated with loss of muscle mass and weakening the muscle structure. Work in the past few years showed that specific targeting of muscle stem cells can increase the regeneration potential of dystrophic muscles. Integrin α7 is a highly specific surface marker of muscle progenitor cells. Thus, the development of strategies able to target integrin in a precise and sensitive manner represents an important challenge to combat against DMD. Herein, we developed an aptamer based novel delivery strategy to specifically target muscle stem cells and increase muscle regeneration in this dreadful disease. We carried out a cross-over SELEX against recombinant integin α7β1 protein and integrin over-expressing C2C12 cell-line simultaneously. After 14 iterative rounds of SELEX, we identified a group of potential DNA aptamer candidates among which NM15.1 showed the highest binding capacity (Kd -130 nM) with the recombinant protein. We have functionalized the NM15.1 aptamer and conjugated it with gold nanoparticle for delivery in the DMD mice model. This aptamer functionalized nanoparticle modality holds a great potential to develop an efficient and affordable therapeutic tool against DMD.

Keywords: DNA aptamer, Duchenne Muscular dystrophy (DMD), theranostic module

P10

BIOPHYSICS OF DNA APTAMERS FOR HUNTINGTIN TARGETING

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Huntington's Disease (HD) is a rare, progressive, autosomal dominant neurodegenerative disorder. HD results in severe movement and thinking disorders, which can eventually lead to death as no effective therapy exists. As for genetics, HD hinges on the CAG triplet expansion within the first exon of the HTT gene, which then encodes for a mutant, polyglutamine-expanded (polyQ) protein (mHTT).
Several recent reviews have described the mHTT biology, the related pathogenesis mechanisms, and the roles of polyQ and N17 in HD onset. We discussed the HTT biophysics and the perspective, innovative aptamer-based approach for its targeting in a recent review[1]. To date, most molecular approaches (e.g. antibodies, intrabodies) failed because of inadequate selectivity, toxicity, poor delivery, and system complexity. Aptamers promise to override these limits thanks to their unmatched specificity (virtually at patient-specific level), easy selection, very low immunogenicity, and broad stability. Four G-rich DNA aptamers able to selectively bind the mHTT and to ameliorate at least one of its aberrant effects were identified in a recent study2. Interestingly, these aptamers were proved to fold into G-quadruplex structures but no conclusive data on their conformation and/or structure-activity relationship was provided[2].
We here present a thorough biophysical characterization of these aptamers. Circular dichroism (CD) was used to assess secondary structures and thermal stabilities. Differential Scanning Calorimetry (DSC) provided fine thermodynamic parameters on the folding/unfolding equilibrium of aptamers and stability in terms of Gibbs energy. CD and DSC analyses also allow determining their kinetics of folding/unfolding under physiological conditions.
The physico-chemical data will lay the basis for the rational design of chemically modified aptamers with improved pharmacokinetic properties, which will be realized by in-house synthesis. The synthetized sequences will be then characterized to evaluate their thermal stability, unfolding/folding reversibility etc., by standard techniques like UV-Vis, CD, fluorescence, DSC, and their interaction with either wtHTT or mHTT with different-length Q-stretches will be studied by biophysical methodologies (ITC, SPR, thermophoresis).
Furthermore, to evaluate the in vivo activity of these aptamers, we will use a well stablished Drosophila melanogaster model for HD (Q128HD - FL) in which the human HTT complementary DNA (cDNA), with 128 glutamine repeats, is expressed in all neuronal tissues.

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P11

APTAMERS IN EDUCATION

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Aptamers have a well-earned place in therapeutic, diagnostic, and sensor applications, and we now show that they provide an excellent foundation for education, as well. Within the context of the Freshman Research Initiative at the University of Texas at Austin, students have used aptamer selection and development technologies in a teaching laboratory to develop technical and soft skills appropriate for research scientists from virtually their first days on campus. In the FRI, course-based undergraduate research experience, approximately 35 freshman-sophomore students a year participate in a yearlong aptamer research experience, learning the theory and techniques to perform in vitro aptamer selections, as well as general aptamer characterization studies and project-specific application development. One of the extraordinary aspects of this program is that students often develop their own projects, and take ownership of their own science in what would otherwise be a conventional teaching lab setting. One of the many successes of this work includes the isolation and characterization a novel calf intestinal alkaline phosphatase (anti-CIAP) aptamer by an undergraduate researcher. This work will highlight the positive student outcomes of the research experience in our Aptamer Stream, identify the use of aptamers in other educational settings, and more particularly present the research findings relative to the anti-CIAP aptamer.

P12

TLR4 INHIBITION USING A SPECIFIC APTAMER AGAINST RECEPTOR TO SUPPRESS TUMOR DEVELOPMENT IN BREAST CANCER CELLS

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There exists a connection between inflammation and tumor development. Numerous studies have shown that tumor inflammation contributes to the initiation and tumor progression. In this context Toll like receptor-4 (TLR4) plays an important role. TLR4 belong to a family of pattern recognition receptors involved in the host defense against microbial infection. However, it expressed in tumor cells, in which TLR4 up-regulation has been detected, correlating positively with cancer progression and metastasis.

In tumor cells this inflammation and tumor development is generated by DAMPs (Damage-associated molecular patterns), molecules present at the tumor that initiate an inflammatory response, promoting downstream changes in signal cascade, in which Akt phosphorylation via PI3K, promote GSK3β phosphorylation, triggering the inhibition of β-catenin phosphorylation for possible degradation by the proteasome promoting its translocation to the nucleus where β-catenin functions as a transcription factor promoting the overexpression of metalloproteins MMP7, MMP9 and growth factors such as VEGFA, that will promote tumor progression and metastasis in breast cancer. The stimulation through the ligand also induces the activation of ERK, promoting cell survival, independently of the phosphorylation mediated by GSK3β. On the other hand, there exist another natural ligand for TLR4, the lipopolysaccharide LPS, which in immune cells is presented together with CD14 to the MD2-TLR4 complex, recognized by proteins with TIR-like domains, activating NFKB, triggering its translocation in the nucleus promoting chronic inflammation.
We study TLR4 up-regulation effect generated by DAMPs stimulation, to investigate the role in breast cancer cells, trying to knock down TLR4 using a specific aptamer, ApTLR#4FT, previously development in our laboratory, which shows antagonistic activity against TLR4. In this study, we analyze the effect that TLR4 have in signal cascade, detecting changes by addition of ApTLR#4FT, resulting in a decrease in phosphorylation de GSK3β via PI3K/Akt and ERK.

P13

MODULAR PLATFORM FOR CHEMICALLY MODIFIED APTAMERS

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Aptamers are becoming more widely used in laboratory research, diagnostic tools development and even as therapeutic/ theranostic agents. The activity of an aptamer is strictly related to its 2D and 3D structure and therefore strongly depends on its building components, i.e. nucleobases, sugars and linkers (e.g. phosphate groups). We believe that the true power of aptamers lies in the possibility of introduction of a whole range of various chemical modifications into their nucleic acid structure by substitution of standard atoms or chemical groups with artificial ones. This in turn can affect the ability of aptamers to specifically interact with their molecular targets and significantly broaden the set of potential binding partners. However, the usability of numerous modifications in currently available aptamer in vitro selection approaches is restricted due to at least a few technological and non-technological reasons, e.g. their polymerase compatibility.

To this end we have developed a modular platform termed PureApta for an easy introduction of new chemical groups into oligonucleotides structure. By modifying deoxythymidine triphosphate analogue with various chemicals using "click-chemistry"-based approach we provide an easy method for generation of fully substituted oligonucleotides. Moreover, in our case, all tested modifications were highly compatible with DNA polymerase-guided incorporation into a single-stranded DNA in a primer extension reaction. In this work, we present selected modified structures and their proven SELEX compatibility.

P14

DEVELOPING FLUORESCENT APTAMER SENSORS USING RNA CAPTURE-SELEX

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One of the main goals of synthetic biology is to re-engineer organisms to produce valuable biomolecules and chemical compounds in a cheaper and more efficient way. It has recently been shown that fluorescent RNA aptamers can be used to screen for efficient production strains in a microfluidic platform[1]. These aptamers are developed using SELEX that generates sequences binding to a wide range of target molecules with high affinity and specificity. Aptamers small size, cheap cost of production, low batch to batch variability and binding properties make them good candidates for the design of functional biosensors to detect and quantify target molecules.

However, development of small molecule aptamers has been challenging throughout the years. Recently, Capture-SELEX was developed as an alternative to circumvent classical SELEX drawbacks for the development of DNA[2] and RNA[3] aptamers. Capture-SELEX does not require any modification of the target molecule and can select structure-switching aptamers during the selection process. This structure-switching property is one of the main characteristic to develop a biosensor as refolding plays a critical role in controlling the fluorescence of the sensing domain.

The aim of this project is to establish the use of fluorescent aptamer biosensors to measure extracellular protein and/or metabolite titers in nanodroplets. This would provide a highly flexible high-throughput screening platform to significantly shorten the timelines needed to select efficient production cell lines at Novo Nordisk. The project will first consist in selecting specific fluorescent aptamer sensors using RNA Capture-SELEX for one or more defined model proteins/metabolites in the Andersen Lab at Aarhus University, then testing their applicability in nanodroplets and finally validating the selected cell lines in the high-throughput microfluidics platform at Novo Nordisk.

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P15

TOWARDS THE ENZYMATIC FORMATION OF ARTIFICIAL METAL BASE PAIRS WITH MODIFIED NUCLEOTIDES

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The expansion of the genetic code is a long-standing aim in synthetic biology that strives to create proteins with new or enhanced properties and structures as well as providing new strategies for the isolation of aptamers with enhanced properties. Here, we have developed new synthetic routes to generate two chemically modified nucleoside triphosphates: 4-carboxyimidazole triphosphate (dImcTP) and an adenine nucleotide analog bearing a pyridine moiety on the nucleobase (dPyATP). Their potency at serving for the enzymatic construction of artificial metal base pairs has been evaluated. Particularly, the dependence of various metals is tested in order to improve the enzymatic construction of the Nu-Mn+-Nu base pair and identify suitable metals cofactors and polymerases. We demonstrate that dImcTP is incorporated with high efficiency and selectivity opposite a templating dPyA nucleotide by the Vent exo□. The presence of Ag+ is strictly required for the formation of the artificial base pair. The latter can also be by-passed by the polymerase.

The compatibility of those artificial nucleotides with enzymatic synthesis is an essential prerequisite for their further use in selection experiments. The final aim of this project is to use this expanded genetic alphabet for the elaboration of modified aptamers. Thus, combinatorial selection process in presence of cancer cells will be carried out on a library of oligonucleotides containing the modified triphosphates. This process would allow the generation of new selective therapeutic aptamers with enhanced properties.

P16

CENTER OF APTAMER RESEARCH AND DEVELOPMENT (CARD)

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Aptamers are excellent tools for the detection of proteins and small molecules due to their small size, chemical stability and cost-effectiveness over conventional bioreceptors such as antibodies. The technology for the development of an aptamer mostly takes several weeks of hands-on work in the laboratory. Here we show, that we can enrich RNA aptamers binding to proteins and small molecules in a few days by using a fully automated system. Aptamers were selected for different proteins and small molecules. CARD is a non-profit center, which offers scientists to access to aptamer enrichment in a rapid and economic manner. Besides the automated Capture-SELEX process, we offer the selection of RNA and 2'-deoxy-2'-fluoro pyrimidine modified RNA aptamers for various targets. In the future, the range of our fully automated selection methods will be expanded for DNA and modified DNA. The Center of Aptamer Research and Development (CARD) is aiming to cover the need of academic and industrial researchers from biomedical, pharmaceutical, and environmental fields for affordable and customized aptamers.

P17

AN RNA LIGHT-UP SENSOR FOR PROBING CELLULAR GLYCOLYTIC-FLUX

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Tools to sense the flux of intracellular molecules are needed to study cellular signaling and physiology. A possible solution could be provided by RNA building blocks, which sense the variation of a specific metabolite and consequently bind and activate the fluorescence of small molecule dyes. These RNA-based sensing systems exhibit compelling attributes for in-vivo applications, such as gene-encodability and real-time signal modulation with logarithmic response. Furthermore, their nucleobase nature presents massive potential with regards to the design and screening of these sensors. We showcase this potential by selecting the first RNA-based sensor for Fructose 1,6-bisphosphate, a key metabolite of glycolytic flux regulation, through ultra-HT droplet screening technology. The identified sensor FBPs shows a 9-fold fluorescence light-up in the presence of FBP and capability of discrimination between highly similar metabolites. We proceed with a full-characterization of the ions and critical region required for the activity of the sensor and outline the molecular mechanism of FBPs on the nucleotide level.
While we anticipate an application of the sensor to probe FBP-flux in cell, we foresee this approach to be a starting point for the design of more sophisticated strategies for the identification of robust light-up sensors for key metabolites of cell signaling pathways.

P18

DETECTION OF TROPONIN I BY SURFACE PLASMON RESONANCE IMAGING BASED ON A SANDWICH ASSAY USING ANTIBODIES AND APTAMERS

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Myocardial infarctions are the leading cause of death in the world. Designing a diagnostic
tool, which is able to predict as fast as possible these cardiac failures is actually an important issue.
In emergency departments, cardiac troponin is the only cardiac biomarker currently measured to diagnose myocardial infarctions and used for therapeutic follow-ups of patients. This protein is the most reliable and relevant biomarker because of its high specificity and its rapid release by cardiac cells into the bloodstream after myocardial infarctions. Even when myocardial infarction is not detectable by electrocardiogram, a positive troponin assay may prove the presence of myocardial infarction. Portable and self-running devices allowing in vitro diagnostics like Point-Of-Care (POC) devices tend to be an interesting method to accelerate and optimize patient's healthcare management. Actual POC devices are not enough sensitive and reliable to rule-out patient in one hour [1]. As a result, reducing the limit of detection of cardiac troponin by developing high sensitivity assays in POC systems currently remains key points to be addressed.
Instead of solid-phase enzyme sandwich immuno-assays, we aim at developing an innovative detection mode consisting of an assay based on a molecular sandwich between cardiac troponin I (cTnI), aptamers and antibodies. We take advantage of the stability of aptamers, their ease of integration into portable microfluidic devices [2], and their capability to be amplified by Polymerase Chain Reaction (PCR) to increase the sensitivity of the detection.
Both cTnI specific reagents have been chosen. Tro4 aptamer selected by a Korean group [3] was used to capture cTnI. The recognition between the target cTnI and both probes (antibodies and aptamers) were validated by Surface Plasmon Resonance Imaging (SPRi) technique, which allowed following in real time kinetics of molecular interactions without any labeling. This technique enables the evaluation of the sensitivity of the detection to check if it is compatible with pathophysiological concentrations of cTnI. The signal amplification by PCR has been validated.

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P19

EVALUATION OF THIOLATED AND PKA-PERTURBED NUCLEOTIDES AS POTENTIAL CANDIDATES FOR AN EXPANSION OF THE GENETIC ALPHABET

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The incorporation of artificial metal base pairs in aptamer selection is an alluring prospect promising expansion of the nucleotide repertoire available in the selection pool. Unfortunately, access to DNA functionalized with metal base pairs is currently granted mainly by solid-phase synthesis. The SELEX process requires that enzymatic incorporation of artificial base pairs is possible via the polymerization of modified nucleoside triphosphates. Here we explore the possibility of using size-expanded and pKa-perturbed nucleotide triphosphates for the enzymatic construction of artificial metal base pairs. The thiolated nucleotides S2C, S6G, and S4T as well as the fluorinated analog 5FU are readily incorporated opposite a templatingS4T nucleotide through the guidance of a metal cation. 3'-end incorporation of modified bases by the polymerase Terminal deoxynucleotidyl Transferase (TdT) is also demonstrated. Thus, sulfur-substitution and pKa perturbation represent alternative strategies for the design of modified nucleotides compatible with the enzymatic construction of metal base pairs and a step towards incorporation of these artificial bases in the SELEX process.

P20

EFFICIENT EXCLUSION OF LOW-AFFINITY BINDERS IN APTAMER SELECTION BY MICROBEADS-ASSISTED CAPILLARY ELECTROPHORESIS (MACE) SEPARATION

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Herein, we successfully identified new classes of thrombin-binding DNA aptamers (TBAs) and tumor necrosis factor-α (TNFα)-binding DNA aptamers with high affinity and pharmacological activity by the systematic evolution of ligands by exponential enrichment (SELEX) with microbeads-assisted capillary electrophoresis (MACE) separation1-3.
MACE is a novel CE-partitioning method for SELEX and able to separate microbead-coupled tar-get/aptamer complexes from a library of unbound nucleic acids by CE, where the aptamer/target complexes can be detect-ed reliably and partitioned with high purity even in the first selection cycle. MACE-SELEX showed a more than 250-fold enrichment of the aptamers relative to that of conventional CE-SELEX. Three selection rounds of MACE-SELEX afforded several TBAs with a nanomolar affinity (Kd = 4.5-8.2 nM) that surpasses the previously reported TBAs such as HD1, HD22, and NU172 (Kd = 118, 13, and 12 nM respectively). One of the obtained aptamer, M08 (70-mer) or the truncated M08 (43-mer), showed a 10 to 20-fold more prolonged clotting time than other anticoagulant TBAs such as HD1, NU172, RE31 and RA36. In addition, toehold-mediated antidotes which enable rapid strand displacement was designed and developed. Anal-yses of the aptamer-thrombin complexes using both of bare and coated capillaries suggested that a large number of effi-cient aptamers can be collected only by MACE-SELEX, due to the increased interaction between the target/aptamer com-plex and the surface of capillary. TBAs discovered in the present study could serve as new

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P21

CIRCULAR BIVALENT THROMBIN APTAMERS HAVING NUCLEASE RESISTANCE AND HIGH ANTI-COAGULANT ACTIVITY

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Aptamers are generated by systematic evolution of ligands by exponential enrichment (SELEX) or by in vitro selection. Usually, SELEX involves repeated rounds of the following steps: (i) incubating a large random sequence library with the target, (ii) partitioning of target-binding sequences, (iii) amplification of the sequences by polymerase chain reaction (PCR), and (iv) sequencing in order to identify sequences of aptamers. Typically, SELEX requires up to twenty selection rounds to furnish aptamers, and is thus very laborious. To shorten the time required to obtain aptamers, and to increase the efficiency of the selection, a number of modifications have been developed that improve the basic procedural steps of SELEX. Among the aforementioned points, the partitioning of the target-aptamer complexes is a particularly critical step for the rapid enrichment of the aptamers in SELEX. Although capillary electrophoresis (CE)-SELEX represents so far the most efficient separation method, its success remains restricted by several limitations. During the CE separation, the identification of the aptamer/target complexes by UV or fluorescence detection is generally difficult due to the low concentrations of aptamer/target complexes. Thus, undetected aptamer/target complexes may be blindly collected within a relatively broad collection window that may also contain low-affinity aptamers or even free oligonucleotides. In addition, target molecules applicable to CE-SELEX are limited, as a large zeta potential shift upon binding is required to separate aptamer/target complexes from free oligonucleotides.

Herein, to acquire thrombin-binding aptamer candidates with higher affinity for anticoagulant therapy rapidly, we have developed a robust SELEX system with microbeads-assisted capillary electrophoresis (MACE).1, 2 During the MACE separation, an incubated mixture of target-coupled microbeads and an oligonucleotide library are directly introduced into a capillary. Since the elution time of the target-coupled microbeads is significantly different from that of the oligonucleotide library, the aptamer/target complexes can be identified by UV detection using the absorbance change that originates from the light scattering of the microbeads. Thus, the target-bound aptamers can be effectively separated and collected even in the first selection round. After three rounds of MACE-SELEX, the aptamer with 10 to 20-hold higher anticoagulant activity than previously reported TBAs was successfully discovered. In addition, we succeeded in the development of bivalent aptamer with the obtained aptamers.

However, aptamers are susceptible to plasma exonucleases, resulting in their lower stability in vivo. Utilizing circularization of aptamers, which were obtained by MACE-SELEX, using T4 DNA ligase, we successfully developed circular bivalent thrombin aptamers having nuclease resistance and high anti-coagulant activity.

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P22

NEW METHOD FOR UNBIASED QUANTIFICATION OF RIBOSWITCH TRANSCRIPTIONAL ACTIVITY

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In bacteria, the expression of significant fraction of genes is controlled by premature transcription termination. An interesting example of such mechanism are riboswitches; regulatory sequences, usually located within the 5' untranslated region (5'UTR) of certain genes, able to bind small cellular compounds, therefore evoking regulatory effect. In vast majority of riboswitches, interaction of ligand-binding domain with a given ligand (metabolite) causes structural rearrangements, resulting in the formation of termination hairpin and consequently, premature transcription termination. Terminated as well as read-through transcripts are detectable by standard experimental procedures (like northern blot or real-time PCR). However, an absolute quantification cannot be easily achieved by currently available methods. This fact was an inspiration for creation of a new method for direct identification and absolute quantification of transcription termination events, as a result of riboswitch activity.

The first step in the developed protocol requires a site-directed RNaseH-induced cleavage (covering transcription termination site), resulting in separation of two transcript's populations: 5' part – corresponding to the sum of terminated and read-through transcripts and 3' part, corresponding to full-length transcripts alone. The cleavage efficiency is then determined by primers flanking the cleavage site. An absolute concentration of terminated and read-through transcripts is quantified using the droplet digital PCR (ddPCR) technology. As it is shown, utilization of our protocol allows for precise and unbiased quantitative analysis.

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P23

PATTERNITY-SEQ V4.0: ANALYZING MOLECULAR EVOLUTION PROCESSES WITH UNPRECEDENTED ACCURACY

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High throughput sequencing (HTS) allows millions of sequences from several in vitro selection cycles to be analyzed. This can make it possible to identify aptamers or ribozymes in fewer cycles than ever before but also to find better ones. However, manipulating such high amount of data needs advanced bioinformatics tools. For this reason, we developed PATTERNITY-seq a bioinformatic pipeline that could extract and graphically present several patterns of molecular evolution. It can analyze classical or doped SELEX, but also branched SELEX.

Here, we will present the last optimization of PATTERNITY-seq©. From raw sequencing data, it extracts the sequences and counts their frequency at each round. Then, the sequences are grouped into families whose frequencies are also counted at each round. The analysis of the evolution of families over several rounds enables them to be ranked by a potential better evolution factor, which is not simply abundance in the last round. For every family, the evolution of several aptamer variants can be used to build an empirical genealogical evolutionary (EGE) tree that can reconstructed the evolution of variations that could appear successively to improve an aptamer. This information is also used to construct a Logo that highlights base positions that are preserved during evolution, suggesting they are crucial for the aptamer. Another Logo presents mutations that can be beneficial for the aptamer. The association of Logos and EGE trees can provide an indication of the best variants of an aptamer that are not the most abundant in the family most of the time. Finally, PATTERNITY-seq is able to predict a possible G-quadruplex structure for each family. It can also search for other possible sub-structural patterns that can be shared by several families (stem-loop, internal loop, bulge). The potential frequency of these sub-structural motifs in each round can subsequently be calculated.

By combining all these processed data, it offers an unprecedented way to better understand the effect of selection pressure and optimize the choice of aptamer or ribozyme candidates.

P24

CHARACTERIZATION OF THE OCHRATOXIN A-BINDING APTAMER AND BINDING TO ITS LIGAND

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NMR spectroscopy, isothermal titration calorimetry (ITC), and UV-Vis spectroscopy are being used to investigate the interaction between the ochratoxin-A-binding aptamer (OTA) and its ligand ochratoxin-A. Ochratoxin A is a mycotoxin produced by certain types of Penicillium and Apsergillus fungi. Found in grain, pork and a number of other sources, ochratoxin-A is one of the most abundant food contaminating mycotoxins. Ochratoxin-A is a strong neurotoxin thought to deplete dopamine levels in brain and cause oxidative damage to DNA. The OTA aptamer is predicted to be a monomolecular antiparallel G-quadruplex with a two G-tetrad core, and two tails extending from the same face of the quadruplex. Initial NMR data shows the quadruplex requires the ligand to form the correct conformation but remains in the same conformation even if the ligand falls away from the aptamer. NOESY data suggests that the two tails of the aptamer may be forming a double helix structure extending off one of the faces of the quadruplex. This hybrid-quadruplex motif has been seen recently in the literature in other aptamers. Initial ITC data shows the binding of the ligand and the aptamer to have a binding affinity (Kd) of 6 μM, binding at a 1:1 stochiometric ratio which is also supported by titration NMR data. Thermal analysis using UV-Vis spectroscopy shows a melting temperature of 55°C for the unbound aptamer, and 50°C for the ligand-bound complex. These data are being used to determine the structure of the aptamer-ligand complex, and to understand the interaction between these two molecules.

P25

HIGH AFFINITY APTAMER FOR THE DETECTION OF BIOGENIC AMINE HISTAMINE

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The importance of histamine in various physiological functions and its involvement in allergenic responses, make this small molecule one of the most studied biogenic amines. Even though a variety of chromatography-based methods have been described for its analytical determination, the disadvantages they present in terms of cost, analysis time and low portability limit their suitability for in situ routine testing. In this work, we sought to identify histamine-binding aptamers that could then be exploited for the development of rapid, facile and sensitive assays for histamine detection suitable for use at the point-of-need. A classic SELEX process was designed employing magnetic beads for target immobilization and the selection was completed after ten rounds. Following Next Generation Sequencing of the last selection rounds from both positive and counter selection magnetic beads, several sequences were identified and initially screened using an apta-PCR affinity assay (APAA). Structural and functional characterization of the candidates resulted in the identification of the H2 aptamer. The high binding affinity of the H2 aptamer to histamine was validated using four independent assays (KD of 3 –34 nM). Finally, the H2 aptamer was used for the development of a magnetic beads-based competitive assay for the detection of histamine in both buffer and synthetic urine, achieving very low limits of detection of 18 pM and 76pM, respectively, whilst no matrix effects were observed. These results highlight the suitability of the strategy followed for identifying small molecule-binding aptamers and the compatibility of the selected H2 aptamer with the analysis of biological samples, thus facilitating the development of point-of-care devices for routine testing. Ongoing work is focused on extending the application of the H2 aptamer to the detection of spoilage in meat, fish and beverages as well as its detection in saliva and blood.

P26

HRP-MIMICKING BASED DNA APTAZYMES IN DIAGNOSTICS: TOP OR FLOP?

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A DNA aptazyme consists of an aptamer domain and a DNAzyme module, in which the DNAzyme activity can be regulated by aptamer-target interaction. A few aptazymes based on HRP (horseradish peroxidase)-mimicking DNAzyme were reported for the detection of proteins such as lysozyme 1,2 or small molecules including ATP 1,2. The advantage of this approach is the autonomous detection of targets with no need for further reprocessing or sophisticated instruments. However, our attempts to repeat those experiments led to inconsistent results and no target concentration dependency was realized; leading us to suspect the reliability of DNA aptazyme as an autonomous biomarker for protein and small molecule detection. In a separate attempt, we designed ca. 200 variously designed aptazymes consisting of an aptamer targeting staphylococcus enterotoxin B (SEB) 3, protein A 4 or ATP 5 and a G-quadruplex as HRP-mimicking DNAzyme. Similarly, our newly-designed aptazymes showed inconsistent results with only a small signal improvement compared to the control. False positive signal, due to the inherent activity of the DNAzyme is generally considered to be the main problem for obtaining functional aptazymes. However, little attention has been put on this question if the affinity of the aptamer after integration into the aptazyme is still reserved. In the design process of aptazymes, part of the aptamer -as well as the DNAzyme- sequence is often blocked by a linker or trapped into a loop to allow for a conformational change upon target recognition. The secondary structure of an aptazyme is often very different from the integrated aptamer, thus the affinity of aptamer might be reduced or even blocked after insertion into the aptazyme. For this aim, we analyzed the affinity of aptazymes using ELONA (Enzyme-linked oligonucleotide assay). Our results demonstrate the reduced affinity of aptazyme to the target compared to the integrated aptamer. Incubation of the aptazyme with the target for a longer time did not recover the affinity.

HRP-mimicking based DNA aptazymes are reliable biosensors for oligonucleotide targets, and under various set of experiments, we observed reliable and design-dependent nucleotide recognition. However, our findings indicate the importance of a sophisticated design of these DNA aptazymes if they should be used as autonomous test system for proteins and small molecules. The decreased affinity of integrated aptamers besides the high background signal due to the inherent activity of the DNAzyme hinder the aptazyme specificity. Finding the golden structure of the aptazyme, in which the aptamer affinity is largely reserved but still allows for substantial conformational change upon target binding is the key. For this aim, it is essential to perform SELEX for screening the aptazyme rather than the integrated aptamer6. This approach allows screening up to 1015 aptazymes compared to few hundreds of structures, which are made through conventional adding of the aptamer to the DNAzyme through linkers.

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P27

ALTERED BIOCHEMICAL SPECIFICITY OF G-QUADRUPLEXES WITH MUTATED TETRADS

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A fundamental motif in canonical nucleic acid structure is the base pair. Mutations that disrupt base pairs are typically destabilizing, but stability can often be restored by a second mutation that replaces the original base pair with an isosteric variant. Such concerted changes are a way to identify helical regions in secondary structures and to identify new functional motifs in sequenced genomes. In principle, such analysis can be extended to noncanonical nucleic acid structures, but this approach has not been utilized because the sequence requirements of such structures are not well understood. Here we investigate the sequence requirements of a G-quadruplex that can both bind GTP and promote peroxidase reactions in the presence oh hemin and hydrogen peroxide. Characterization of all 256 variants of the central tetrad in this structure indicates that certain mutations can compensate for canonical G-G-G-G tetrads in the context of both GTP-binding and peroxidase activity. Furthermore, the sequence requirements of these motifs are significantly different, indicating that tetrad sequence plays a role in determining the biochemical specificity of G-quadruplex activity. Our results provide insight into the sequence requirements of G-quadruplexes, and should also facilitate the analysis of such motifs in sequenced genomes.

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P28

SELECTION OF APTAMERS AGAINST FRAGMENTS OF CIGUATOXIN FOR THE DEVELOPMENT OF APTAMER BASED SANDWICH ASSAY

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Ciguatera is a human intoxication caused by the ingestion of a variety of reef fish. The disease is characterized by severe neurological, gastrointestinal, and cardiovascular disorders. Globally, more than 50,000 people are estimated to suffer annually from this type of poisoning, making it one of the largest scale food poisonings of nonbacterial origin. Whilst the gold standard for the detection of ciguatoxins is a mouse bioassay of lipid extracts of potentially contaminated fish, an immunoassay kit was recently launched for the detection of ciguatoxin. Aptamers have emerged as versatile molecular recognition agents offering a very attractive alternative to antibodies. The extremely low levels of ciguatoxin found in contaminated fish, coupled with an extremely laborious and inefficient extraction methods, as well as a lack of any commercial source of ciguatoxin, has hampered the development of aptamers against this important target. For this reason synthethic non-toxic haptens were used for the selection of aptamers that recognize the specific wing of ciguatoxins. Four synthetic ciguatoxin haptens conjugated to keyhole limpet hemocyanin were used for aptamer selection. For each hapten, an individual specific library was used in order to minimize possible cross-contamination during the selection process. Two different SELEX strategies using microtiter plates and magnetic beads were explored. Results obtained through the entire selection process indicate selection of aptamers that bind to each wing of a ciguatoxin, which will be implemented in analytical tools that can be deployed for use at the point of need.

P29

STRUCTURE-SWITCHING APTAMERS BINDING THE STEROID HORMONE TESTOSTERONE

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Since their discovery almost thirty years ago, aptamers have been widely exploited for the detection of a variety of targets ranging from small molecules to whole cells. The classic SELEX strategy typically used is suitable for many targets but there are many challenges associated with the discovery and validation of aptamers binding small molecules. The immobilization of these small molecules to facilitate the selection process can lead to significant structural changes of the targets, thus hindering the detection of the native, unmodified molecules. On the other hand, even when the selection is successful, there are limited assays which are suitable for the characterization of potential aptamer candidates, whereas the development of assays compatible with these targets is also quite challenging. One of the approaches used to overcome these limitations is the Capture-SELEX strategy. Using the library immobilized via DNA hybridization and the unmodified target in solution, it is possible to promote significant structural changes of the ssDNA sequences forcing them to displace to enable target binding. This property is quite advantageous since displacement-based assays can be designed for target detection. Here we present the selection of aptamers binding the steroid hormone testosterone based on Capture-SELEX in combination with high-throughput sequencing. Testosterone is the main male sex hormone and an anabolic androgenic steroid. It is involved in the regulation of various physiological processes, and its accurate detection is extremely important for general health monitoring or for doping detection. Enriched sequences after counter selection with a mixture of different steroids were identified as potential candidates and were selected for further characterization using different approaches.

P30

ANALYSIS OF LIGAND-INDUCED FOLDING AND APTACHAIN SELF-ASSEMBLY IN THE COCAINE-BINDING APTAMER

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The cocaine-binding aptamer has become a widely employed model system for the development of aptamer-based biosensors for two main reasons: (i) it has a structure-switching mechanism that depends on the length of one stem, and (ii) it binds ligands, such as quinine, tighter than its originally selected cocaine ligand. This work presents progress on two aspects of the cocaine-binding aptamer. First, we present the dynamics of the free and ligand-bound of two constructs of the cocaine-binding aptamer linked to stilbene employing fluorescence decay kinetics and fluorescence anisotropy of stilbene conjugates. Conventional fluorescence sensing of aptamers depends on emission measurements by comparing signals in the presence and absence of analytes. However, conventional techniques do not provide information on whether an aptamer is undergoing a structure-switching binding mechanism. Photoisomerization reaction mechanisms are powerful means in the development of aptamer sensors, where the absorption or emission of light is converted to detectable electrical signals. We utilized the cis-trans photoisomerization and fluorescence anisotropy of 4-acetamido-4′-isothiocyanato-2,2′-stilbenedisulfonic acid (SITS) conjugated with two constructs of the cocaine-binding aptamer. We compared the dynamics of the free and ligand-bound MN19-SITS and MN4-SITS aptamers employing cocaine and quinine as ligands. By comparing the kinetics of MN19-SITS and MN4-SITS, we demonstrate that cocaine and quinine induce a structural switching folding mechanism for MN19-SITS but not MN4-SITS. Additionally, we show the optimization of aptachain. This is where the cocaine-binding aptamer is split into two overlapping strands and assembles into an extended oligomer complex upon ligand binding. We use size-exclusion chromatography and UV thermal melts to show that the quinine-bound oligos form a larger assembly of aptachain units than in the absence of ligand. We propose that splitting aptamers into overlapping strands that form oligomers in the presence of a ligand will be generally applicable to aptamers and prove useful in a variety of biotechnology applications. Finally, we think that the change in the fluorescence decay kinetics of MN19-SITS is a powerful and sensitive tool to study the ligand-induced folding mechanism of this aptamer as well as other aptamers that also have a ligand-induced structure-switching mechanism.

P31

TWO-STATE BINDING MODEL OF DNA APTAMERS FOR THERAPEUTIC ANTHRACYCLINES

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Anthracyclines like daunorubicin (DRN) and doxorubicin (DOX) play an undisputed key role in cancer treatment, but their chronic administration can cause severe side effects. For precise anthracycline analytical systems, aptamers are preferable recognition elements. Here, we describe the detailed characterisation of a single-stranded DNA aptamer DRN-10 and its truncated versions for DOX and DRN detection. The binding affinities were determined from surface plasmon resonance (SPR) and microscale thermophoresis (MST) and combined with conformational data from circular dichroism (CD). Both aptamers displayed similar nanomolar binding affinities to both anthracyclines DRN and DOX, even though their rate constants differed as shown by SPR recordings. In addition, the formation of the aptamer-anthracycline complex seems to be made by a two-state binding model. CD recordings have supported this specific binding model and the data has suggested a specific G-quadruplex as structural basis for anthracycline binding. We concluded that the aptamer DRN-10 is a promising recognition element for anthracycline detection systems and further selected aptamers can be adequately characterised with the combined methodological approach presented here.

P32

APTAMER-MEDIATED INHIBITION OF BACTERIAL INITIATION FACTOR IF3

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Bacterial translation initiation factor IF3 is an essential protein that promotes 30S initiation complex (IC) formation. IF3 binds the 30S ribosomal subunit and modulates the fidelity and speed of the codon-anticodon interaction between the mRNA and initiator tRNA. Although IF3 functions are well understood, the role of the N terminal domain (IF3N) of the factor remains debated. We use a SELEX technique that includes modified nucleotides to develop aptamers for IF3N. Biophysical methods were applied to study aptamer-mediated inhibition of 30S IC assembly. Purified IF3N from Escherichia coli was used as target for aptamers selection. Five potential aptamers were identified and chemically synthesized. Pull-down assays using target-immobilized magnetic particles suggested four molecules as potential binders. Three aptamers showed dissociation constants in the range of 100-400 nM, as assayed by Microscale Thermophoresis (MST). Intramolecular Förster Resonance Energy Transfer (FRET) showed that all three aptamers are able to bind a double labeled IF3; however, only one molecule was able to drastically reduce the speed of IF3 binding to the 30S subunit. The aptamer affected IF1- and IF2-depended conformational accommodations of IF3 on the 30S subunit. Furthermore, the canonical codon¬-anticodon association appeared to be affected by the aptamer as assayed by rapid kinetics and MST. Altogether, our results suggest that IF3N positioning contributes with the accommodation of initiator tRNA and therefore, with canonical 30S IC formation. Additionally, the experimental scheme presented here provides a workflow to develop aptamers for studying bacterial protein synthesis targeting specific factor domains.

P33

FLEXIBLE MONITORING OF SMALL MOLECULES WITH APTAMER AFFINITY REAGENTS

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There is an increasing need to reliably detect and monitor levels of small molecules in all areas of the life sciences. This need ranges from monitoring individual patient drug levels, to food or environmental contaminant, detection. In every case, more favourable outcomes are achieved when tests are performed 'on-site' or at 'point-of-care' as immediate action can be taken. Monitoring small molecules often relies on expensive laboratory based methods such as chromatography and mass spectrometry. Simple field based diagnostic devices, such as lateral flow devices or ELISA-like assays, are widely employed as a first-pass assay to determine if more thorough analysis is required.

At Aptamer Group, we have developed an automated selection approach using non-immobilized small molecules, to generate structure-switching 'Displacement aptamers'. This method has been successfully employed to generate numerous aptamers which are highly specific to their small molecule targets. By taking advantage of the conformational change in these aptamers, various diagnostic assays and biosensor applications have been developed for high-through screening, sample detection and characterization of aptamer-target interaction.

Recently, Aptamer Group have also introduced an additional screening approach to allow rapid identification of the minimal functional binding fragment for aptamers. This approach allows us to supply our customers with the best performing 'OptimerTM'; with improved kinetic parameters and that can further reduce the cost of downstream production. We will present data to show the versatility of various assay platforms with example data from a number of different aptamer based applications; with a focus on small molecule detection and quantification.

P34

SELECTION AND CHARACTERIZATION OF CHEMICALLY MODIFIED APTAMERS TARGETING CXCL9 AS A DIAGNOSTIC TOOL FOR RENAL ALLOGRAFT REJECTION

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Rejections of renal allografts is a common poster-surgery risk factor. If diagnosed early, the successful treatment is promising. The chemokine CXCL9 (CXC-Ligand 9) is one early onset marker for the rejection of the donor organ1. Therefore, we want to develop a highly sensitive diagnostic tool for renal rejection which can be used as an early POC (point of care) test. In contrast to most antibodies, aptamers have a high thermostability and no batch-to-batch variations, thus they are an interesting tool for diagnostic tests.

One drawback of the selection of conventional aptamers is their limited chemical diversity, hence, targeting difficult molecules can be challenging 2. To overcome this drawback, we developed a novel selection technique by introducing chemical residues as modular building blocks into the DNA. The so-called click-SELEX makes use of alkyne-modified nucleotides, which can be functionalised via copper catalysed alkyne-azide cycloaddition (CuAAC) with different azide bearing building block3,4. With this method hydrophobic side chains can be introduced, and thus DNA-protein interactions are more favoured. As for most targets the best type of DNA modification is unknown, we developed a multimodal click selection which enables us the selection for one target with up to five different modifications.

Here, we demonstrate the successful selection for the human chemokine CXCL9 with a chemically modified DNA library. Two high affine binding sequences could be identified, one which binds solely if modified with aromatic residues (I29). The other sequence (G123) is binding as DNA but also with different modifications. Furthermore, enhanced binding abilities can be detected for G123 if modified with indole residues. Both sequences show also a similar affinity for CXCL11 as for CXCL9, but no binding to CXCL10, although all three chemokines bind to the same receptor (CXCR3).

Next steps include binding performance in human serum and urine as well as further characterisation of the non-conventional binding behaviour of G123. Last, we want to confirm that both aptamers may bind to different epitopes, which would be important for the development of a sandwich ELONA (enzyme linked oligonucleotide assay). This opens the perspective for a point-of-care diagnostic test, to detect low amounts of the chemokine in urine and serum of kidney allograft patients.

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P35

CHARACTERIZATION OF CARDIAC TROPONIN I SPECIFIC SPIEGELMERS

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Troponins are regulator proteins of striated muscle; thus, their appearance in the circulation indicates muscle cell damage. The troponin complex is composed of three protein subunits and two of those have cardiac muscle specific isoforms hence could be applied as highly-selective markers of myocardial infarction. Accordingly, various systems have been developed for fast and sensitive detection of cardiac Troponin I (cTnI) and T (cTnT) proteins.

The common feature of the various troponin level measuring methods is their antibody demand.

We aimed to pave the way for development of a completely spiegelmer-based sandwich assays for cTnI detection. To this end, spiegelmers were selected for a N and a C terminal epitope of cTnI previously in our laboratory. Following screening of candidate oligonucleotides, three of the most promising spieglemers were synthetized with biotin labeling.

Further characterization of the spiegelmer candidates was accomplished by using the label free Bio-Layer Interferometry (BLI) technology. The obtained data demonstrated that the Kd values of analyzed oligonucleotides are in the low nanomolar range.

Next, AlphaLisa technology was leveraged to demonstrate if the selected spiegelmers are suitable for cTnI detection in a sandwich type arrangement. These studies showed that spiegelmers, which were generated for the two different epitopes of cTnI protein could selectively detect the cardiac muscle specific Troponin I protein. Furthermore, the spiegelmers remained to be selective even in human plasma containing samples.

These results suggest that spiegelmers could replace antibodies in the next generation of cardiac Troponin I diagnostic devices.

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P36

THIAMINE RIBOSWITCH FOR CONTROLLING TRANSGENE EXPRESSION IN THE GREEN ALGA *CHLAMYDOMONAS REINHARDTII*

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The green alga Chlamydomonas reinhardtii is an attractive host organism for the production of high value compounds. For it to reach its full potential as biotechnological host, the repertoire of genetic tools to engineer this organism needs to be expanded. Our aim is to develop novel conditional systems for controlling gene expression in Chlamydomonas. Recent work from our group has shown that the CrTHI4 thiamine riboswitch can be used to repress expression of a reporter gene (in this case an antibiotic selection marker) when thiamine is added to the culture (Mehrshahi et al., submitted). Furthermore, Mehrshahi et al. constructed chimeric riboswitches by swapping the aptamer of CrTHI4 for that of other eukaryotic riboswitches, and showed that these devices are functional, supporting the idea that riboswitches have a modular nature. In this poster, we present follow-up work that aims to further characterize this riboswitch as a molecular tool. Using fluorescent proteins as reporters, we have quantitatively measured how this device responds to thiamine supplementation. In addition, with a series of mutant constructs containing deletions in the introns, we are studying the functional mechanism of this regulatory element in more detail. We believe that our efforts in developing aptamer-based conditional systems for photosynthetic eukaryotes will generate useful knowledge for the RNA biology community, as well as tools for plant biotechnologists.

P37

GENERATION OF RSV F PROTEIN SELECTIVE APTAMERS

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The rapid identification of viral infections is essential for the appropriate treatment of patients. Current viral diagnostics heavily depend on the selective detection of viral proteins, using antibodies in most cases, or the quantification of viral nucleic acid using qPCR. Both methods have significant drawbacks due to their time-consumption and cost. Using aptamers could provide an answer to the need for a receptor that is suitable for rapid virus detection. Aptamers rival antibodies in target selectivity and binding affinity, and excel in terms of robustness and cost of synthesis.

Our aim was to generate aptamers with therapeutic potential for respiratory syncytial virus (RSV) infection. RSV is the most common cause of acute lower respiratory tract infections during infancy and late elderhood. The target molecule in our experiment was one of RSV's main surface proteins, the so-called protein F, which is responsible for the host cell and the virus membrane fusion. During fusion, protein F goes under a conformational change thus two forms of the protein F can be distinguished i.e. prefusion and postfusion form. We hypothesize that RSV infection of host cells can be prevented by binding of an aptamer to the prefusion form. These two forms of protein F were immobilized onto paramagnetic beads and used as targets during SELEX. The SELEX steps were monitored for enrichment by qPCR with melting curve analysis. As the aptamer pool after the 5th selection round showed enrichment, the binding affinities of the selection steps were determined by biolayer interferometry and then Sanger sequenced. In silico analysis of the aptamer sequences were performed.

We demonstrated that the monitoring of SELEX could aid the success of aptamer selection process. The monitoring can conveniently be performed by qPCR and melting curve analysis. Our SELEX protocol yielded an aptamer pool that selectively binds the prefusion form of RSV surface protein F that was demonstrated by BLI. Further experimental work is needed to pinpoint the most promising aptamer candidate, whereas the interaction between the target protein and all of the individual sequences needs to be assessed.

The RSV protein F samples were kindly provided by Peter D. Kwong and the Structural Biology Section of Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA.
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P38

IDENTIFICATION OF NUCLEASE-RESISTANT G-QUADRUPLEX DNA APTAMER WITH VEGF A-LIKE ACTIVITY

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Vascular endothelial growth factors (VEGFs) are hypoxia-inducible secreted proteins to promote angiogenesis, in which VEGF-A is an important molecule that binds and activates vascular endothelial growth factor receptor-1 (VEGFR-1) and VEGFR-2. Most recently, it has been reported that VEGF-A is one of the essential molecules for inducing differentiation of endothelial cells from human induced pluripotent stem cells. Replacing expensive and unstable VEGFs with synthetic alternatives is important in the biomedical fields, especially stem cell-based regenerative medicines. DNA aptamers are single-stranded oligonucleotides with high affinity to specific targets such as proteins and small molecules. DNA aptamers are attractive materials that mimic the biological activity of a native protein owing to their low cost of production, thermal stability, and ease of chemical modification.

In this study, two DNA aptamers, Apt01 and Apt02, with VEGF-A-mimic activity were successfully isolated by alternating consecutive systematic evolution of ligands by exponential enrichment (SELEX) against VEGFR-1 and -2 using deep sequencing analysis in early selection round. To the best of our knowledge, this is the first report of identification of DNA aptamers with function similar to VEGF-A, which bind to multiple targets, VEGFR-1 and -2. From the results of circular dichroism spectra and ultraviolet melting curves, it was confirmed that Apt01 had a stem loop structure, while Apt02 had a G-quadruplex structure. Due to the G-quadruplex structure, Apt02 showed higher nuclease resistance than Apt01. Also, Apt02 promoted tube formation of human umbilical vein endothelial cells (HUVECs) on Matrigel as well as VEGF165. These results indicated that Apt02 has a potential to be non-protein cytokine that functions as an angiogenesis promoter.

P39

IN VIVO LOCALIZATION OF FLUORESCENTLY LABELLED APTAMERS FOR INFORMED TUMOR TARGETED DRUG DELIVERY

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A key hurdle for aptamer mediated delivery of chemotherapeutics is localization. While their smaller size potentially allows for increased penetration of the intratumoral space than bulkier targeting moieties, such as antibodies, it also results in increased renal clearance. Further consideration of potential targets, cargo, and linking groups is needed. A powerful tool to help assess the potential of certain aptamers for tumor targeting is in vivo fluorescent imaging1. As imaging techniques become more powerful and accessible, molecules of interest can be screened in real time by monitoring their localization within a live subject. Utilizing basic bioconjugation chemistry, we have stably linked near-infrared dyes to aptamers targeting prostate tumor cells. Using an IVIS imager, we have examined the clearance and localization of aptamers in multiple mouse xenograft cancer models. To further facilitate our understanding of the clearance and stability of these molecules, we have run preliminary pharmacokinetics and serum stability assays. A primary goal of these studies is to create a more informed pipeline to determine which aptamer-target combinations are likely to yield efficacious delivery.

1 Powell Gray, B. et al. Tunable cytotoxic aptamer-drug conjugates for the treatment of prostate cancer. Proc Natl Acad Sci U S A 115, 4761-4766 (2018).

P40

SELECTIVE TARGETING OF MELANOMA BIOMARKERS WITH DNA APTAMERS

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Aptamers are oligonucleotides obtained by a powerful combinatorial method known as Systematic Evolution of Ligands by Exponential Enrichment (SELEX). Aptamers are generated against a wide variety of targets. It binds to its target through 3D conformational structural forms with high affinity and specificity.
Discoidin Domain Receptors (DDRs) have been proposed as a marker of tumor proliferation and invasion in melanoma. Our collaborators demonstrated that DDR1 and DDR2 are over-expressed in A375 melanoma cells in comparison to primary human melanocytes. These receptors are involved in the activation of Matrix Metalloproteinases (MMP2/MMP9) which are responsible for cell invasion, thus involve in metastasis. In our lab, we have successfully generated DNA aptamer (C25) by Cross-Over SELEX, which specifically binds to DDR1. We further performed a SELEX against DDR1 with doped library of C25. After 5 rounds of Doped SELEX, a strong enrichment of sequences was observed. Based on this result, we selected few candidates which are under evaluation. We are also implementing various SELEX methods in order to generate In-Vivo target specific and resistant aptamer against DDR2.

P41

HIGH-THROUGHPUT SCREENING OF FLUORESCENT RNA APTAMERS

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The discovery of Green Fluorescent Protein few decades ago has revolutionized the way we do molecular biology research. Today we are witnessing a similar process in the RNA world with the development and utilization of various RNA mimics of the GFP such as Spinach and Broccoli, which bind different chemical fluorophores with high affinity and induce their fluorescence. They can be fused to RNAs expressed in the cell and used for investigation of their localisation and stability in vivo. Here we show how we repurposed commercially available gene expression microarray for high-throughput screening of fluorescent RNAs. We designed a library of all possible single and double mutants of the Broccoli RNA aptamer and a fraction of mutants of Spinach RNA, fused to a different sequence complementary to a specific probe on the microarray.

We used microscope imagining to measure fluorescence of each mutant from library in various conditions: ranges of magnesium, potassium or fluorophore (DFHBI) concentrations, pH and temperature. Collected data allowed us to recapitulate 2D structure of Broccoli - different from prediction by free energy minimization, identify crucial positions involved in forming of G-quadruplexes containing structure (responsible for fluorophore binding) and mutants with shifted emission spectrum. We also noticed interesting lack of correlation between affinity to DFHBI and brightness of the mutants. Among other possibilities such approach will potentially provide a set of well characterised aptamers which can serve as fluorescent intracellular sensors of pH or ions concentration.

P42

DEVELOPMENT OF APTASENSORS BASED ON DNA KISSING COMPLEX FORMATION

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Several reports have described the use of oligonucleotide aptamers as sensors. Some aptasensors change their structure from an unfolded to a folded state upon the specific binding of their target (usually a small molecule). The conformation switch of the aptamers signs the presence of the target and allows its quantification. Such aptaswitches derived from previously identified aptamers were reported. We developed aptaswitches taking advantage of loop-loop interaction with a hairpin named aptakiss. Both RNA-RNA and RNA-DNA combinations were previously described (Durand et al. Angewandte Chemie 2014; Durand et al. Nucleic Acids Research 2016). The aim of the present study was to develop DNA-DNA aptakiss aptaswitch complexes for nanotechnological applications.

For this purpose, we carried out in vitro selection of DNA kissing complexes using two different procedures, starting from randomized libraries containing either single hairpins or dual hairpins connected by an oligo(dA) tether. High throughput sequencing and bioinformatic analysis of these two selections allowed the identification of DNA hairpins able to form highly stable DNA kissing complexes.
Using RNA-DNA and DNA-DNA aptakiss-aptaswitch designs we engineered two combinations leading to the formation and to the dissociation of kissing complexes, respectively, upon addition of adenosine. These building blocks are of particular interest for triggered assembly or disassembly of supramolecular nucleic acid scaffolds.

P43

APTAMER DEVELOPMENT AGAINST PLASMODIUM VIVAX LACTATE DEHYDROGENASE FOR THE SELECTIVE DETECTION OF MALARIA SUBSPECIES

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Malaria, a major infectious disease caused by Plasmodium parasites, remains a major cause of morbidity in developing countries and a health risk to millions travelling through endemic regions each year. Among the five Plasmodium species known to infect humans, P. vivax and P. falciparum are the most widespread causes of malaria. While P. vivax is rarely life-threatening, liver dormant parasites lead to relapse infections not prevented by typical malaria prophylaxis. Separately, P. falciparum malaria may be fatal if treatment is delayed after the onset of symptoms. Treatment of each infection type requires rapid, accurate and inexpensive diagnostic testing able to differentiate between Plasmodium species.

Antibody-based rapid diagnostics tests (RDT) developed to detect malarial metabolic enzymes: aldolase, histidine-rich-protein-2 (HRP-2) and Plasmodium-specific lactate dehydrogenase (pLDH) offer a realistic point-of-care approach to malaria diagnosis at low cost. Separate from aldalose- and HRP2-based tests, pLDH RDTs detect all human-infecting malaria species, are not limited by antigen expression or genetic polymorphism and may be used to measure drug sensitivity against malaria. In addition, sufficient differences in P. falciparum and P. vivax LDH antigenicity have allowed for the differential detection of the two malarial species. Current pLDH RDTs utilizing antibody-based detection are not without limitations, exhibiting decreased sensitivity at low parasite densities, low thermal stability, and a high cost of production.

Oligonucleotide aptamer-based recognition of pLDH has made strides to overcome these difficulties and currently enable preferential detection of P. falciparum LDH over P. vivax variants. To extend aptamer-based differentiation of pLDH detection, the work herein describes the generation of aptamers evolved against a P.vivax LDH species-specific peptide epitope and whole recombinant protein. Aptamer selection combined stringent counter-selection SELEX, high-throughput sequencing of each enrichment round, and magnetic-bead binding assays to identify sequences of high affinity and specificity to P.vivax LDH. The selected aptamers hold promise as biorecognition elements in malaria diagnostic devices for the detection and differentiation of P. vivax malaria infections.

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A NOVEL SINGLE DNA APTASENSOR FOR WATER QUALITY MONITORING

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As water pollution is ever increasing, and emerging contaminants are added to the European Community watch list, novel methods for water quality monitoring are needed. Sensitivity, robustness, rapidity and multiplex functionality are among the most crucial criteria for the detection and monitoring of these contaminants. With a view to fulfill these needs, we develop an aptasensor combining our original single DNA biochip with aptamers.

This biochip is based on the Tethered Particle Motion technique. Beads tethered to spots patterned on a glass slide through single DNA molecules undergo Brownian motion in a flow cell. The amplitude of motion of the beads, which is function of the DNA tether effective length, is monitored using video-microscopy and image analysis. This powerful tool is used to study the conformational dynamics of DNA molecules (1, 2).

Combined to recognition molecules our biochip could constitute a specific and sensitive biosensor. We chose to work with aptamers for their numerous advantages over other molecules like antibodies, namely that they are more stable and easily functionalized.

To obtain a first proof-of-concept we chose the two thrombin aptamers, HD1 and HD22, both thoroughly described in the literature and widely used for the development of biosensors. We designed different configuration assays, inspired by ELISA tests. The HD22 aptamer is grafted on the DNA tether, and the beads coated with either the HD1 aptamer for a "sandwich" assay, or a partially complementary oligonucleotide to HD22 in a "competition" test. As the specific target is recognized, a significant change in the motion amplitude of the beads is expected.

We obtained the full validation of both assays. This marks a decisive step towards the development of a generic platform with a broad panel of possible uses in the fields of water pollution monitoring, defense, food safety, and health care.

(1): Plénat T, Tardin C et al., High-throughput single-molecule analysis of DNA-protein interactions by Tethered Particle Motion. Nucleic Acids Research 40, e89 (2012).

(2): Patent n° FR 1057031 filed September 3rd, 2010,
"Biopuces pour l'analyse de la dynamique de molécules d'acide nucléique" ; extension PCT/EP 2 611 940 B1, on Apr 8, 2015

P45

TARGETING DISCOIDIN DOMAIN RECEPTORS WITH DNA APTAMERS

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Melanoma is the cause of the majority of deaths from skin cancers. The prognosis for patients with a metastatic evolution of the disease is poor. Moreover, according to projections melanoma incidence will increase in the coming decades. It is crucial to find new targets and develop alternative strategies to treat patients.

Melanoma aggressiveness is mostly consecutive to tumor invasion of the neighboring parenchyma. During invasion, cancer cells use invasive and proteolytic structures called invadosomes to invade the surrounding extracellular matrix (ECM)–rich stroma and tissues. Invadosomes are dynamic subcellular structures composed of actin microdomains formed at the contact of ECM and able to degrade ECM elements. Collaborators have recently highlighted a new, linear, organization of invadosomes that form when cells are seeded on type I collagen fibrils, a major component of dermis. It has been also demonstrated that the discoidin domain receptor 1 (DDR1) controls the formation and activity of linear invadosomes in different cancer cells, independently of its tyrosine kinase activity.

The DDR receptor family belongs to the large group of receptor tyrosine kinases and is composed of two members, DDR1 and DDR2. DDRs only bind collagens in their native physiological triple-helical conformation. The DDRs are considered as collagen sensors, but also participate in many cellular processes including cell proliferation and adhesion. A number of recent studies have shown that DDRs are often upregulated in various cancers, such as breast, lung or colorectal cancers, making them a target of interest to develop theranostics tools.

Our team has developed a DNA aptamer targeting DDR1 extracellular domain. The communication will present our selection protocol, which involves alternative supports for candidate sorting and the use of massive parallel sequencing and doped-selex to shorten the selection progress.

P46

A NEW SURFACE DNA-BASED BIOSENSOR ASSAY FOR THE KINETIC CHARACTERIZATION OF APTAMER FOLDING AND INTERACTIONS

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Therapeutic and diagnostic nucleic acid aptamers are designed to bind tightly and specifically to their target. To obtain a deeper understanding of the binding behavior of aptamers, structural and kinetic analyses of aptamer interactions gain more and more importance. Here, we show how the DNA-based switchSENSE biosensor enables the detailed kinetic characterization of aptamer folding and interactions with proteins, small molecules and even ions (kon, koff, KD) using the well-characterized thrombin-binding-aptamer (TBA) as a model system.
By observing the conformational change induced by cations in real time, we resolve the folding kinetics of the aptamer, and find that the affinity for potassium (kon = 15 M-1s-1, koff = 0.1 s-1, KD = 8mM) is one magnitude higher than for other ions. We find that thrombin binds to its aptamer with an extremely fast on-rate and a KD in the pM range (kon = 4 x 108 M-1s-1, koff = 6 x 10-2 s-1, KD = 154 pM) in the presence of potassium. Thereby, we achieve an unprecedented fit quality by applying very fast flow rates and low surface densities, which prevents artifacts from mass-transport limitation. In agreement with expectations from literature and our ion-induced folding observations, we find a strong dependence of the thrombin-TBA interaction on type and concentration of the present cations.
Easy regeneration of the sensor surface by hybridization and a quick exchange of analytes allows to test the structure and sequence specificity of protein targets. Reversing the assay by immobilizing the target protein, further facilitates aptamer screening. In conclusion, we show that switchSENSE technology can provide detailed kinetic and structural insights about aptamer folding and interactions.

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NANOPARTICLE-APTAMER CONJUGATES AS A TOOL FOR HEAVY METALS DETECTION

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In recent years, oligonucleotides have been widely used as the receptor molecules for heavy metals detection in environmental monitoring. However, the question of their selectivity is still open especially when detection of target ions happens in presence of other ones. In present work, analytical methods based on the use of conjugated aptamers with gold nanoparticles were characterized on specificity. To provide experimental work, the chosen oligonucleotides (poly-C, poly-T and G,T-rich aptamers) were conjugated with gold nanoparticles with average diameter of 31.1±4.0 nm. The resulting conjugates interacted with heavy metal ions. The interaction was confirmed by spectrophotometry (changing optical density of aggregated gold nanoparticles at 595 and 620 nm) and transmission electron microscopy. Spectrophotometry was chosen as the registration method due to surface plasmon resonance of gold nanoparticles. The selectivity of lead determination has been established by the replacement of the target ions with other ions at the concentrations close to the real environmental conditions (from 10 ng/mL to 1 mg/mL). The advantages of the proposed approach, which determine its practical relevance, are rapidity (duration of the analysis is 1 min), easy analysis and simple visual evaluation of the obtained results.

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DISCOVERY OF TUMORICIDAL DNA APTAMERS BY EFFECT-DIRECTED IN-VITRO EVOLUTION

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Our current model of drug discovery is challenged by the relative ineffectiveness of drugs against highly variable and rapidly evolving diseases and their relatively high incidence of adverse effects due to poor selectivity. Here we describe a robust and reproducible platform which could potentially address these limitations. The platform enables rapid, de-novo discovery of DNA aptamers evolved in-vitro to exert specific biological effects on target cells. Unlike conventional aptamers, which are selected by their ligand binding capacity, this platform is driven directly by therapeutic effect and selectivity towards target vs negative target cells. The process could, therefore, operate without any a-priori knowledge (e.g. mutations, biomarker expression, or known drug resistance) of the target. We report the discovery of DNA aptamers with direct and selective cytotoxicity towards several tumor cell lines as well as primary, patient-derived solid and hematological tumors, some with chemotherapy resistance. Aptamers discovered by this platform exhibited favorable biodistribution in animals, persistence in target tumors up to 48 hours after injection, and safety in human blood. These aptamers showed remarkable efficacy in-vivo as well as ex-vivo in freshly obtained, 3D cultured human tumors resistant to multiple chemotherapies. With further improvement, these findings could lead to a drug discovery model which is target-tailored, mechanism-flexible, and nearly on-demand.

This work has been submitted to Science and is available on:
https://www.biorxiv.org/content/10.1101/629105v1.full

P49

MULTIPLEX DETECTION OF SMALL MOLECULES COMBINING APTAMERS KISSING COMPLEX AND SURFACE PLASMON RESONANCE IMAGING TECHNOLOGY: CHALLENGING ON THE RAPIDITY AND THE SENSITIVITY

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Detection of small molecules such as toxins and pesticides, can have high impact on the quality control process in agri-food industry and by consequence on the human being health. Food sample is one of the most challenging material to be analysed. Many analytical approaches are investigated in order to reach more and more sensitivity in the shortest time.
In the following work a proof of concept has been established to demonstrate the detection of small molecules in multiplex format and in label-free way. Indeed, an aptaswitch designed to be specific to the adenosine nucleoside was immobilized on a SPRi-Biochip and analysis of its binding with adenosine and its specific aptakiss was monitored by a Surface Plasmon Resonance imaging (SPRi) system. Thanks to the multiplexing feature of this technique, it can easily be extended to multiple different aptaswitches immobilized on a single biochip.

Aptaswitch is a special aptamer design that folds into a hairpin shape upon binding to its target molecule. The aptaswitch-target complex is specifically recognized by an RNA hairpin named aptakiss through loop-loop or kissing interactions.
The choice of SPRi technique is mainly driven by the advantages to study molecule binding in label-free and multiplex conditions. This leads to reduce the time analysis and thus the cost of the measurement. Nevertheless, its performances are limited for small molecules detection and very low target concentration. Aptamer kissing complexes strategy can especially help SPRi in increasing the sensitivity detection, as the formation of the complexes will amplify the signal by adding molecular weight.

Key words: aptamers, kissing interactions, adenosine, Surface Plasmon Resonance imaging (SPRi).

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IDENTIFICATION OF DNA APTAMERS AGAINST PLASMODIUM FALCIPARUM

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Malaria still remains as one of the main causes of mortality in many developing countries. Caused by parasitic infection of Plasmodium species, the current diagnostic gold standard is light microscopy of peripheral blood smears, which is time and labor intensive, and needs prepared personnel. Alternatively, antigen-based rapid tests have limited sensitivity and do not provide quantitative measure; and PCR-based molecular methods, although sensitive, demand for highly trained personnel and costly reactives. Accordingly, malaria massive screening will require new rapid, sensitive, simple and economically affordable methods, able to detect even asymptomatic infected patients and low-density infections, and such goals demand screening for new bioreceptors. Antibody production often involves the use of laboratory animals and is time-consuming and costly, especially when the target is Plasmodium, whose variable antigen expression complicates the development of long-lived biomarkers. To circumvent these obstacles we have applied the Systematic Evolution of Ligands by EXponential enrichment (SELEX) method to the rapid identification of DNA aptamers against Plasmodium-infected red blood cells (pRBCs). Aptamers are expected to display higher dry-storage and lyophilisation stability than antibodies, being certainly suitable biomarkers for diagnostic applications. Five 70 bp-long ssDNA sequences with highly specific binding of fixed pRBCs versus non-infected erythrocytes have been identified.

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THE USE OF HT-SELEX TO INVESTIGATE THE EFFECT OF DIFFERENT SELECTION STRATEGIES ON THE DEVELOPMENT OF APTAMERS FOR SMALL MOLECULES

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Selecting high-affinity nucleic acid aptamers for small molecule targets is inherently challenging. The development of high-throughput sequencing technologies has enabled researchers to investigate the effect of different selection parameters on the in vitro evolution of a nucleic acid library. Herein, we use high-throughput SELEX (HT-SELEX) to elucidate the effect of selection pressures on a random library over the course of SELEX when targeting a small molecular target. To achieve this, we compared seven different selection strategies across a stringency gradient and assessed the effect of each strategy on the resultant products of SELEX using bioinformatics analyses. The selection pressures tested included incubation temperature, mutagenesis PCR, washing stringency and negative and counter selection steps. Briefly, seven aliquots of the same library were subjected to seven rounds of selection against a small molecule environmental contaminant under the selected strategies. The libraries were sequenced using Illumina Next Seq at every round (including before selection) and the resultant reads were analyzed to assess the number of unique sequences, the nucleotide composition and the presence of highly enriched candidates. Enrichment patterns were analyzed for all strategies. There were significant differences within the enriched candidate aptamers in terms of the frequency of enrichment and the types of predicted secondary structures. The highest stringency strategies (those which included counter selection, negative selection or both as well as higher stringency washing) showed far greater enrichment across the final two SELEX rounds compared to low stringency strategies which did not include these pressures. For example, the average enrichment fold-change for the two most stringent strategies was 718.19 ± 157.61 and 592.16 ± 118.69 respectively, while the corresponding values for the two lowest stringency schemes were 147.43 ± 20.80 and 96.03 ± 21.01 respectively. There were also differences in the average delta Gibbs free energy of the predicted secondary structures. Low stringency strategies produced aptamer candidates with average delta-G values ranging from -2.27 to -3.95 kJ mol-1 while high stringency strategies produced candidates with average values from -4.58 to -5.34 kJ mol-1. These results indicate that higher stringency strategies produce aptamer candidates that are able to form more stable secondary structures. Furthermore, lessons regarding the effect of selection events, library quality control and controls for experimental design, were identified for future investigation. The overall results from this study have elucidated the most efficient methodology for selection of aptamers for small molecules.

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NEW OPTICAL METHODS FOR DETECTING APTAMER TARGETS AND CONTROLLING THEIR CATALYTIC FUNCTION

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We report on two new optical approaches for detecting the presence of, or controlling the catalytic function of, aptamer targets. In each case, we utilise well-known thrombin-aptamer interactions to demonstrate these effects.

The first of these uses the technique of Linear Dichroism (LD) spectroscopy in which an LD signal can be obtained by the alignment in flow of anisotropic particles such as the M13 bacteriophage, (ca. 1μm in length by ca. 7 nm in width).[1] The coat protein of the M13 phage was first covalently linked to multiple copies of a 21-mer strand of DNA, with these then annealed to functionalised strands of either TBA or HD22, DNA aptamers that bind predominantly to thrombin exosites I and II respectively. Subsequent studies indicated that alignment in flow of these functionalized M13 phages was dramatically affected by the presence of thrombin, thus allowing thrombin to be sensed at low (pM to nM) concentrations.

The second approach involves functionalisation of the aptamer TBA with two anthracene groups to enable reversible photocontrol over the catalytic clotting function of thrombin.[2] Various modifications of TBA are known to allow external control over thrombin function but until now, anthracene photochromism has not been studied with aptamer-based systems.[3] Light-triggered anthracene photodimerisation was found to distort the aptamer structure, which inhibited the binding of thrombin and in turn triggered thrombin catalysis and the resulting clotting process. Various spectroscopic, gel and clotting studies will be presented in support of these findings.

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IMPROVEMENT OF SPECIFIC LOADING AND DRUG RELEASE USING APTAMER-BASED LIPOSOMES

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The major limitation of liposome technology is to encapsulate and deliver sufficient therapeutics to reach therapeutic dose at the target site. In this study, we demonstrate drug-binding aptamers can actively load drugs into liposomes. We designed a series of DNA aptamer sequences specific to doxorubicin, displaying multiple binding sites and various binding affinities. The impact on drug loading, drug release and therapeutic efficacy was investigated. This proof-of-concept was first demonstrated with doxorubicin and applied to tobramycin, a hydrophilic drug suffering from low encapsulation into liposomes.

Methods. A series of aptamers was designed and custom synthesized. Affinity constants were measured by the quenching of doxorubicin's fluorescence upon addition of aptamer. Cationic liposomes (DOTAP/cholesterol/DSPE-PEG2000) were incubated with each aptamer at various charge ratios to form lipoplexes. Aptamer encapsulation efficiency and stability was determined by fluorescence assay. The drug loading capacity was determined by fluorescence after incubation at various aptamer/drug ratios, for doxorubicin and tobramycin-Cy5, respectively. In vitro release of doxorubicin from lipoplexes was studied at pH 5 and pH 7.4. In vitro therapeutic efficiency was evaluated by cell viability measurements on HeLa cells.

Results. The aptamers displayed an affinity constants ranging from 68 to 380 nM for doxorubicin, and 1.15 nM for tobramycin, showing the specificity of the aptamer sequence to the drug. The binding ability of aptamers was preserved when incorporated into cationic liposomes, and resulted in a 16 and 6-fold improvement of encapsulation efficiency of doxorubicin and tobramycin, respectively, as compared to classical liposomes. Encapsulation efficiency reached 85%, which is similar to the pH-gradient active loading strategy. In addition, kinetic release profiles and cytotoxicity assay on HeLa cells demonstrated that the release and therapeutic efficacy of liposomal doxorubicin could be controlled by the aptamer's structure.

In conclusion, we demonstrate in this study that aptamers specific to a drug can be designed to improve the loading capacity of liposomes and enhance their therapeutic efficiency. Since aptamer can be synthetically prepared against about any target, this strategy may be applied to a large variety of drugs.

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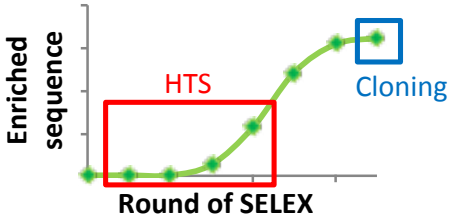

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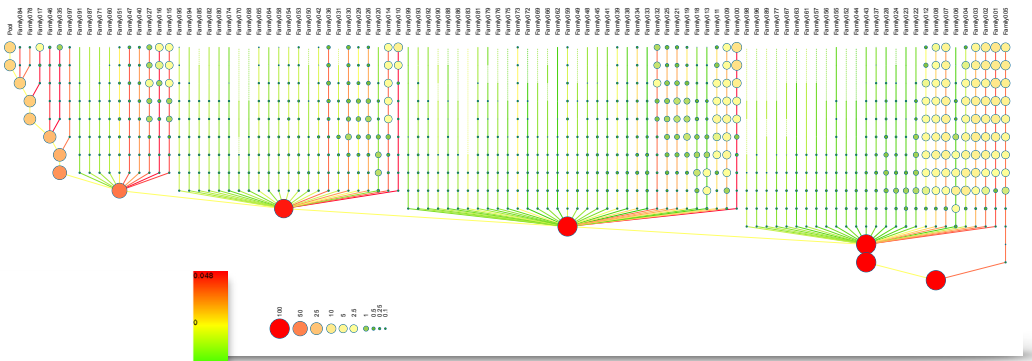

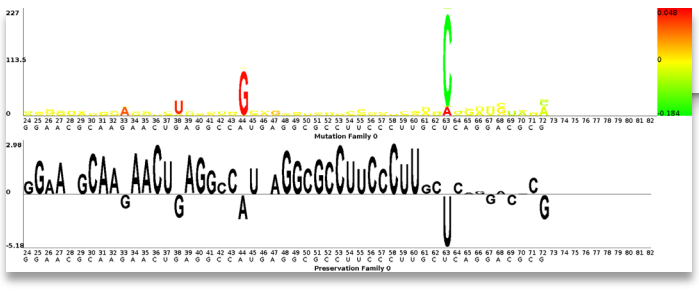

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