Biochimie 145 (2018) 2–7

Contents lists available at ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Meeting Report

Aptamers in Bordeaux 2017: An exceptional *"millésime"*

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ARTICLE INFO

Article history:
Received 8 November 2017
Accepted 22 November 2017
Available online 24 November 2017

Keywords:
Aptamer
Oligonucleotide
Riboswitch
Biosensor
Diagnostic
Pre-clinical and clinical

ABSTRACT

About 150 participants attended the symposium organised at the *Palais de la Bourse* in Bordeaux, France on September 22–23, 2017. Thirty speakers from all over the world delivered lectures covering selection processes, aptamer chemistry and innovative applications of these powerful tools that display major advantages over antibodies. Beyond the remarkable science presented, lively discussion and fruitful exchange between participants made this meeting a great success. A series of lectures were focused on synthetic biology (riboswitches, new synthetic base pairs, mutated polymerases). Innovative selection procedures including functional screening of oligonucleotide pools were described. Examples of aptasensors for the detection of pathogens were reported. The potential of aptamers for the diagnostic and the treatment of diseases was also presented. Brief summaries of the lectures presented during the symposium are given in this report. The third edition of this symposium will take place in Boulder, Colorado in Summer 2018 (information available at http://www.aptamers-in-bordeaux.com/).

1. Introduction - aim and scope of the symposium

A bit more than one year after the first edition of "Aptamers in Bordeaux" [1] aptamer experts from all over the world aggregated again in the reknown city of Bordeaux. The many facettes relevant to this promising class of oligonucleotides were covered, from "aptamerology" -the way to select, characterize and improve aptamers- to the most advanced applications in human health. Following a session dedicated to artificial riboswitches we heard on the one hand about the selection of aptamers that included modified bases, making the aptamer repertoire more and more diverse, and on the other hand about a bunch of processes derived from regular SELEX: SELkiss, Aptaswift, LIGS …. Characteristics and properties of aptamers raised against a very diverse range of targets (metal cations, opioids, influenza hemagglutinin, histones, factor IXa, etc …) were described. These aptamers and others were converted into biotechnological tools or integrated into devices for analytical or diagnostic purposes. Therapeutic applications were also described for cardiac surgery or for the treatment of age related macular degeneracy, multiple organ degeneracy, non-Hodgkin lymphoma or glioblastoma. Remarkably, 28 countries were represented this year by at least one participant. Thirty PhD students or post-docs competed for the three poster awards sponsored by "Novaptech" and by "Biomedicines". In addition about 40 participants from 26 biotech or pharma companies attended the symposium, a marked increase compared to the 2016 meeting. Larry Gold might be right in writing in his abstract that *"aptamers […] are approaching their moment in the sun"*.

2. Riboswitches and synthetic biology

The meeting started with talks dealing with the use of aptamers in synthetic biology and more specifically with the design of novel riboswitches to artificially regulate gene expression. Indeed engineered regulation of genetic circuits is a very exciting and active field. Among the regulatory elements for circuit design, riboswitches are prominent and elegant examples. Riboswitches consist in RNA motifs comprising a so-called "aptamer domain". The binding of a small molecule to this domain results in a conformational change which then determines the output of the downstream "expression platform". **Prof Beatrix Suess** (Darmstadt

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https://doi.org/10.1016/j.biochi.2017.11.015
0300-9084/

University, Darmstadt, Germany) described the use of capture SELEX [1] to select aptamers targeting small molecules such as antibiotics that can be used for riboswitch design. RNA-aptamer libraries were then screened *in vivo* in yeast, with a method based on fluorescence readout, to identify sequences that control translation in a ligand-dependent fashion. In the second part of her talk, she introduced new data about the design of a light responsive riboswitch. **Dr Sven Feindeiss** (Leipzig University, Leipzig, Germany) took another approach to engineer artificial riboswitches. He presented a new technique based on *in silico* prediction to transform a known aptamer sequence into a functional riboswitch. As a proof of concept, he applied his bioinformatic tool to transform the theophylline aptamer into a synthetic riboswitch able to control gene expression at the transcriptional level in *Escherichia coli*. Furthermore, he showed that these elements can be combined in tandem or tridem arrangements, which strongly reduced the background activity. He then concluded by discussing various limitations of *in silico* approaches and more specifically what could be improved to increase the fold change of regulation in response to the ligand. **Dr Alexander Taylor** (MRC, Cambridge, UK) described a series of synthetic alternatives to DNA composed of non-natural building blocks, termed 'xeno nucleic acids' (XNA), to evolve functional aptamers (XNA aptamers) and enzymes (« XNAzymes ») such as endonucleases and DNA ligases [2]. He also presented a series of applications for these non-natural compounds, for example the development of new material and in particular their use in nucleic acid nanotechnology, such as self assembly of 3D-structures [3]. Finally his presentation highlighted the great potential for XNAs, including resistance to nucleases, to provide novel tools in aptamer research as these modified nucleic acid analogs can be manipulated by recombinant enzymes such XNA polymerases or XNA-dependent DNA polymerases. A selected FANA aptamer was active against the Ebola virus. **Prof Steven Benner** (Foundation for Applied Molecular Biology, Alachua FL, USA) gave a fascinating talk on the development of artificially expanded genetic information systems (AEGIS) [4]. Due to the limited number of functional groups from the four standard nucleotides, standard RNA/DNA (collectively named NA) cannot yield a rich enough diversity for binding and catalysis. Moreover, the low information density of the four letter genetic system appears to diminish the performance of NA molecules due to ambiguous/alternative inactive folding. In order to overcome these limitations of natural NA, both modified NA bases with additional functional groups and new base pairs were synthesized in Benner's lab over the last 25 years. In parallel, enzymes to replicate, transcribe, reverse-transcribe AEGIS oligonucleotides were developed as well as molecular tools for sequencing them. Having every tool in hands, SELEX with AEGIS libraries was carried out against several targets. MDA-MD-231 breast cancer cell line, the toxic form of anthrax protective antigen [5] and Glypican 3 expressing liver cancer cells were targeted. The results clearly demonstrate that incorporating AEGIS nucleotides helps both improving the specificity of the aptamers and decreasing the number of selection rounds needed. Moreover, ambiguous folding is reduced leading to a better functionality of these AEGIS aptamers.

Dr Juewen Liu (University of Waterloo, Waterloo, Canada) presented an interesting work on the selection of metal-binding aptamers, a challenging task as metal ions cannot be immobilized without impacting their coordination potential. For circumventing this limitation, Dr Liu made use of catalytic DNA selection. Using this process, he was able to select after a few rounds the first RNA-cleaving DNAzyme using cerium, a trivalent lanthanide metal ion, as the sole metal co-factor. Interestingly, this lanthanide-dependent DNAzyme needs also Na^+ to work. Using 2-aminopurine as a label, Na^+ concentration was monitored [6]. Moreover, a phosphorothioate modification at the cleavage site allows this lanthanide-dependent DNAzyme to detect specifically thiophilic metals. In addition to the lanthanide-dependent DNAzyme story, Juewen Liu also presented a catalytic DNA selection process that leads to the obtention of a silver DNAzyme with an impressive detection limit of 25 nM [7]. He is developping aptamers against a wide range of lanthanides, performing only 5 SELEX rounds.

3. Selection methodology and chemically modified aptamers

Researchers at SomaLogic, demonstrated the benefit of introducing functional groups mimicking amino acid side chains at one of the four nucleic acid bases (uridine) for the obtention of SOMAmers (Slow Off- rate Modified Aptamers) [8]. **Prof Larry Gold** (Somalogic, Inc., Boulder, CO, USA; see Fig. 1) indicated that 5000 somamers were identified and that the structure of three crystallized aptamer-protein complexes was solved. Modified nucleotide side chains create generally intramolecular motifs (benzyl zipper, hydrophobic cluster) [9]. The association with the target protein is not driven by electrostatic interactions. Surprisingly somamers are poorly structured and show few, even no Watson Crick pairs. Larry Gold then elaborated on globularity, a characteristic of enzymes and a challenge for catalytic RNAs. He described the properties of short hairpins containing benzyl-dU in their loop that turn out to be extremely stable, even more than the tetraloops GNRA and UNCG. **Dr Bharat Gawande** (Somalogic, Inc., Boulder, CO, USA) presented a new development of the SOMAmer selection. The possible benefit of the introduction of two different modifications on two bases was questioned. Several selections were performed against proprotein convertase subtilisin/kexin type 9 (PCSK9) with libraries containing modifications at the C5 position of either dC or dU or both dC and dU [10]. It was demonstrated that some doubly modified SOMAmers (hydrophobic side chain on cytidine combined with a tyrosine-like side chain on uridine) resulted in a high affinity (Kd lower than nanomolar). Moreover, two modified SOMAmers exhibited a large target epitope coverage thus improving sandwich pair identification. Among the sandwich pairs tested, the best exhibited an affinity in the picomolar range.

Several talks were dedicated to new selection methodologies. Light up aptamers are aptamers that, upon binding, dramatically increase the fluorescence quantum yield of fluorogenic molecules that are non fluorescent in the free state. However a very limited fraction of aptamers to fluorogens translate their association into fluorescence emission. **Dr. Michael Ryckelynck** (University of Strasbourg, Strasbourg, France) described a powerful functional screening based on microfluidic-assisted compartmentalization for the selection of RNA light up aptamers. Each candidate is individually encapsulated into picoliter water-in-oil droplet where it is PCR-amplified and transcribed. After addition of the fluorogen molecule into each droplet, light-up RNA aptamers are collected in a high throughput manner (up to several millions variants are analyzed per day). Mutants of iSpinach and Mango light up aptamers with improved fluorescent properties were uncovered using this approach [11]. Moreover, an efficient iSpinach-derived biosensor for theophylline detection was obtained thus demonstrating the efficiency of this approach for the selection of light up aptamers. Microfluidic-assisted selection constitutes an efficient and quick method for the identification of functional aptamers as far as a fluorescent reporter is available. However pre-selection is required as the method does not allow handling the complexity of 10^{14} candidates in starting pools routinely used for selection. This is actually also the case for the method called APTASWIFT described by **Dr Christin Rath** (University Freiburg, Freiburg, Germany) based on affinity screening without prior need for cloning or sequencing.