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Account/Revue

Nucleic acid based tools for pharmacology and nano-engineering☆

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ABSTRACT

We have developed modular assays based on aptamer displacement or protein-dependent reporter ribozymes for the screening of small molecule inhibitors. Thereby, chemical space can be explored in a rapid, focused, and modular manner, by indirectly taking advantage of the highest molecular diversity currently amenable to screening, namely that of 10^{16} different nucleic acid sequences. In this account, we discuss the application of these approaches to find new inhibitors for target proteins. Examples showing that these modulators can be used as tools for gaining novel biological insight are provided. We also discuss some recent data on interlocked DNA architectures such as entirely double-stranded DNA rotaxanes.

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1. Introduction

Aptamers were first described in 1990 [1,2] and named with a neologism built up from the Latin expression “aptus” (to fit) and the Greek word “meros” (part) [1]. Aptamers are short single-stranded nucleic acid molecules that can fold into complex, well-defined three-dimensional shapes [1,2]. They form binding pockets and clefts for the specific binding of any given molecule. Targets that are recognized by aptamers can range from metal ions and small chemicals to proteins, viruses or whole cells [3]. These target molecules are bound with high specificity and affinities in the nano- to picomolar, and sometimes even femtomolar range [4]. Aptamers therefore constitute a class of functional nucleic acids with molecular recognition properties comparable to monoclonal antibodies or Fab-fragments.

Nucleic acid aptamers are accessible by enzymatic procedures or by entire chemical synthesis. *In vitro*, aptamers can be obtained by searching mixtures or so-called “libraries” of many billions of different nucleic acid sequences through a process called *in vitro* selection or SELEX, an acronym for Systematic Evolution of Ligands by Exponential enrichment. A library of up to 10^{15}–10^{16} different nucleic acid sequences is generated by automated chemical synthesis. Aptamer libraries display a much higher diversity than any other combinatorial library and thus have an increased probability to successfully isolate a target-binding sequence. The challenge is to select active nucleic acid molecules from this huge diversity. Following combinatorial solid-phase synthesis, the aptamer library is incubated with the immobilized target molecule and tightly binding sequences are separated from unbound ones (Fig. 1). Subsequently, bound aptamers are collected and amplified enzymatically. This library, enriched for target-binding sequences, can then be used for the next selection cycle. Depending on the nature of the target molecule, the selection procedure has to be repeated up to 15 times. Individual aptamer sequences can be obtained from the enriched library by cloning and standard sequencing techniques [5] (Fig. 1). The SELEX process has successfully been used to select aptamers against many different targets but has also some inherent

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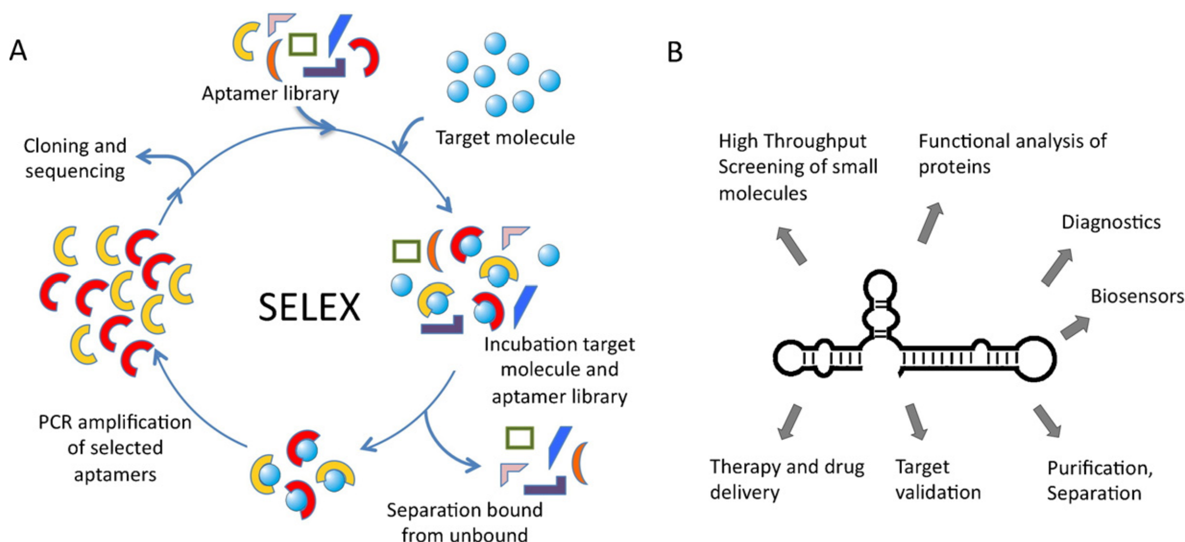

Fig. 1. SELEX procedure and aptamer applications. A. Nucleic acid libraries are incubated with the target molecule, binding sequences are separated from unbound and amplified by PCR. Selected sequences can be used in additional cycles. B. Aptamer applications ranging from basic research to medicinal therapy.

disadvantages. One is the uncertain prediction of the success of the selection. This is mainly due to conformational instability of target proteins, since aptamers recognise defined 3D-shapes [6]. Another problem, reducing *in vivo* applications so far, is aptamer stability under physiological conditions. To tackle this problem, aptamers can be chemically modified with a variety of functional groups that improve their stability in various biological compartments, enhance the chemical diversity and increase the capacity to recognize their targets [7–10]. Modifications of aptamers are introduced either at the phosphate/ribose backbone or at the nucleobases. Phosphothioates in the DNA backbone enhance the stability against nucleases and the cellular availability. RNA aptamers are most prominently modified at the 2'-ribose, as this position strongly contributes to aptamer stability [11]. Recently the use of locked nucleic acids (LNA) for PCR and *in vitro* transcription has been described. LNAs contain a methylene ether that bridges the 2'-oxygen atom and the 4'-carbon atom, thereby fixing the ribose in *endo* conformation [12]. The development of enzymes that allow the adaptation of LNAs for SELEX process will enable the selection of LNA-based aptamers and thereby further expand the chemical diversity and binding capabilities.

Over the last several years, aptamers have become increasingly important in both basic research and biomedical application and are expected to have an even higher impact in the future [5,6,13–15]. Aptamers have been used for various purposes in diagnostics, biosensing, chemistry, and molecular biomedicine. These include the application of aptamers as inhibitors of target molecules, which is particularly important for target validation and functional characterisation in cellculture experiments and *in vivo* [5]. In addition, aptamers are applied in drug discovery, purification and separation purposes and for method development (Fig. 1). Aptamer-ligand binding is often attended by structural alterations of the aptamer that can be converted into a detectable signal. Through direct coupling of molecular recognition to signal generation, aptamers can be enabled to function as versatile reporters [13,15]. It is also envisioned to utilize aptamers as drugs or as tools for drug-delivery [6,16].

The following chapters address two recent examples from our research, FACS-based SELEX and aptamer displacement, demonstrating how aptamers can be applied in screening procedures. In the final part, we extend our research to nano-engineering based on interlocked, entirely double-stranded DNA architectures.

2. Aptamer displacement

To identify molecules of biological or medicinal interest, our previous and current aim is to develop approaches that combine evolution of biomolecules with small molecule screening. Using the SELEX process, aptamers are isolated *in vitro* out of the highly diverse nucleic acid libraries by adaptation to external conditions [7,16]. *In vitro*, aptamers can serve as specific inhibitors of any protein target. While the delivery of aptamers into cells is as straightforward as analogous delivery methods for other functional nucleic acids, particularly siRNAs, the delivery-problem of functional aptamers into tissues or whole organisms is still largely unsolved, hampering their broad therapeutic or clinical application for intracellular targets. This renders aptamer approaches suited only for limited clinical use. Compared to nucleic acid or biopolymer-based inhibitors, small molecules are highly advantageous with respect to cell permeability and chemical diversity. On the other hand, direct screening of small molecules can be tedious and often screening methods need to be tailored to the corresponding drug target [16]. To combine the advantages of small molecules and the aptamer selection process, we developed methods to convert an aptamer-protein complex into a small molecule

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