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Customised nucleic acid libraries for enhanced aptamer selection and performance

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Aptamers are short single-stranded oligo(deoxy)nucleotides that are selected to bind to target molecules with high affinity and specificity. Because of their sophisticated characteristics and versatile applicability, aptamers are thought to become universal molecular probes in biotechnological and therapeutic applications. However, the variety of possible interactions with a putative target molecule is limited by the chemical repertoire of the natural nucleobases. Consequently, many desired targets are not addressable by aptamers. This obstacle is overcome by broadening the chemical diversity of aptamers, mainly achieved by nucleobase-modifications and the introduction of novel bases or base pairs. We discuss these achievements and the characteristics of the respective modified aptamers, reflected by SOMAmers (slow off-rate modified aptamers), clickmers, and aptamers bearing an expanded genetic alphabet.

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Introduction

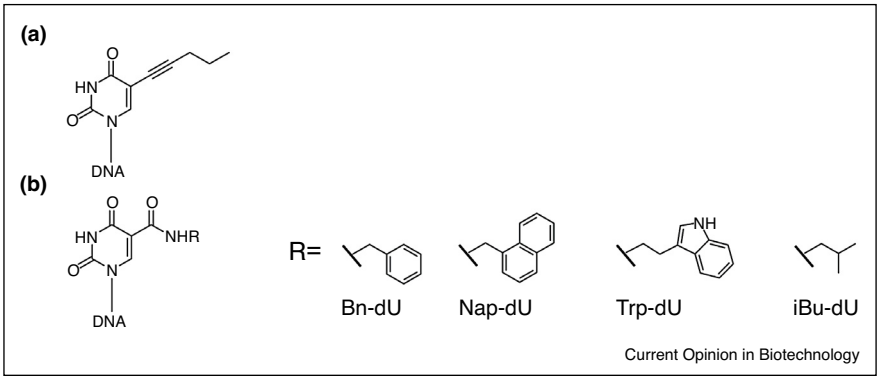
Aptamers are short single stranded oligo(deoxy)nucleotides that bind to their target molecules with high affinity and specificity [1,2]. With possible targets ranging from small molecules over proteins and peptides to whole cells, they are regarded as an alternative to antibodies in many biotechnological and molecular biology applications. In contrast to antibodies, aptamers are lowly immunogenic and thermostable [3]. The fact that aptamers are identified by an *in vitro* process, together with the possibility to synthesize aptamers chemically allows their cheap production and avoids problems like batch-to-batch variations, which are known for antibodies [4]. As RNA and DNA aptamers are prone to degradation by endogenous nucleases early efforts focused on modifications of the sugar-phosphate backbone to increase aptamer stability [5]. Common variations are 2′-modifications, 2′-4′-bridging, 2′-3′-ring opening, and phosphorothioate backbone modifications [6]. We refer to current excellent reviews on this topic for further reading [5,7•,8,9].

Aptamers have a limited chemical repertoire as they consist of only four different nucleobases, which are quite similar in regard to biophysical properties and a sugar-phosphate backbone. In contrast, proteins are built from 21 different amino acids with a heterogeneous chemistry set, which enables a multitude of interaction possibilities with a target of interest and the creation of specific microenvironments, for example hydrophobic pockets and low or high pH-value clefts. While ionic and metal ion bridged interactions have also been observed in aptamer-target complexes [10], hydrophobic interactions and those with negatively charged sites are rare. These observations may illustrate the limited success rate of selection experiments in regard to protein targets [11••]. In the following, we will give an overview on techniques that have recently been developed to enhance the interaction possibilities of the natural nucleobases, namely chemical modifications and aptamers with an expanded set of nucleotides.

Aptamers with modified nucleobases

To overcome the limited chemical repertoire of nucleic acids, modifications of nucleobases have been developed that are compatible with the enzymatic steps of a selection experiment, that is polymerase chain reaction (PCR). These modifications increase the chemical diversity of nucleic acid libraries and, hence, the interaction properties of resultant aptamers. The first nucleobase-modified aptamer was described by Latham *et al.* in 1994 [12]. In this important study, a DNA library in which thymidine was replaced by 5-(1-pentynyl)-2′-deoxy-uridine was employed for the selection of aptamers binding to thrombin (Figure 1a). Although the resultant aptamer was found to interact with a lower affinity with thrombin compared to a previously identified native DNA aptamer [13], its interaction properties were shown to be related to the presence of the pentynyl moiety [12,14].

Figure 1



Structures of modified nucleobases. **(a)** 5-(1-pentynyl)-2'-deoxyuridine was the first modified nucleobase which was used for aptamer selection. Here, the C5-position is modified with a 1-pentynyl side chain [12]. **(b)** Examples of deoxyuridine building blocks used for the generation of SOMAmers. Modifications are attached at the C5-position via an amide linker [11••].

Hydrophobic contacts, which are frequently observed in protein–protein interactions [15] are rare between aptamers and their target molecules. This limitation has been overcome in an innovative selection process that utilizes so-called SOMAmers (Slow Off-rate Modified Aptamers) as a new generation of aptamers. SOMAmers have chemically modified deoxyuridine residues that bear amino acid like side chains [11••]. A series of artificial nucleobases that bear amide-linked side chains differing in size, polarity and interaction capability at the C5 position of deoxyuridine has been synthesized (Figure 1b). However, the direct amplification of these modified DNA templates with nucleotide mixtures also containing the respective modified dUTP leads to shortened DNA fragments. This drawback was resolved by applying a two-step PCR-amplification procedure, in which the modified DNA template is initially amplified using the canonical set of nucleotides, that is dATP, dGTP, dTTP and dCTP. The resultant dsDNA is then converted into modified DNA by a subsequent PCR, in which dTTP is substituted by a particularly modified dUTP [9]. In this way, the amplification of randomised and modified DNA libraries has been made possible. In a seminal study, Gold *et al.* performed selection experiments comparing enrichment performances of DNA libraries that contained either the natural set of nucleotides or a modified set [11••]. This study revealed that the probability of identifying an aptamer targeting a set of supposedly difficult target proteins is significantly increased when using modified nucleobases [11••,16]. SOMAmers can also be further modified post-selectively, for example by replacing a modification at a specific nucleotide position with a slightly different chemical entity leading to SOMAmer variants with optimized properties [17–19]. For now, aromatic hydrophobic side chains have been found to be the most successful modifications in SELEX experiments [16].

Artificial nucleotides also enable the formation of novel three-dimensional structures of aptamers [16,20]. In comparison to conventional aptamers, the amount of polar contacts of SOMAmers to target molecules has been found to be decreased [20] and instead architectures with hydrophobic networks within a SOMAmer are formed, thus enabling the adoption of binding modalities that mimic those found in proteins. In these, the artificial nucleotides largely contribute to the interaction with the respective target protein [17,20]. The amide linker has been observed to contribute to the folding of SOMAmers and to the interaction with the target protein, that is by hydrogen-bonding [18,20,21•].

The SOMAmer binding to the nerve growth factor (NGF) adopts a completely new folding motif, previously not found in nucleic acid structures (Figure 2). This SOMAmer folds into an S-shaped structure, composed of three aligned strands connected by tight backbone turns (Figure 2). In contrast to other SOMAmer structures, only a few modified nucleotides interact directly with the protein. Most of them are involved in forming hydrophobic clusters (Figure 2a,b) [21•].

Recently SOMALogic reported an approach that allowed the introduction of multiple protein-like modifications into randomized regions of the applied nucleic acid libraries. Herein, the C5-position of both pyrimidines (dC and dU) is modified with different building blocks. In a first SELEX experiment, they compared several starting libraries bearing no, one, or two different modifications. The target molecule was human PCSK9 (proprotein convertase subtilisin/kexin type 9). It became apparent that the presence of two different modifications allowed the selection of ligands with higher affinities, compared to ligands with only a single modification. Additionally, the second modification enhanced the nuclease resistance of the ligands and truncation of