Aptamers for Nanostructured Biosensors

Beate Strehlitz, Christine Reinemann, Regina Stoltenburg

Helmholtz-Centre for Environmental Research – UFZ, Permoserstr. 15, D-04318 Leipzig, Germany
beate.strehlitz@ufz.de

Aptamers are ssDNA or RNA oligonucleotides featured by a very high affinity of their target binding, offer new possibilities for nanotechnological applications. Aptamers are suitable as molecular recognition elements in a wide range of analytical systems with the goal of separation and purification of molecules from complex mixtures or of detection of molecules in complex matrices. In this context, aptamers will play an important role as new receptors in biosensors [1, 2]. Moreover, aptamers have a great potential for their use in the field of medical and pharmaceutical basic research as well as clinical diagnostic and therapy (e.g. inhibition of enzyme activities, blocking of receptor binding sites).

Aptamers as nucleic acids are very attractive compounds for combinatorial chemistry. They are able to fold into defined secondary and tertiary structures, and they can easily be amplified by PCR. Very complex libraries of random sequence oligonucleotides with about 10^{15} different molecules can be produced by chemical synthesis and screened in parallel for a particular functionality, such as recognition and high affinity binding to a given target or catalytic activity. In 1990, three laboratories independently described a method for the identification of nucleic acid sequences, exhibiting predetermined properties, within large pools of randomised synthetic oligonucleotides [6-8]. This method is known as SELEX (Systematic Evolution of Ligands by Exponential enrichment) and is now widely used for the selection of aptamers, which bind their target with high affinity and specificity.

The functionality of aptamers is based on their stable 3D-structure, which depends on the primary sequence, the length of the nucleic acid molecule (smaller than 100 nt) and the ambient conditions. Typical structural motives are stems, internal loops, bulges, hairpin structures, tetra loops, pseudoknots, triplicates, kissing complexes, or G-quadruplex structures. In presence of the target, the aptamers undergo adaptive conformational changes and their three-dimensional folding creates a specific binding site for the target. The intermolecular interactions between aptamer and target are characterised by a combination of complementarity in shape, stacking interactions between aromatic compounds and the nucleobases of the aptamers, electrostatic interactions between charged groups, and hydrogen bondings [6-8].

The aptamer selection process (SELEX) is an iterative process (Fig. 1). Starting point is a chemically synthesised random DNA oligonucleotide library consisting of about 10^{13} to 10^{15} different sequence motifs. Several rounds (typically 6 to 20 rounds) of *in vitro* selection (binding, partitioning) and enzymatic amplification of oligonucleotide variants result in the enrichment of relatively few sequence motifs with the highest affinity and specificity for the target. This evolution is driven by the selection conditions (target features and concentration, buffer, temperature, incubation time, efficiency of the partition method, negative selection steps ...). The stringency strongly affects the affinity and specificity of the aptamers to be selected, and is typically progressively increased in the course of a SELEX process.

The SELEX technology has been applied to different classes of targets. Inorganic and small organic molecules, peptides, proteins, carbohydrates, antibiotics as well as complex targets like target mixtures or whole cells and organisms were used for an aptamer selection [9-13]. Aptamers can also be selected for toxic or non-immunogenic targets. Once selected, they can be produced by chemical synthesis in high amount and with high reproducibility. Multiple modifications are possible e.g. to enhance their stability or to permit the quantification and immobilisation of the aptamers. Denatured aptamers can be

regenerated. Because of these properties aptamers represent an alternative to antibodies regarding analytical applications.

With this presentation we will show several results of our aptamer selections and some examples of their application in biosensors and assays.

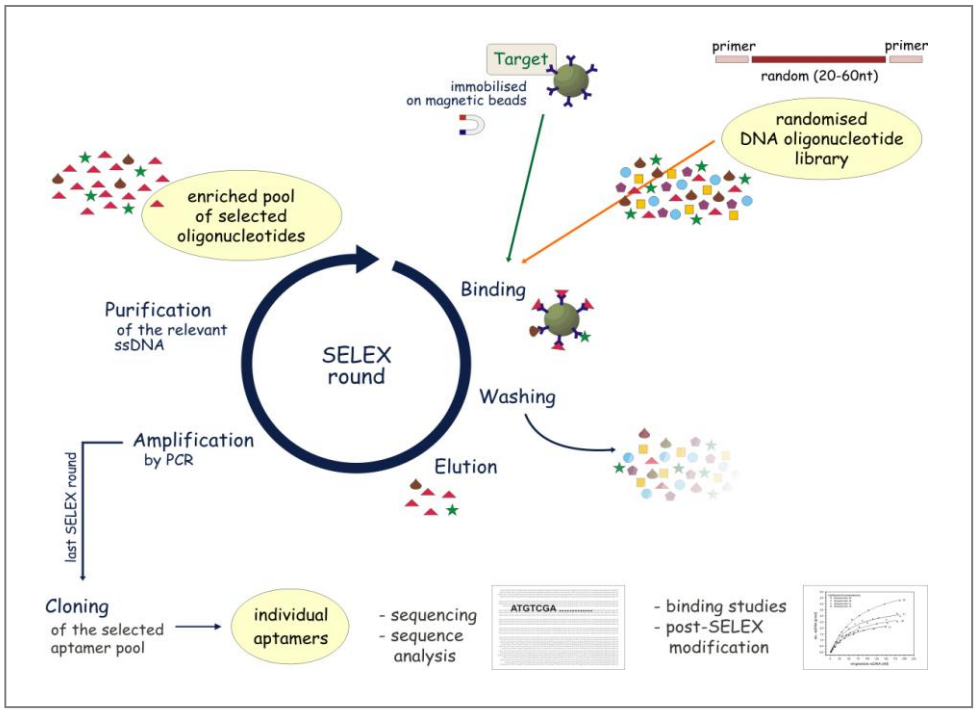

Fig. 1: SELEX process for the selection of target-specific DNA aptamers. The starting randomised oligonucleotide library is directly used for binding with the target molecules (in this case immobilised on magnetic beads) in the first SELEX round. Unbound oligonucleotides are removed by several stringent washing steps of the binding complexes. The target-bound oligonucleotides are eluted and subsequently amplified by PCR. A new enriched pool of selected oligonucleotides is generated by purification of the relevant ssDNA from the PCR products. This selected new oligonucleotide pool is then used for the next selection round. If an enrichment of target-specific oligonucleotides is observed the last SELEX round is finished after the amplification step. The enriched aptamer pool is cloned and several individual aptamers have to be characterised.

References

[1] O'Sullivan, C. K., Anal. Bioanal. Chem. **372** (2002) 44-48.
[2] Tombelli, S., Minunni, A.Mascini, A., Biosens. Bioelectron. **20** (2005) 2424-2434.
[3] Ellington, A. D., Szostak, J. W., Nature **346** (1990) 818-822.
[4] Tuerk, C., Gold, L., Science **249** (1990) 505-510.
[5] Robertson, D. L., Joyce, G. F., Nature **344** (1990) 467-468.
[6] Hermann, T., Patel, D. J., Science **287** (2000) 820-825.
[7] Patel, D. J., Curr. Opin. Chem. Biol. **1** (1997) 32-46.
[8] Patel, D. J., Suri, A. K., Jiang, F., Jiang, L. C., Fan, P., Kumar, R. A.Nonin, S., J. Mol. Biol. **272** (1997) 645-664.
[9] Famulok, M. Curr. Opin. Struct. Biol. **9** (1999) 324-329.
[10] Göringer, H. U., Homann, M.Lorger, M., Int. J. Parasitol. **33** (2003) 1309-1317.
[11] Klussmann, S. (2006) The Aptamer Handbook. Functional Oligonucleotides and Their Applications, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.
[12] Stoltenburg, R., Reinemann, C., Strehlitz, B., Biomol. Eng. **24** (2007) 381-403.
[13] Wilson, D. S., Szostak, J. W., Annu. Rev. Biochem. **68** (1999) 611-647.