Aptamer-based scaffolds for developments in nanotechnology.

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Aptamers are oligonucleotides identified in large randomly synthesized libraries containing up to 10^{15} different oligomers, through in vitro selection, a process known as SELEX (Systematic Evolution of Ligands by EXponantial enrichment). Aptamers have been successfully raised against a wide range of targets: amino acids, nucleic acid bases, proteins, intact viruses and live cells. These molecules display generally a high affinity for their target, characterized by Kds in the nanomolar range for macromolecules and in the micromolar range for small molecules. In addition they show a high specificity of recognition and can discriminate between closely related molecules. Aptamer oligonucleotides are easy to synthesize on solid support by phosphoramidite chemistry; they can be easily chemically modified, conjugated to different pendant groups that provide them with new functionnalities or grafted on various surfaces. They presently rival antibodies for many different applications in the field of bio- and nanotechnologies, including for diagnostic or therapeutic applications.

We have been working in this area for many years for different purposes (artificial regulation of gene expression, design of probes for imaging or of biosensors) [1] . We recently worked on several aspects that are of interest for aptamer developments in nanotechnology. SELEX is a tedious and repetitive process. The identification and characterization of aptamers may require several months. In order to take full advantage of aptamers it is necessary to implement methodologies that speeds up the selection procedure and increases the throughput of the screening. To this end we assembled a robot that allows the parallel selection of DNA, RNA or chemically-modified oligonucleotides against several targets. Moreover we developped a new screening procedure.

Selection is generally followed by cloning and sequencing of the enriched pool of oligonucleotides to enable the bioinformatic comparison of selected sequences. The most represented candidates are then synthesized and their binding properties are individually evaluated thus leading to the identification of aptamers. These post-selection steps are time consuming and introduce a bias to the expense of poorly amplified binders that might be of high affinity and are consequently underrepresented. We described a novel homogeneous solution-based method for screening large populations of oligonucleotide candidates generated from SELEX [2]. This approach, based on the AlphaScreen® technology, is carried out on the exclusive basis of the binding properties of the selected candidates without the needs of performing *a priori* sequencing. It therefore enables the functional identification of high affinity aptamers. We validated the HAPIscreen (High throughput APtamer Identification screen) methodology using aptamer (R06) targeted to the RNA hairpin (TAR). HAPIscreen can be adapted to any type of tagged target (figure 1) and is fully amenable to automation.

Hairpins resulting from intramolecular complementarity between two neighbour regions are recurent RNA motifs. Beyond the biological function of these structures they are also of interest for the construction of scaffolds. Indeed the apical loop at the top of the double-stranded stem is prone to interactions with single RNA regions of another molecule. Using SELEX we identified RNA hairpin aptamer (R06) that recognize a target RNA (TAR) through so-called kissing interactions between complementary loops of both the target and the aptamer. Such a loop-loop complex is characterized by a strong binding (Kd = 20 nM) and a very high specificity of recognition. The structure of the complex has been fully characterized by molecular dynamics, X-ray crystallography and NMR [3, 4, 5]. Kissing complexes can be arranged in more complex associations; we demonstrated that dimeric aptamers give rise to extremely stable complexes with a target containing two tandem hairpins [6]. This has been further developed by others for generating 3D scaffolds, taking advantage of the well defined relative geometries of the different helices (stems and loop-loop) [7].

Scaffolds can also be generated by the association of aptamers with organized synthetic polymers (foldamers). We raised aptamers against a cationic octameric oligoamide O2N–(**Q+**)8–OH which folds into a helix spanning over three turns in the solid state and in solution. This approach proved to be very fruitful. It confirmed from an independent and unbiased assay the prevalence of a specific interaction between multiturn (**Q+**)n oligomers and G-quadruplex DNA, a motif of increasingly recognized biological relevance. We demonstrated that this interaction can be made completely diastereoselective with one-

handed helices. We identified a foldamer that selectively binds to one quadruplex sequence and not to others and identified the first example of a DNA- *vs* RNA-selective G- quadruplex synthetic ligand [8]. Such a foldaptamer complex can constitute the basis for three-dimensional networks which can be further on grafted with various functional groups.

Aptamers are of interest for multiple applications; we indeed generated ^{99}Tc-aptamer probes for imaging human brain tumors [9], micro-arrays for detecting viral proteins [10], or targeting nanoparticles for theranostic treatment. The work presented here on aptamer-based nanoscaffolds will extend the potential of these tools.

References

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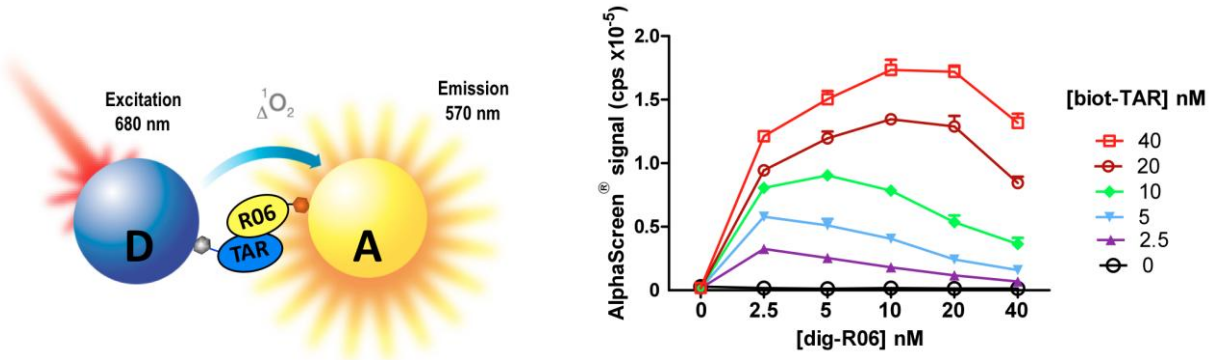
Figures



(**Left**) Scheme of the assay setup using a digoxigenin–tagged aptamer (R06) and a biotinylated target RNA hairpin (TAR). The association of the two components is detected by using both Donor streptavidin (D) and Acceptor anti-digoxigenin (A) coated AlphaScreen® beads. The production of singlet oxygen upon laser excitation by D-phtalocyanin is monitored by the fluorescence emission of A-rubrene beads. (**Right**) Results obtained when increasing concentrations of dig-R06 were added to A and D beads for different biot-TAR concentrations.