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Selection of DNA aptamers for drug detection

Fabio Spiga, Anna Ferretti, Carlotta Guiducci

Laboratory of Life Science Electronics (CLSE) - Ecole Polytechnique Fédérale de Lausanne (EPFL)

INTRODUCTION

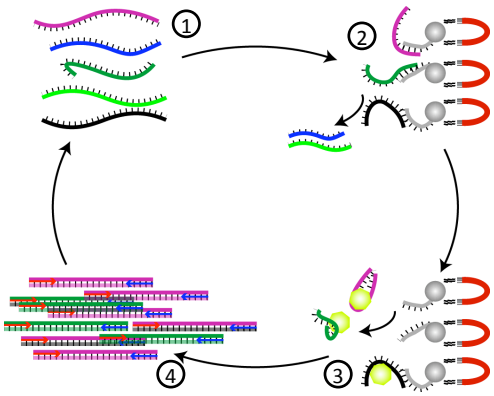
Aptamers are nucleic acid sequences with the ability to specifically bind to a target molecule against which they have been selected; they can be either RNA or DNA molecules. With respect to antibodies, aptamers present all the advantages that an oligonucleotide has over a protein: higher stability to different conditions of incubation, longer shelf life, and, once the sequence is established, the production based on chemical synthesis is highly accurate and reproducible, which is not the case for antibody.

They can also be targeted against small molecules (<800 Da), as in the case of most of the drugs, which are not immunogenic.

Moreover, they can be easily functionalized with a vast range of modifications (fluorescent labels, spacers, thiol groups, biotin…), becoming a very versatile probe.

SELEX PROCEDURE

Aptamer production is based on SELEX (Systematic Evolution of Ligands by Exponential Enrichment) technology: from a library of random sequences the one/ones with the best affinity for the target molecule are selected through a series of cycles with increasing stringency conditions. Our SELEX approach is based on Morse protocol [1], modified to be applied to DNA sequences, and our target molecule is tobramycin.



Step 1: an initial library of random sequences is digested to obtain the single strand oligonucleotide pool for the selection

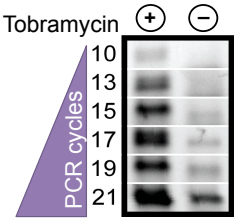
Step 2: the single strand sequence are put on magnetic beads through hybridization with a short oligonucleotide immobilized on the surface

Step 3: upon incubation with the target, aptamers that undergo a conformational change due to the interaction with the target are displaced from the beads and collected

Step 4: the so selected aptamer are amplified by PCR and used as starting material for the following cycle

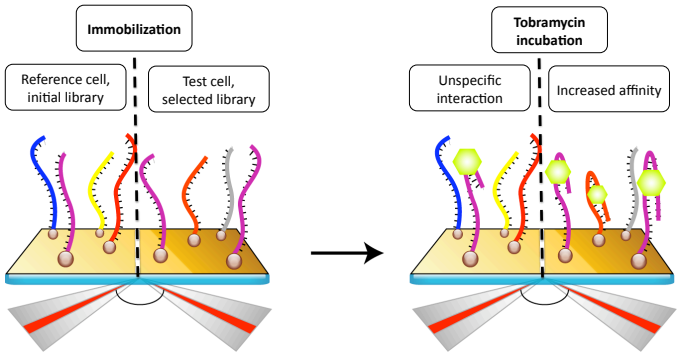
To monitor the SELEX process, during step 3 a negative control is performed by incubating the beads in absence of the target.

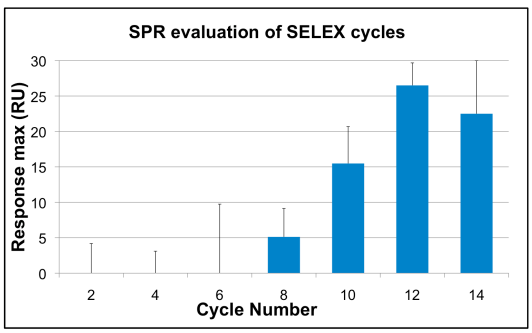
The difference in yield between positive and negative control is assessed by semi-quantitative PCR



SPR EVALUATION

To evaluate the outcome of the SELEX we employ Surface Plasmon Resonance (SPR). By immobilizing on one cell the investigated cycle and in the reference cell the starting library, we are able to see an increase in the differential signal with the proceeding of the selection.





Cycle 12 library has been sent to sequencing. After sequence analysis some aptamer candidate will be tested singularly on SPR to check for the most performing ones which will be used as probe molecules in sensors application.

Bibliography

Morse, D. P. (2007). "Direct selection of RNA beacon aptamers." Biochemical and Biophysical Research Communications 359(1): 94-101