Heavy Metals Biosensing

Pakistan – Chile iGEM Collaboration

Here we describe two methods of heavy metal cell-free biosensing based on DNA sequences known as "aptamers", which bind with high affinity and specificity to target molecules in the same way as antibodies. We want to show you some elements of these systems that we used to build a free cell genetic machinery because we think that eventually they could help you to improve your biosensing or solve some troubles that you have in your project.

I. CATIONIC SURFACTANT BIOSENSING.

The first system we want to describe consists in an aptamer arsenic-specific and a cationic surfactant (Hexadecyltrimethylammonium bromide "CTAB") assemble that form a supramolecule, which prevent AuNPs (gold nanoparticles) from aggregating due to the exhaustion of cationic surfactant. The introduction of As (III) specifically interacted with the arsenic- binding aptamer to form the aptamer–As(III) complex, releasing the cationic surfactant and allowing AuNPs to be added and cause the remarkable change in color [1]. Thus, in absence of As(III) the color is blue and in presence of it the color is red.

The sensitivity of the biosensor for As (III) detection using CTAB to aggregate AuNPs is well seen in figure 2. Color change is detected with naked eye among the solutions treated with multiple part per billion concentrations of As (III).

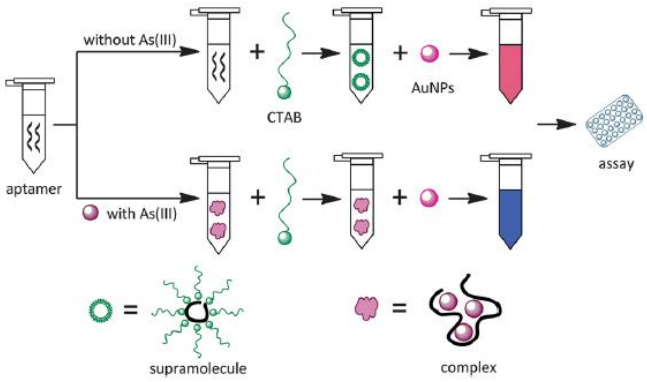

Figure 1. As (III) biosensing scheme.

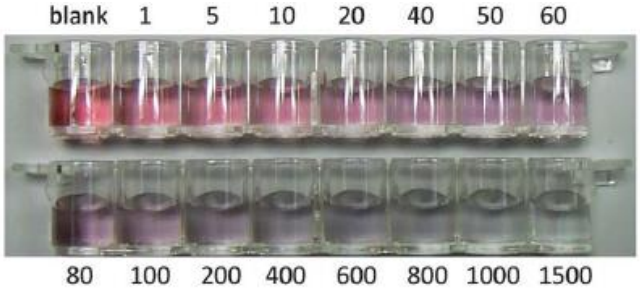

Figure 2. Colorimetric response by different concentrations of As (III) cationic surfactant biosensing.

Similar systems have been described for cadmium [2] (Cd II), where the gold nanoparticules are sequestered by the formation of a complex with a complementary sequence for the aptamer hybridized with it (figure 3).

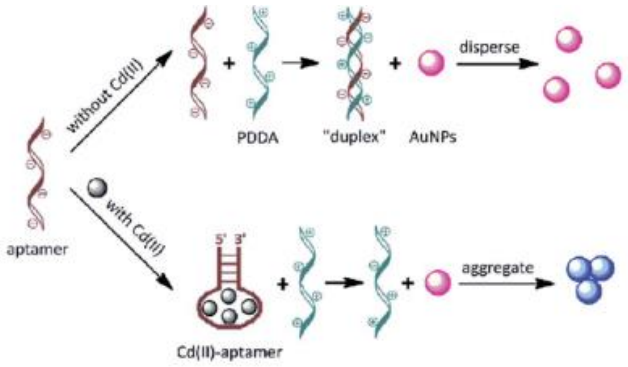

Figure 3. Cd (II) biosensing scheme.

II. DNAZYME-APTAMER BIOSENSING

DNAzymes are DNA sequences that have catalytic activity when are well folded. DNazyme-Aptamer systems have been reported for the biosensing of AMP and lysozyme [3]. This system consists of a DNAzyme with peroxidase activity (blue in figure 4), which is sequestered by the interaction with a complementary DNA sequence containing part of the aptamer required (orange) and part of the linker sequence (grey). When the target (AMP) binds to the aptamer, the complete DNA oligomer suffers a conformational change that release the DNAzyme so it can fold correctly. Finally, the DNAzyme can catalyze the oxidation of ABTS, a compound that emits a cyan color in his reduced form.

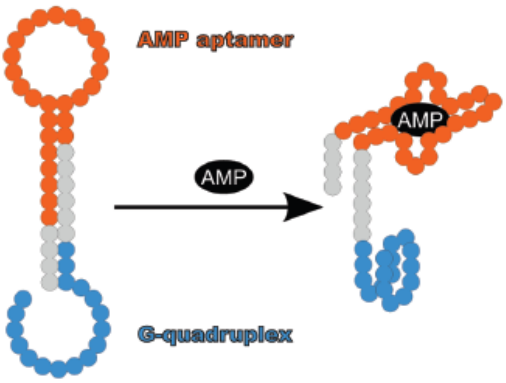

Figure 4. Dnazyme-Aptamer system for AMP biosensing.

The advantage of this system is that you can build a DNAzyme-Aptamer sequence with any molecule that have a great affinity aptamer for it, like the heavy metals aptamers that we show below.

III. Arsenic Aptamer

The major affinity sequence is [4]:

5'GGTAATACGACTCACTATAGGGAGATACCA GCTTATTCAATTTTACAGAACAACCAACGTCG CTCCGGGTACTTCTTATCGAGATAGTAAGTGC AATCT-3'.

IV. Cadmium Aptamer

The major affinity sequence is [2]:

5' GGACTGTTGTGGTATTATTTTTGGTTGTGC-3'.

V. Zinc Aptamer

The major affinity sequence is [5]:

5'GCATCAGTTAGTCATTACGCTTACGGCGGCT CTATCCTAACTGATATATTGTGAAGTCGTGTCC C- 3′

VI. Mercury Aptamer

The major affinity sequence is [6]:

5′- TTTTTTTTTT-3′

VII. Solubilizing Insoluble Heavy Metals

In several papers hydrophobic target aptamers have been reported. In 2012 a RNA aptamer was designed to bind to the insoluble form of prion protein PrP (infectious form) which causes the Creutzfeldt–Jakob disease [7]. Also, in 2014 celiac disease-triggering hydrophobic proteins aptamers where used to build a sensitive gluten detection approach [8]. The method to can select the aptamers consists in a Systematic Evolution of Ligands by Exponential Enrichment (SELEX). With this tool, the sequences with more affinity expected to the substract are selected from a pool of random sequences. Thus, you can eventually build an aptamer which binds with high affinity to the insoluble forms of the heavy metals and use it with any of the biosensing systems which we have described above.

VIII. References

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