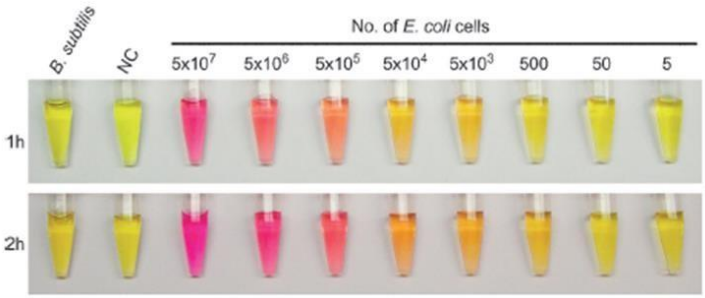
The AptaReport™

Forget Antibodies. Use Aptamers!

SPRING 2016

Bacterial Detection Litmus Test

The need to establish a cost-efficient and easy-to-use pathogen detection method is increasingly present. Early detection of microbial pathogens could be the difference between life and death for many people in resource-limited regions. An existing DNAzyme was modified to give a visible color change in the presence of the *E. coli* pathogen. This was achieved by coupling the DNAzyme to a urease enzyme. Urease is a very efficient and readily-available enzyme that produces a basic environment in the presence of urea. This DNAzyme-urease complex is immobilized on a magnetic bead, but only in the absence of the target. In this study, the target is the crude cellular extract left behind by *E. coli*. In the presence of this extract, the DNAzyme-urease complex binds to the target rather than to the magnetic bead. After magnetic separation, the solution, which no longer contains the beads, is exposed to urea and a litmus dye.



If *E. coli* was present in the initial solution, the litmus dye will change color to indicate a basic environment. *B. subtilis* was used to demonstrate that this DNAzyme only responds to *E. coli*. However, this technique can be modified using any aptazyme and litmus dye combination.

-M.E.H.

*Reference: Apta-Index™ ID #596

Real-time detection of an unlabled analyte

Creating a flow-through biosensor that allows for real-time detection of an unlabeled analyte is of great use in a variety of fields. Using quantum dots (QD) as a source of fluorescence and detection, such a system was created using aptamers for both Botulinum Neurotoxin A (BoNTA) and Ricin Toxin Achain (RTA). A flow cell containing a replaceable quartz window was coated internally with an anchor oligonucleotide. A sequence complementary to part of this anchor was added to the 5′ end of the aptamer, allowing for it to be immobilized on the tube. A detector oligonucleotide was designed to be partially complementary to the aptamer sequence, and was 5′ labeled with biotin. The last component of the biosensor is the QD-streptavidin conjugate which can bind to the detector oligo, due to the interaction between biotin and streptavidin. In the absence of the target, the aptamer will stay bound to the biotin-QDstreptavidin complex. In the presence of the target, the aptamer will release this complex in order to bind its target. Therefore, the level of fluorescence, which is easily detectable, will decrease in the presence of the target molecule. Additionally, it was shown that both BoNTA and RTA could be detected separately using one quartz window by immobilizing both aptamers on its surface and using two distinct QDs.

-M.E.H.

*Reference: Apta-Index™ ID #600, #601

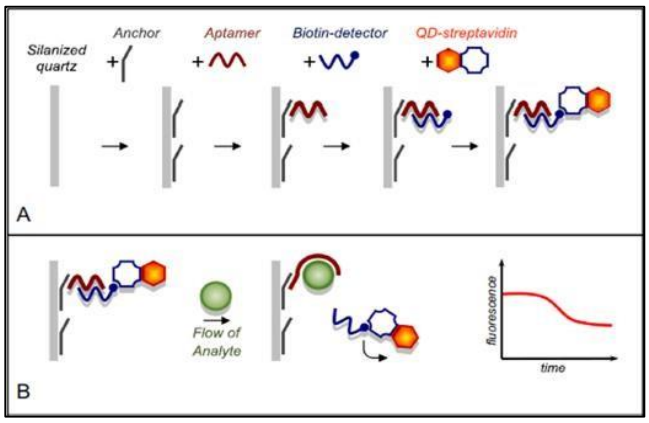


Figure 2 — Bogomolova, A., Aldissi, M. "Real-time and label-free analyte detection in a flow-through mode using immobilized fluorescent aptamer/quantum dots molecular switches." Biosensors and Bioelectronics, 66 (2015); 290296

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A PH DEPENDENT "CATCH-AND-RELEASE" SYSTEM

A 'chemomechanical sorting system' was designed that utilizes pH dependence of both an aptamer and a versatile hydrogel. The aptamer-target complex undergoes reversible binding and releasing, dependent on the pH of its environment. Similarly, the hydrogel undergoes reversible volume change based on the pH of the solution surrounding it. In basic conditions, the hydrogel is swollen and the aptamer is bound to its target. In acidic conditions, the hydrogel contracts and the aptamer releases its target. The hydrogel contains arms that are topped with the aptamer; the arms protrude up when the gel is swollen and the arms are bent when the gel is contracted. A basic solution containing the target passes over the swollen hydrogel, allowing the aptamer to 'catch' its target. An acidic solution is then passed over the hydrogel, causing contraction and 'release' of the aptamer into a separate reservoir. In this study, thrombin was the target and a well-known thrombin aptamer was bound to the hydrogel arms. However, this technology could be utilized for a wide range of targets and aptamers, lending to a variety of applications.

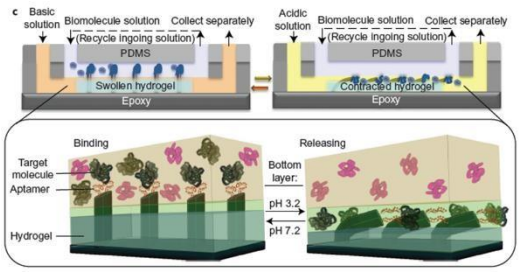

Figure 3 — Shastri, A, et al. (2015) Nature Chem.

-M.E.H.

*Reference: Apta-Index™ ID #64

ENZYME LINKED APTAMER ASSAY DETECTS AND TRACKS PARASITIC INFECTION IN MICE

Chagas Disease (CD), also known as trypanosomiasis, is a potentially lethal disease caused by the parasitic *Trypanosoma cruzi*, a protozoan common to Central and South America. Spread through insect bites, transmission of the disease is relatively common in tropical areas, and can go undetected for years. With only two decades-old drugs currently available to combat *T. cruzi* infection, scientists at the FDA sought to develop an assay that would enable researchers to detect and track infections to enable drug development. To that end, they selected several aptamers specific to the *T. cruzi* excreted secreted antigen (TESA) and conducted an Enzyme Linked Aptamer Assay in order to detect infection and track the progress of treatment of infected mice. With the specificity of the aptamers, the scientists were readily able to detect the presence of the parasite from blood samples taken from the mice. Additionally, upon treating the mice with Benznidazole (one of the two drugs currently available for treating CD) the scientists were able to track the amount of parasite remaining in various tissues of the mice, and determine whether the mice had been cured, or the parasite had gone into a chronic stage of infection. This aptamer based assay demonstrates promise for the use of aptamers in diagnostic testing, and offers researchers a reliable way to track the effectiveness of new therapeutic agents during drug discovery.

-C.B.

*Reference: Apta-Index™ ID #597

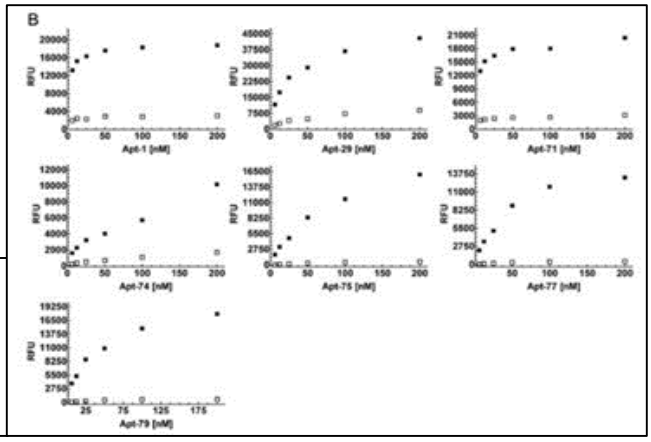

Figure 4 — Serial dilutions from 200 nM to 6.25 nM of 59-biotinylated monoclonal aptamers, Apt-1, 29, 71, 74, 75, 77, and 79, were used to perform ELA assays on polystyrene 96 well plates coated with 50 ng/ml/well, of TESA (filled squares) and BSA (open squares). Relative fluorescence units (RFU) obtained were plotted on the Y axis and each point represents the mean of duplicate values. All the aptamers showed a dose of dependent saturable binding to TESA but not to BSA. Data shown is representative of at least three independent experiments.

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