The AptaReport™

Newsletter

Fall 2020

Aptamer-Nanozyme mediated sensing platform for the rapid detection of *Escherichia Coli* in fruit juice

A new aptamer-Nanozyme mediated detection platform has been constructed to combat this problem. Previous Polymerase Chain Reaction (PCR) and ELISA (Enzyme linked immunosorbent assay), however requires trained users and complex machines making it inherently time and cost intensive. The need for a field ready and cost-effective device is evident and this application may be the solution. This device is colorimetric and can be seen with the naked eye, and costs ~$2 to produce.

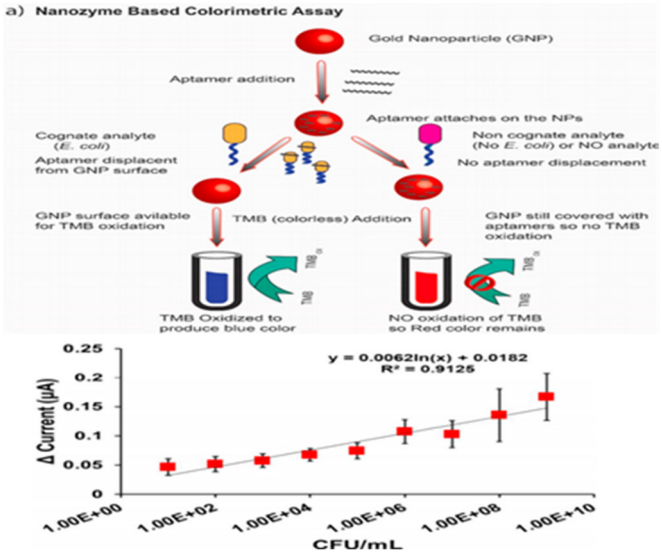

Figure (adapted) A) The colorometric assay changes color based on the addition of E.Coli C) Linear relationship with increased concentration of E.Coli. LOD: of 10 is comparable to previous studies.*

The assay is twofold in its approach: Nanozyme based colorimetric and electrochemical assay. Both use a Gold Nanoparticle (GNP) to adhere to the EC 12-31 aptamer. The assay takes advantage of TMB (tetramethylbenzidine) oxidation due to the GNP's peroxidase activity. The aptamer has high affinity and selectivity and releases from the GNP to bind to E.Coli, allowing the bare GNP surface to oxidize, creating a change from red to blue. The electrochemical assay quenches TMB with H_2SO_4 to produce an electrochemically active form and is measured using amperometry. The study tests this assay in apple juice and with an R^2 = .931, proved effective which is a promising result in the battle against food and waterborne illness caused by *E.Coli.*

*Reference: Apta-Index™ ID #7061 -J.G

Discovery of Aptamers Targeting Receptor-Binding Domain of the SARS-CoV-2 Spike Glycoprotein

SARS CoV-2 is a novel coronavirus which is responsible for tens of thousands of deaths around the world and halting life as we know it. Thus, there is an urgent need to find a vaccine to prevent further spread of this deadly virus. This study by Yang et al. has taken a promising step by discovering two new aptamers, CoV2-RBD-1C and CoV2-RBD-4C, with high affinity (5.8 nM and 19.9 nM respectively) to the receptor binding domain (RBD) of the CoV2 virus. The target for this selection was recombinant SARS CoV2 RBD. Aptamers developed for this target were discovered through competitive SELEX and then analyzed by a machine learning screening algorithm.

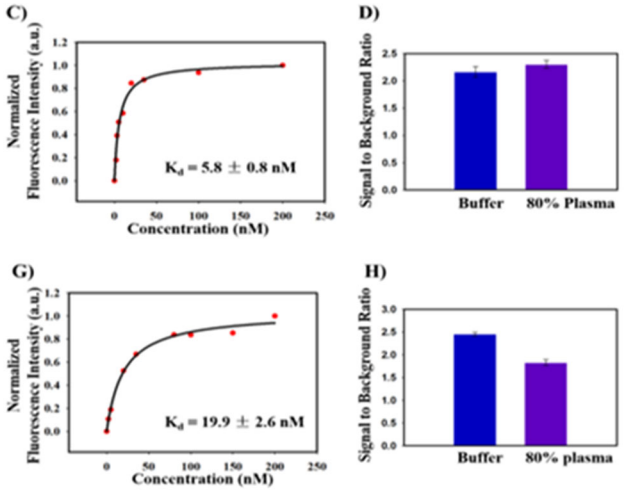

Figure (adapted) C) Affinity of truncated CoV2-RBD-1C aptamer and G) Truncated CoV2-RBD-4C aptamer using fluorescence intensity. D) Signal to background ratio of aptamer Cov2-RBD-1C and H) CoV2-RBD*

The virus causes severe acute respiratory syndrome (SARS) by binding to the Angiotensin-converting enzyme II (ACE2) present in human respiratory epithelial cells. The S-glycoprotein (S) of the RBD specifically binds to the ACE2 receptor. Therefore, the aptamers produced in this study have the potential to block the binding which causes the illness and the knowledge of this can be used in developing therapeutics or diagnostics. The aptamers tested showed high affinity in not only the selection buffer, but also 80% plasma which is a practical result for use in vivo down the line.

*Reference: Apta-Index™ ID #7058 -J.G.

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Novel System for Detecting SARS Coronavirus Nucleocapsid Protein Using an ssDNA Aptamer

A newly identified β-Coronavirus, SARS-CoV-2, receiving worldwide attention, had a recent outbreak in Wuhan, China. This is the third introduction of a highly pathogenic novel Coronavirus. This recent coronavirus uses the same receptor as SARS-CoV-1, which SARS-CoV-1 had it's first outbreak in 2002. The coronavirus encodes structural proteins that modulate innate immune signaling, alter host expression, and antagonize epigenetic regulation of hosts' cells. The nucleocapsid, N protein, stimulates an immune response in the host which indicates that it has strong antigenicity, making it a good diagnostic marker and potential target for vaccine development.

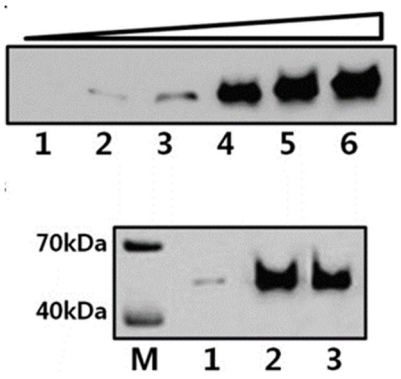


Figure (adapted) **Western blot analysis** with ssDNA aptamer, His-probe HRP, and anti-N protein. (A) Western blot analysis using aptamer 1. Various amounts of N protein were separated by SDS-PAGE and incubated with 5'-biotinylated aptamer 1 followed by streptavidin-HRP. The bands were visualized using the ECL reaction. Lane 1, 0 µg; lane 2, 0.92 µg; lane 3, 1.84 µg; lane 4, 4.6 µg; lane 5, 9.2 µg; and lane 6, 18.4 µg N protein. (B) Western blot analysis was performed to compare the efficacy of the His-probe HRP, anti-N protein, and aptamer 1. 9.2 µg of N protein was separated by SDS-PAGE and incubated with His-probe HRP (lane 1), anti-N protein (lane 2), and 5'-biotinylated aptamer 1 (lane 3), respectively. Lane M is the molecular weight marker.*

Cho et al. identified a ssDNA aptamer from an 88-mer library which was screened by SELEX using nickel sepharose beads and His-tagged N protein. After 12 cycles, 15 aptamers were identified and binding affinity measurements were carried out by ELISA assays. Based on these results aptamer 1 was chosen and a western blot analysis was conducted to determine if the selected aptamer 1 can be a substitute for the N protein antibody. The results showed that aptamer 1 bound to the N protein in dose dependent matter indicating that it could be used as an alternative to antibodies for detection of N protein. This aptamer establishes a fast and reliable detection of N protein making it useful for a more sensitive diagnosis of SARS.

*Reference: Apta-Index™ ID #7060 -B.A.

Novel Breast Cancer Aptamer Specific for Capturing Circulating Tumor Cells

Metastatic breast cancer is the leading cause of death among breast cancer patients, and there are few strategies to detect early metastasis and predict recurrence. Primary tumors release circulating tumor cells (CTCs) into the bloodstream. These CTCs in the bloodstream are related to the risk of metastasis and recurrence, and may serve as potential biomarkers. In order to capture CTCs, epithelial cell adhesion molecule (EpCAM) is the most commonly used marker for CTC detection. However, this method cannot detect EpCAM negative CTCs, which could result in false negatives. Therefore, a more sensitive and specific system is needed to detect CTCs.

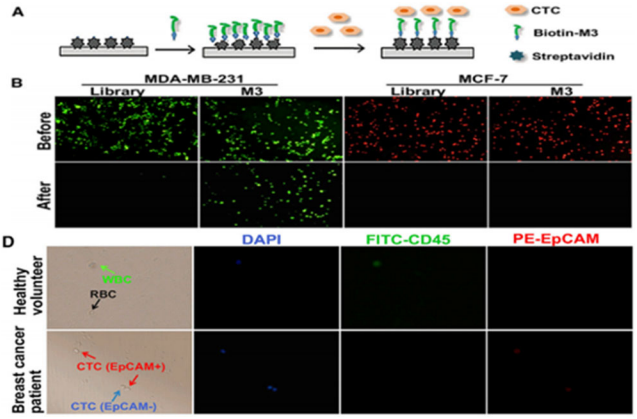
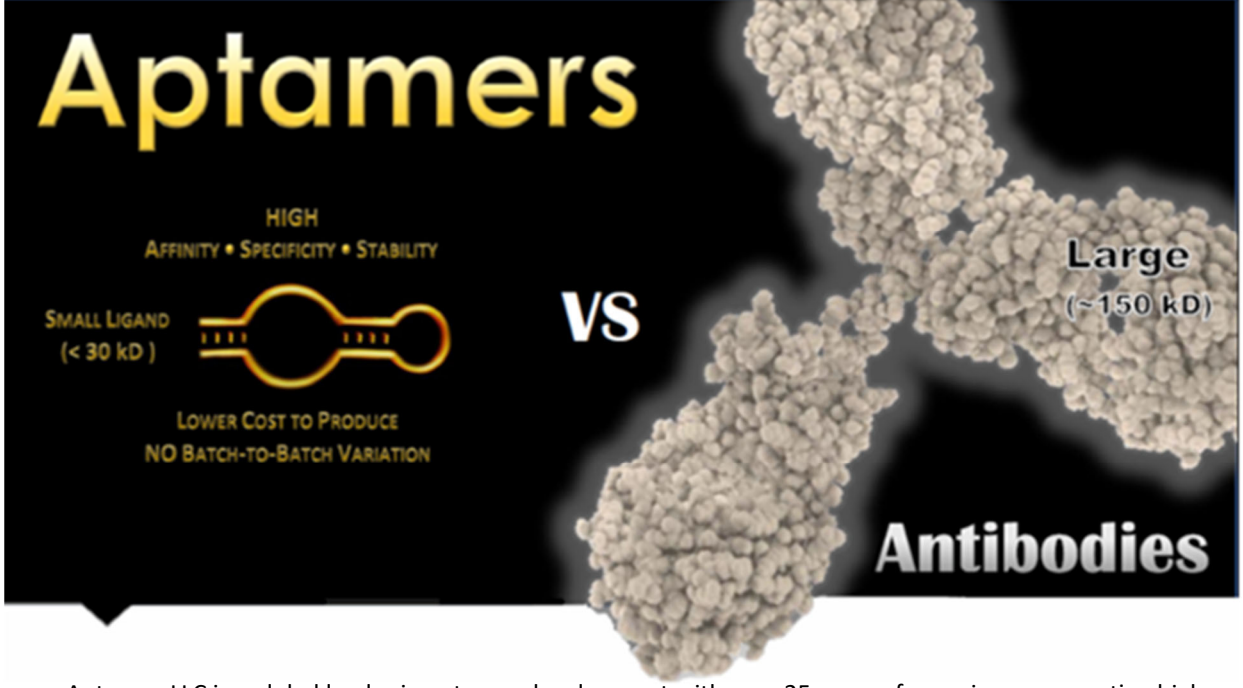


Figure (adapted) (A) Schematic diagram of the CTC capturing system. (B) The selective capture of MDA-MB-231 cells (green) on M3-coated 96 well-plate. MCF-7 cells (red) were not captured and washed away. The ssDNA library was used as a negative control. Fluorescence microscopy was used to image the results. (C) The Capture of CTCs in patient blood cells. Cells were stained with DAPI, FITC-labeled anti-CD45, or PE-labeled anti-EpCAM. The cells were imaged and identified with a fluorescence microscope at 400× magnification.*

Aptamers were selected against metastatic breast cancer MDA-MB-231 cells via Cell SELEX. Out of five candidate aptamers, aptamer M3 had the best binding affinity (45.6 +/- 1.2 nM), and was used for further evaluation. A capture system was created with M3 by immobilizing the aptamer to a 96 well-plate (Figure 1A). First, MDA-MB-231 cells (target labeled green) and MCF-7 cells (non-target labeled red) were added to the wells. The system only captured the MDA-MB-231 cells and not the MCF-7 cells; therefore, M3 was specific to MDA-MB-231(Figure 1B). In order to investigate the clinical ability of the system, real blood samples were used. The samples were obtained from 6 healthy volunteers and 25 breast cancer patients. CTCs (DAPI stained) were only detected in the patient samples and not in the healthy samples (Figure 1C). This aptamer-capturing system has the potential to clinically detect CTCs to determine the risk of metastasis.

*Reference: Apta-Index™ ID #7059 -M.D.



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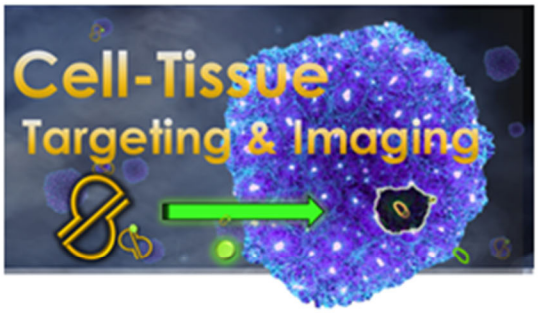

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