The AptaReport™
Newsletter
Fall 2020

Binding Detection of SARS Coronavirus Nucleocapsid Protein using RNA Aptamer-Based Detection

Reverse transcription-PCR has been used to detect the virus, but can result in many false-positives. The most accurate detection method is ELISA, this method detects the nucleocapsids (N) antibodies from samples, but this test can not detect antibodies until 2-3 weeks after infection. For earlier detection, an ELISA technique was developed that directly tested the SARS-CoV N protein. This indicated that targeting the N protein within SARS-CoV was a good diagnostic indicator. Ahn et al. has developed an RNA aptamer that specifically bound to the SARS-CoV N protein.

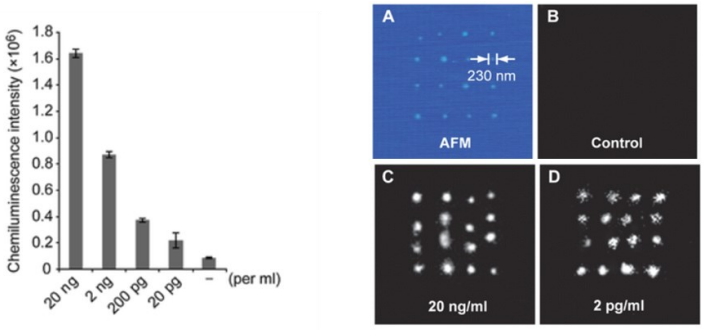

Figure (adapted) (1) Chemiluminescence immunosorbent assay of serially diluted SARS-CoV N protein bound to aptamer 1 (2) Nanoarray aptamer chip analysis using serially diluted SARS-CoV N protein bound to aptamer 1*

The N proteins were purified and bound using NTA-agarose, heparin-agarose and SP-Sepharose columns. The purified protein were used in conventional SELEX along with a RNA library to produce aptamer 1 and aptamer 2 with a high specificity (1.65nM and 3.35nM respectively). To exhibit the abilities of aptamer 1 for diagnostic testing, this study coated a streptavidin-coated 96-well plate with aptamer 1 bound to SARS-CoV N-protein and the captured SARS-CoV N-protein was detected using chemiluminescence immunosorbent assay (Figure 1). Detection of the SARS-CoV N protein could be made at as little as 420 fM. This was detection limit was further improved by using a aptamer-based nanoarray chip, where the SARS-CoV N protein could be detected at a concentration of 42 fM (Figure 2). Overall indicating that the RNA aptamer 1 could be useful in detecting the N protein in SARS-CoV with a high sensitivity level. .

*Reference: Apta-Index™ ID #7086 -M.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.

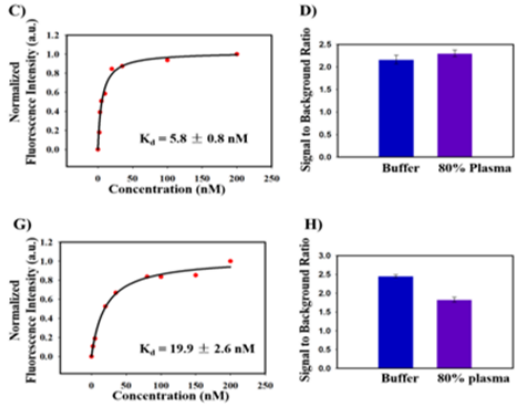

Figure (adapted) C) Affinity of truncated CoV2-RBD-1C aptamer and G) Truncated CoV2-RBD-4C aptamer using fluorescence intensity. D) Signal to background ratio of aptamer Cov2-RBD-1C and H) CoV2-RBD-4C in buffer and 80% plasma.*

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.

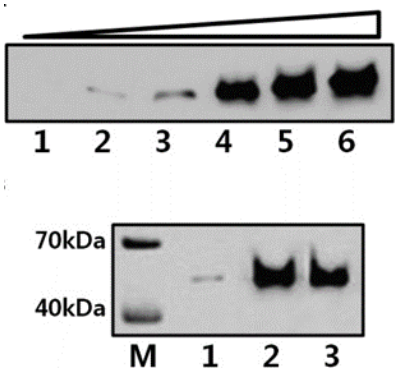


Figure (adapted) Western blot analysis with ssDNA aptamer, His-probe HRP, and anti-N protein. (A) Western blot analysis using aptamer 1. Various amounts of N protein were separated by SDS-PAGE and incubated with 5'-biotinylated aptamer 1 followed by streptavidin-HRP. The bands were visualized using the 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 Differential Inhibitory Activities and Stabilization of DNA Aptamers against the SARS Coronavirus Helicase

In order to replicate, is dependent on the helicase protein for nucleic acid unwinding and RNA capping. Shum and Tanner outline their process of selecting ssDNA aptamers that target this viral helicase for use in inhibiting viral replication and proliferation. The ssDNA aptamer candidates were found through 20 rounds of Ni-NTA magnetic bead SELEX. Inhibitory effects were evaluated, and the IC_{50} and K_m values were determined by a Fluorescence Resonance Energy Transfer (FRET) assay, . Non-G-quadraplex (NG) aptamers were found to inhibit helicase activity more efficiently than those with G-quadraplex (G) structures. The NG aptamers also exhibited much lower K_m values than both of the G aptamers. The specificity was then investigated by repeating the previous procedure with a helicase protein derived from *Escherichia coli*. An aptamer concentration of 1 µM did not inhibit the bacterial helicase while this same concentration did inhibit the viral helicase; this suggests that the aptamers were not general helicase inhibitors, but rather specific to the SARS-CoV-1 helicase.

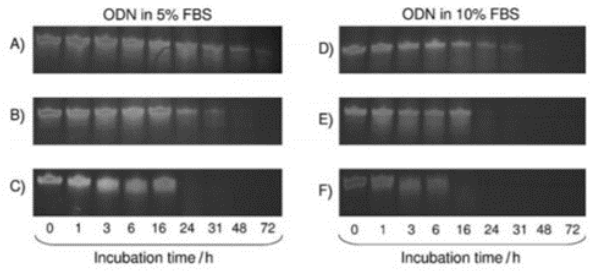
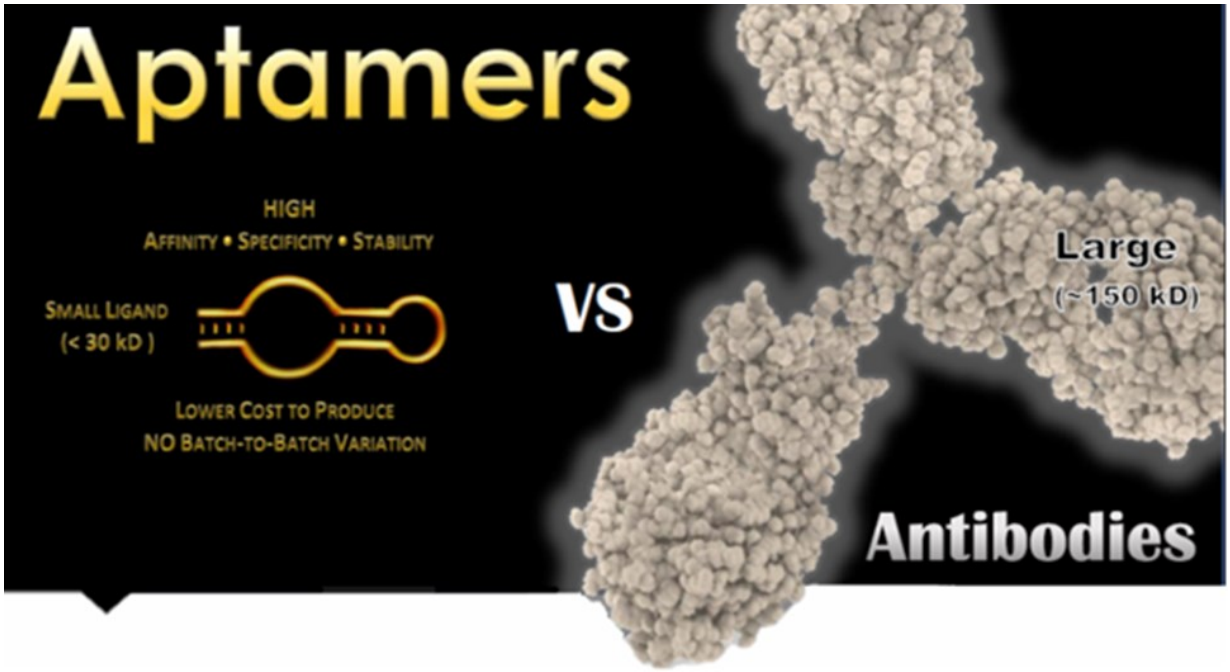


Figure (adapted) Stability of modified and unmodified aptamer NG8. Aliquots loaded onto 20% denaturing urea PAGE. Rows A and D represent 3'-inverted thymidine NH8, rows B and E represent 3'-biotin NH8, and rows C and F represent unmodified NG8 in either 5% or 10% FBS.*

The best aptamer candidate, NG8, was then chemically modified to increase its stability in serum. As seen in Adapted Figure 2, the unmodified NG8 was digested easily in 5% and 10% FBS; however, the modified 3'-biotin NG8 and the 3'-inverted thymidine NG8 were found to be resistant to nuclease attack. These modifications were also confirmed not to have any effect on the specificity previously evaluated. It was concluded that the 3'-inverted thymidine NG8 was the best overall aptamer candidate to be utilized for inhibiting the viral helicase, and in turn, the replication and proliferation of SARS-CoV-1.

*Reference: Apta-Index™ #7085 -J.R.



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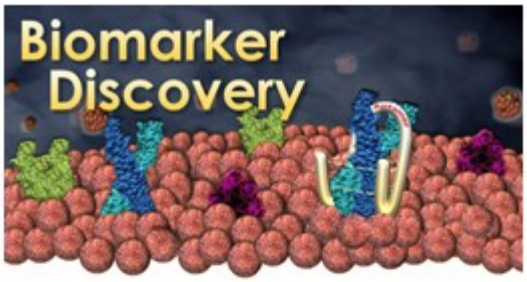

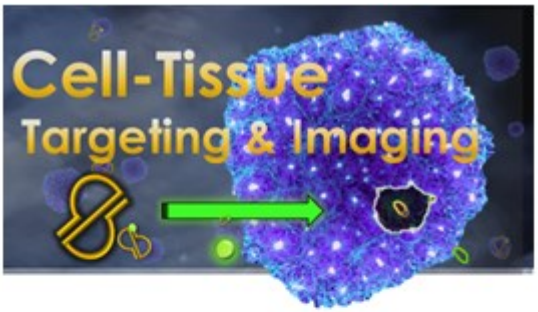

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