Aptamer-Powered Surveillance of SARS-CoV-3

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Supporting Information

Supporting Figures:

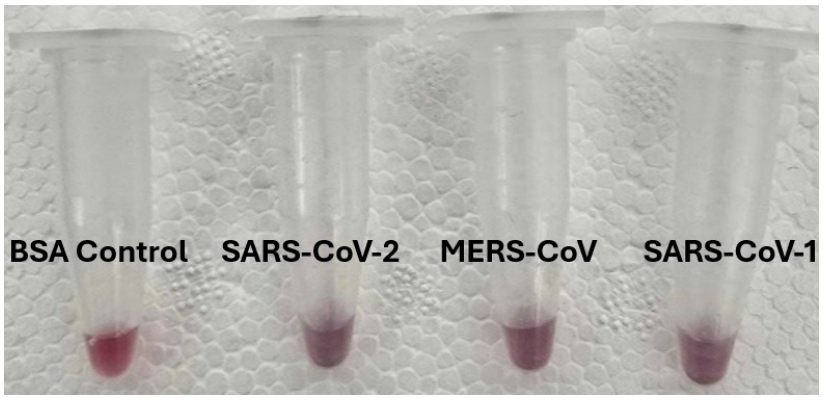

Figure S1. Specificity test for the S2A2C1 aptamer using an AuNPs-based colorimetric assay.

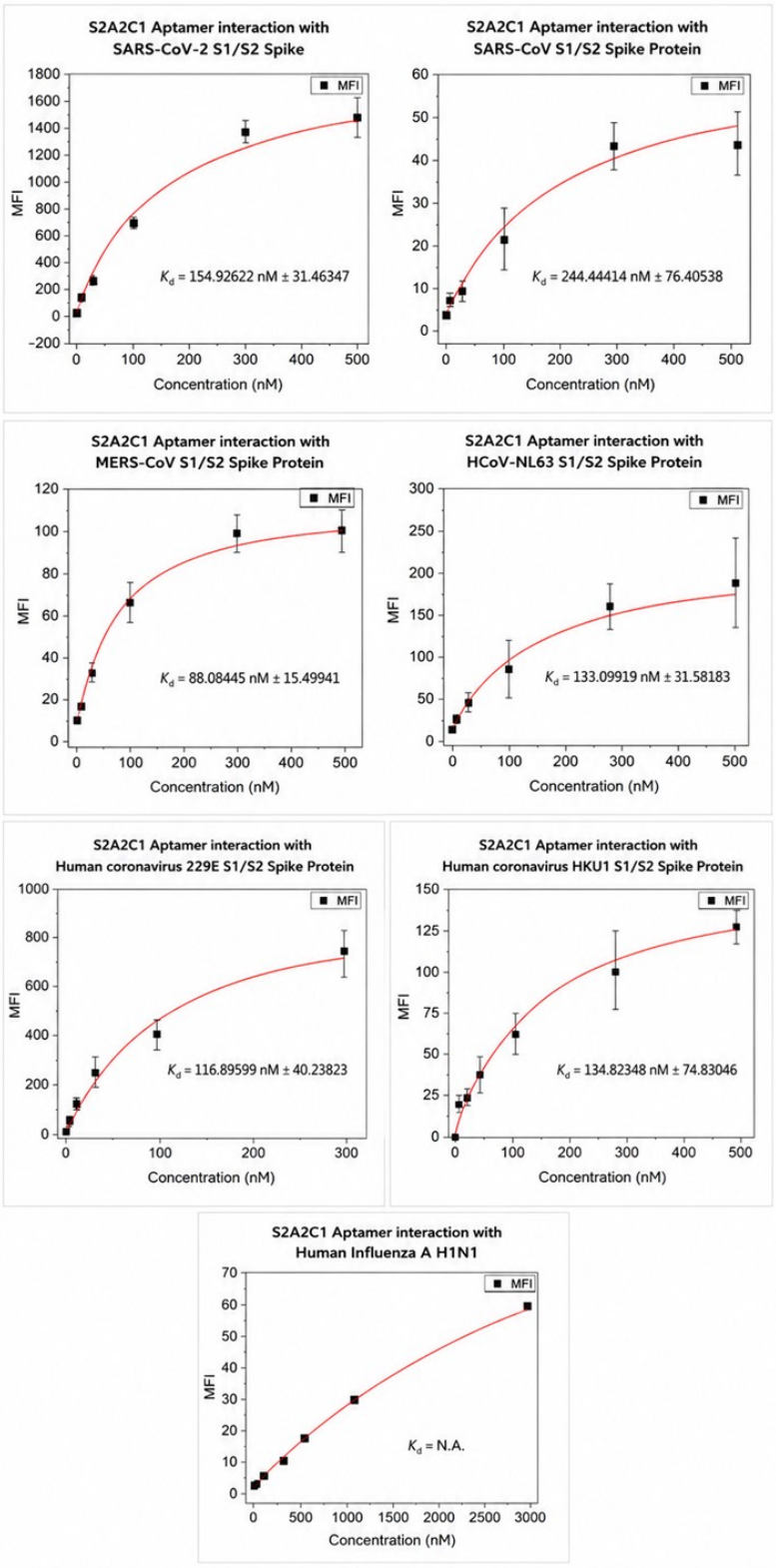

Figure S2. The binding affinity curves. The error bar indicates the standard deviation of the absorbance from their mean observed trial. The error bars represent the standard deviation of the fluorescence emission intensity values obtained from three independent trials.

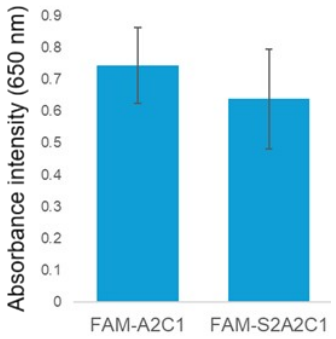

Figure S3. The ELISA assay to measure the detection efficacy of two FAM labelled aptamers toward SARS-CoV-2 WT spike protein. The absorbance is measured by employing a Clariostar microplate reader at 650 nm. The error bars represent the standard deviation of the absorbance intensity values obtained from three independent trials.

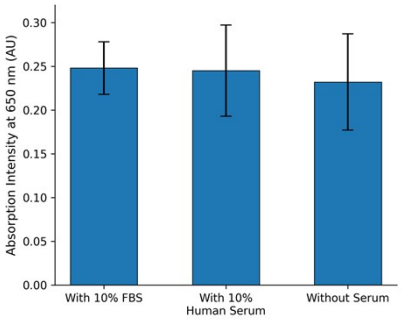

Figure S4. The ELISA assay to measure the detection efficacy of the FAM labelled S2A2C1 aptamer toward SARS-CoV-2 WT spike protein, with or without 10% serum (fetal calve or human). The absorbance is measured by employing a Clariostar microplate reader at 650 nm. The error bars represent the standard deviation of the absorbance intensity values obtained from three independent trials.

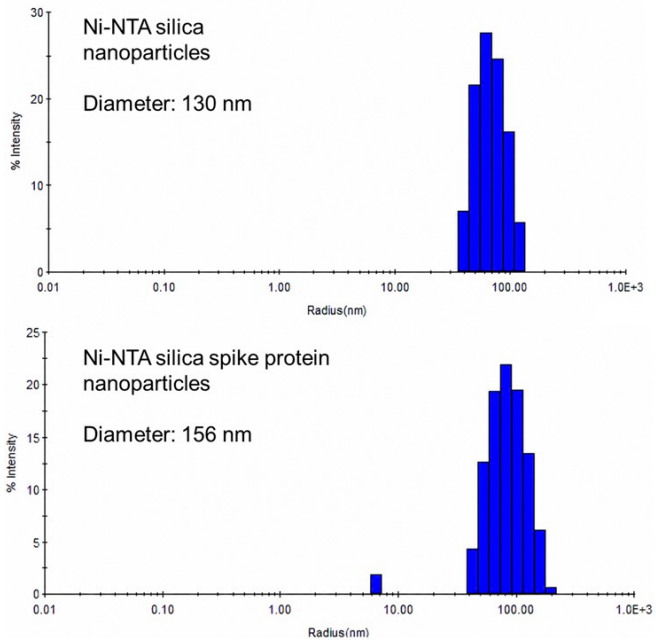

Figure S5. Dynamic light scattering (DLS) characterization of Ni-NTA silica spike protein nanoparticles compared to unmodified Ni-NTA silica nanoparticles. The +26 nm shift in diameter confirms that the bulky and long trimeric spike protein complexes have successfully immobilized onto the surface of the silica Ni-NTA core.

Materials and Methods

Chemicals and reagents. All proteins used in this work, including virus spike-proteins, were purchased from Sino Biological and used without further purification. All aptamers and other nucleic acids were obtained from Integrated DNA Technologies, Inc. (www.idtdna.com) as lyophilized powders and were dissolved in nanopure water upon receipt. All chemicals were purchased from Sigma unless mentioned otherwise.

AuNPs assay. A three-necked round bottom flask was cleaned with freshly prepared aqua regia (concentrated HNO_3 and HCl in 1:3 molar ratio), rinsed with nuclease-free water, and perfectly dried before use. The AuNPs colloid was synthesized from $KAuCl_4$ (Ref. # 334545-1G; Sigma Aldrich) precursor using the classical citrate reduction method. Briefly, a 100 mL of 1 mM $KAuCl_4$ solution was heated to boiling. Then, 2 mL of 194 mM sodium citrate solution (CAS # 1545801, Sigma Aldrich) was added and boiled for an additional 15 min with good stirring. The color of the solution changes from yellow, clear/gray, and finally

to dark wine-red. After 15 min of boiling the reaction, the flask was taken out and cooled slowly to room temperature. The wine-red color of the AuNPs colloids was intact for more than 48 hours in 1.5 M NaCl and 250 nM S2A2C1 aptamer. However, it dramatically changed into blue or purple within 5 minutes of adding 250 nM of spike-proteins.

ELISA assay with HRP anti-FAM antibody. The 0.5 µg spike protein in 50 µL 0.1 M $NaHCO_3$ (pH 8.6) was added to the high-binding 96 well plates (Ref# 12565501; Fisher brand) and incubated overnight. The solution was removed and incubated with 100 µL of 5 mg/mL BSA in 0.1 M $NaHCO_3$ for 1 h at RT and washed 3 times with 200 µL SELEX buffer containing Tween 20. Then, a 50 µL solution of 100 nM FAM-S2A2C1 in PBS buffer (with 1 mM $MgCl_2$) was incubated at RT for 1 h. The plate was then washed six times with 200 µL PBS-Mg buffer to remove the unbound aptamer. Then, 50 µL of 2000 times diluted anti-fluorescein (FAM) HRP antibody (catalog# ab6656; Abcam) in buffer was added, which bound to the remaining FAM-S2A2C1. The wells were incubated at RT for 30 min and washed six times. Finally, 50 µL of TMB substrate solution was added to the well and incubated for 30 minutes at RT. The intense blue color produced in this step was caused by the strong S2/aptamer interaction. The absorbance of the blue product was measured at λ_{max} = 650 nm using the Clariostar microplate reader (BMG LABTECH).

Flow cytometry experiment to measure the binding affinity of the aptamers. The 100 µL of 200 nM His-tagged spike-protein was prepared in PBS-Mg buffer and incubated with 1 µL of Ni-NTA magnetic beads (Ref# 062N-A; G-Biosciences), rotating for 1 h at RT. The Ni-NTA bead/spike-protein complex was washed twice with 200 µL of SELEX buffer and incubated with 100 µL of 3, 10, 30, 100, 300, and 1000 nM of 6-FAM-labeled aptamer prepared in PBS-Mg buffer for 1h at RT with rotation. After incubation, the beads were washed two times with 200 µL of PBS-Mg buffer and finally resuspended with 100 µL of PBS-Mg buffer. The 6-FAM-labeled aptamers bound to the protein/bead complex were analyzed by Flow cytometry (BD Accuri C6 Plus), counting approximately 5000 events. Each experiment was run for three trials to calculate the mean fluorescence intensity (X_C) and standard error. The binding affinity (K_d) of the 6-FAM-A1C1 aptamer against spike-protein was determined by an intensity vs. concentration plot using the Origin software.

Capture and detection assay using mimic viruses. The buffer used in this assay was PBS-Mg supplemented with 1% biotin-free casein. The 100 nm silica Ni-NTA beads (ABSI-0010-Ni, Abvigen Inc.) were gently swirled to obtain a homogeneous suspension. A 10 µL aliquot of bead slurry was mixed with 2 µg of spike protein in 140 µL of buffer and incubated for 30 min at room temperature on a rotator. The beads were then washed twice with buffer. A BCA assay confirmed that more than 95% of the protein was successfully coated onto the beads. Meanwhile, the streptavidin-coated ELISA plate (SP-14,

ACROBiosystems) was washed two times with buffer. Then, 50 µL of 1 µM biotin-labeled aptamer was added to each well and incubated for 15 min. Biotin-labeled T22 was used as a control aptamer. After incubation and washing twice, 50 µL of virus-mimic particles was added and incubated for 30 min at room temperature. Following another wash step, 1 µM FAM-labeled aptamer was added and incubated for 30 min at room temperature. After washing, 50 µL of anti-fluorescein (FAM) HRP-conjugated antibody (ab6656, Abcam), diluted 1:2000 in buffer, was added and incubated for 30 min at room temperature. The wells were then washed six times. Finally, 50 µL of TMB substrate solution was added and incubated for 30 min at room temperature. The resulting blue color reflected the S2–aptamer interaction. The absorbance was measured at 650 nm using a CLARIOstar microplate reader (BMG LABTECH).