Supporting Information

Aptamer Conjugated Gold Nanostar Based Distance Dependent Nanoparticle Surface Energy Transfer Spectroscopy for Ultrasensitive Detection and Inactivation of Corona Virus

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Methods

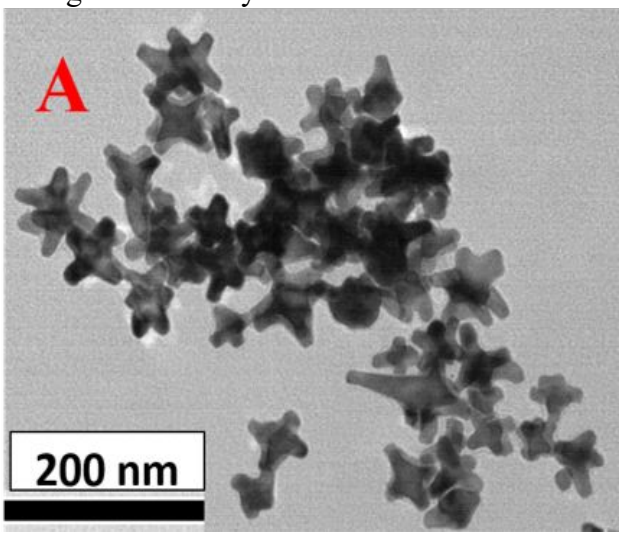
Gold chloride, citric acid, sodium borohydride, aptamer, and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). We have purchased SARS-CoV-2 spike protein recombinant antigen from Sigma-Aldrich (St. Louis, MO, USA). We have purchased the HEK293T cell line from the American Type Culture Collection, which were grown according to an ATCC procedure**.** The SARS-CoV-2 spike protein pseudotyped baculovirus (#C1110G, Montano Molecular, Bozeman, MT) was purchased from Montano Molecular, Bozeman, Montana 59715. We have purchased artificial nasal mucus fluid from Biochemazone, Ontario, Canada.

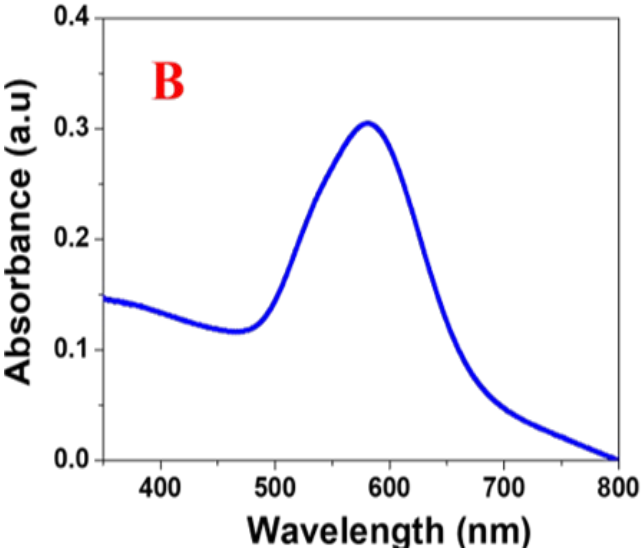
Synthesis of Gold Nanostar (GNS)

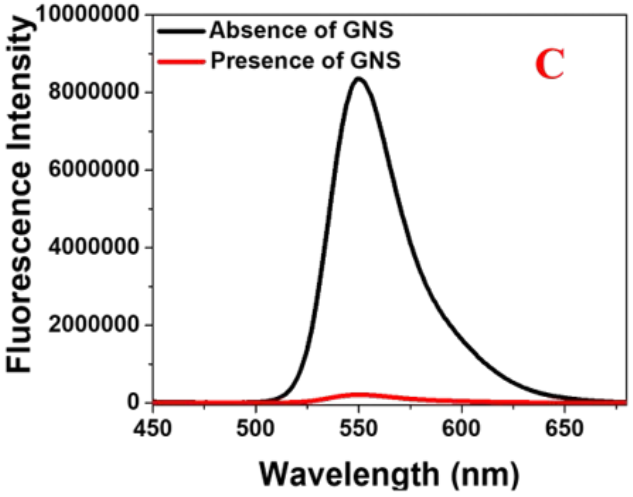
The gold nano-stars (GNSs) were synthesized by using silver assisted seedless growth method, as we and others have reported before [23-25]. For this process, initially the stock solution of HEPES (100 mM) buffer was adjusted to pH 7.4±0.1 using 1.0 M NaOH with double distilled water. In the next step, HAuCl4 (20 µL, 50 mM), 9 µL of 1 mM aqueous silver nitrate and 3 ml of distilled water were mixed gently for 5 minutes. Then, 2 ml of 100 mM HEPES was added to the solution and kept without stirring for another 25 minutes at room temperature. After that, aqueous solution of (600 ml, 25 mM) polyvinylpyrrolidone (PVP, average Mw ~ 10,000) was added gently to the greenish-blue solution and left to stand for 12 hours at room temperature. After that, the final solution was centrifuged for an hour at 3500 rpm speed. The precipitation was collected and redispersed in double distilled water for further use.

Synthesis of spike protein specific DNA aptamer attached GNS

5′-Rh-6G-ATCCAGAGTGACGCAGCATTTCATCGGGTCCAAAAGGGGCTGCTCGGGATTGCGGATATGGACACGT-SH-3′ was attached to GNS via thiol-gold chemistry.







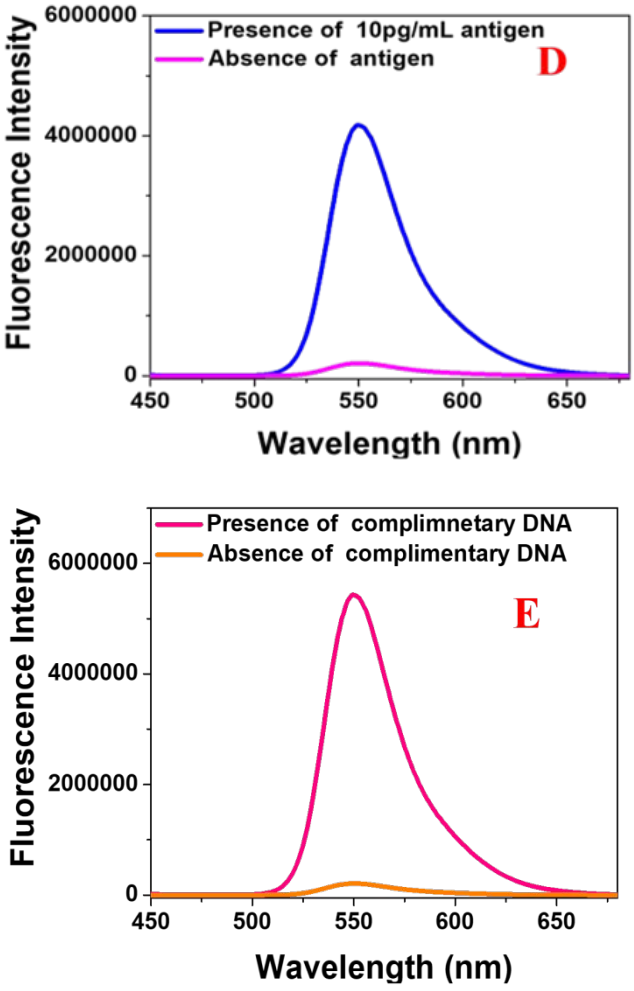


Figure S1: A) TEM shows the morphology of freshly prepared GNS using HEPES buffer. B) Absorption spectra from freshly prepared GNS using HEPES buffer. C) Fluorescence spectra from Rh-6G conjugated DNA aptamer and GNS attached Rh-6G conjugated DNA aptamer. D) Fluorescence spectra from Rh-6G conjugated DNA aptamer attached GNS in the presence and absence of COVID-19 antigen. E) Fluorescence spectra from Rh-6G conjugated DNA aptamer attached GNS in the presence and absence of complementary DNA.

For this purpose, –SH modified aptamer were gradually exposed to GNS in the presence of sodium dodecyl sulfate, sodium chloride and PBS buffer for about 12-14 hours. After attachment, unbound DNA strands were removed by centrifugation of the solution at 6,000 rpm for 20 minutes.

NSET Spectroscopy using aptamer attached GNS

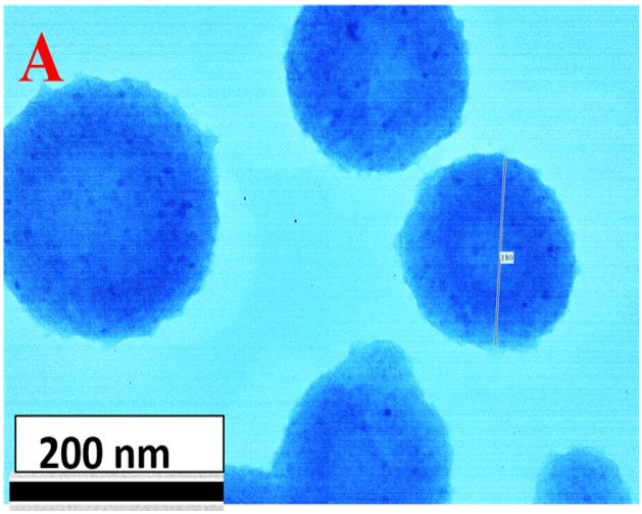
For the binding between antigen and aptamer, we have used binding buffer (PBS, pH =7.4, including 136.8 mM NaCl, 10.1 mM Na_2HPO_4, 2.7 mM KCl, 1.8 mM KH_2PO_4, 0.55 mM $MgCl_2$). For the detection of COVID-19 antigen and virus using NSET, we have used portable fluorescence spectrometer developed by us with laser excitation of 480 nm/532 nm[18-19,21]. We have used solid state high stability DPSS laser with adjustable power of up to 50 mW laser power. InPhotonics fiber optic probe has been used for excitation and data collection. For NSET signal collections, we have used miniaturized QE65000 spectrometer from Ocean Optics which is responsive from 200 nm to 1100 nm [18-19,21]. QE65000 spectrometer provides 90 percent quantum efficiency with high signal-to-noise, which is excellent for low-light level applications[18-19,21]. The whole system is equipped in a small box and attached with a laptop computer for data acquisition[18-19,21]. The total price including spectrometer, fiber optics, laser source and computer is ~$16000. Fluorescence spectrum was collected with Ocean Optics data acquisition software. All measurements were performed with 5 ms integration time with 5 spectra averaging using the software.

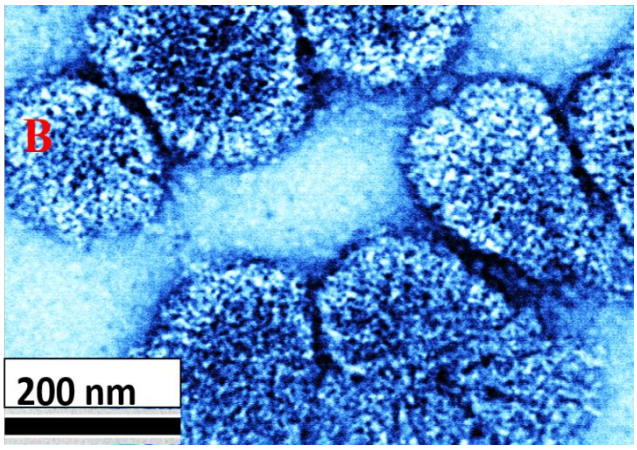
Fluorescence Imaging of Virus

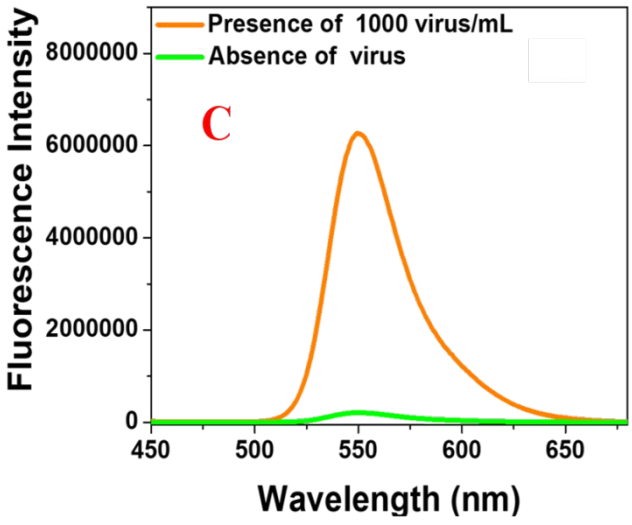
For the fluorescence imaging of an Olympus IX71 inverted confocal fluorescence microscope fitted with a SPOT Insight digital camera was used.

SARS-CoV-2 pseudotype particles

The SARS-CoV-2 spike protein pseudotyped baculovirus (Cat #C1110G) was purchased from Montana Molecular, Bozeman, Montan0 59715.







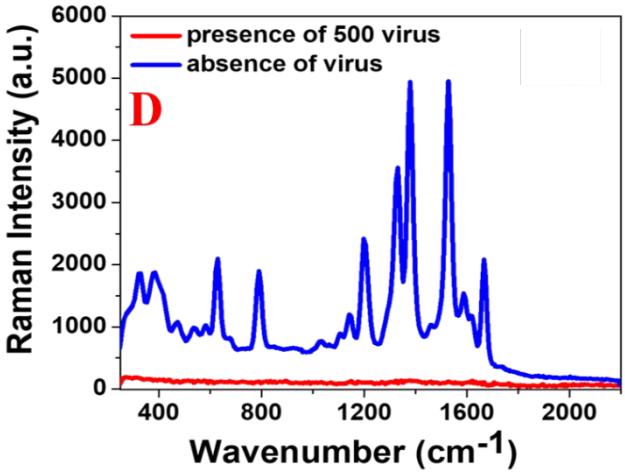


Figure S2: A) TEM image shows the morphology of SARS-CoV-2 spike protein pseudotyped baculovirus, without GNS. B) TEM image shows the morphology of SARS-CoV-2 spike protein pseudotyped baculovirus attached GNS C) Fluorescence spectra from Rh-6G conjugated DNA aptamer attached GNS in the presence and absence of SARS-CoV-2 spike protein pseudotyped baculovirus. D) Surface enhanced Raman spectra (SERS) from Rh-6G conjugated DNA aptamer attached GNS in the presence and absence of baculovirus.

The HEK293T cells (ATCC # CRL3216) plated in twelve well tissue culture dishes were infected with the pseudotyped virus with a dilution range of 10^2 to 10^7 and virus titers were calculated by counting the green fluorescent protein (GFP) positive cells under a fluorescent microscope [27-28].

Virus inhibition assays

HEK293T cells (ATCC # CRL3216) were plated on a 96-well plate in complete media (DMEM + 10% FBS) and incubated under normal growth conditions (5% CO_2 and 37°C, protected from light) for 12-24 hours[27-28]. These HEK293T cells express low levels of ACE2 receptor that is sufficient for SARS-CoV-2 entry. Dilutions of

test nanoparticles were made in DMEM with an end volume of 100 µl each[27-28].

Figure S3: SARS-CoV-2 pseudovirus GFP expression in infected HEK293T cells in the presence of 100 ng/mL spike protein specific ss DNA aptamer attached GNS, when Virus and bio-conjugated nanoparticles are incubated for an hour.

The pseudovirus stock (2.5 µl of the $2X10^{10}$ units/ml stock) was mixed with the diluted nanoparticles and incubated for 1 h at 37°C, then laid over HEK293T cells plated in the 96-well tissue culture dishes, along with 0.6 µL of the 500 nM sodium butyrate (to give a final concentration of 2mM). Plates were incubated at 37°C and 5% CO_2 for 48 h. Cells were fixed in 3.7% formaldehyde and the assay was read on Cytation 5 automated fluorescent microscope (BioTek Instruments, Inc., Winooski, VT, USA) [27-28]. The total number of cells per well were counted and data was analyzed in Prism 8 (Graphpad Inc) [27-28]. Triplicate samples were used and standard error of mean was plotted as error bars[27-28].

Finding the concentration of virus

To determine the concentration of the virus stock, HEK293T cells were plated in 12 well tissue culture dishes and infected with serial dilutions of the virus stock (range of 10^2 to 10^7), as we have reported before [27-28]. After that, virus titers were calculated by enumerating the GFP positive cells in the dilution producing 20 to 100 GFP-positive cells, and multiplying with the dilution factor, as we have reported before[27-28].

Finding the concentration of antigen

We have used Enzyme-linked Immunosorbent assay (ELISA) assay to find the concentration of antigen as we have reported before [27-28]. For this purpose, 384-well MaxiSorp plates (Thermo Fisher Scientific) were coated with recombinant protein as we have reported before [27-28]. Next, we have incubated those plates overnight at 4 °C. After that plates were washed with PBS-Tween 20 and blocked with PBS containing 3% dry milk for an hour at room temperature[27-28]. In the next step, horse radish peroxidase conjugated to anti-human IgM, purchased from Southern Biotech company, was diluted 1:2000 in PBS containing 1% dry milk and added to the wells[27-28]. After that, we have incubated them at room temperature for an hour. In the next step, plates were washed five times and were developed with tetramethyl-benzidine [27-28]. After 30 minutes, development was stopped by adding 25 µl of 2 N H_2SO_4 to each well[2]. At the end, we have measured the absorbance at 450 nm [27-28].

Limit of Detection (LOD) Determination

The limit of detection was determined from the NSET titration data as reported in Figures 2D and 3C. For this purpose, in each concentration, at least five experiments were carried out. For LOD determination we have used the following equation[16-25].

$$LOD = 3\sigma/S \quad \textbf{(1)}$$

where, σ is the standard deviation of the blank in the absence of the target or presence of negative

control (buffer) and S is the slope of the calibration curve. For this purpose, we have performed three independent measurements of the blank signal.