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

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ABSTRACT: The ongoing outbreak of the coronavirus infection has killed more than 2 million people. Herein, we demonstrate that Rhodamine 6G (Rh-6G) dye conjugated DNA aptamer-attached gold nanostar (GNS)-based distance-dependent nanoparticle surface energy transfer (NSET) spectroscopy has the capability of rapid diagnosis of specific SARS-CoV-2 spike recombinant antigen or SARS-CoV-2 spike protein pseudotyped baculovirus within 10 min. Because Rh-6G-attached single-stand DNA aptamer wrapped the GNS, 99% dye fluorescence was quenched because of the NSET process. In the presence of spike antigen or virus, the fluorescence signal persists because of the aptamer–spike protein binding. Specifically, the limit of detection for the NSET assay has been determined to be 130 fg/mL for antigen and 8 particles/mL for virus. Finally, we have demonstrated that DNA aptamer-attached GNSs can stop virus infection by blocking the angiotensin-converting enzyme 2 (ACE2) receptor binding capability and destroying the lipid membrane of the virus.

The respiratory syndrome coronavirus-2 (SARS-CoV-2) epidemic has spread worldwide very rapidly, and it threatens the world economy, health, and social life.1–10 As per the world health organization (WHO), more than 106 million people around the world have suffered the effects of the coronavirus disease of 2019 (COVID-19), and around 2.3 million have died worldwide.1–5 For any disease, the immediate requirement is a fast and effective diagnostic of the virus, which is the key to prevent infection in the society.5–15 In the current Letter, we report that spike protein-specific aptamer-attached gold nanostars (GNSs) can be used for rapid diagnosis of specific SARS-CoV-2 spike recombinant antigen or virus itself via a distance-dependent nanoparticle surface energy transfer (NSET) process.16–22

Because gold nanoparticles exhibit 9–10 orders of magnitude higher quenching efficiency than typical small molecule dye–quencher pairs,16–24 NSET spectroscopy using GNSs has the capability to be used as a biophysical tools beyond Förster resonance energy transfer (FRET).16–22 It is now well-documented that because gold nanostars exhibit very high extinction coefficient, single GNS-based optical nanorobes are comparable with an optical organic probe made with more than 10⁶ dye molecules together.16–27 Recent published data indicate that the receptor-binding domain (RBD) of the SARS-CoV-2 spike glycoprotein (S protein) is responsible for virus entry and disease pathogenesis.3–15 Therefore, we have used spike protein-specific aptamer-attached GNSs for specific diagnosis and effective inhibition of the virus. As shown in Figure 1A, in the absence of spike protein, Rh-6G attached to single stand (ss) DNA aptamer-wrapped gold nanoparticle. Because of the above fact, the fluorescence signal from the Rh-6G dye is quenched by GNS via the NSET process. In contrast, as shown in Figure 1B,C, when SARS-CoV-2 antigen or virus particles are added, because of the aptamer–spike protein binding, the distance between the GNS and dye increases, and as a result, the fluorescence signal persists. We have used the observed NSET signal change in the absence or presence of antigen or virus for the detection purpose. Reported data show that spike protein-specific aptamer-attached GNSs can be used for the diagnosis of COVID-19 spike antigen at 130 fg/mL concentration level and virus at 8 particles/mL level.

For the detection and inactivation, SARS-CoV-2 spike protein pseudotyped baculovirus has been used.27,28 Because the S protein of the virus and host angiotensin-converting enzyme 2 (ACE2) binding is the key for infection spread, we have used aptamer-attached GNSs as an inhibitor for blocking virus spread.28–30 In the current Letter, we demonstrate...
that the spike protein-specific DNA aptamer-attached GNSs (without Rh-6G) can be used to block the viral entry into cells. For this purpose, we have used ACE2 expressing HEK293T cell line.\(^\text{27,28}\) Reported scanning and transmission electron microscopy and fluorescence microscopy data show that spike protein-specific DNA aptamer-attached GNSs can be used to stop the spread of the virus.

For the design of Rh-6G conjugated ss-DNA aptamer-attached GNS, we have used \(5\prime\)-ATCCAGAGTGACGCAGCATTTCAT-CGGGTTCAAAAGGGGCTGCTCGGGATTGCGGATA-TGGACACGT-3\prime\), DNA aptamer which has been reported recently to be specific for the SARS-CoV-2 spike glycoprotein.\(^\text{12}\) As shown in the Figure 1A, ss-DNA (\(\text{HS}-(\text{CH}_2)_n\)-Oligo-Rh-6G) was attached to GNS via thiol-gold chemistry, as we and others have reported before.\(^\text{16-22}\) For this purpose, initially we have synthesized GNS using silver assisted seedless growth method in the presence of HEPES buffer.\(^\text{23-25}\) We report the synthesis details in the Supporting Information. After that, GNSs were characterized by UV–vis spectroscopy and TEM as reported in Figure S1A,B in the Supporting Information.

TEM data show that for GNS particles the spherical core diameter is around \(15 \pm 3\) and branch length is around \(12 \pm 4\) nm. The extinction spectra show that freshly prepared GNSs exhibit strong plasmon band with \(\lambda_{\text{max}} \approx 560\) nm.\(^\text{23}\) In the next step, ssDNA (\(\text{HS}-(\text{CH}_2)_n\)-Oligo-Rh-6G) aptamer was attached to GNS via thiol-gold chemistry. We have reported the synthesis details in the Supporting Information. Next, to determine how many aptamers are bound to GNS, we have separated aptamer from GNS by dissolving nanostar in \(10\) \(\mu\)M potassium cyanide. In the next step, from fluorescence recovery intensity measurement, we have found that around \(60-80\) aptamers are attached to each GNS. As reported in Figure S1C in the Supporting Information and Figure 2A, the fluorescence signal from the Rh-6G attached aptamer is totally quenched when it has been attached to a GNS. As shown in Figure 1A, when a Rh-6G conjugated ss DNA aptamer is attached to GNS surface via -SH linkage, the \(5\prime\)-Rh-6G ends of ss DNA loop back onto the GNS surface.\(^\text{16-22}\) In this condition, strong NSET occurs between dye donor and GNS acceptor, which provided 99% of quenching efficiency, as reported in Figure 2A. As we and others have reported before, when Rh-6G is placed at a short distance on GNSs, which possesses a strong plasmon field, the electrons of Rh-6G participating in the excitation/emission process will interact with the field.\(^\text{16-22}\) As a result, we have observed strong NSET, and it is because each GNS has the
enhanced Raman intensity (SERS) enhancement experiment, aptamers are adsorbed onto the GNS surface, a surface. The rhodamine 6G at the 3′ end can loop back onto GNS, we have added complementary DNA to the genome and the distance between GNS and Rd-6G dye increases. As a result, we have observed a decrease in fluorescence after the formation of -ds DNA, as reported in Figure S1E in the Supporting Information. The above experimental data is clear evidence that in ss DNA aptamer, the distance-dependent NSET intensity change in the presence of different amounts of spike antigen, we have determined the NSET intensity variation for different amounts of spike antigen, although we have used 100 pg/mL antigen. On the other hand, excellent distance-dependent NSET intensity variation for different amounts of spike antigen is observed. As reported in Figure 2B, in the presence of spike antigen, we have observed an increase in fluorescence after the formation of ds DNA, as reported in Figure 2B, excellent distance-dependent NSET intensity change in the presence of different amounts of spike antigen. As shown in Figure 2C, the distance-dependent NSET assay can recognize COVID-19-specific antigen even at the concentration of 100 fg/mL. As reported in Figure 2D, the log of NSET intensity difference (intensity after and before COVID-19 spike antigen) varies linearly with the concentration of COVID-19 spike antigen. We used eq 1 for finding the LOD for the distance-dependent NSET assay.

\[ \text{LOD} = 3\sigma / S \]  

(1)

In our experiment the standard deviation of the blank (\( \sigma \)) has been measured in the absence of antigen. The slope of the calibration curve (S) has been calculated from the linear curve reported in Figure 2D. The LOD for the distance-dependent NSET assay has been determined to be \( \sim 130 \) fg/mL for spike antigen. Selectivity of the 5′-Rh-6G conjugated DNA-attached GNS-based distance-dependent NSET assay has been demonstrated using flu virus antigen and rotavirus antigen separately. As shown in Figure 2A, we have not observed any distance-dependent NSET intensity variation for flu or rotavirus antigen, although we have used 100 pg/mL antigen. On the other hand, excellent distance-dependent NSET intensity...
variation can be noted when only 10 pg/mL spike antigen has been added. The above experimental data clearly indicate that the NSET assay can be used for specific recognition of SARS-CoV-2 spike recombinant antigen.

As shown in Figure 1 C, spike protein-specific DNA conjugated GNS-based NSET diagnosis of virus is based on the binding affinity of virus spike protein and aptamer. As reported in Figures 3 B–D and S2C in the Supporting Information, because of the above interaction, the distance-dependent NSET intensity enhances abruptly. The reported distance-dependent NSET intensity variation has been used for the detection of the virus. Because the size of the virus (120–160 nm) is much greater than that of the GNS, electron microscopy images reported in Figures 3 B and S2A,B in the Supporting Information show that several aptamer-attached GNSs are bound on the virus. To understand better whether 5′-Rh-6G conjugated ss DNA aptamer-attached GNS have been used. For this experiment, each of them were separately incubated with virus for an hour.

Sensitivity of the distance-dependent NSET assay for specific virus detection has been determined by varying the virus amount from 10 to 500 virus/mL. As shown in Figure 3 B, distance-dependent NSET assay has the capability to identify coronavirus even at the 10 virus/mL concentration level. As reported in Figure 3 C, the log of NSET intensity difference (intensity before and after virus addition) varies linearly with the log of the concentration of the virus. Using the linear curve reported in Figure 3 F and eq 1, the LOD for distance-dependent NSET assay has been determined to be ~8 virus/mL.

To understand whether the Rh-6G conjugated spike protein-specific DNA aptamer-attached GNS-based NSET assay can be used for the detection of COVID-19-specific antigen or virus, we have performed an experiment using antigen or virus-infected artificial nasal mucus fluid samples. Artificial nasal mucus fluid was purchased from Biochemazone, Canada, and the nasal matrix was infected by adding different concentrations of COVID-19-specific antigen or virus, separately.
shown in Figure 4A, we have not observed NSET intensity change in the presence of only nasal matrix or nasal matrix infected with flu virus antigen. On the other hand, as reported in Figure 4B, the Rh-6G conjugated spike protein-specific DNA aptamer-attached GNS-based NSET assay has the capability to detect COVID-19-specific antigen even in the concentration of 100 fg/mL. Similarly, as reported in Figure 4C, the Rh-6G conjugated spike protein-specific DNA aptamer-attached GNS-based NSET assay has the capability to detect virus even in the concentration of 20 virus/mL.

Next, we have determined whether spike protein-specific ss DNA aptamer-attached GNS can be used to inhibit viral replication. For this purpose, ACE2 expressing HEK293T cell line has been used. In this experiment, we have used spike protein-specific ss DNA aptamer-attached GNS without Rh-6G. We have discussed experimental details in the Supporting Information. Reported virus inactivation data in Figure 5A–C and S3 in the Supporting Information indicate that ss DNA aptamer-attached GNS can block viral replication. 100% inhibition efficiency was observed for ss DNA aptamer-attached GNSs at the concentration of 100 ng/mL. On the other hand, less than 1% inhibition efficiency was achieved for only GNSs or aptamer at the same concentration level. The observed excellent inhibition efficiency for aptamer-attached GNSs can be because of the binding of the aptamer-attached GNSs to virus, resulting in the inability of the virus to bind with ACE2. Experimental data shown in Figure 5B indicate that aptamer-attached GNS can destroy the lipid membrane of pseudo baculovirus, so that the virus particle collapses, and as a result, it stops spreading.

In conclusion, in this work we report that spike protein-specific aptamer-attached GNSs can be used for detection and inactivation of corona virus. Our finding indicates that rhodamine 6G (Rh-6G) dye conjugated DNA aptamer-attached GNSs can effectively detect the viral level for SARS-CoV-2 spike recombinant antigen, and in the case of virus, the LOD is only 8 particles/mL. Finally, we demonstrate that 100% virus inhibition efficiency for aptamer-attached GNSs occurs via blocking the cell attachment process and damage of the lipid membrane. Although reported experimental data indicate that the aptamer conjugated GNSs have the capability for diagnosis and inhibition of corona virus, we are in the infancy of this research. In the next phase, we plan to move beyond the phase of demonstrations in the laboratory and find a way to move toward clinical applications.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jpcl.0c03570.

Synthetic details for aptamer conjugated GNS formation and their characterization as well as other experiments (PDF)

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**Notes**

The authors declare no competing financial interest.

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