ABSTRACT: Since the angiotensin-converting enzyme 2 (ACE2) protein is abundant on the surface of respiratory cells in the lungs, it has been confirmed to be the entry-point receptor for the spike glycoprotein of SARS-CoV-2. As such, gold nanorods (AuNRs) functionalized with ACE2 ectodomain (ACE2ED) act not only as decoys for these viruses to keep them from binding with the ACE2-expressing cells but also as agents to ablate infectious virions through heat generated from AuNRs under near-infrared (NIR) laser irradiation. Using plasmid containing the SARS-CoV-2 spike protein gene (with a D614G mutation), spike protein pseudotyped viral particles with a lentiviral core and green fluorescent protein reporter were constructed and used for transfecting ACE2-expressing HEK293T cells. Since these viral particles behave like their coronavirus counterparts, they are the ideal surrogates of native virions for studying viral entry into host cells. Our results showed that, once the surrogate pseudoviruses with spike protein encounter ACE2ED-tethered AuNRs, these virions are entrapped, resulting in decreased viral infection to ACE2-expressing HEK293T cells. Moreover, the effect of photothermolysis created by ACE2ED-tagged AuNRs under 808-nm NIR laser irradiation for 5 min led to viral breakdown. In summary, ACE2ED-tethered AuNRs with dual functions (virus decoy and destruction) could have an intriguing advantage in the treatment of diseases involving rapidly mutating viral species such as SARS-CoV-2.

KEYWORDS: SARS-CoV-2, D614G spike protein, pseudovirus, gold nanorod, ACE2, NIR laser, photothermal heating
it should be noted that very high concentrations (up to 200 μg/mL) of sACE2 were required to inhibit SARS-CoV-2 infection\(^6\) because low levels of sACE2 could enhance cellular entry of sACE2-SARS-CoV-2 complexes through receptor-mediated endocytosis via the cell surface receptor AT1.\(^8\) Such high ACE2 concentrations in blood circulation might lead to increased vulnerability to COVID-19 and cardiovascular disease patients who are on renin–angiotensin–aldosterone system inhibitors.\(^9\)–\(^12\) Moreover, according to pharmacokinetic studies, hrsACE2 exhibits a fast clearance rate with a short half-life of only a few hours.\(^13\)–\(^15\) In addition to using sACE2 as the decoy, it has been functionalized with an Fc fragment to inhibit viral replication,\(^16\) trigger antibody-dependent cellular cytotoxicity,\(^17\) and neutralize the virus.\(^18\) An engineered sACE2 decoy with three amino acid mutations has recently demonstrated to enhance binding affinity for SARS-CoV-2 spike protein.\(^19\) Furthermore, extracellular vesicles expressing ACE2 were demonstrated to be much more efficient decoys than sACE2,\(^20\)–\(^22\) suggesting tethering ACE2 on nanomaterials could be a potential approach to prevent SARS-CoV-2 infection.

Although vaccines and monoclonal antibody (mAb) treatments for SARS-CoV-2 have been developed, ever-evolving SARS-CoV-2 variants continue posing a great threat to people’s health worldwide. In particular, the fast-spreading Omicron BA.4 and BA.5 subvariants jeopardize the efficacy of currently approved vaccines and mAb treatments. Thus, there is an urgent need to explore and evaluate alternative prophylactic and therapeutic approaches. Nanomaterials have been proposed and utilized to fight SARS-CoV-2 infection.\(^23\)–\(^25\) Among them, lipid nanoparticles have enabled the success of two leading COVID-19 mRNA vaccines (i.e., Pfizer/BioNTech and Moderna). In addition, ACE2 nanodecays released from human lung spheroids delivered via inhalation could neutralize SARS-CoV-2 and protect the host lung cells from infection.\(^26\) Membrane nanoparticles derived from ACE2-rich cells efficiently suppressed SARS-CoV-2 spike protein pseudovirion entry into host cells and blocked viral infection.\(^27\) Using ACE2-funionalized nanoparticles to capture virions via their spike proteins and therefore block their cell entrance is the main strategy; however, it might not thoroughly prevent infection unless the bound virions are further inactivated in the extracellular milieu. In this study, we functionalized gold nanorods (AuNRs) with ACE2 ectodomain (ACE2ED) not only for preventing viral entry to ACE2-expressing HEK293T cells but also for deforming the virus using heat released from AuNRs exposed to near-infrared (NIR) laser irradiation. Due to a surface plasmon resonance effect,\(^28\) gold nanorods (AuNRs) synthesized by seed-mediated growth\(^29\)–\(^31\) and tuned by the aspect ratio can convert photon energy absorbed from near-infrared light sources into localized heating.\(^32\)–\(^34\) NIR transmission with minimal attenuation by water and hemoglobin makes AuNR-based plasmonic photothermal therapy a promising modality to induce hyperthermia of malignant cells located in deeper tissue.\(^35\)–\(^37\)

Based on the molecular mechanism of ACE2 blockade of SARS-CoV-2 attachment to target cells, the ectodomain (ED) of human ACE2 could have the capability to achieve that role. The plasmid encoding human ACE2ED and core-streptavidin (coreSA) was transformed into T7 expression competent Escherichia coli for protein expression and purification of the ACE2ED-coreSA fusion protein. The biotinylated AuNRs were then bound with the recombinant ACE2ED-coreSA fusion protein via biotin–streptavidin affinity. Plasmid co-transfection technology was used to generate D614G S protein pseudotyped virions with a lentiviral core and green fluorescent protein (GFP) reporter for biosafety level-2 operation. The S protein and infectivity of SARS-CoV-2 surrogates were

Figure 1. Schematic illustration of the mechanism of blocking and inactivation of SARS-CoV-2 spike protein pseudotyped lentivirus using ACE2ED-tethered AuNRs under NIR laser irradiation. (A) HEK293T-ACE2 cell treated with GFP-encoding lentivirus shows the expression of GFP 48-h postinfection; (B) HEK293T-ACE2 cell treated with GFP-encoding lentivirus and ACE2ED-tethered AuNRs under NIR laser exposure for 5 min reveal no expression of GFP after 48-h infection.
verified. Then, the effect of ACE2ED-tagged AuNRs as decoys on inhibiting SARS-CoV-2 surrogates’ entry into ACE2-expressing HEK293T cells by associating with S proteins was evaluated. Moreover, the degree of photothermalysis on SARS-CoV-2 surrogates by bound ACE2ED-tagged AuNRs under 808-nm NIR laser irradiation was examined. The mechanism of action is schematically illustrated in Figure 1. Our results indicated that the expressed and purified ACE2ED-coreSA fusion protein bound with biotinylated AuNRs mitigated viral spread by blocking the entry of SARS-CoV-2 surrogates into ACE2-expressing HEK293T cells and, ultimately, facilitated the impairing of virions under 808-nm NIR laser irradiation.

RESULTs AND DISCUSSION

Expression, Purification, and Characterization of ACE2-coreSA Fusion Protein. As shown in Figure 2A, (A) Circular vector map shows multiple cloning sites (MCS) for ACE2ED-coreSA fusion gene. CoreSA was inserted between EcoRI (forward primer) and XhoI (reverse primer); the extracellular domain (ED) of human ACE2 was inserted between KpnI (forward primer) and EcoRV (reverse primer); (B) DNA gel electrophoresis of PCR products cloned from pET-30a(+)→ACE2ED-coreSA: DNA ladder (lane 1), coreSA (~0.4 kb, lane 2), and ACE2ED (~1.8 kb, lane 3).

Figure 2. Construction of plasmid pET-30a(+)→ACE2ED-coreSA. (A) Circular vector map shows multiple cloning sites (MCS) for ACE2ED-coreSA fusion gene. CoreSA was inserted between EcoRI (forward primer) and XhoI (reverse primer); the extracellular domain (ED) of human ACE2 was inserted between KpnI (forward primer) and EcoRV (reverse primer); (B) DNA gel electrophoresis of PCR products cloned from pET-30a(+)→ACE2ED-coreSA: DNA ladder (lane 1), coreSA (~0.4 kb, lane 2), and ACE2ED (~1.8 kb, lane 3).

ACE2ED-coreSA encoding recombinant plasmid (pET30-ACE2ED-coreSA) was constructed by inserting the gene sequences of coreSA and ACE2 into the vector pET30a(+). The gene sequences encoding the angiotensin-converting enzyme 2 ectodomain (ACE2ED) and core streptavadin (coreSA) from pET30a(+) cloned by polymerase chain reaction (PCR) were verified by DNA gel electrophoresis. As displayed in Figure 2B, ACE2ED PCR product showed the size of ~1.8 kb, and coreSA PCR product showed the size of ~0.4 kb on an agarose gel. These PCR results confirm that both gene sequences were successfully cloned into pET-30a(+). The orientation of the insertions examined by restriction enzyme analysis was correct, and the open reading frames of recombinant plasmids were verified by DNA sequencing. According to the sequence size of ACE2ED and coreSA, ACE2ED-coreSA fusion protein has an estimated molecular weight around ~81 kDa. A sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis of cell lysates indicated a protein band with molecular weight (MW) of 81 kDa (Figure 3B). The wash and elution profile of the reading at OD280 shows the peak at the elution 2 fraction indicating most of the ACE2ED-coreSA fusion protein was eluted (Figure 3A). The elution 2 fraction was concentrated using a protein concentrator and quantified through the bicinchoninic acid (BCA) assay, and the concentration of purified protein was calculated as 270 μg/mL. A Western blot analysis against anti-ACE2 mAb and anti-SA mAb showing clear bands in Figure 3C further confirmed the purified ACE2ED-coreSA fusion protein.

Production and Characterization of D614G Spike-Pseudotyped Lentivirus Tethered with AuNRs. Instead of conducting an authentic SARS-CoV-2 virus study in a biosafety level 3 lab, D614G S-pseudotyped lentivirus was produced for biosafety level 2 operation because pseudotyping moiety gives the replication-deficient lentivirus a similar host range as the authentic SARS-CoV-2 virus. To construct SARS-CoV-2 Spike protein pseudotyped viruses, the producer cell expression system was co-transfected with three monocistronic vectors. Using cationic polyethylenimine (PEI), human embryonic kidney (HEK) 293T cells were co-transfected with pCMV-dR8.2 packaging plasmid for virus structure expressing human immunodeficiency (HIV) proteins (Tat, Gag-Pol, and Rev), pLJM1-EGFP plasmid for GFP expression, and pcDNA3.1 SARS-CoV-2 S D614G plasmid for expressing the mutant D614G spike protein on viral capsid. Then, the produced D614G S-pseudotyped lentivirus can be used to infect ACE2-expressing HEK293T (HEK293T-ACE2) cells. The expression of D614G spike protein on the lentivirus surface was verified by the dot blot analysis and dynamic light scattering. The dot blot analysis that was performed using anti-SARS-CoV-2-S1 mAb confirmed the existence of spike protein on the virus surface (Figure 4B). Native lentivirus without spike protein was used as a negative control (Figure 4A). The level of GFP expression (i.e., viral infectivity) in HEK293T-ACE2 cells revealed a dose-dependent augmentation with an increasing dose of soluble ACE2ED-coreSA. The half-maximal inhibitory concentration (IC50) of ACE2ED-coreSA was ~25 μg/mL, comparable to the IC50 value of ACE2-Fc previously reported for the neutralization of SARS-CoV-2 S pseudovirions. The size of S-pseudotyped lentivirus determined by dynamic light scattering was shown in Table 1. The median size of D614G S-pseudotyped viruses (124.48 nm) was ~20 nm larger than the one of native lentivirus without spike protein (99.5 nm) indicating that the median diameter of spike protein is about 12.5 nm, comparable to the reported 10 nm. The zeta potentials of both types of viral particles were measured to be negative. The sizes of AuNR-capped D614G S-pseudotyped viruses were slightly larger than the D614G S-pseudotyped viruses because AuNRs (38 nm in length and 10 nm in diameter) were laterally tethered on viruses. Since the biotinylated AuNR is negatively charged (~22 mV provided by the vendor), the charge of AuNR-virus hybrids (~24.23 mV) is more negative than the D614G S-pseudotyped viruses (~19.53 mV). The UV–vis spectra shown in Figure 5 illustrate two absorption peaks at 520 and 808 nm of AuNR-biotin-coreSA-ACE2ED, AuNR-biotin-coreSA-ACE2ED-D614G S-pseudotyped lentivirus, while no peak was revealed for ACE2ED-coreSA fusion protein and D614G S-pseudotyped lentivirus alone. No significant change was noticed in the absorbance amount and spectra shape of AuNRs before and after functionalization. Moreover, the spectra did not exhibit any red shift of both
transversal and longitudinal absorption peaks of biotinylated AuNRs after binding ACE2ED and D614G S-pseudotyped virus, respectively. This indicates that there was no aggregation of gold nanorods after tethering with protein and virus because aggregation would lead to the broadening of plasmon resonance peaks and a red shift of the absorption spectrum.\textsuperscript{39}

**Affecting the Entry of S-Pseudotyped Lentivirus Tethered with AuNRs.** As shown in Figure 6, stable expression of ACE2 receptors on the outer cell membrane of HEK293T-ACE2 cells was confirmed by immunofluorescence detection (Figure 6A) and Western blot analysis (ACE2 \(\sim 75\) kDa, Figure 6B), in comparison with non-susceptible parental HEK293T cells, which have no expression of ACE2 receptors. HEK293T-ACE2 cells were treated with virus only (as the positive control) and AuNR-capped D614G S-pseudotyped lentivirus for 24 h, respectively. As demonstrated in Figure 7, HEK293T-ACE2 cells treated with AuNR-capped D614G S-pseudotyped lentivirus (Figure 7-B-iv) revealed less GFP protein expression than the cells infected by D614G S-pseudotyped lentivirus only (Figure 7-A-i). This is quantitatively verified by the fluorescent intensities (Figure 7-D) measured by the plate reader. When HEK293T-ACE2 cells are treated with D614G S-pseudotyped lentivirus, the corresponding relative green fluorescent intensity determined in Figure 7-D-i revealed a median value of about 470. For HEK293T-ACE2 cells treated with AuNR-capped D614G S-pseudotyped lentivirus, the relative green fluorescence (shown in Figure 7-D-iv) had a median value of 175, which is an \(\sim 62\%\) decrease from 470 mentioned above. A similar tendency was obtained by the flow cytometric analysis shown in Figure 8-i (27.5\% GFP-expressing cells) and 8-iv (16.8\% GFP-expressing cells). This indicates that ACE2ED-tagged AuNRs were able to bind some of the D614G spike protein on the pseudovirions,

![Figure 3](image1.png)

**(A)** Wash and elution profile of ACE2ED-coreSA fusion protein purification with collected 1 mL fractions detected at OD\(_{280}\), **(B)** SDS-PAGE image of protein markers (M), crude proteins (lane 1), and purified ACE2ED-coreSA fusion protein (lane 2), **(C)** Western blot images of purified ACE2ED-coreSA fusion protein against anti-ACE2 and anti-SA (M = protein markers, E = eluted fusion protein).

![Figure 4](image2.png)

**Figure 4.** Dot blot analysis of lentivirus (as a negative control denoted as A) and D614G spike protein pseudotyped lentivirus (denoted as B) against anti-SARS-CoV-2-S1 mAb.

| Table 1. Dynamic Light Scattering Measurement of the Size, Polydispersity Index (PDI), and Zeta Potential of Native Lentivirus, D614G S-Pseudotyped Lentivirus and AuNR-Capped D614G S-Pseudotyped Lentivirus |
|---------------------------------|------------------|------------------|------------------|
| size (nm)                                                                 |
| native lentivirus               | 99.50 ± 12.2     | 0.28 ± 0.035     | −17.88 ± 2.0     |
| D614G S-pseudotyped lentivirus  | 124.48 ± 12.7    | 0.261 ± 0.036    | −19.53 ± 2.1     |
| AuNR-capped D614G S-pseudotyped lentivirus | 132.88 ± 13.3 | 0.290 ± 0.033 | −24.23 ± 2.3     |
thereby blocking viral entry to HEK293T-ACE2 cells to some extent. Our results indicate that AuNR-capped D614G S-pseudotyped lentivirus without NIR laser treatment could inhibit viral infection to some level. However, according to flow cytometry analysis (given in Figure 8-iv), there were still 16.8% of HEK293T-ACE2 cells that showed GFP expression (i.e., viral infection). This is probably due to the endocytosis of AuNR-virus hybrids, thereby leading to ensuing infection. HEK293T-ACE2 cells without viral treatment were used as the negative control group. As illustrated in Figure 7-C-vi and Figure 8-vi, there was nominal or no green fluorescence detected by fluorescent microscopy and flow cytometry.

AuNRs Photothermal Heating with Collimated Laser Beam. Figure 9 shows the experimental setup for the cell culture system photothermal heating by collimated laser beam output at 808 nm. The side-view IR thermal images displayed in Figure 10 are of a 24-well culture plate inoculated with HEK293T-ACE2 cells and then overlaid with the virus-containing medium (panel A) and plasmonic AuNR-capped virus-containing medium (panel B), respectively. Before the laser was turned on, it showed a uniform temperature distribution around 20 °C. After laser illumination on the virus-containing medium for a duration of 1, 3, and 5 min (panel A) at power density of 2 W/cm², there was only a slight temperature increase (from 20 to 20.5 °C) of the culture medium containing only D614G S-pseudotyped lentivirus. However, the infrared thermal images (panel B) for the culture medium containing AuNR-capped virions under an 808-nm laser exposure with increasing the irradiation time are significantly different from the images shown in panel A. A rapid heating of a localized hot zone was observed upon exposure with an NIR collimated laser beam for 1 min (27.2 °C) and 3 min (35.7 °C). After 5 min of illumination, the temperature reached 41.1 °C and started to level off toward steady-state equilibrium as reported in the literature. Upon removal of NIR laser treatment, the temperature profile displayed expected Newtonian cooling behavior. It should be noted that the elevated temperature below the circle of Figure 10 was caused by heating up an adjustable stainless steel lab platform used to support the cell culture plate.

It should be noted that a thin layer of culture medium (5.26 mm) was used to minimize the temperature gradient within
the culture well. Moreover, the temperature recorded by the IR thermal camera was actually lower than the real value, due to the infrared absorption and emission by the front wall of the culture plate. The side-view images, however, provide the relative temperature profiles of the fixed portion of the medium inside the 24-well culture plate at different times. Since at temperatures greater than 43 °C, disruption of cellular membrane is known to occur and ablation of tumor tissues
has been shown in numerous cases,\textsuperscript{43,44} it was determined for this study to use 5 min laser illumination (with recorded 41.1 °C), which might have higher real temperature but less than 43 °C to avoid damage of HEK293T-ACE2 cells. It should be noted that the local temperature near the AuNR surface would be much higher than the detected bulk temperature of the culture medium due to temperature gradients caused by the photothermal heating generated by AuNRs dissipated to the surrounding medium.\textsuperscript{45}

**Inactivation of S-Pseudotyped Lentivirus Capped with AuNRs under NIR Laser Irradiation.** HEK293T-ACE2 cells, right after they were overlaid separately with medium containing virus only (as the positive control) and AuNR-capped virus, were irradiated with an 808-nm NIR laser up to 5 min. As shown in Figure 7-B-v, HEK293T-ACE2 cells treated with AuNR-capped D614G S-pseudotyped lentivirus under 808-nm NIR laser exposure for 5 min revealed nominal or no detection of GFP protein expression. This is verified by the relative fluorescent intensity (see Figure 7-D-v) measured by the plate reader and flow cytometric analysis of GFP expression in HEK293T-ACE2 cells (2.7% shown in Figure 8-v). When HEK293T-ACE2 cells were treated with D614G S-pseudotyped lentivirus under NIR laser irradiation, the corresponding relative fluorescent intensity determined in Figure 7-A-ii reveals a median value of about 465, which is similar to the one (i.e., 470) obtained without NIR laser irradiation. Likewise, the percentage of GFP-expressing cells determined by flow cytometry for cells challenged with virus under NIR laser illumination (26.2% in Figure 8-ii) is close to the one without NIR laser exposure (27.5% in Figure 8-i).

Another control experiment was conducted by decorating biotinylated AuNRs with coreSA protein only (i.e., no ACE2ED protein) to further confirm the importance of ACE2ED protein tethered on biotinylated AuNRs via the fused coreSA protein for the blocking of viral infection. Figure 7-A-iii demonstrated that AuNRs functionalized with coreSA protein only were not able to attach on D614G S-pseudotyped lentivirus due to the lack of ACE2ED-spike protein binding, thereby resulting in similar GFP expression levels illustrated in Figure 7-A-i and 7-A-II after NIR laser treatment. This is confirmed with similar percentages of GFP-expressing cells and mean fluorescence intensities shown in Figure 8-A (i, ii, and iii) and Figure 8-B (i, ii, and iii), respectively. For HEK293T-ACE2 cells treated with AuNR-capped D614G S-pseudotyped lentivirus under the exposure of 808-nm NIR laser, the relative green fluorescence (shown in Figure 7-D-v) has a median value of 5, which is an ∼99% decrease from 465 mentioned above. This indicates that ACE2ED-tagged AuNRs decorated on the virus surface via the binding between ACE2ED and D614G spike protein on viral capsid were able to thoroughly destroy viral infectivity via the localized hypothermia (temperature much higher than the detected 41.1 °C in the bulk medium) generated by AuNRs under NIR laser irradiation, thereby entirely blocking viral entry to HEK293T-ACE2 cells. It is noteworthy that the bright-field images of cells shown in Figure 7 reveal that the HEK293T-ACE2 cells were grown in a normal cell morphology indicating the temperature was not rising higher than 43 °C, which could destroy the cell membrane as stated above. However, it is surmised that local changes in temperature were not sufficient to cause such destruction.

**Figure 9.** Experimental setup with photothermal heating by collimated laser beam output at 808 nm.

**Figure 10.** Infrared thermal images of HEK293T-ACE2 cells in culture medium (A) containing D614G S-pseudotyped lentivirus and (B) containing AuNR-capped D614G S-pseudotyped lentivirus under 808-nm NIR laser illumination for 1, 3, and 5 min, respectively. The cell culture medium was irradiated by the 808-nm NIR diode laser at 2 W/cm² with a beam area of 0.785 cm².
hyperthermia could increase oxygen consumption and make surrounding cells under hypoxia, thereby resulting in less attachment of the spike protein of SARS-CoV-2 to lung epithelial cells by the diminution of ACE2 expression as reported previously.\textsuperscript{16}

The current COVID-19 vaccine may lose its effectiveness against new coronavirus variants. In this study, biotinylated AuNRs functionalized with ACE2ED-coreSA fusion protein, acting as decoys, bind onto the spike protein of D614G COVID-19 variant and successfully prevented the infection of D614G S-pseudovirulent lentivirus to HEK293T-ACE2 cells when irradiated with an 808-nm NIR laser. Since near-infrared laser with a wavelength of 808 nm can cross the brain skull and target deeper tissues,\textsuperscript{47} if one could deliver ACE2ED-tagged AuNRs to the lungs and target the SARS-CoV-2 viral surface via ACE2ED-spike protein affinity, we may be able to irradiate the NIR laser directly on the lung tissue from the chest or from the back area to generate sufficient amount of heat to ablate infectious virions. Alternatively, the ACE2ED-tagged AuNRs and NIR laser light could be delivered internally to the coronavirus-infected respiratory airways via well-tolerated flexible bronchoscopy, as was previously proposed.\textsuperscript{48}

\section*{CONCLUSIONS}

In this study, we demonstrated that an effective cell entry inhibitor could be established to bind spike protein on viral capsid, thereby blocking the spike protein interaction with the cellular ACE2 receptor. Accordingly, AuNRs functionalized with target ACE2ED molecules, by efficiently augmenting their affinity toward D614G spike protein pseudovirions and behaving as decoys, can block viral interaction with ACE2 present on the outer cell membrane and therefore arrest infection at an early stage to an adequate extent. Additionally, the photothermal effects of ACE2ED-tethered AuNRs generated under 808-nm NIR laser irradiation for 5 min can completely inhibit the infectivity of SARS-CoV-2 surrogates to susceptible cells. Overall, by harnessing the dual functions—virus decoy and destruction—of ACE2ED-tagged AuNRs, this study presents a promising approach to tackling SARS-CoV-2 as well as other diseases involving rapidly mutating viral species.

\section*{MATERIALS AND METHODS}

\textbf{Materials.} Dulbecco's modified Eagles' medium (DMEM) culture medium, 0.25% trypsin-EDTA, fetal bovine serum (FBS), protein concentrator PES (molecular weight cutoff (MWCO) = 50 kDa), phosphate buffer saline (PBS), bacterial protein extraction reagent (B-PER), agar, UltraPure agarose, protease inhibitor ethylenediaminetraacetic acid (EDTA) free, bicinchoninic acid (BCA) protein assay kit, streptavidin monoclonal antibody, bovine serum albumin (BSA), RIPA buffer, HisPur cobalt-NTA resin, enhanced chemiluminescence (ECL) Western blotting substrate, methanol, anti-ACE2 monoclonal antibody (\#x), antistreptavidin mAb, and anti-SARS-CoV-2 spike protein S1 mAb were all purchased from Thermo Fisher Scientific. Dimethyl sulfoxide (DMSO), kanamycin sulfate, and mouse IgG kappa binding protein (sc-516141) were obtained from Santa Cruz Biotech. Mouse IgG HRP (horseradish peroxidase)-conjugated antibody was bought from R&D Systems. Isopropyl-$\beta$-thiogalactoside (IPTG), imidazole, lysogeny broth (LB) media, 2-mercaptoethanol, penicillin-streptomycin, hexamethidine bromide (Polybrene), polyethyleneimine (PEI), and G418 disulfate salt were purchased from Sigma-Aldrich. Acetic acid was bought from J. T. Baker. HEK293T cell line was obtained from ATCC. High-fidelity Phusion polymerase, restriction enzymes (KpnI, EcoRV, EcoRI, XhoI), T4 DNA ligase, Blunt/TA Ligase Master Mix, Monarch PCR & DNA cleanup kit, T7 Express Lemo21(DE3) competent E. coli, NEB5a competent E. coli (subcloning efficiency), and L-ribonuclease were all purchased from New England BioLabs (NEB). Plasmid pET-30a (+) was purchased from GenScript. Rapid Coomassie stain was purchased from Research Products International. Plasmid Maxi kit was obtained from QIAGEN. Plasmid pCDNA3.1-hACE2, pCMV delta R8.2, plJMJ1-EGFP, and pCDNA3.1 SARS-CoV-2 S D614G were purchased from Addgene. Forward and reverse primers were all ordered from Integrated DNA Technologies. Tris-hydrochloride (Tris-HCl) was obtained from Promega. Tris-buffer saline, Laemmli sample buffer, nitrocellulose membrane (0.45 μm), TGY FastCast acrylamide solutions, Tris/glycine/SDS buffer, and Tween-20 were purchased from Bio-Rad. Biotinylated gold nanorods with aspect ratio of 3.8 were purchased from Nanopartz (diameter = 10 nm, length = 38 nm).

\textbf{Construction of Human ACE2 Ectodomain-Core Streptavidin (ACE2ED-coreSA) Encoding Plasmid.} The ectodomain of angiotensin-converting enzyme 2 (ACE2ED, ranging from 18 to 615 residues) cDNA was cloned from pCDNA3.1-hACE2 by polymerase chain reaction (PCR) with forward primer S′-AATGGTGTA-CCACTCTGCTGCTAGTCCACC-3′ and reverse primer S′-GAGAGATATCATATGGACTCCAGTCGGT-3′. The ACE2ED PCR product was generated in a T-100 thermocycler using high-fidelity Phusion polymerase (NEB) with initial denaturation at 98 °C for 30 s, 30 cycles of denaturation at 98 °C for 20 s, annealing at 62 °C for 30 s, extension at 72 °C for 15 s, and final extension at 72 °C for 5 min. The PCR product of core-streptavidin (coreSA) cDNA was achieved with forward primer S′-AGATCGAATTCCGTCCTGCTCGAAGCAGGT-3′ and reverse primer S′-ATTTACCTCGAGGGAGGCGGGCGACGGCTT-3′ using pST2E–215 (yol) plasmid\textsuperscript{57} as a template DNA, with an initial denaturation at 98 °C for 30 s, followed by 35 cycles of denaturation at 98 °C for 10 s, annealing at 62 °C for 15 s, extension at 72 °C for 15 s, and final extension at 72 °C for 5 min. Both PCR products (ACE2ED and coreSA) were then purified through the Monarch PCR & DNA cleanup kit (NEB) and run on 1% agarose gel electrophoresis to confirm. The coreSA product (insert) was cut with restriction enzymes Xhol/EcoRI and ligated by T4 DNA ligase (NEB) in pET-30a (+) to get pET-30a (+)-coreSA. The ACE2ED PCR product was digested with KpnI/EcoRV and ligated in pET-30a (+)-coreSA to get pET-30a (+)-ACE2ED-coreSA. This final ligation product was amplified by transformation with NEB5a competent E. coli and purified using Plasmid Maxi kit (QIAGEN).

\textbf{Expression of ACE2ED-coreSA Fusion Protein.} The constructed pET-30a (+)-ACE2ED-coreSA vector was transformed into Lemo21(DE3) competent E. coli (NEB) for protein expression according to previously reported method.\textsuperscript{50} Briefly, after bacterial transformation the cells were spread on an agar plate (supplemented with 50 μg/mL kanamycin), and incubated overnight at 37 °C. A single colony was resuspended in 5 mL of Luria broth (LB) media (supplemented with kanamycin 50 μg/mL) and grown overnight at 37 °C to produce a starter culture. 0.5 mL of starter culture was diluted to 500 mL of LB media (supplemented with 500 μM of L-ribonuclease and kanamycin 50 μg/mL). When the optical density at 600 nm (OD\textsubscript{600}) reached at least 0.6, cell culture was induced with 400 μM IPTG and kept shaking overnight at 22 °C with 225 rpm. Bacterial cells were then harvested by centrifugation for 15 min at 4500g. The cell pellet was resuspended in 40 mL of lysis buffer (proprietary nonionic detergent B-PER supplemented with 50 mM Tris-HCl and protease inhibitor EDTA-free, pH = 7.4) and incubated at room temperature for 15 min. To maximize the protein extraction efficiency, the bacterial lysate was sonicated on ice for 30 min with power output set at three (Misonix). Finally, the lysate was centrifuged at 18 000g for 15 min, and the supernatant (i.e., crude protein) was collected for purification.

\textbf{Purification of ACE2ED-coreSA Fusion Protein.} ACE2ED-coreSA fusion protein was separated through immobilized metal affinity chromatography from crude fractions. First, the crude protein was mixed with equilibrium buffer (10 mM imidazole in 1X PBS, pH = 7.4) at 1:1 ratio and added to equilibrated HisPur cobalt resin,
followed by incubation at 4 °C for 1 h under gentle shaking condition. The mixture was loaded onto a column, and the flow through was collected. The resin was rinsed with washing buffer (10 mM imidazole in 1× PBS, pH = 7.4) for at least five resin bed volumes, and finally ACE2ED-coreSA fusion protein was eluted with three resin bed volumes of elution buffer (250 mM imidazole in 1× PBS, pH = 7.4). The absorbance OD was followed by incubation at 4 °C for 30 min. Then, the mixture was centrifuged at 10 000×g for 10 min at 4 °C. The lentivirus pellet was finally resuspended in 1 mL of PBS and stored at −80 °C for later use. For the control group, lentivirus without D614G spike protein was prepared as the aforementioned procedures except the SARS-CoV-2 S D614G plasmid was not included in the co-transfection process.

**D614G Spike-Pseudotyped Lentivirus Characterization.** For the characterization of spike protein pseudotyped lentivirus, 5 μL of D614G S-pseudotyped lentivirus and native lentivirus were each loaded onto a nitrocellulose membrane to conduct a dot blot assay. The membrane was blocked with a blocking buffer (5% w/v BSA in TBST) at room temperature for 1 h with mild shaking. The membrane was then incubated in primary antibody solution (1:1000 dilution of anti-SARS-CoV-2-S-2-S1 mAb in blocking buffer) for 1 h at room temperature followed by washing three times with TBST. Next, the membrane was mixed with a secondary antibody solution (1:1000 dilution of mouse IgG HRP-conjugated antibody in blocking buffer) for 1 h at room temperature. The image was detected using ECL substrate.

**Construction and Characterization of ACE2-Expressing HEK293T Cells.** HEK293T cells (originally negative for ACE2) were seeded into a T-25 flask at cell density of 2 × 10⁴ cells/cm² and maintained in culture medium. Next day, the cells were transfected with 5 μL of pcDNA3.1-hACE2 plasmid (1 μg/μL) premixed with 0.1 mL of PEI (200 μg/μL) for 30 min, and the culture media was replaced. After 48 h post-transfection, the cells were selected with 500 μg/mL antibiotic G418 disulfate for four weeks to obtain a stable HEK293T cell line expressing hACE2 (HEK293T-ACE2). To verify the level of ACE2 expressed on the outer cell membrane surface, HEK293T-ACE2 cells were incubated with anti-ACE2 primary antibody for 1 h at room temperature. After three washes with PBS, the cells were incubated with antimouse IgG kappa binding protein conjugated with red fluorescent dye CruzFluor™ S94 as a secondary antibody for 30 min at room temperature. Cells were imaged using a Leica DMi8 microscope equipped with Leica EC3 camera (Leica Microsystems). HEK293T-ACE2 cells were also lysed in RIPA buffer for a Western blot analysis. Briefly, SDS-PAGE was carried out for the lysate and transferred to the blot as stated above. Anti-ACE2 mAb was used as a primary antibody, and antimouse IgG HRP-conjugated antibody was used as a secondary antibody. Virus titer of D614G S-pseudotyped lentivirus was calculated by averaging 1–20% HEK293T-ACE2 cells expressing GFP, measured by a flow cytometer (Accuri C6 Plus, BD Biosciences), after 3 d postinfection of 1 mL of virus with 10-fold serial dilutions ranging from 10⁻¹ to 10⁻⁵. To determine the ability of soluble ACE2ED-coreSA fusion protein to neutralize the pseudotyped virus with D614G spike protein, serial dilutions of the soluble ACE2ED-coreSA proteins was conducted for their capability of blocking the entry of D614G S-pseudovirions into HEK293T-ACE2 cells.

**Preparation and Characterization of ACE2ED-Tagged AuNPs and AuNR-Labeled S-Pseudotyped Lentivirus.** 0.1 mL of purified ACE2ED-coreSA fusion protein (100 μg/mL) was mixed with 6 × 10⁵ particles of biotinylated AuNPs for 30 min at 4 °C. After biotin–streptavidin affinity, ACE2ED-tagged AuNPs were obtained by centrifugation at 1000g for 3 min. Unbound ACE2ED-coreSA protein was removed by washing two times with 1× PBS. The absorption spectra of biotinylated AuNPs, purified ACE2ED-coreSA, and ACE2ED-tethered AuNPs were detected by a microplate reader (SpectraMax M2e; Molecular Devices). The size of the ACE2ED-tagged AuNPs were determined by dynamic light scattering using a NanoBrook 90Plus PALS (Brookhaven Instruments Corporation). The spin-down ACE2ED-tagged AuNPs were further mixed with 1 mL of culture medium containing S-pseudotyped lentivirus (6 × 10⁵TU/mL) for 1 h to facilitate the binding between ACE2ED and spike protein. After that, the mixture was centrifuged at 1000g for 3 min to
remove unbound virions in the supernatant and procure AuNR-labeled S-pseudotyped lentivirus. The absorption spectra, sizes, and charges of native lentivirus, D614G S-pseudotyped lentivirus, and AuNR-capped D614G S-pseudotyped lentivirus were measured by SpectraMax M2e and NanoBrook 90Plus PALS, respectively.

**NIR Laser Irradiation.** HEK293-ACE2 cells were inoculated on a 24-well culture plate at $1 \times 10^5$ cells per well using DMEM supplemented with 10% FBS. The cells were cultivated in 5% CO$_2$ humidified incubator at 37 °C. After overnight culture, the conditioned medium was replaced with 1 mL of AuNR-capped virus containing medium at a multiplicity of infection (MOI) of 6 and then subjected to the collimated 808-nm NIR continuous wave diode laser beam (Onset Electro-Optics) at an intensity of 2 W/cm$^2$. The collimated beam area (0.785 cm$^2$) is smaller than the culture plate cross-sectional area (1.9 cm$^2$). The distance between the laser pointer lens and the top surface of the culture medium containing AuNR-capped virus was fixed at 3 cm. A thermal infrared camera with sensitivity of 0.1 °C (CS, Teledyne FLIR) was positioned 20 cm away to capture thermal images (160 × 120 pixel) of the culture plate and laser. The camera setting was adjusted to measure temperature at a fixed spot on the culture well. NIR laser irradiation continued until 5 min was reached; after that, the culture plate went through a cooling-down process (no radiation). With additional 48 h of incubation, virus infectivity was determined by GFP expression in the transduced cells using a Leica DMi8 fluorescent microscope, SpectraMax M2e plate reader, and BD Accuri C6 flow cytometer. Control experiments were conducted following a similar procedure using virus only with and without laser treatment, virus-treated coreSA-tethered AuNRs (i.e., no ACE2ED on AuNRs) with NIR laser treatment, and AuNR-capped virus without laser treatment for comparison. In each group, the experiment was performed in triplicate.

**Statistical Analysis.** All data were presented as mean ± standard deviation from three independent experiments and analyzed using GraphPad Prism version 8.0 via one-way analysis of variance (ANOVA) test. A $p$-value less than 0.05 was considered statistically significant.

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