Supporting Information

Blocking SARS-CoV-2 Delta Variant (B.1.617.2) Spike Protein RBD Binding with ACE2 Receptor of the Host Cell and Inhibiting Virus Infections Using Human Host Defense Peptides Conjugated Graphene Quantum Dots

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Methods

Gold chloride, citric acid, sodium borohydride, HNP1(ACYCRIPACIAGERRYGTCIYQGRLW AFCC) and LL-37 (LLGDFFRKSKEKRIVQRIKDFLRNLVPRTES) peptides, and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). We purchased the HEK293T cell line and HEK293T cell line from the American Type Culture Collection, which were grown according to an ATCC procedure**.** The GFP tagged Baculovirus pseudotyped with a SARS-CoV-2 Delta Variant (B.1.617.2) spike protein (#C1123G, Montano Molecular, Bozeman, MT) was purchased from Montan0 Molecular, Bozeman, Montana 59715.

Synthesis of Graphene Oxide: Graphene oxide nanoparticles were synthesized using natural Graphite powder with an average size of <150 µm using according to modified Hummer's method which was also described in our previous work [22-23], as shown in Figure S1. In brief, .501 g of graphite, 0.503 g of sodium nitrate, and 23 mL of concentrated sulfuric acid were added into a 500 ml beaker and kept under ice bath (below 5°C) with constant stirring. The mixture was stirred for 20 minutes and then 3.0 gm of potassium permanganate was added to the mixture. The next batch of permanganate was added after the green color disappeared. Then the mixture was maintained under stirring at the same condition. 3 mL of concentrated sulfuric acid was used to wash down the graphite on the inside of the beaker after each addition. Then 75 ml of deionized water was further added to the above solution. Next the H_2O_2 (50 %) was added to the mixture till there was no gas generated (~ 3 mL). There was a distinct change in color was observed from dark brown to golden yellow which is the visual marker for the formation of graphene oxide. color. Then, the yellow mixture was washed with 5% HCl followed by ethanol for remove the metal impurities. Finally, the GO was purified by centrifugation at 10000 rpm speed for 60 min repeatedly until the pH was near neutral (pH ~ 6.5-7.0).

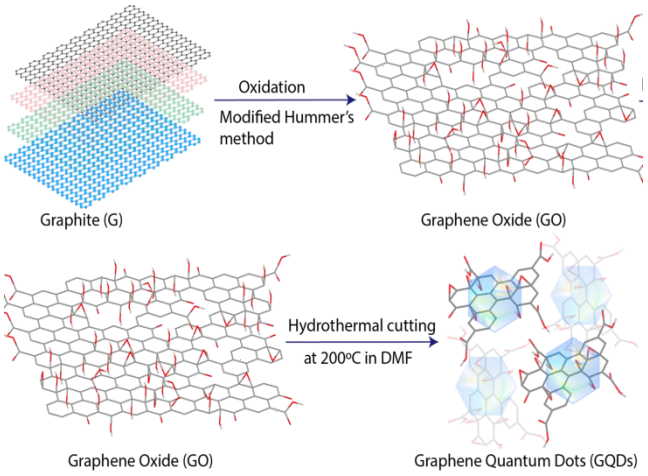


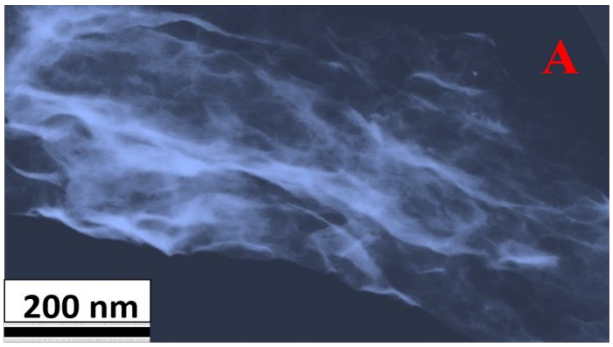
Figure S1: Scheme shows the design of graphene quantum dots (GQDs) from graphite using modified Hummer's method and hydrothermal cutting method

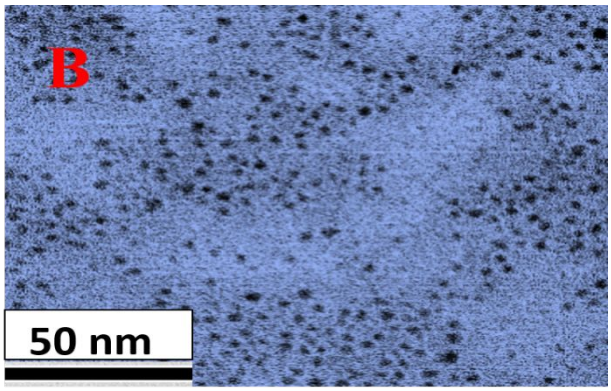
The GO was finally dried by lyophilization at -20°C and characterized by spectroscopic and imaging analysis.

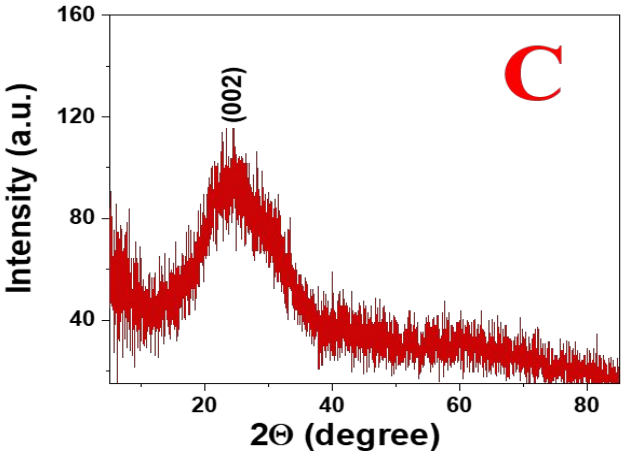
Synthesis of Graphene Quantum Dots (GQDs): Graphene Quantum Dots (GQDs) also synthesized using our previously reported procedure developed by our group[22-23], as shown in Figure S1. In a typical synthesis, 100 mg of GO was added in 100 ml of Dimethyl formamide

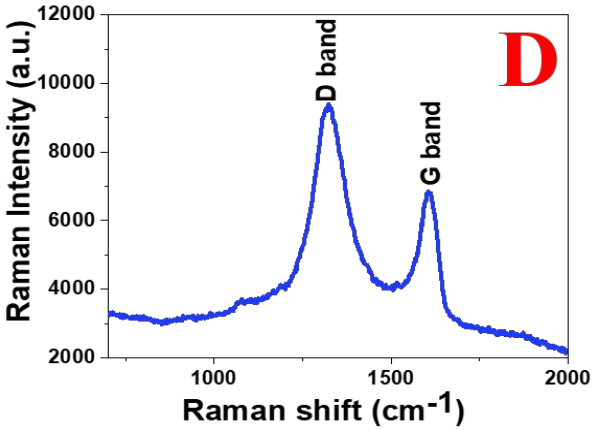
(DMF) to produce GO-DMF suspension. Then the suspension was transferred into a Teflon lined autoclave, and it was heated 200°C for overnight. Then the mixture was cooled down to room temperature and filtered using 0.5-micron filter paper to remove black precipitate. Lastly, the solid product was obtained by rotary evaporation under reduced pressure at 80°C.

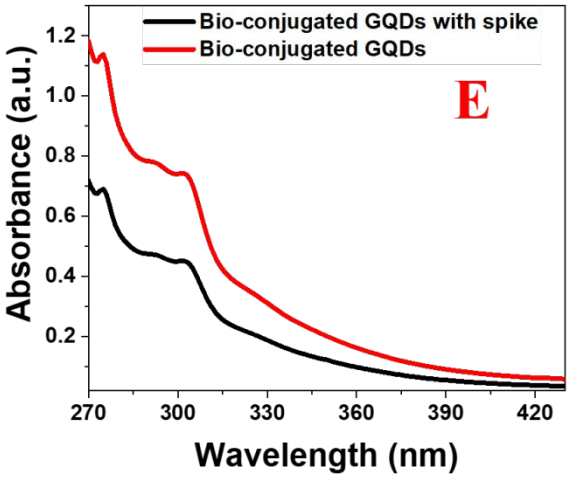
Synthesis of HNP-1 and LL-37 Human Host Defense Peptide attached Graphene Quantum Dots (GQDs): For the development of HNP-1, and LL-37 human host defense peptide attached GQDS, we have used carbodiimide coupling chemistry between carboxy group of graphene oxides and amine group of peptides [17,23].











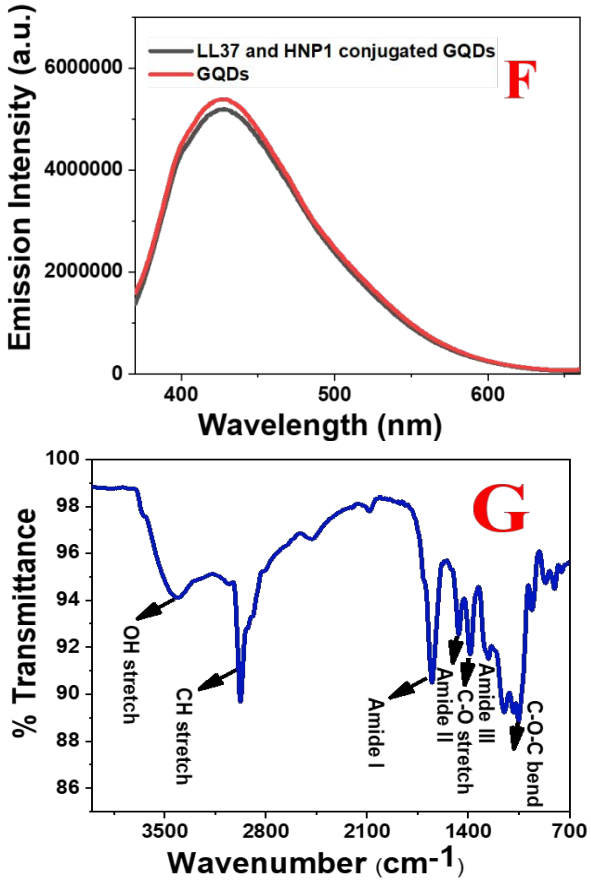


Figure S2: A) TEM shows the morphology of freshly prepared graphene oxide. B) TEM shows the morphology of freshly prepared graphene quantum dots (GQDs), which indicates that GQDs sizes are 4 ± 2 nm C) XRD spectra from LL-37 and HNP1 attached GQDs shows diffractions peaks due to the graphite (002) planes. D) Raman spectrum from LL-37 and HNP1 attached GQDs shows the presence of D and G bands due to the GQD. Higher D bands indicate the high defect due to the presence of peptides. E) Absorption spectra from freshly prepared LL-37 and HNP1 attached GQDs. F) Fluorescence spectra from freshly prepared GQDs and LL-37 and HNP1 attached GQDs. G) FTIR spectra from freshly prepared GQDs and LL-37 and HNP1 attached GQDs show characteristic amide − O−H, −C-H, amide-I, amide-II, C = O and − C−O-C- peaks.

As shown in Figure 1, for this purpose, initially, carboxyl groups from GQDs were activated using 1-ethyl-3(3-(dimethylamino)-propyl)carbodiimide·HCl (EDC) and *N*-hydroxysuccinimide (NHS) coupling agents for 30 min[17-23]. In the next step, HNP-1, and LL-37 human host defense peptides, were added at different weight ratios under stirring for 24 h. After that, we have performed centrifugation at 10 000 rpm for 30 min, followed by decantation.

At the end high-resolution transmission electron microscopy (HRTEM), X-ray diffraction (XRD) spectroscopy, Nano ZS, Energy-dispersive X-ray (EDX), Fourier transform infrared (FTIR) spectroscopy and Raman spectroscopy have been used for characterization[14,15,17,22-23], as shown in Figure S2.

SARS-CoV-2 Delta Variant (B.1.617.2) spike protein RBD and HNP-1, and LL-37 human host defense peptides attached GQDs binding studies using peptide attached GQDs based luminescence

For the binding between, SARS-CoV-2 Delta Variant (B.1.617.2) spike protein RBD and HNP-1, and LL-37 human host defense peptides attached GQDs, 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 $MgCl_2$).

Table S1: Size of GQDs, HNP-1, and LL-37 human host defense peptides attached GQDs and bio-conjugated GQDs bound virus, using dynamic light scattering (DLS) and TEM techniques

System	Size measured by	Size measured by

	DLS	TEM
GQDs	5 ± 2 nm	4 ± 2 nm
Peptides attached GQDs	7 ± 3 nm	6 ± 2 nm
Virus	100 ± 30 nm	100 ± 30 nm
GQDs bound virus	130 ± 30 nm	120 ± 30 nm

For the detection of luminescence from HNP-1, and LL-37 human host defense peptides attached GQDs, in the presence and absence SARS-CoV-2 Delta Variant (B.1.617.2) spike protein RBD, we have used portable fluorescence spectrometer developed by us with laser excitation of 360 nm diode laser [14-15,22-23]. In our design, 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[14-15,22-23]. For emission signal collections, we have used miniaturized QE65000 spectrometer from Ocean Optics[14-15,22-23]. All measurements were performed with 5 ms integration time with 5 spectra averaging using the software[14-15,22-23].

ELISA like assay for determining the binding between Delta Variant (B.1.617.2) spike protein peptides attached GQDs

For this purpose, we have used ELISA-like assay using His-tag Delta variant SARS-CoV-2 S1 protein, which were adsorbed to a 96-well plate overnight at 4 °C.

After that the plate was blocked using blocking buffer and then different concentration peptide attached GQDs were incubated on the plate. In the next step, we have used streptavidin protein that is covalently conjugated to horseradish peroxidase (HRP) enzyme for determine the binding. We have also used TMB (3,3',5,5'-tetramethylbenzidine) for colorimetric assay detection. Signal intensity was recorded using plate reader. From the binding curve, we have estimated the binding constants (K_D) using non-linear curve fitting as reported before.

In Vitro experiment for blocking ACE2 binding with Baculovirus pseudotyped with a SARS-CoV-2 Delta Variant (B.1.617.2) spike protein using Fluorescence Imaging:

For this experiment we have used human embryonic kidney-239T cells with a high expression of ACE2 (HEK-293T). Since Delta variant SARS-CoV-2 is a biosafety-level-3 virus, for our experiment we have used GFP tagged Baculovirus pseudotyped with a SARS-CoV-2 Delta Variant (B.1.617.2) spike protein (catalog number #C1123G). For this experiment, HEK293T cells 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[14-15,27-28]. The pseudovirus stock (2.5 µl of the $2X10^{10}$ units/ml stock) was mixed with the HNP-1, and LL-37 human host defense peptides attached GQDs at different concentration 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. Plates were incubated at 37°C and 5% CO_2 for one day[14-15,27-28]. Cells were fixed in 3.7% formaldehyde for fluorescence imaging experiment[14-15,27-28]. For the fluorescence imaging of an Olympus IX71 inverted confocal fluorescence microscope fitted with a SPOT Insight digital camera was used[14-15,27-28].

Virus inhibition for HNP-1, and LL-37 human host defense peptides attached GQDs

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[14-15,27-28]. These HEK293T cells express low levels of ACE2 receptor that is sufficient for delta variant SARS-CoV-2 entry. Dilutions of test HNP-1, and LL-37 human host defense peptides attached GQDs were made in DMEM with an end volume of 100 µl each[14-15,27-28]. The GFP tagged Baculovirus pseudotyped with a SARS-CoV-2 Delta Variant (B.1.617.2) spike protein stock (2.5 µl of the $2X10^{10}$ units/ml stock) was mixed with the HNP-1, and LL-37 human host defense peptides attached GQDs at different concentrations 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) [14-15,27-28]. 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) [14-15,27-28]. 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[14-15,27-28].

Finding the concentration of virus

To find out the concentration of virus, HEK293T cells were plated in 12 well tissue culture dishes and then the cells were infected with the virus with a dilution range of 10^2 to 10^7, as we have reported before [14-15,27-28]. After that, the virus titers were calculated by counting the GFP positive cells, as we have reported before[14-15,27-28].

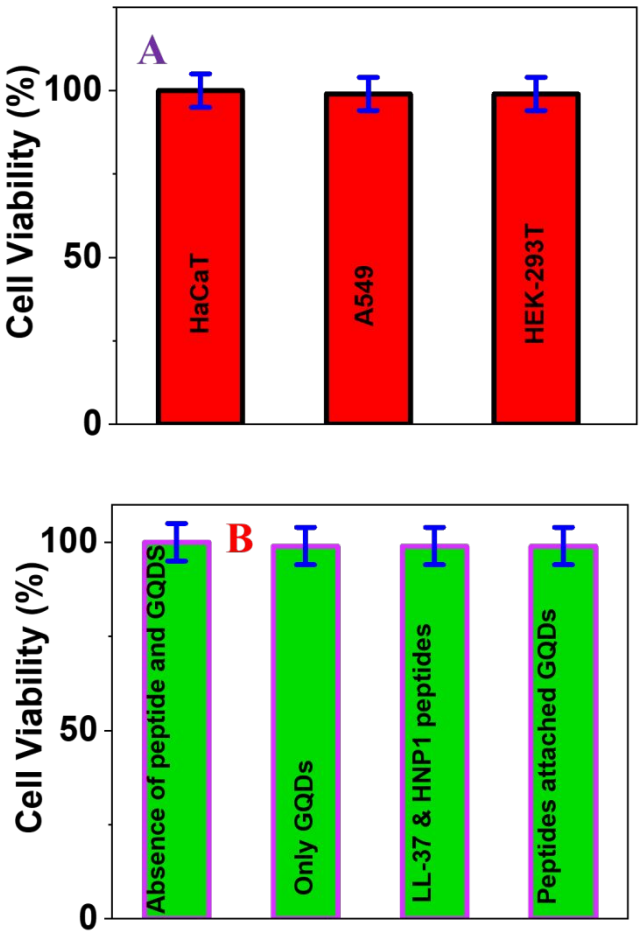


Figure S3: A) Cell viability of normal skin HaCaT cells, lung cancer A549 cells and human embryonic kidney-239T cells when they are treated with 60 µg/mL GQDs + 4 µg/mL LL-37 + 4 µg/mL HNP1 for 48 hours. B) Cell viability of HEK-239T cells in the absence of peptide with GQDs and when they are treated with 60 µg/mL GQDs, 4 µg/mL LL-37 + 4 µg/mL HNP1 peptides and 60 µg/mL GQDs + 4 µg/mL LL-37 + 4 µg/mL HNP1, for 48 hours.