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Supplementary Information

A heparan sulfate proteoglycan-mimicking AIE fluorescent probe for SARS-CoV-2 detection

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1 Materials

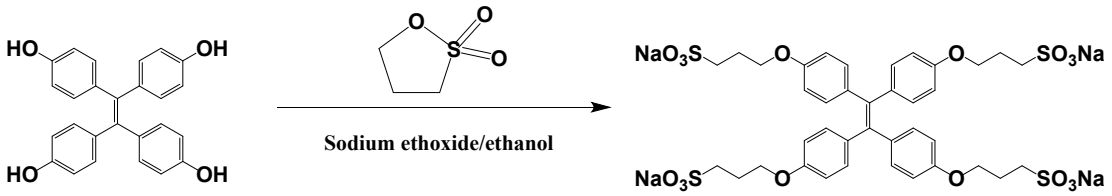
Tetra-(4-hydroxyphenyl)ethylene (TPE-OH) was purchased from Leyan Co., Ltd (Shanghai, China). Sodium ethoxide and 1,3-propanesultone were bought from Aladdin Reagent Co., Ltd (Shanghai, China). Pseudovirus SARS-CoV-2 Omicron variant (B.1.617.2) was purchased from PackGene Biotechnology (Guangzhou, China). Pseudovirus SARS-CoV-2 Omicron variant (B.1.1.529) and Pseudovirus VSV were purchased from Future Biotherapeutics (Suzhou, China). SARS-CoV-2 spike protein was afforded by HuicH (Shanghai, China). Simulated body fluid (SBF) was purchased from Yuanye Bio-Technology Co., Ltd (Shanghai, China). All other solvents and chemicals were of analytical purity and used without further purification.

2 Methods

2.1 Synthesis and characterizations

The water-soluble tetrasulfonated tetraphenylethene (TPES) was synthesized according to previously published experimental procedures.[1] TPE-OH (200 mg, 0.5 mmol) and sodium ethoxide (170 mg, 2.5 mmol) were dissolved in 10 mL ultra-dry anhydrous ethanol. The reaction was carried out at 45℃, 550 rpm, and under argon protection for 1 h to convert TPE-OH to its sodium salt, resulting in a dark green solution. Excess 1,3-propane sultone (0.5 mL) was added, and the reaction continued for 12 h. Then, the solution turned orange-yellow and white solid continuously precipitated, which was collected from acetone and reprecipitated for three times. The precipitate was dried by rotary evaporation to obtain a white powder (TPES, 210 mg, 54%).

The ^{1}H NMR spectra were measured on a 500 MHz NMR spectrometer (Bruker DMX500) (Fig. S1). ^{1}H NMR (D_2O, δ), 7.07 (*d*, *J*=8.6 Hz, 8H), 6.83 (*d*, *J*=8.6 Hz, 8H), 4.14 (*t*, *J*=6.3 Hz , 8H), 3.12–3.06 (*m*, 8H), 2.20 (*dt*, *J*=13.7, 6.3 Hz, 8H). UV-vis spectra were measured by a UV-vis spectrometer (UV-2550, Shimadzu) (Fig. S2).



Scheme S1 Synthetic route of TPES

2.2 Fluorescence measurement

To simulate the virus detection environment, TPES was dissolved in H_2O to obtain stock solution of 1 mg mL^{-1}, 0.2 mg mL^{-1}, 0.1 mg mL^{-1}, 0.05 mg mL^{-1}, 0.01 mg mL^{-1},

0.001 mg mL^{-1}. The fluorescence emission spectra were determined by a microplate reader (BioTek, Synergy H1). Different concentration gradients were measured at 350 nm excitation light and the emission range was 390-600 nm. The highest emission peak was observed at 482 nm. To investigate the AIE effect of TPES, a mixed solvent of DMSO and H_2O was used to dissolve it.

2.3 Virus detection

The Pseudovirus SARS-CoV-2 Delta variant was dissolved in H_2O and stored at 4℃ after being taken out from the -80℃ freezer. A solution of TPES (0.1 mg mL^{-1}, 100 μL) in the H_2O was added into a 96-well costar black/clear bottom plates, followed by the addition of Pseudovirus SARS-CoV-2 Delta variant (4×10^7 TU mL^{-1}, 100 μL). After shaking for 15 seconds and incubated for 5 min, a solution of TPES (0.05 mg mL^{-1}) and Delta variant (2×10^7 TU mL^{-1}) in H_2O was obtained. In the quantitative assay experiment, the mixture of Pseudovirus SARS-CoV-2 Omicron variant (10-1×10^6 TU mL^{-1}) and TPES (0.03 mg mL^{-1}) was obtained. To exclude the influence of concentration on fluorescence intensity, an equal amount of H_2O was blank control. Mixture of TPES and SARS-CoV-2 were allowed to incubated for 5 min consistently before fluorescence spectrum and intensity (λ_{ex}=350 nm, λ_{em}=482 nm) were measured. The stability of the detection probe was investigated by measuring the fluorescence intensity of mixture of TPES and virus after being stored at thermostatic refrigerator for 30 days. Quantitative data was rendered as average ± SD (n=3).

2.4 spike protein detection

SARS-CoV-2 spike protein solutions were prepared as before and their fluorescence characterizations were determined similarly. The solutions of TPES (0.01 mg mL^{-1}) and spike protein (0.0001-0.5 mg mL^{-1}) were shaked for 15 seconds and incubated for 5 min before fluorescence spectrum and intensity (λ_{ex}=350 nm, λ_{em}=482 nm) were measured.

2.5 Interference experiment

The solutions of TPES (0.01 mg mL^{-1}) and pseudovirus SARS-CoV-2 Omicron variant (1×10^7 TU mL^{-1}), pseudovirus VSV (1×10^7 TU mL^{-1}) were measured under the same testing conditions as described in the previous section. The mixture of TPES (0.05 mg mL^{-1}), pseudovirus SARS-CoV-2 Omicron variant (1×10^7 TU mL^{-1}), different ion solution (1 mg mL^{-1}) were measured under the same testing conditions as described in the previous section. The ions included: Cl^-, $CH_3CH_2COO^-$, $H_2PO_4^-$, SO_4^{2-}, SO_3^{2-}, HCO_3^-, Mg^{2+}, NH_4^+.

3 Results and discussion

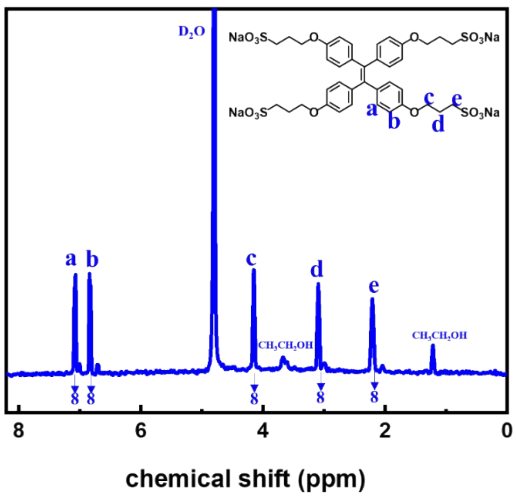


Fig. S1 ^{1}H NMR spectrum of TPES (D_2O, 500MHz)

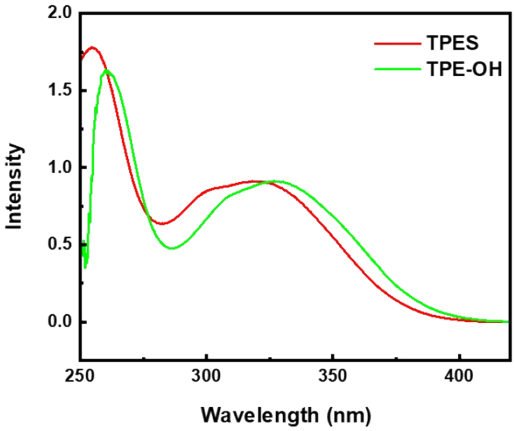


Fig. S2 UV absorption spectra of TPES and TPEOH

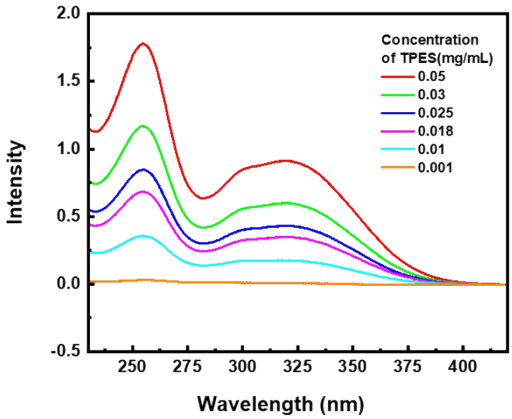


Fig. S3 UV absorption spectra of different concentrations of TPES (concentration of TPES, 0.05 mg mL^{-1}, 0.03 mg mL^{-1}, 0.025 mg mL^{-1}, 0.018 mg mL^{-1}, 0.01 mg mL^{-1}, 0.001 mg mL^{-1})

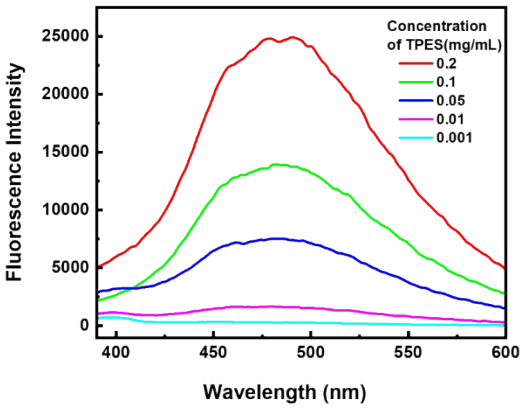


Fig. S4 Fluorescence spectra of different concentrations of TPES (concentration of TPES, 0.2 mg mL^{-1}, 0.1 mg mL^{-1}, 0.05 mg mL^{-1}, 0.01 mg mL^{-1}, 0.001 mg mL^{-1}) λ_{ex}=350 nm, λ_{em}=482 nm

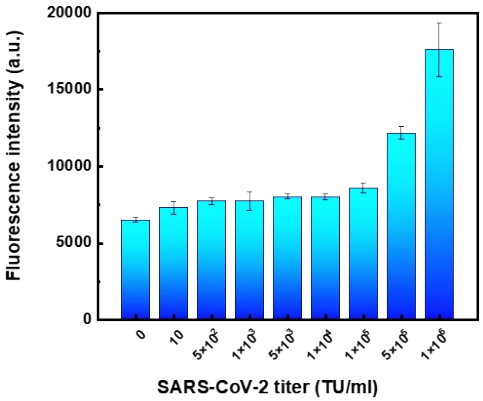


Fig. S5 Fluorescence intensity of TPES mixed with different titers of pseudovirus SARS-CoV-2 Omicron variant (concentration of TPES, 0.03 mg mL^{-1}. titer of virus, 1×10^6 TU mL^{-1}, 5×10^5 TU mL^{-1}, 1×10^5 TU mL^{-1}, 1×10^4 TU mL^{-1}, 5×10^3 TU mL^{-1}, 1×10^3 TU mL^{-1}, 5×10^2 TU mL^{-1}, 10 TU mL^{-1}). λ_{ex}=350 nm, λ_{em}=482 nm

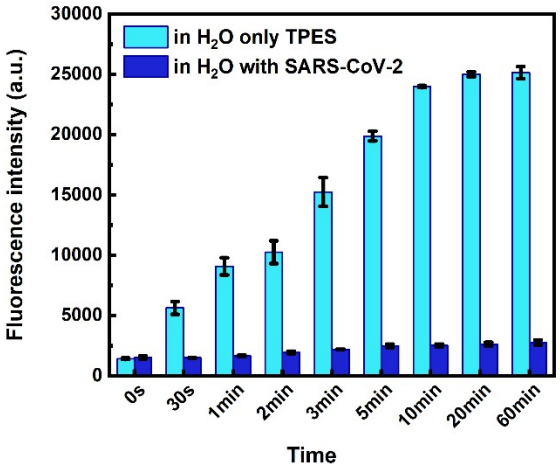


Fig. S6 Fluorescence intensity of TPES after mixing with pseudovirus SARS-CoV-2 Delta variant in water for different time intervals (concentration of TPES, 0.1 mg mL^{-1}. titer of virus, 5×10^5 TU mL^{-1}). λ_{ex}=350 nm, λ_{em}=482 nm

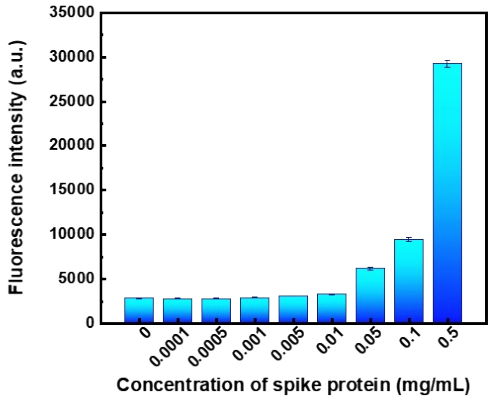


Fig. S7 Fluorescence intensity of TPES mixed with different concentrations of spike protein (concentration of TPES, 0.01 mg mL^{-1}. concentration of spike protein, 0.5 mg mL^{-1}, 0.1 mg mL^{-1}, 0.05 mg mL^{-1}, 0.01 mg mL^{-1}, 0.005 mg mL^{-1}, 0.001 mg mL^{-1}, 0.0005 mg mL^{-1}, 0.0001 mg mL^{-1}) . λ_{ex}=350 nm, λ_{em}=482 nm

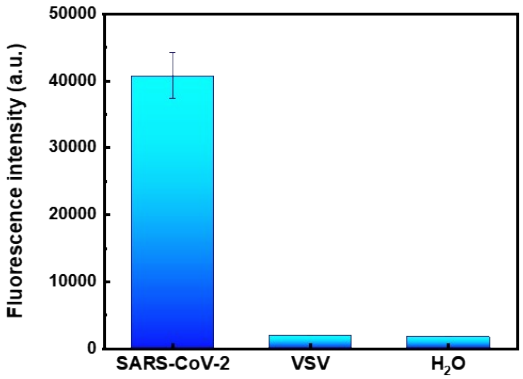


Fig. S8 Fluorescence intensity of TPES mixed with pseudovirus SARS-CoV-2 Omicron variant and pseudovirus VSV (concentration of TPES, 0.01 mg mL^{-1}. titer of virus, 1×10^{7} TU mL^{-1}). λ_{ex}=350 nm, λ_{em}=482 nm

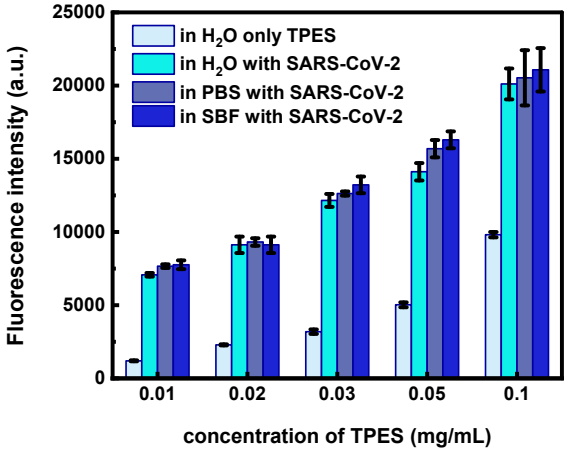


Fig. S9 Fluorescence intensity of TPES mixed with pseudovirus SARS-CoV-2 Delta variant in water, PBS or SBF (concentration of TPES, 0.01 mg mL^{-1}, 0.02 mg mL^{-1}, 0.03 mg mL^{-1}, 0.05 mg mL^{-1}, 0. 1 mg mL^{-1}. titer of virus, 5×10^{5} TU mL^{-1}). λ_{ex}=350 nm, λ_{em}=482 nm

References

1. H. Tong, Y. Hong, Y. Dong, M. Häussler, Z. Li, J. W. Y. Lam, Y. Dong, H. H.-Y. Sung, I. D. Williams and B. Z. Tang, *J. Phys. Chem. B,* 2007, **111**, 11817–11823.