Supporting Information

Materials

Maleic anhydride (MAH), styrene (St), divinyl benzene (DVB), azodiisobutyronitrile (AIBN), bovine serum albumin (BSA), and human serum albumin (HSA) were purchased from J&K Scientific Ltd. Avidin, biotin, PBS buffer solutions and BCA Protein Assay Kit were obtained from Sangon Biotech (Shanghai) Co., Ltd. BSA-avidin was obtained from Beijing Solarbio Science & Technology Co., Ltd.. SARS-COV-2 IgM and SARS-COV-2 IgG were obtained from Hangzhou Sonice Biotechnology Co. Ltd.. SARS-COV-2 Nucleocapsid protein (His tag) was purchased from Sino Biological Inc. Lateral flow assay strips were supported by ZHONGSHAN BIO-TECH Co., LTD.. All of the agents were used as received without further purification. Solvents, including ethyl alcohol, ethyl butyrate (EB), and ethyl acetate (EA) were utilized as received.

Experimental Procedures

Preparation of FPPs

TPE-1VBC was synthesized according to our previous work.[1]

FPPs were prepared though a typical self-stabilized precipitation (2SP) polymerization. In brief, TPE-1VBC (12.50 mg, 0.020 mmol), MAH (200 mg, 2.040 mmol), St (207.90 mg, 1.996 mmol), and AIBN (16.50 mg, 0.101 mmol) were completely dissolved in 20.00 mL EB solution. After excluding oxygen with nitrogen in the reaction for 20 min, the monomer solution was polymerized under nitrogen atmosphere at 60 ^{o}C. PL spectra and DLS analysis of obtained FPPs were carried out at designated time, and the polymerization quenched by quickly cooling under ice water.

For preparation of crosslinked FPPs, crosslinker of DVB was added into the polymerization together with MAH, St, TPE-1VBC and AIBN. A typical procedure of polymerization reaction was exhibited as followed. TPE-1VBC (12.50 mg, 0.020 mmol), MAH (200 mg, 2.040 mmol), St (188.88 mg, 1.816 mmol), DVB (13.26 mg, 0.10 mmol, 5%) and AIBN (16.50 mg, 0.101 mmol) were dissolved in 20.00 mL EB solution and polymerized at 60 ^{o}C with the exclude oxygen procedures.

FPPs with objective sizes were obtained by controlling the concentration of monomers and the reaction time.

Conjugation of BSA and biotin to FPPs

FPPs obtained from 2SP polymerization were employed to conjugate BSA or biotin through the rapid reaction of anhydride group and amine group. After polymerization, FPPs was centrifuged and harvested from EB solution, and they were washed sequentially by ethyl alcohol and water. After that, FPPs were redispersed into 1 mL PBS buffer solution (pH 8.0, 1mM) with the final concentration of 1mg/mL. For BSA conjugation, 1 mg BSA was added into the FPPs dispersed solution, and it was vibrated at 25 ^{o}C for 8 h. The amount of BSA loaded was measured by BCA Protein Assay Kit

by detecting the absorbance at 562 nm. As biotin conjugated to FPPs, a mixture solution of biotin/BSA was utilized with the concentration of 0.1 mg/mL for biotin and 0.9 mg/mL for BSA. After cultured for 8 h, FPPs with BSA or biotin attached was washed by PBS buffer solution and harvested by centrifuging.

Determining flowing rate and fluorescence intensity in FLFA testing

FLFA testing of the FPPs with different sizes was conducted through the specific binding of biotin on FPPs and avidin on FLFA testing strips. With immersing the bottom of FLFA testing strips into the biotin conjoined FPPs, FPPs infiltrate the strips and flowed to the head of the strips owing to the absorbance from absorbent pad. Flowing time of FPPs consumed from start line to test line was recorded, and the flowing rate was calculated by the simple division operation of distance and time.

Fluorescence intensity of FPPs with different sizes was detected by PL spectrophotometer as the strips were dried thoroughly.

SARS-COV-2 detection

FPPs was centrifuged and harvested from the polymerization solution. After polymerization, FPPs were washed sequentially by ethyl alcohol and water, and diluted into PBS buffer solution (pH 7.4, 10 mM) at the final concentration of 20 μg/mL. 20 μg of SARS-COV-2 Nucleocapsid protein (His tag) and 100 μg of HSA were added into the obtained FPPs dispersed solution and stored at room temperature for 8 h for preparing FPPs indicator.

For detecting SARS-COV-2 IgG and IgM, 10 μg of SARS-COV-2 IgG and 10 μg of SARS-COV-2 IgM was added into 200 uL FPPs indicator solution, which composed the reaction solution. After that, FLFA strips was immersed into the reaction solution and incubated for 15 min. The resulted strips were photographed under UV light at 365 nm.

Characterization

The photoluminescence spectrum was measured using an Edinburgh FS5 fluorescence spectrophotometer. Time resolve emission scan was conducted by Edinburgh FS980 fluorescence spectrophotometer with 377 nm laser as the exciting light. Morphology of nanoparticles was investigated by scanning electron microscopy (SEM, JMS-7800F, JEOL, Japan). Size distribution of nanoparticles were detected on dynamic light scattering (DLS) (NanoBrook Omni, Brookhaven Instruments Co. USA) at a fixed angle of 90^{o} at 25 ^{o}C. UV absorption spectra were taken on a SHIMADZU UV-2600 spectrophotometer.

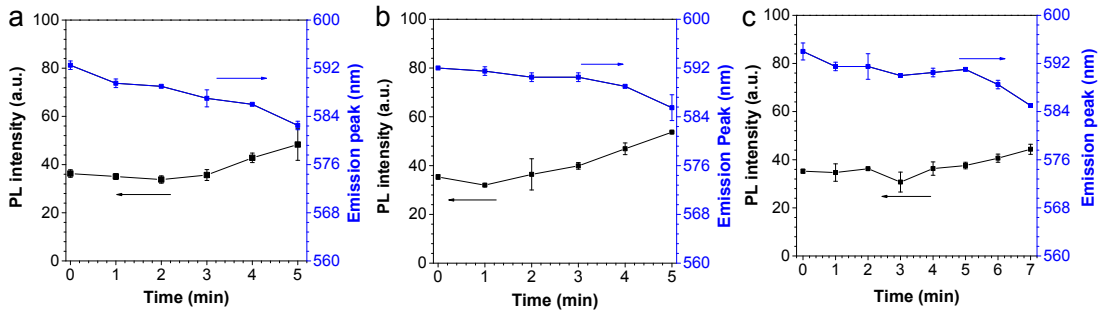

Fig. S1. Plots of PL intensity and emission peak versus polymerization time at the initial of the polymerization, a) in the absence of DVB, with b) 5% and c) 10% DVB as crosslinker. Polymerization was conducted in EB solution at 60 °C, and the wavelength of the excitation is 420 nm.

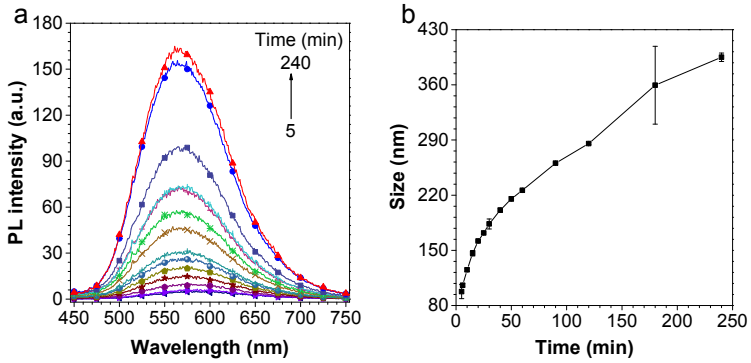

Fig. S2. a) PL spectra and b) FPPs sizes of polymerization at 5, 10, 20, 30, 40, 50, 60, 90, 120, 180, and 240 min in EB solution in the absence of DVB.

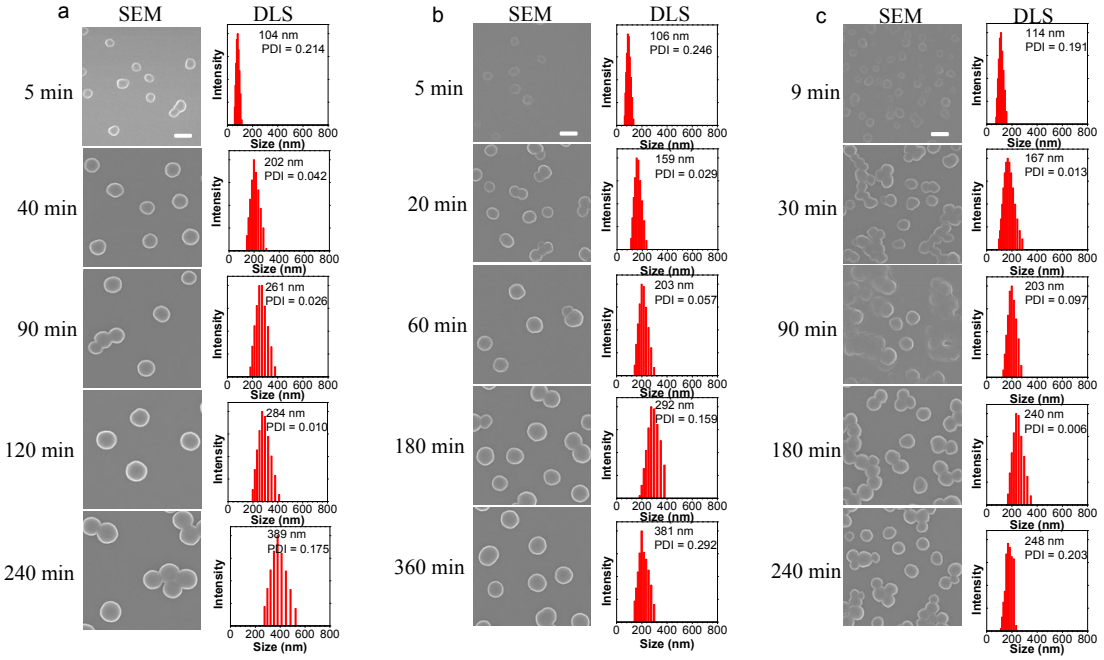

Fig. S3. SEM images, and DLS analysis of FPPs obtained at different time in EB solution a) in the absence of DVB, b) with 5% DVB as crosslinker, and c) with 10%

DVB as crosslinker. Scale bars in SEM images are 400 nm.

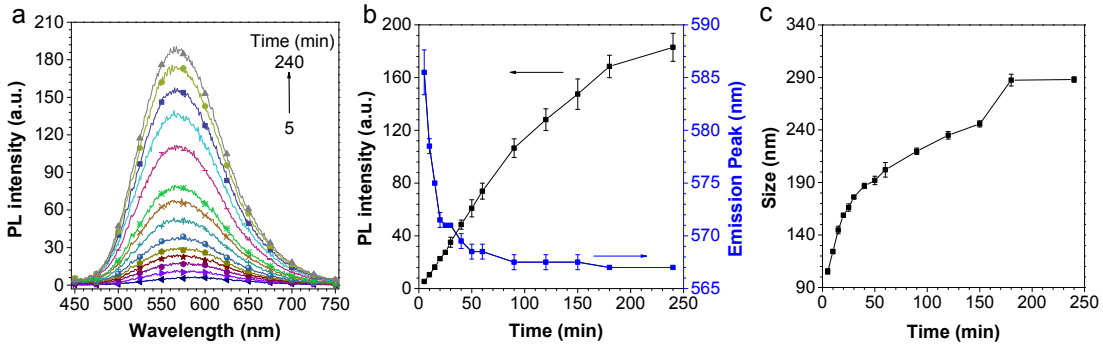

Fig. S4. a) PL spectra, b) plots of PL intensity, and emission peak and c) FPPs sizes of polymerization at 5, 10, 20, 30, 40, 50, 60, 90, 120, 180, and 240 min in EB solution with 5% DVB as crosslinker.

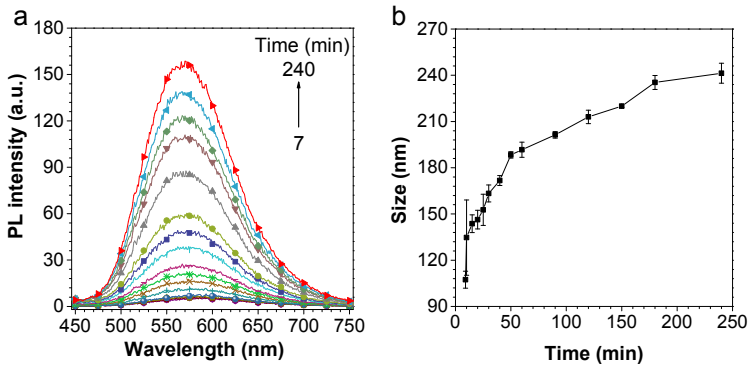

Fig. S5. a) PL spectra and b) FPPs sizes of polymerization at 7, 10, 20, 30, 40, 50, 60, 90, 120, 180, and 240 min in EB solution with 10% DVB as crosslinker.

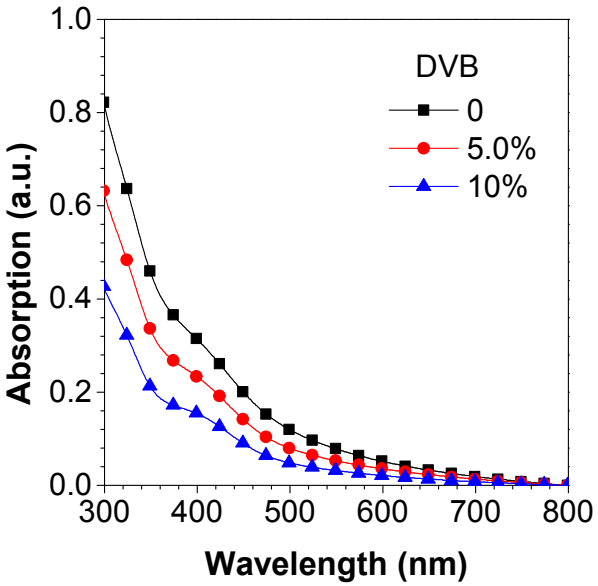

Fig. S6. UV-vis absorption spectra of FPPs in EB solution under different crosslinking (0, 5.0, and 10%). The final concentrations of TPE-1VBC are 2.55 mM.

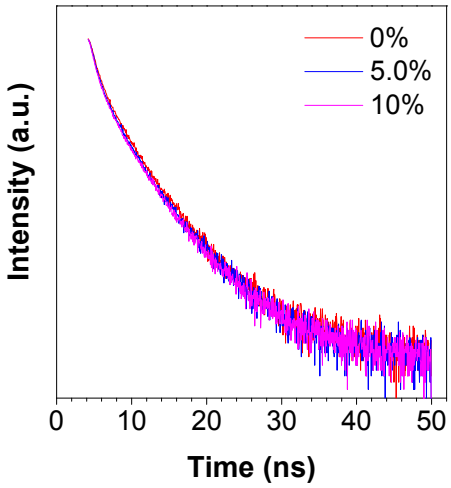

Fig. S7. Time delay PL spectra of the FPPs at 560 nm in EB solution with different degrees of crosslinking. Laser with the wavelength of 377 nm was employed as the excitation light source.

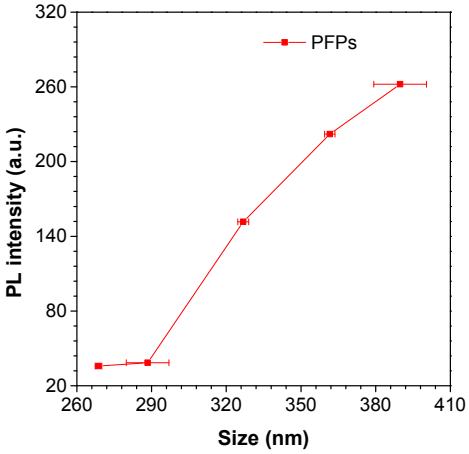

Fig. S8. Plots of PL intensity versus FPPs size in EB solution in the absence of DVB.

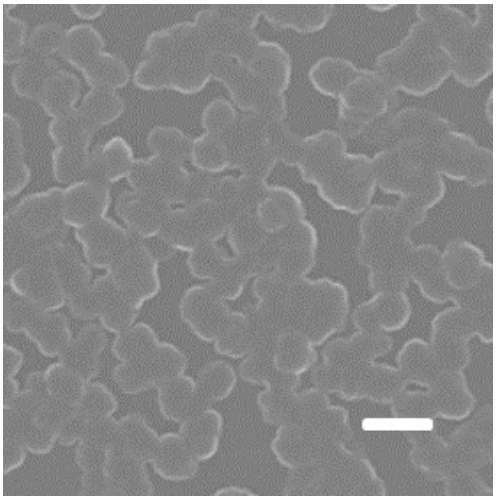

Fig. S9. SEM images of the FPPs obtained from the polymerization with the 20% DVB

utilized. The scale bar is 100 nm.

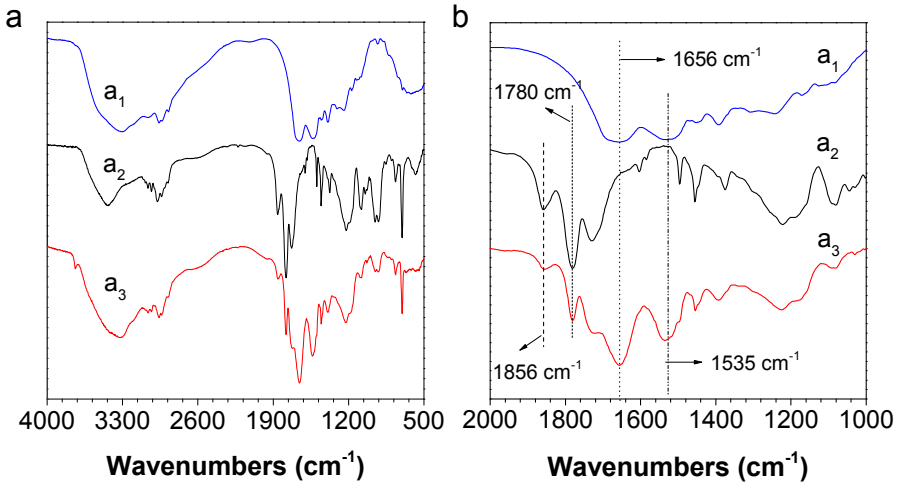

Fig. S10. (a) Overall and (b) partial FTIR spectra of (a_1) BSA, (a_2) FPPs, and (a_3) BSA conjugated FPPs.

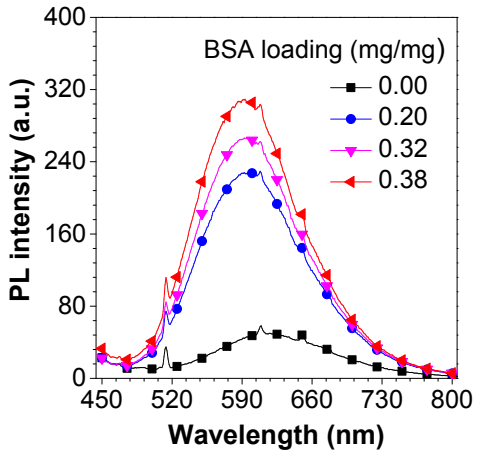

Fig. S11. PL spectra of FPPs in PBS buffer solution (1M, pH 8.0) with various amounts of BSA loading on the FPPs.

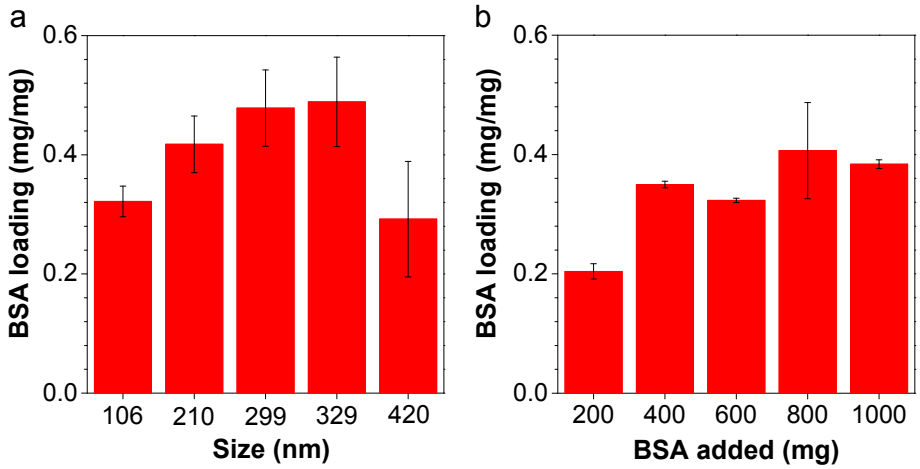

Fig. S12. (a) BSA loading efficiency of FPPs with different sizes. (b) BSA loading

efficiency of FPPs@210 with different initial amounts of BSA added.

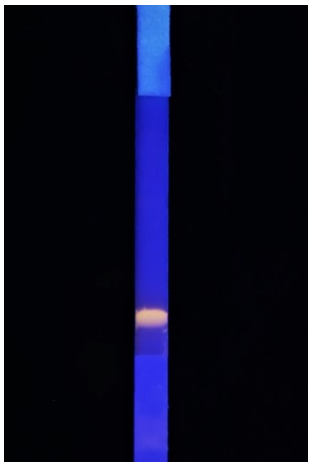

Fig. S13. BSA conjugated FPPs employed for FLFA testing strips.

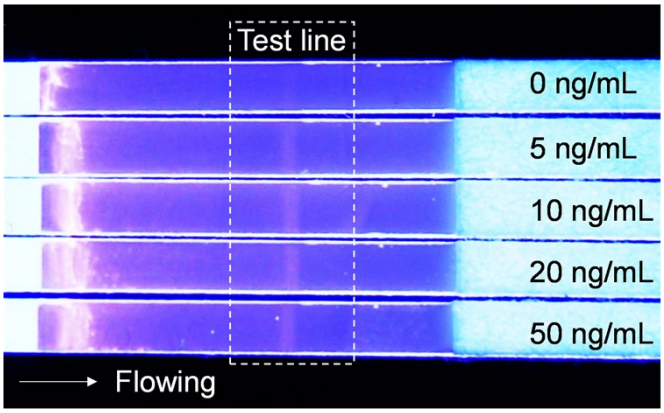

Fig. S14. Fluorescent photos of strips under UV light on detection of analytes containing 0−50 ng/mL biotin.

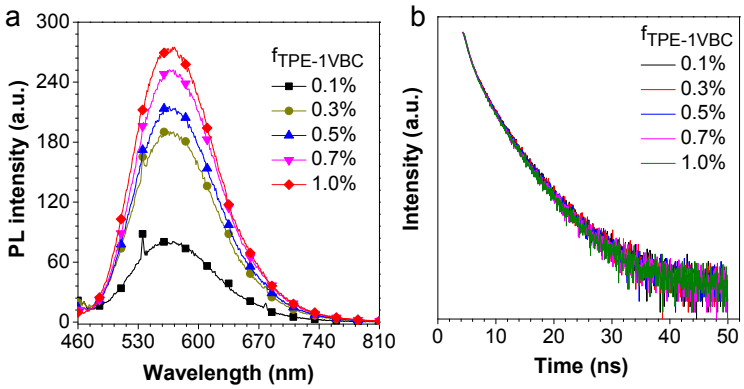

Fig. S15. a) PL spectra and b) time delay PL spectra of the polymerizations in EB solutions with the amount of TPE-1VBC from 0.1% to 1.0%.

Reference:

1. G. Wang, L. Zhou, P. Zhang, E. Zhao, L. Zhou, D. Chen, J. Sun, X. Gu, W. Yang and B. Z. Tang, Fluorescence Self-Reporting Precipitation Polymerization Based on Aggregation-Induced Emission for Constructing Optical Nanoagents, *Angew. Chem., Int. Ed.*, 2020, **59**, 10122-10128.