Electronic Supplementary Information (ESI)

for

Two-photon Fluorescence Silica Nanoparticles-Based FRET Nanoprobe Platform for Effectively Ratiometric Bioimaging of Intracellular Endogenous Adenosine Triphosphate

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1. Apparatus

The transmission electron micrographs (TEM) were acquired from a JEM-2100 transmission electron microscope (JEOL, Japan) at 200 kV, a drop of the diluted samples of two-photon silica nanoparticles and the nanoprobe were placed on the surface of the copper grid, respectively. Dynamic Light Scattering (DLS) and Zeta potential analysis were performed from a Zetasizer Nano ZS90 (Malvin Instruments, Ltd., Malvin, UK) at room temperature, to ensure the accuracy of DLS measurement, be very careful to eliminate dust from the samples. The absorption spectra of samples were recorded by a UV-1800 spectrophotometer from Shimazu Co. Ltd (Kyoto, Japan). Fluorescence measurements were recorded by a RF-6000 spectrophotometer from Shimazu Co. Ltd (Kyoto, Japan) was used to obtain the one-photon excited fluorescence signal. Silica gel 60 F254 was used to carry out thin layer chromatography (TLC), and silica gel (200-300 mesh) was used for column chromatography. Both silica gel 60 F254 and silica gel (200-300 mesh) were obtained from Qingdao Ocean Chemicals (Qingdao, China). The Cell Counting Kit-8 (CCK-8) assay was performed using Varioskan Flash microplate reader (ThermoFisher Scientific) at 490 nm. one-photon excited fluorescence images were obtained by using LEICA TCS SP8 laser scanning confocal microscope. Two-photon excited fluorescence images were obtained by using Olympus FV1200MPE multiphoton laser scanning confocal microscope.

2. Synthesis of COOH-TP-SiNPs

The 2-naphthalenecarboxylic acid (215.2 mg, 1.0 mmol) was added to DMF (5.0 mL) containing 0.5 ml of DIPEA and HATU (456.2 mg, 1.2 mmol). After 30 min of reaction in an ice bath, the APTES (221 mg, 1.0 mmol) was added and stirred for 1 h at room temperature. The mixture was poured into ice water and the crude product was observed to be precipitated. The crude product was separated by vacuum filter and purified by silica gel column chromatography (DCM/EtOH = 50: 1, v/v) to obtain solid. The TEOS (1.0 mL) was dissolved in ethanol of 1.0 mL, then 20 mg of the above solids were added to the mixture one by one, then a few drops of triethanolamine were added drop by drop, and stirred for 1 hour. Thereafter, the product was collected by centrifugation and washed several times with ethanol to remove the residual reactants. The surface of silica nanoparticles were functionalized with carboxyl groups by co-condensation method. The reactants were fully dissolved in ethanol, then glutaric anhydride was added, stirred for 3 hours, centrifuged and washed off the unreacted glutaric anhydride, and freeze-dried to obtain carboxyl functional two photon fluorescence silica nanoparticles COOH-TP-SiNPs.

3. Cell Culture and Cytotoxicity Assays.

The MCF-7 cell line was cultured in DMEM medium supplemented with 10% FBS at 37°C in a humidified atmosphere containing 5% CO_2. The Cell Counting Kit-8 (CCK-8) assay is technique used to determine the cytotoxicity of the nanoprobe. cells were seeded in 96-well plates at a density of 1 × 104 cells per well. Then， cells were incubated in 90 μL of DMEM (10% FBS) for 24 h at 37°C in a humidified atmosphere containing 5% CO_2. Different concentration of nanoprobes (1.0-4.0 mg/mL) was added in 100 μL of DMEM. the cell medium was removed and washed with Dulbecco's phosphate buffered saline (DPBS) after incubation at different times (12 and 24 h), 100 μL of fresh medium and 10 μL of CCK-8 in DMSO were added and incubated for 30 min. The cytotoxicity was obtained by recording the absorbance at 450 nm.

4. Stability experiments

In order to study the stability of the DNA chains on the surface of the our nanoprobe, the UV-Vis absorption spectrometer was used to conduct stability experiments and the FAM is the detection target. As a control, the solution containing the nanoprobe was detected by UV-Vis absorption spectrometer, and centrifuged. Then, the supernatant was used for the detection UV-Vis absorption spectrum. the nanoprobe (10 μg/mL) was blended with DNase (2 U/mL) and RNase (2 U/mL) at 37℃ for 4 h, the mixture was centrifuged and the supernatant was used for the detection UV-Vis absorption spectrum.

5. Location experiments

For the investigation of localization of TP-SiNPs in living cells, MCF-7 cells were seeded on glass-bottom dishes for 24 h. These cells were incubated with 100 μg/mL TP-SiNPs at 37℃ for 4 h in culture medium. After washed three times with PBS, lyso

tracker green (50 nM) was incubated with cells at 37℃ for 15 min. CLSM images were obtained by using Olympus FV1200MPE. The fluorescence imaging of TP-SiNPs was collected in channels, 420-480 nm, under excitation at 450 nm. The fluorescence imaging of lyso tracker was collected in channels, 520-580 nm, under excitation at 488 nm. Image J was used to calculate the Pearson correlation coefficient (P).

6. Anti-interference performance of nanoprobe

In order to research the impact of different ions on nanoprobe, representative interfering ions (5.0 mM Ca^{2+}, Na^{+}, Cl^{-}, NO^{3-}) was selected was blended with the nanoprobe (500 μg/mL) at 37℃ for 60 min. We measure the signal intensity at 445 nm and 520 nm, respectively.

7. FRET Efficiency

FRET efficiency was deduced from the fluorescence emission spectra of TP-SiNPs@FAM-aptamers by the following equation:

$$E = 1 - F_{TP\text{-}SiNPs@FAM\text{-}aptamers} / F_{TP\text{-}SiNPs} \quad (1)$$

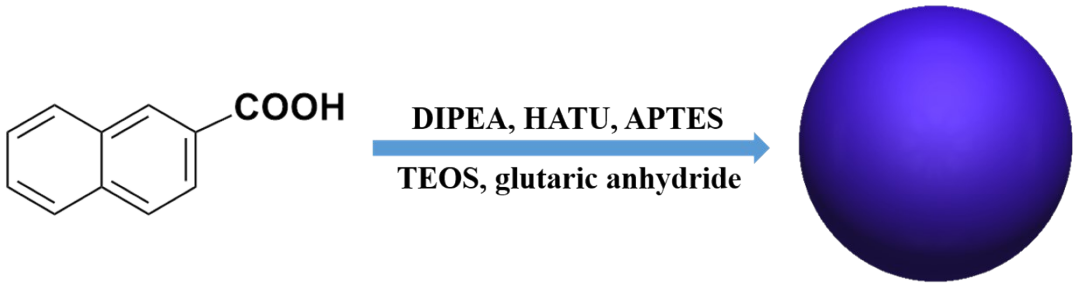
E: FRET efficiency

$F_{TP\text{-}SiNPs@FAM\text{-}aptamers:}$ Fluorescence Intensity of TP-SiNPs@FAM-aptamers

$F_{TP\text{-}SiNPs}$: Fluorescence Intensity of TP-SiNPs

E_m=450 nm

8. Supplementary Scheme and Figures



Scheme S1 Synthesis schematic of TP-SiNPs.

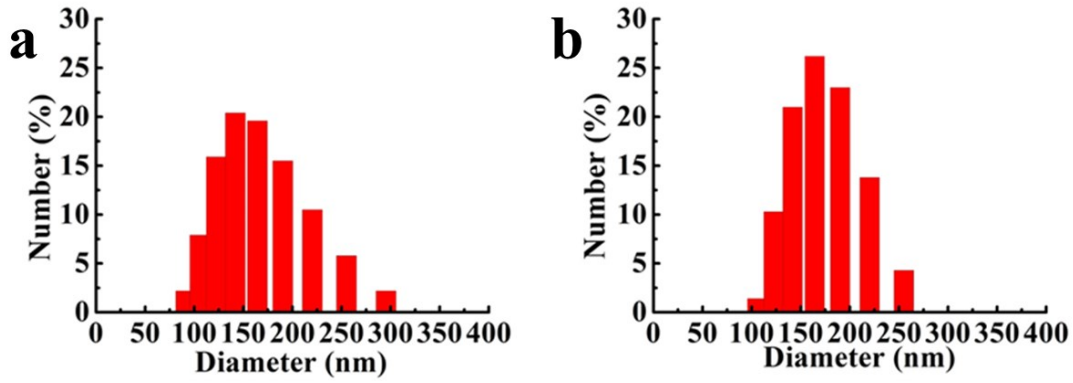


Fig. S1 DLS of TP-SiNPs (a) and TP-SiNPs@FAM-aptamer (b) in deionized H_2O.

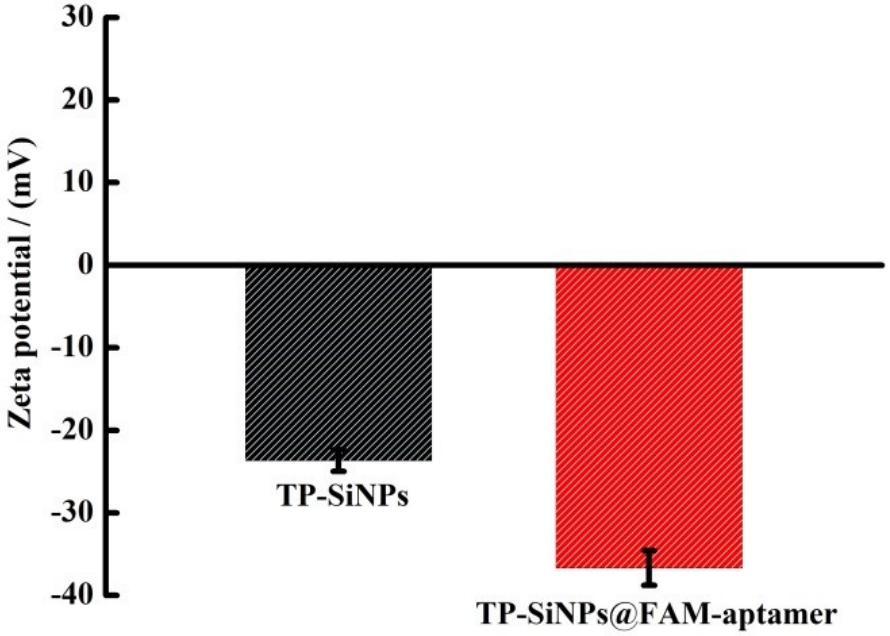


Fig. S2 Zeta potential of TP-SiNPs (-23.6 ± 1.3 mV) and TP-SiNPs@FAM-aptamer (-36.7 ± 2.1 mV) in deionized H_2O.

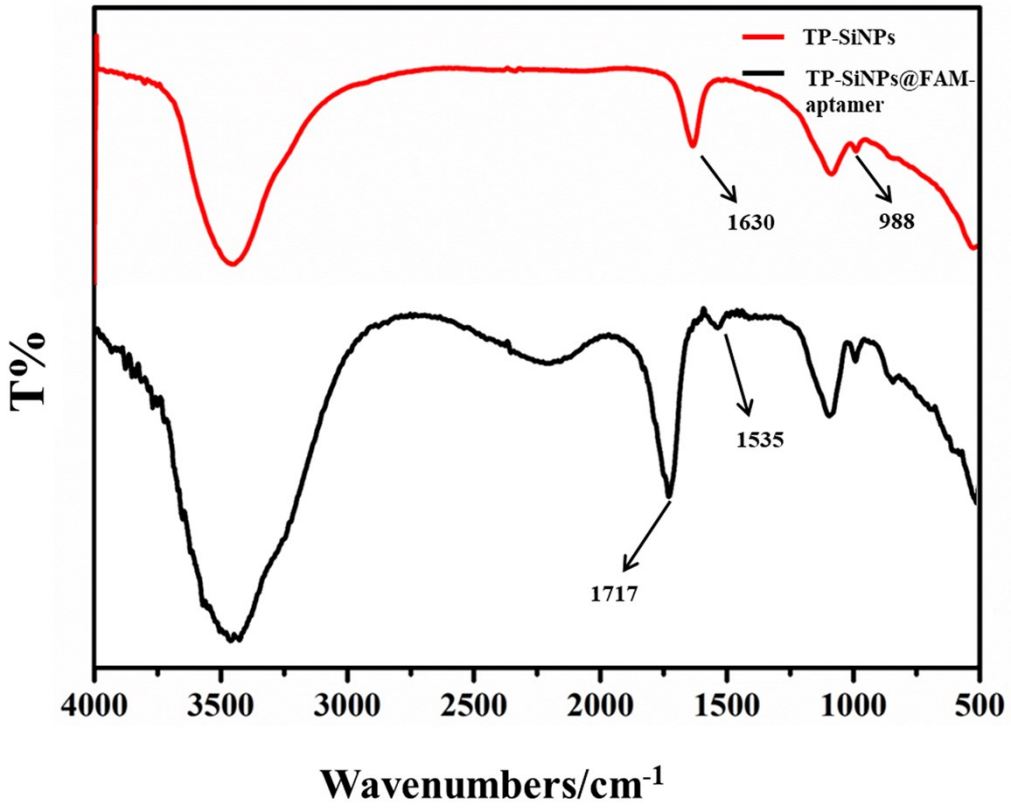


Fig. S3 FTIR spectra of TP-SiNPs, TP-SiNPs@FAM-aptamer.

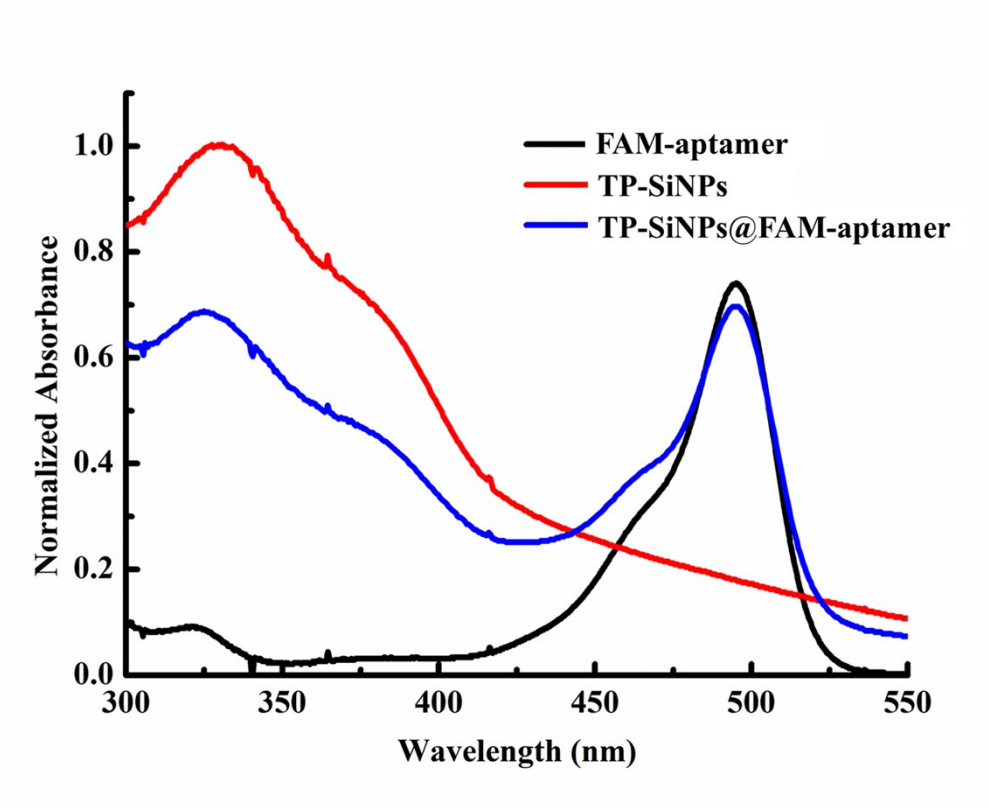


Fig. S4 The normalized absorption spectra of FAM-modified primer, TP-SiNPs and TP-SiNPs@FAM-aptamer.

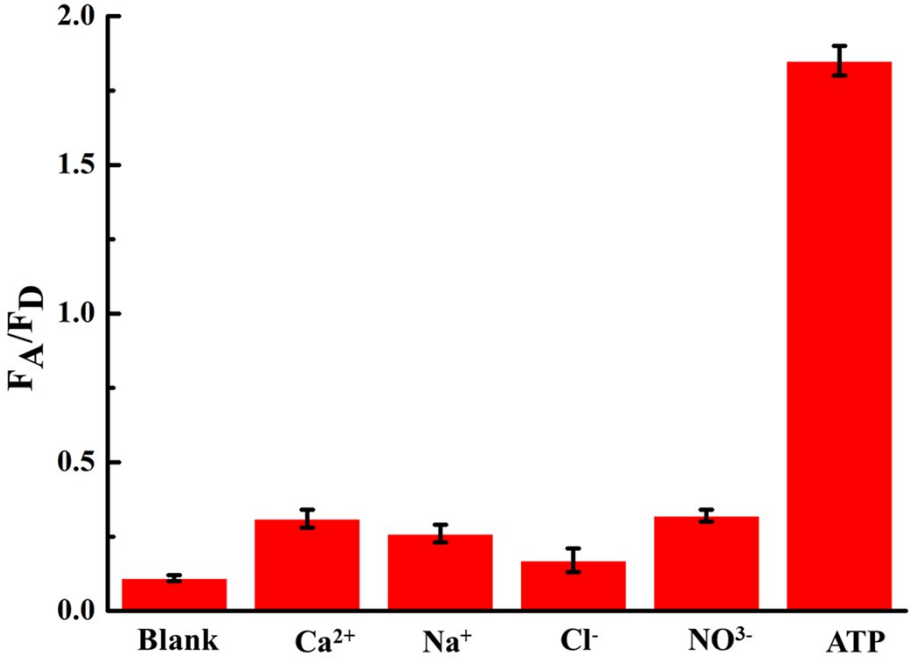


Fig. S5 Ratiometric fluorescent responses of TP-SiNPs@FAM-aptamer (500 μg/mL) for Ca^{2+}, Na^+, Cl^-, NO^{3-} and ATP (5.0 mM**).** (F_D: fluorescence donor, F_A: fluorescence acceptor)

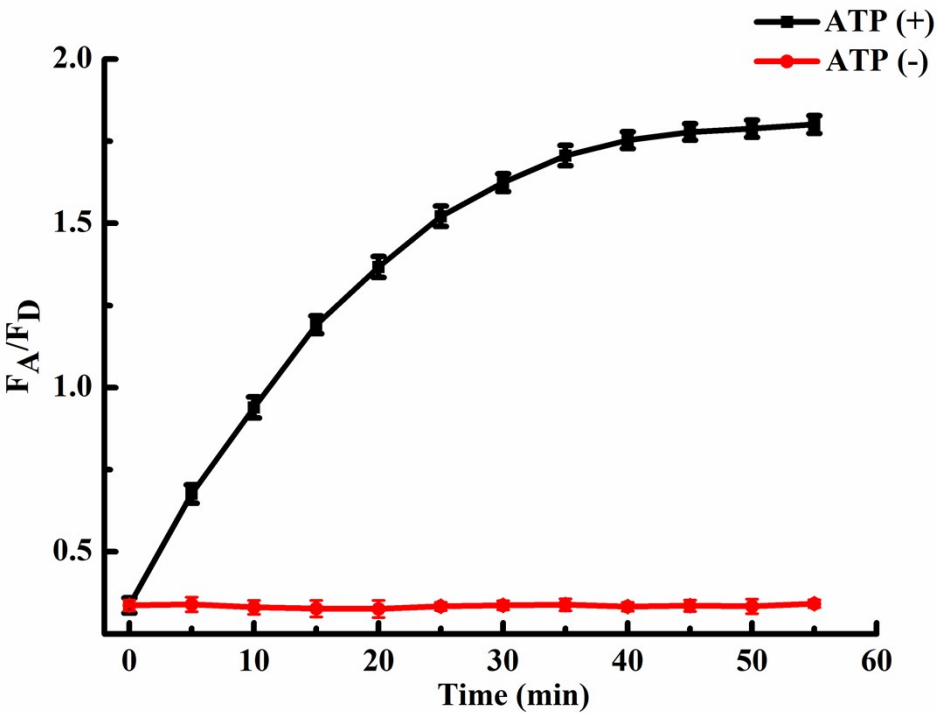


Fig. S6 Time-dependent fluorescence spectral changes of TP-SiNPs@FAM-aptamer (500 μg/mL) in HEPES buffer (0.1 M, pH 7.4). Time points represent 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 min. (F_D: fluorescence donor, F_A: fluorescence acceptor)

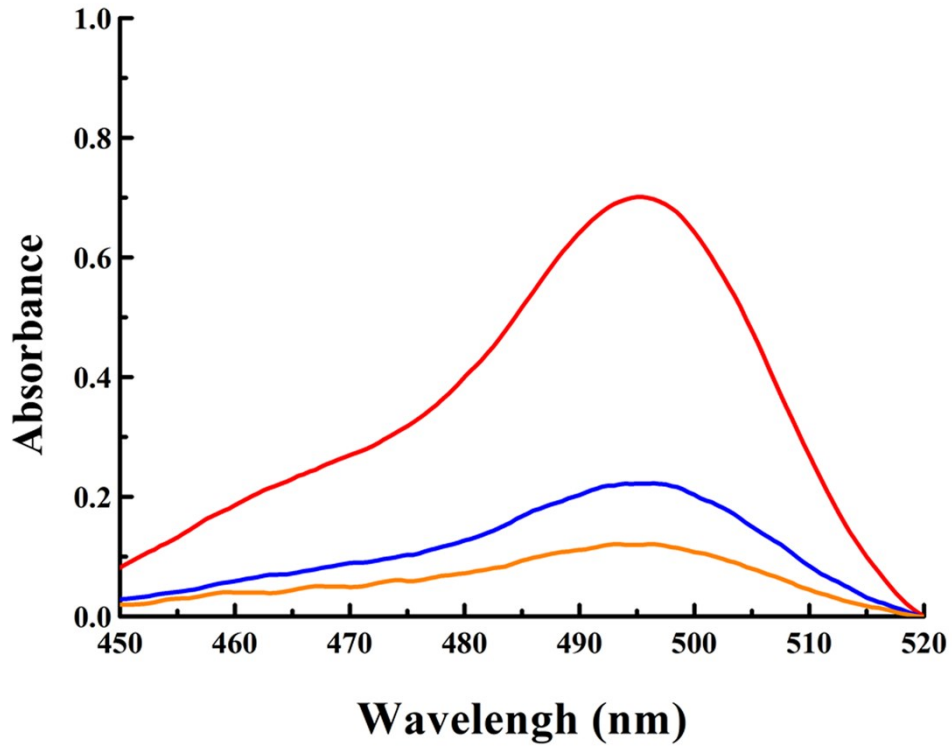


Fig. S7 UV-Vis absorption spectroscopy of TP-SiNPs@FAM-aptamer, supernatant of TP-SiNPs@FAM-aptamer (10 μg/mL) blended with DNase (2 U/mL) and RNase (2 U/mL), supernatant of TP-SiNPs@FAM-aptamer without DNase and RNase. the red line represents the signal of FAM on the surface of nanoprobe, the blue and yellow lines represent FAM signals of supernatant, which came from the nanoprobe solution with enzymes incubation and without enzymes incubation, respectively.

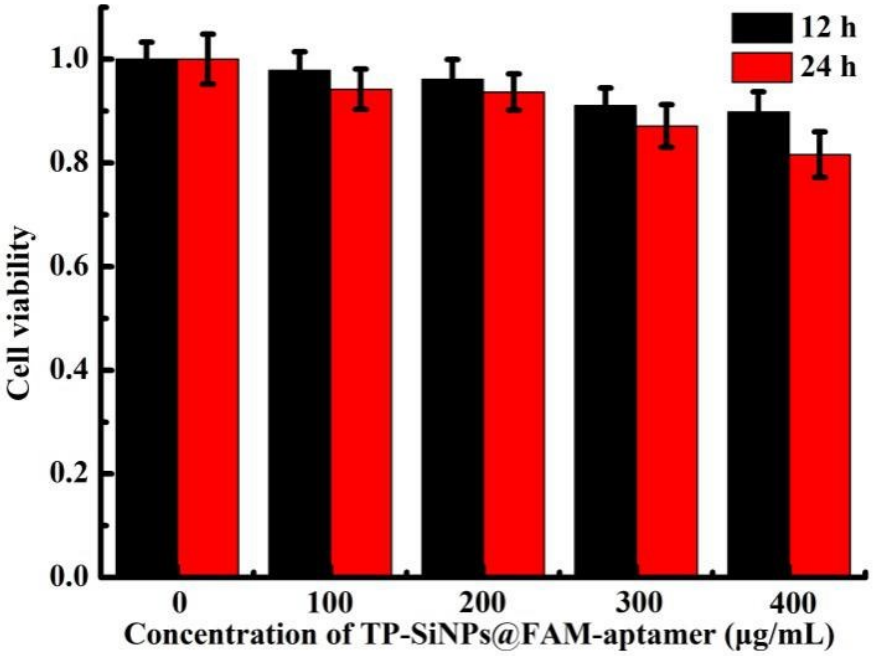


Fig. S8 Cytotoxicity assay of MCF-7 cells treated with different concentrations of nanoprobes TP-SiNPs@FAM-aptamer (100~400 μg/mL) with 12 h and 24 h.

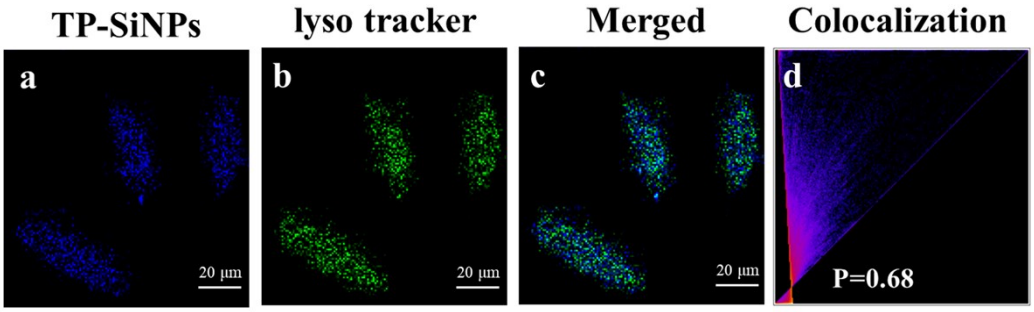


Fig. S9 Fluorescence confocal imaging of living MCF-7 cells were obtained after incubation with TP-SiNPs (a), lyso tracker (b), merged (c) and colocalization (d, P: the Pearson correlation coefficient). The living MCF-7 cells were incubated with TP-SiNPs (100 μg/mL, 4 h). After washed three times with PBS, lyso tracker green (50 nM) was incubated with cells for 15 min. Images were collected in separate channels, 420-480 nm and 520-580 nm, under excitation at 450 nm and 488 nm, respectively. Scale bar = 20 μm.