Supporting information for

A sensitive fluorescent nanoprobe for sulfatase detection and imaging in living cells and in vivo

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Experimental Section

Materials and instruments

No additional purification was required and all of the reagents and solvents were utilized just as they were. Perform TLC analysis on the silica gel plate. The quadrupole mass spectrometry provided the MS spectrum. The Bruker Advance 300 MHz spectrometer was used for conducting ^{1}H-NMR and ^{13}C-NMR experiments. The absorption measurement was conducted using an Ultraviolet-visible spectrophotometer UV-2550 (Shimadzu, Japan). The fluorescence spectral detection was carried out with a Fluorescence spectrophotometer from Hitachi, Japan. For cell and tissue imaging, the Zeiss Axio Observer A1(Leica, Germany) was utilized. Additionally, the IVIS Lumina XR (PerkinElmer, USA) was used for animal imaging.

Synthesis of probe TCF-SULF

Compound TCF-OH was synthesized according to the reported procedure[19].

Compound TCF-SULF. Dissolved the fluorophore TCF-OH (94 mg, 0.312 mmol) in tetrahydrofuran (5 mL), then slowly added sodium *tert*-butoxide (30 mg, 0.312 mmol) dissolved in tetrahydrofuran (1 mL) to the solution. Stirred at room temperature for 15 minutes, then added the trimethylamine-sulfur trioxide copolymer (56.1 mg, 0.406 mmol) and stirred at room temperature for 30 minutes. The reaction solution was concentrated under decreased pressure once the reaction was finished, and the crude product was purified using column chromatography to yield the red solid **TCF-SULF** (10 mg, 10%). ^{1}H NMR (300 MHz, DMSO-*d*6) δ 7.97 (s, 1H), 7.89 (d, J = 8.4 Hz, 2H), 7.30 (d, J = 8.5 Hz, 2H), 7.14 (d, J = 16.3 Hz, 1H), 1.79 (s,6H). ^{13}C NMR (75 MHz, DMSO-*d*6) δ 177.73, 176.07, 157.89, 148.01, 131.47, 129.31, 120.60, 114.11, 113.30, 112.46, 111.53, 99.81, 98.53, 29.50, 25.65. TCF-SULF HRMS (ESI) m/z calculated, for $[M-H]^-$ 382.0503, found 382.0488.

Absorption spectrum studies of TCF-SULF and TCF-OH

Used a UV-Vis spectrophotometer to measure the absorption spectra of **TCF-SULF** and the compound TCF-OH. Absorption spectra were obtained using a 5.0/5.0 nm slit width in the 350-800 nm range. In a PBS solution (1% DMSO, 10 mM, pH=7.4),

the absorption spectra of the chemical TCF-OH (10 μM) and the probe **TCF-SULF** (10 μM) were captured. Furthermore, the absorption spectra of **TCF-SULF** (10 μM) were examined in the presence of sulfatase.

Fluorescence response studies of TCF-SULF to sulfatase

A fluorescence spectrophotometer was used to measure the fluorescence levels in the samples. Under illumination at 580 nm, emissions from solutions with a slit width of 10.0/10.0 nm and a wavelength range of 600-750 nm were gathered. Following the addition of different sulfatase concentrations to the **TCF-SULF** solution, the fluorescence spectra were captured. In particular, 10 μM of **TCF-SULF** was already present in a PBS solution (including 1% DMSO, 10 mM, pH = 7.4) when several concentrations of sulfatase (0-45 U/mL) were added. The mixture was then incubated at 37 °C for 30 minutes. After that, the fluorescence reaction was closely observed and recorded.

To verify the specificity of **TCF-SULF**, additional biorelevant species were introduced to test its selectivity for sulfatase. The following species are biorelevant: HPO_4^{2-}, SO_4^{2-}, H_2O_2, GSH, Cys, Gly, BSA, Arg, GOX, LOX, SULF, Cl^-, Mg^{2+}, K^+, and CO_3^{2-}. Sulfatase or other biorelevant species were incubated with the probe **TCF-SULF** (10 μM) in PBS solution (1% DMSO, 10 mM, pH = 7.4) at 37 °C for 30 minutes. After that, all of the fluorescence spectra were collected.

Fluorescence quantum yield calculation

A series of dilute solutions of standard substances and test substances were prepared, resulting in a gradient of UV visible absorbance at a certain wavelength λ. We tested the fluorescence emission peak generated by the excitation light λ, integrated the peak area, and linearly fitted the absorbance with the integrated area to obtain two straight lines passing through the origin, and then calculated the quantum yield.

The fluorescence quantum yield (Φ) is calculated by the following formula,

$$\Phi_X = \Phi_{ST}\left(\frac{k_X}{k_{ST}}\right)\left(\frac{\eta_X^2}{\eta_{ST}^2}\right)$$

The subscripts ST and X represent the standard and tested fluorescent dyes, respectively; Φ is the fluorescence quantum yield; k is the slope of the linear fitting

line; η is the refractive index of the solvent. The reference system used is Rhodamine 6G solution in ethanol (Φ_{ST}=94%).

Preparation of Nanoprobes TCF-SULF NPs

The stock solution was obtained by dissolving **TCF-SULF** (1 mg) and DSPE-PEG_{2000} (20 mg) in 200 μL of DMSO. Add the solution gradually to the 3 mL of distilled water that has been forcefully mixed, and stir for three hours. To obtain nanoparticles, sonicate for approximately five minutes using a 50 Hz ultrasonic cleaner. Finally, the particle size and shape were characterized using a laser dynamic light scattering particle size analyzer and a high-resolution transmission electron microscope.

Cell culture

Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), penicillin (80 mg/L), and streptomycin (80 mg/L) was used to cultivate MCF-7 cells in a CO_2 incubator set at 37 °C and 5% CO_2.

MTT assay

To investigate the cytotoxicity of the nanoprobes **TCF-SULF** NPs, an MTT assay was conducted on MCF-7 cells. Transferred the MCF-7 cells in the logarithmic growth phase to different 96-well plates and cultured them (1×10^4 cells per well). Afterward, added probes **TCF-SULF** NPs (10 μM, 20 μM, 30 μM, 40 μM, and 50 μM) to the wells, and incubated in a CO_2 incubator (37°C, 5% CO_2). Following a 24-hour incubation period, each well was filled with 20 μL of MTT solution (5 mg/mL) and put the well back in the CO_2 incubator to continue incubating. After four hours, the 96-well plate was taken out, thrown away the culture medium, and then filled each well with 150 μL of DMSO. After shaking for 15 minutes at room temperature in the dark, a microplate reader was used to evaluate the cell viability at 490 nm.

Cell imaging

MCF-7 cells were cultivated in confocal culture dishes to enable confocal fluorescence imaging. After two hours of incubation, treat MCF-7 cells with **TCF-SULF** (0, 80, or 100 μM) and discard the combined medium. The cells underwent three PBS solution washes, a 4% paraformaldehyde fixation, a six-minute staining with 50μL

DAPI, and two additional PBS washes. Next, image the cells using the FV 1000 laser scanning confocal microscope. It gathers emission between 520 and 830 nm and is excited between 500 and 630 nm.

Fluorescent imaging in living mice

The animals involved in the experiment were all purchased from the Qinglongshan Animal Breeding Center (Nanjing, China). All animals were subjected to quality testing by China Pharmaceutical University before use and were handled in strict accordance with the management regulations of the National Animal Protection Center.

To construct a subcutaneous tumor model in mice, MCF-7 cells were inoculated at a density of 2×10^6 cells/mouse in the left axilla of the nude mice. The subcutaneous tumor model was created and the probe **TCF-SULF** was injected into the tumor after it had grown for four to six weeks. Fluorescence imaging was performed on mice at different time points. After the shooting was completed, the mice were dissected, and the other organs and tumor tissues were removed. The organs and tumor tissues were photographed under fluorescence. Imaging was performed on the small animal imaging system using 580 nm excitation light and a 620 nm emission filter.

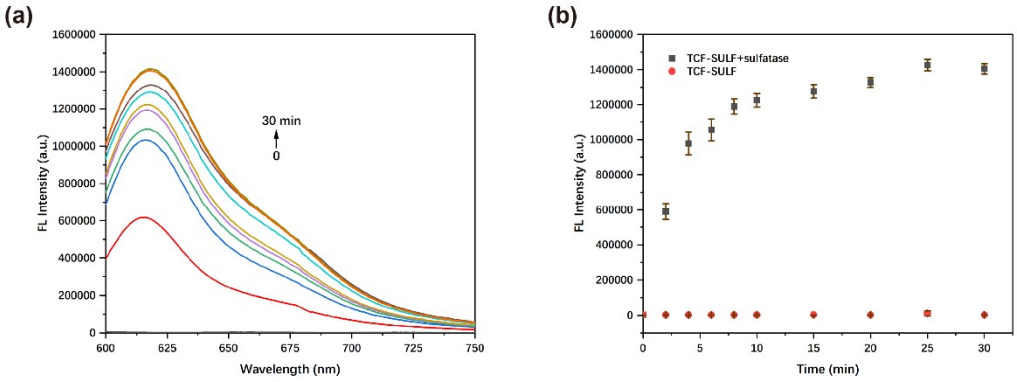

Figure S1. (a) Time-dependent fluorescence intensity of TCF-SULF (10 μM) containing sulfatase (20 U/mL) in PBS buffer (1% DMSO, pH = 7.4). λex/λem = 580 nm/620 nm； (b) Variation of fluorescence intensity at 620 nm at different times.

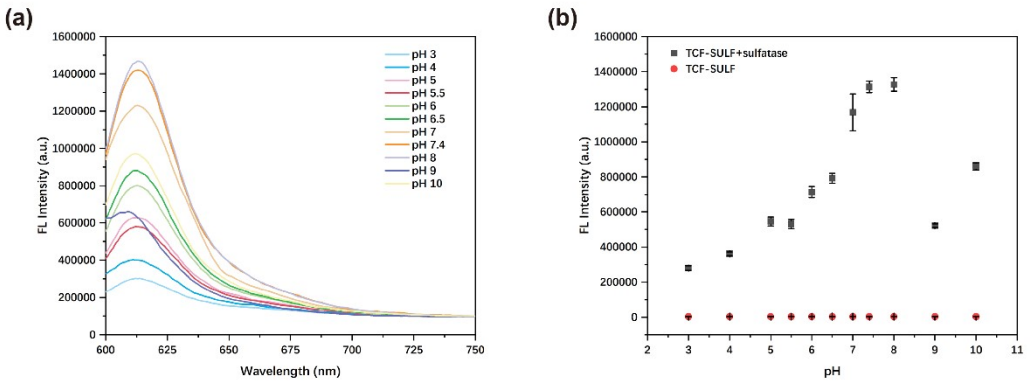

Figure S2. (a) Fluorescence spectra of TCF-SULF (10 μ M) in response to sulfatase under different pH conditions； (b) Fluorescence intensity at 620 nm in the absence and presence of sulfatase (20 U/mL).

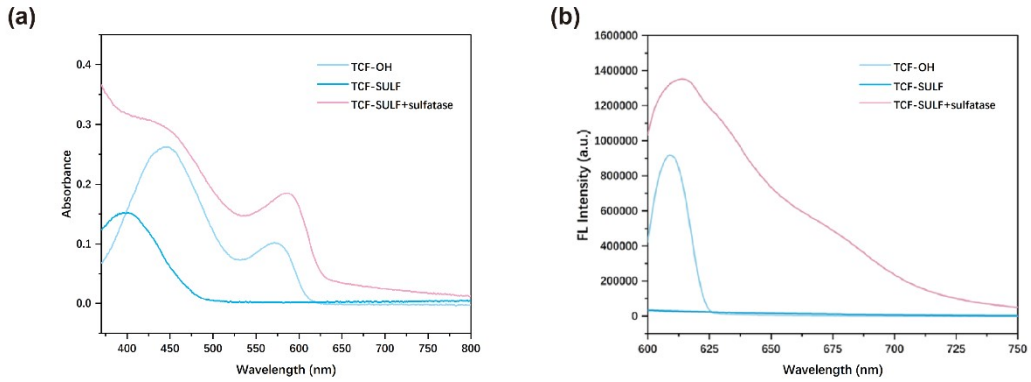

Figure S3. (a) Absorption spectra of **TCF-OH** (10 μM) and **TCF-SULF** (10 μM) in the absence and presence of sulfatase (20 U/mL) in PBS buffer (1% DMSO, 10 mM, pH = 7.4). (b) Fluorescence spectra of **TCF-OH** (10 μM) and **TCF-SULF** (10 μM) in the absence and presence of sulfatase (20 U/mL) in PBS buffer (1% DMSO, 10 mM, pH = 7.4), λ_{ex} = 580 nm.

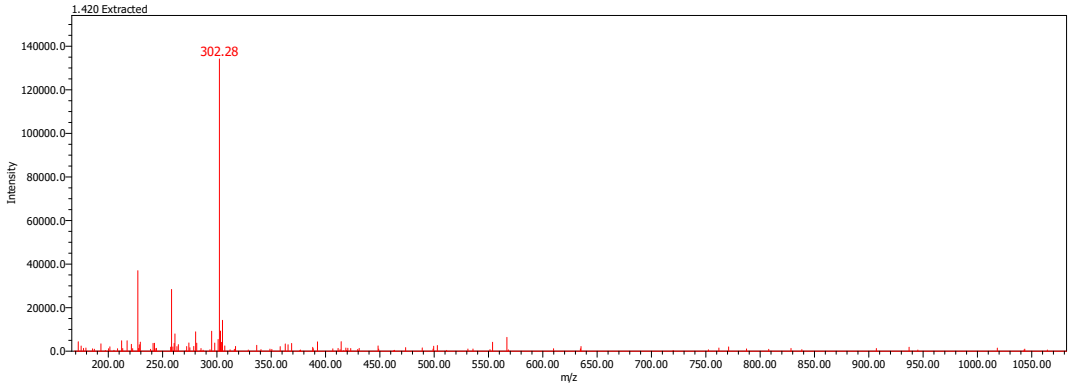

Figure S4. Mass spectrum of the solution of probe **TCF-SULF** after treated with sulfatase.

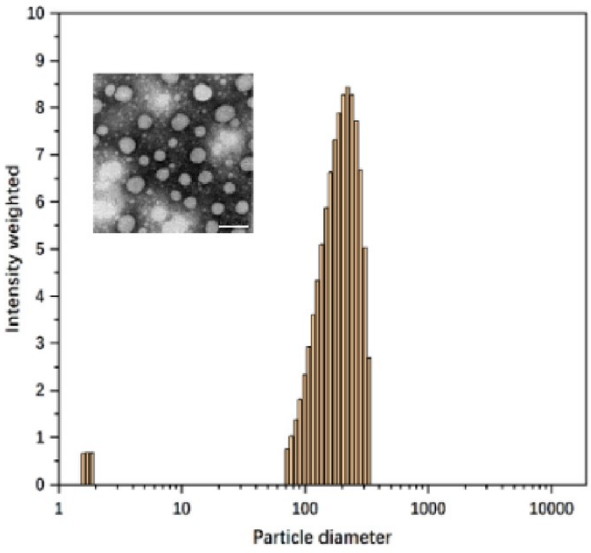

Figure S5. The particle size and electron microscope images of the nanoprobes TCF-SULF. Scale bar = 100 nm.

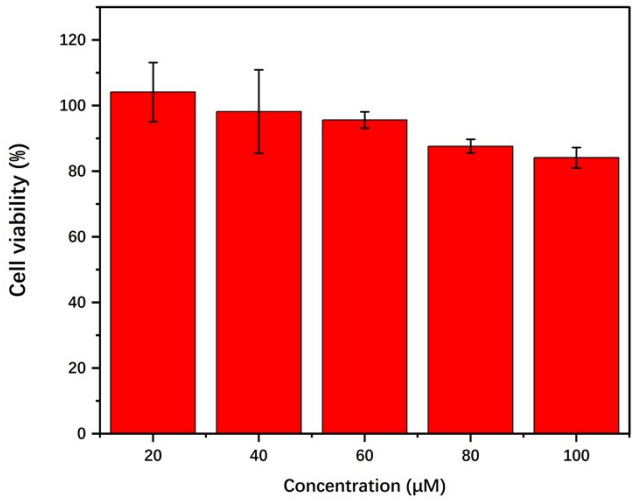

Figure S6. Cell viability of MCF-7 cells in the presence of **TCF-SULF** NPs with indicated concentrations determined by MTT.

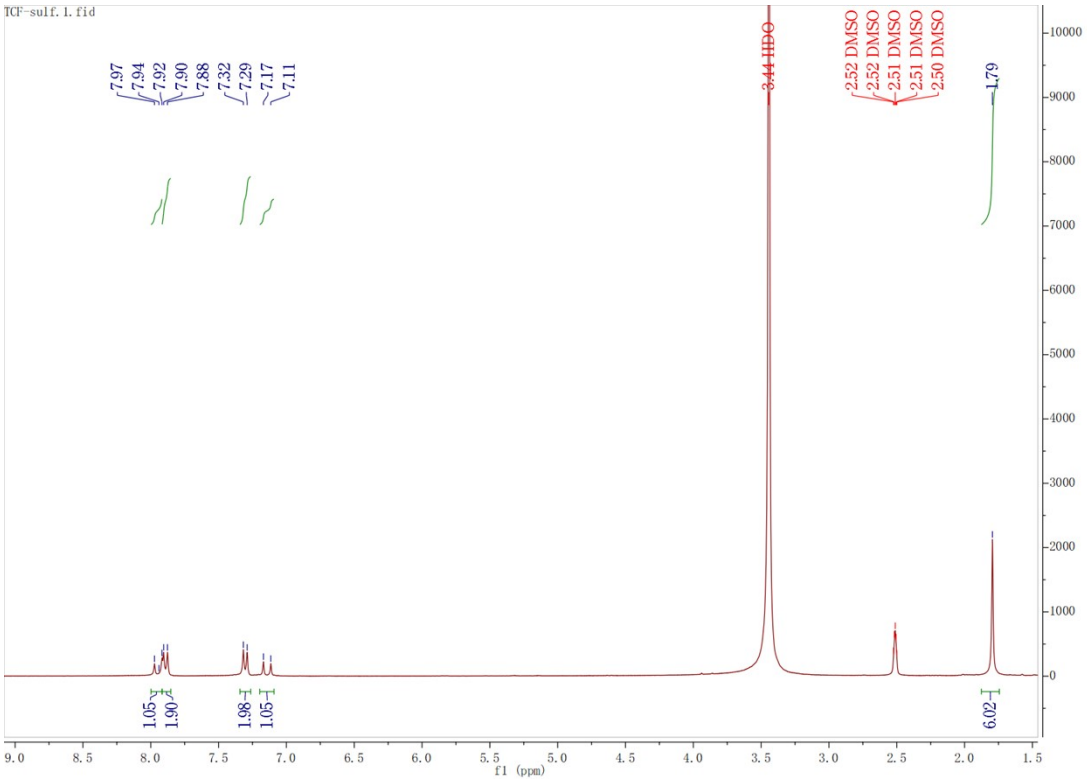

Figure S7. ^{1}H NMR spectrum of **TCF-SULF**

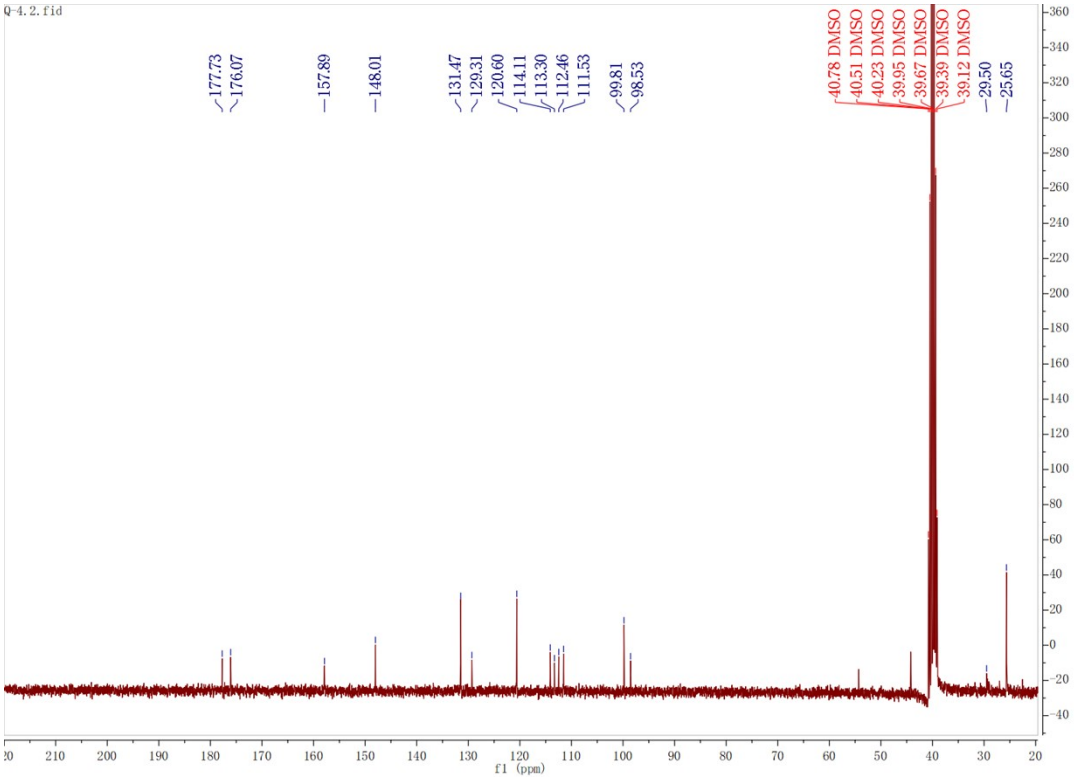

Figure S8. ^{13}C NMR spectrum of compound **TCF-SULF**

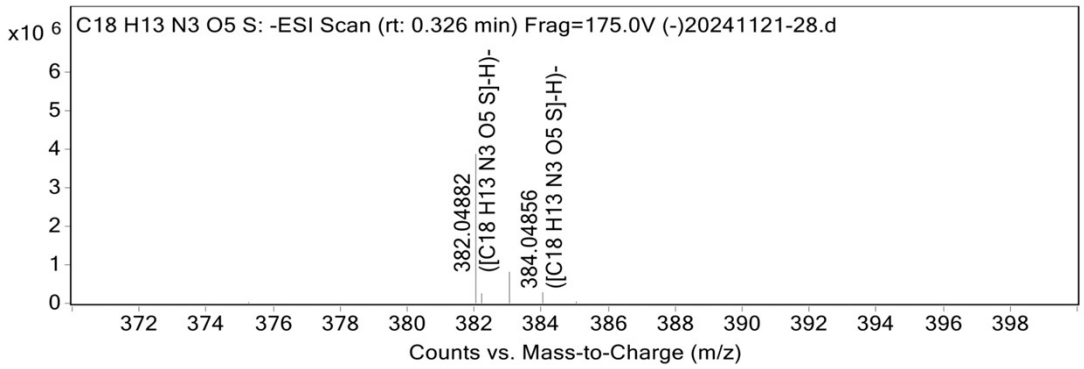

Figure S9. HRMS of compound **TCF-SULF**