Colorimetric and fluorescent detection of GSH with the assistance of CTAB micelles

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Supporting Information

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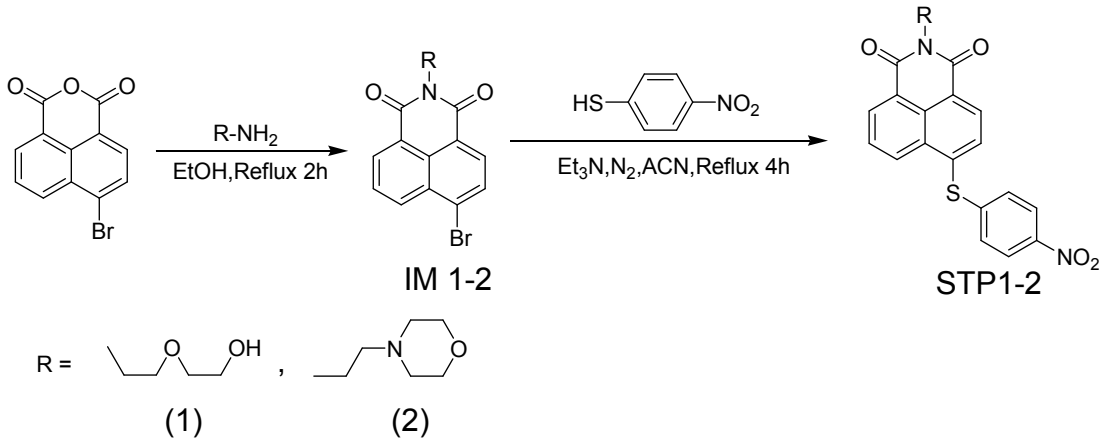
Experimental

Synthesis



Scheme S1 The synthesis procedure of STP1 and STP2

The synthesis of IM 1-2:

A mixture of 4-bromo-1,8-naphthalic anhydride (5 g, 18 mmol) and 18 mmol of 2-(2-aminoethoxy)ethanol/N-(2-Aminoethyl)morpholine in 100 mL ethanol was refluxed under a nitrogen atmosphere for 2 h. After kept at the room temperature for 12 h, the precipitate was collected and recrystallized with ethanol.

IM1 was obtained as white powder (5.23 g, yield 80%), ^{1}H NMR, (400 Hz, $CDCl_3$), δ (ppm): 8.66 (d, J = 7.3 Hz, 1H),8.58 (d, J = 8.5,1H), 8.42 (d, J = 7.9,1H), 8.04 (d, J = 7.9,1H), 7.86 (t, J = 7.6,1H), 4.45 (t, J = 5.6,2H), 3.87 (t, J = 5.6,2H), 3.64-3.72 (m, 4H), 2.42 (s, 1H). **IM2**: white powder (5.45 g, yield: 78%), ^{1}H NMR, (400 Hz, DMSO), δ (ppm): 8.48-8.60 (m, 2H), 8.31 (d, J = 7.8,1H), 8.20 (d, J = 7.8,1H), 8.00 (t, J = 7.7,1H), 4.17 (t, J = 6.6,2H), 3.53 (s, ,4H), 2.58 (t, J = 6.7,2H), 2.47 (s, 2H).

The synthesis of STP1-2

STP1: 363 mg of IM1(1.0 mmol) was dissolved in 100 mL acetonitrile, 4-nitrothiophenol (2.0 mmol, 310 mg) and 6 drops of Et_3N were added to the above acetonitrile solution. The mixture was stirred at room temperature for 1 h, followed by refluxed for 4 h. After evaporated the solvent, the residue was purified by column chromatography on silica (DCM/MeOH = 50/1 as eluent) to give **STP1** as light yellow powder (302 mg, yield 69%). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 8.68 (dd,

J = 6.4, 2H), 8.61 (dd, J = 7.6, 1H), 8.58(d, J = 7.6, 1H), 8.14 (t, J = 2.6, 1H), 8.11 (t, J = 2.0, 1H), 7.92(s, J = 7.7, 1H),7.78-7.84 (m, 1H), 7.30 (t, J = 2.6, 1H), 7.27 (t, J = 2.0,1H), 4.47 (t, J = 5.5, 2H), 3.88 (t, J = 5.5, 2H), 3.65-3.73 (m, 4H), 2.43 (s, 1H). ^{13}C NMR (400 MHz, $CDCl_3$), δ(ppm): 163.93, 163.79, 146.42, 144.55, 137.58, 133.16, 132.24, 131.72, 131.33, 130.99, 128.98, 128.87, 128.19, 124.51, 123.46, 123.31,72.23, 68.35,61.83, 39.68. HRMS (ESI) m/z: found 461.0781, calcul for $C_{22}H_{18}N_2O_6S$ $(M+Na)^+$: 461.0783.

STP2 was obtained by the same procedure with IM2 instead of IM1 (light yellow powder, 24 mg, yield: 70%). ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 8.67 (dd, J = 6.3, 1H), 8.61 (dd, J = 7.6, 1H), 8.57 (d, J = 7.6, 1H), 8.14 (t, J = 2.6, 1H), 8.11 (t, J = 2.0, 1H), 7.39 (d, J = 7.6, 1H), 7.78-7.95 (m, 1H), 7.30 (t, J = 2.6, 1H), 7.28 (t, J = 2.0, 1H), 4.36(t, J = 6.8, 2H), 3.69 (t, J = 4.5, 4H), 2.73 (t, J = 6.9, H), 2.56-2.64(m, 4H). ^{13}C NMR(400MHz, $CDCl_3$), δ(ppm): 163.64, 163.50, 146.42, 144.63, 137.36, 133.20, 132.06, 131.79, 131.20, 130.80, 129.00, 128.87, 128.19, 124.52, 123.66, 123.50, 67.05, 56.13, 53.83, 37.40. HRMS (ESI m/z, calcd for $C_{24}H_{21}N_3O_5S$ $(M+H)^+$: 464.1280, found 464.1279.

Determination of the detection limit

The detection limit (LOD) was obtained by $3S_b/k$, where S_b is the standard deviation of 10 blank measurements, and k is the slope of the fitted line.

Living cell culture and fluorescence imaging

HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37 °C and under 5% CO_2 in a CO_2 incubator. The cells were washed with phosphate buffered solution (PBS) and then incubated with STP1/ STP2 (15 μM) in DMEM for 90 min at 37 °C and washed 3 times with PBS. For the control experiment, the cells were pretreated with 0.5 mM maleimide (or GSH) for 30 min followed by incubated with 15 μM of **STP1**/ **STP2** for 90 min. Cell imaging was carried out after washing cells with PBS for 3 times. Emission was collected at 460–480 nm for the blue channel, and the excitation wavelength was set at 404 nm.

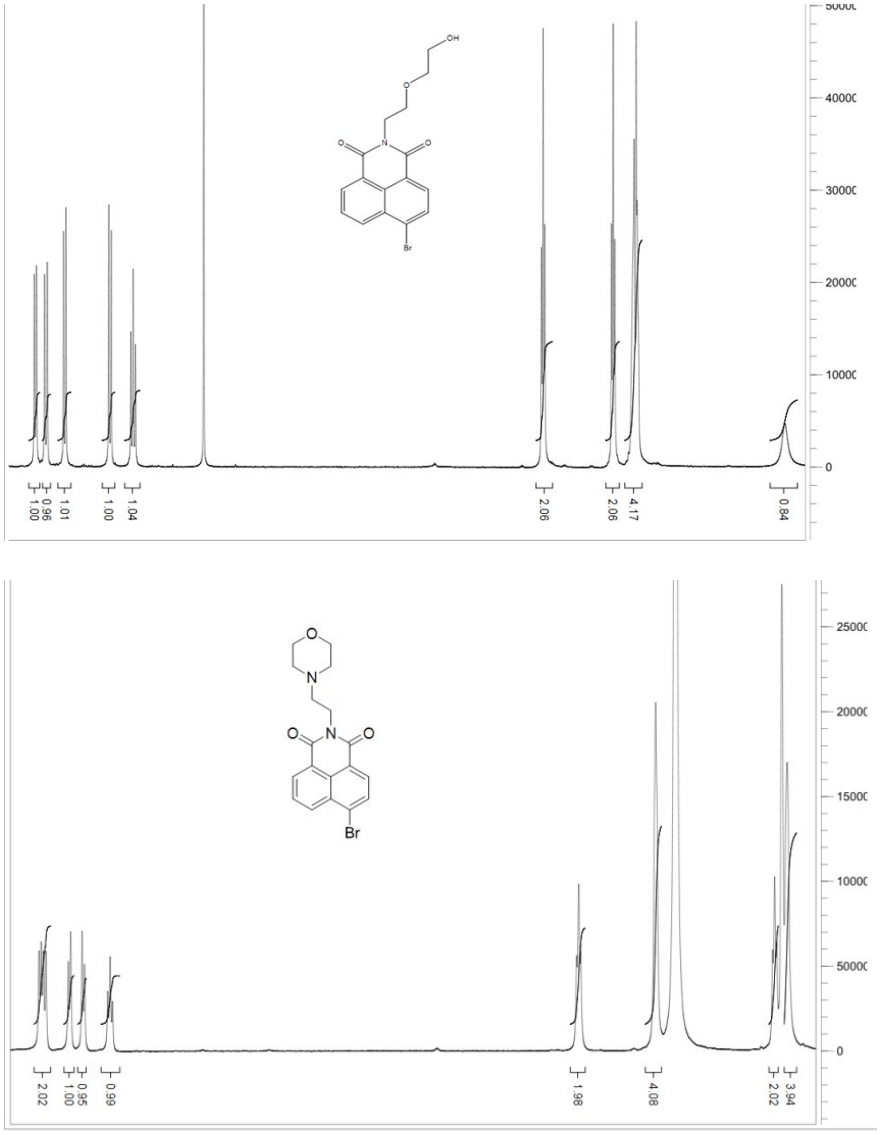
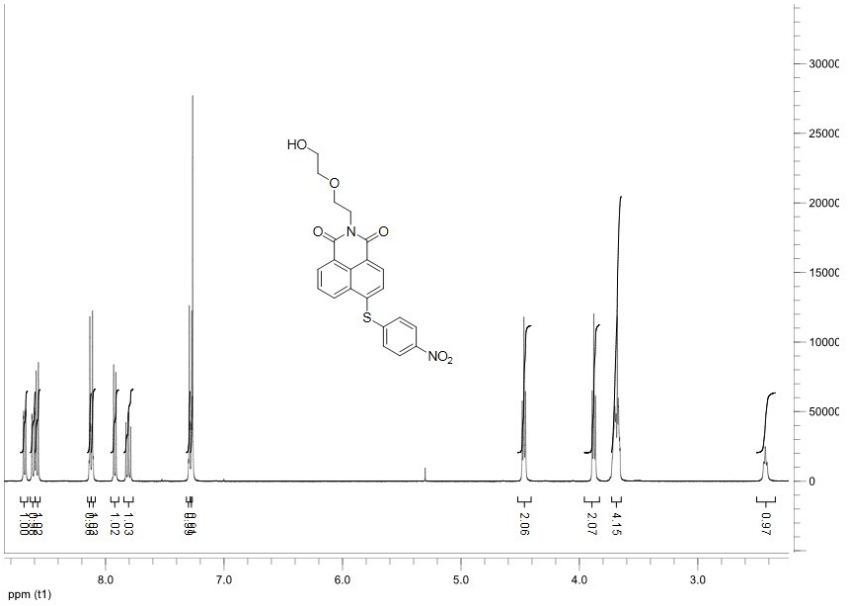
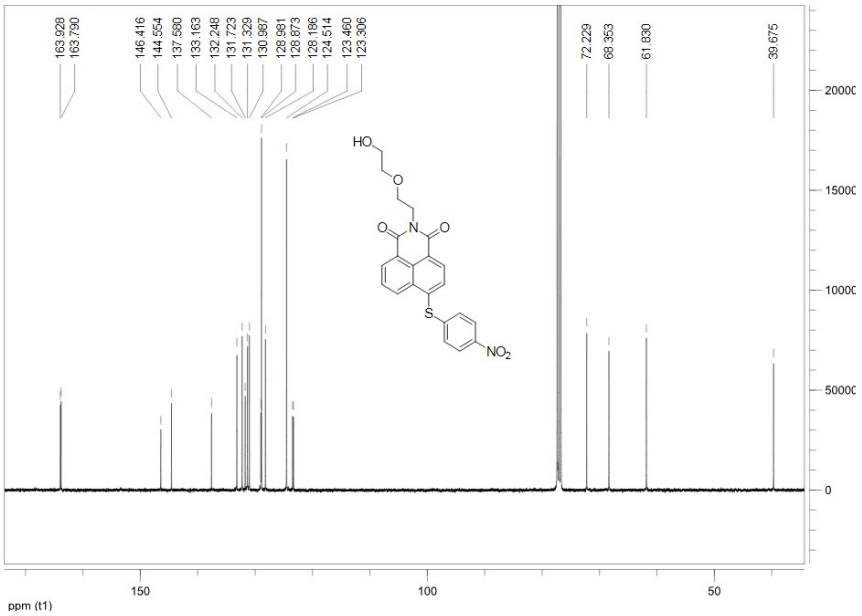
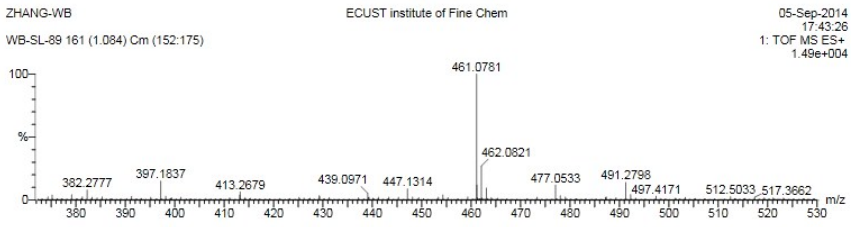


Fig. S1 [1] H NMR spectra of **IM1** (top) and **IM2** (bottom).





Monoisotopic Mass, Even Electron Ions
545 formula(e) evaluated with 26 results within limits (up to 1 best isotopic matches for each mass)
Elements Used:
C: 0-54 H: 0-50 N: 1-3 O: 0-6 S: 0-2 Na: 0-1



Minimum:				-1.5			
Maximum:		30.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
461.0781	461.0783	-0.2	-0.4	14.5	239.8	0.0	C22 H18 N2 O6 S Na

Fig. S2 ^{1}H NMR, ^{13}C NMR and ESI spectra of **STP1**

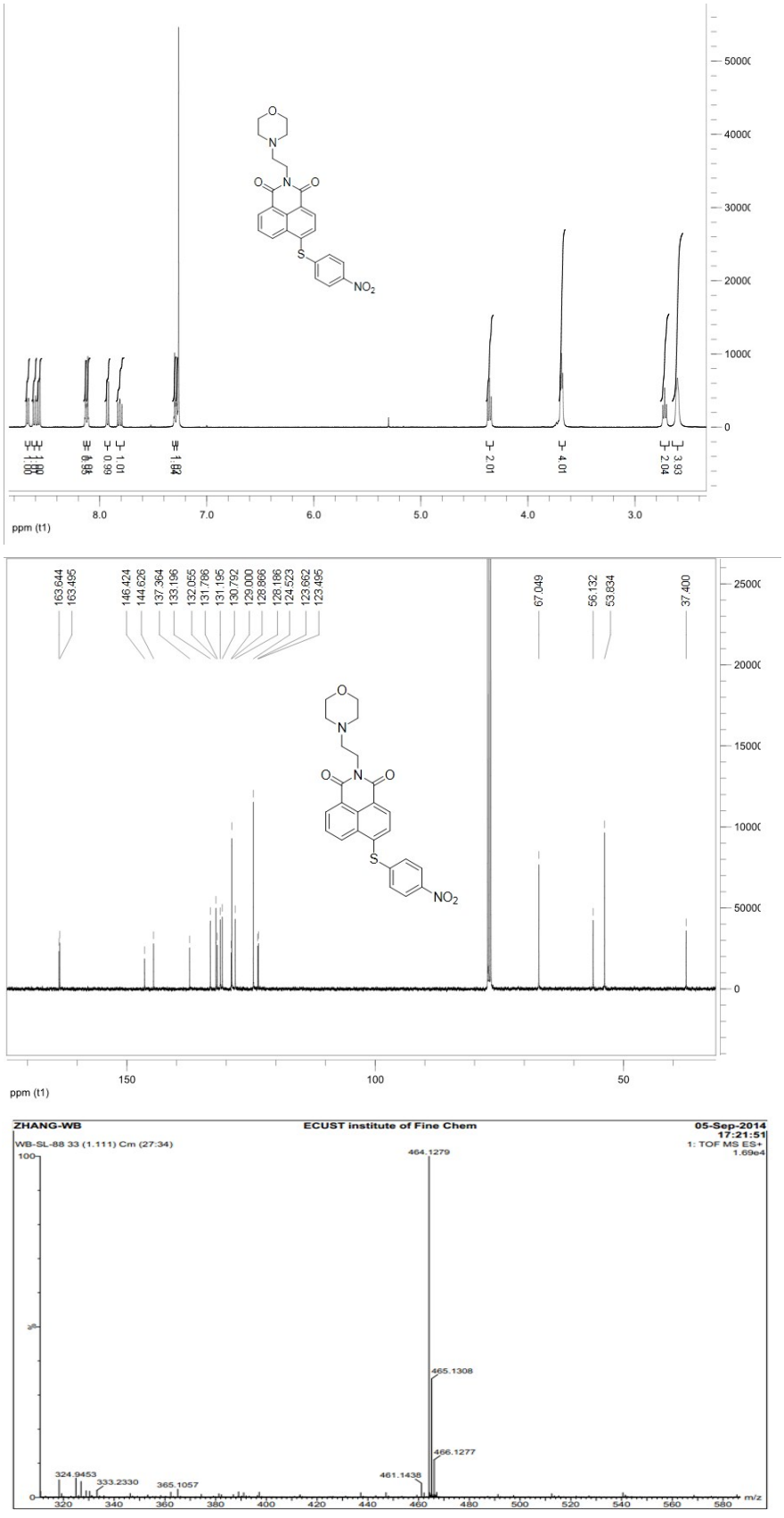


Fig. S3 ^{1}H NMR, ^{13}C NMR and ESI spectra of **STP2**

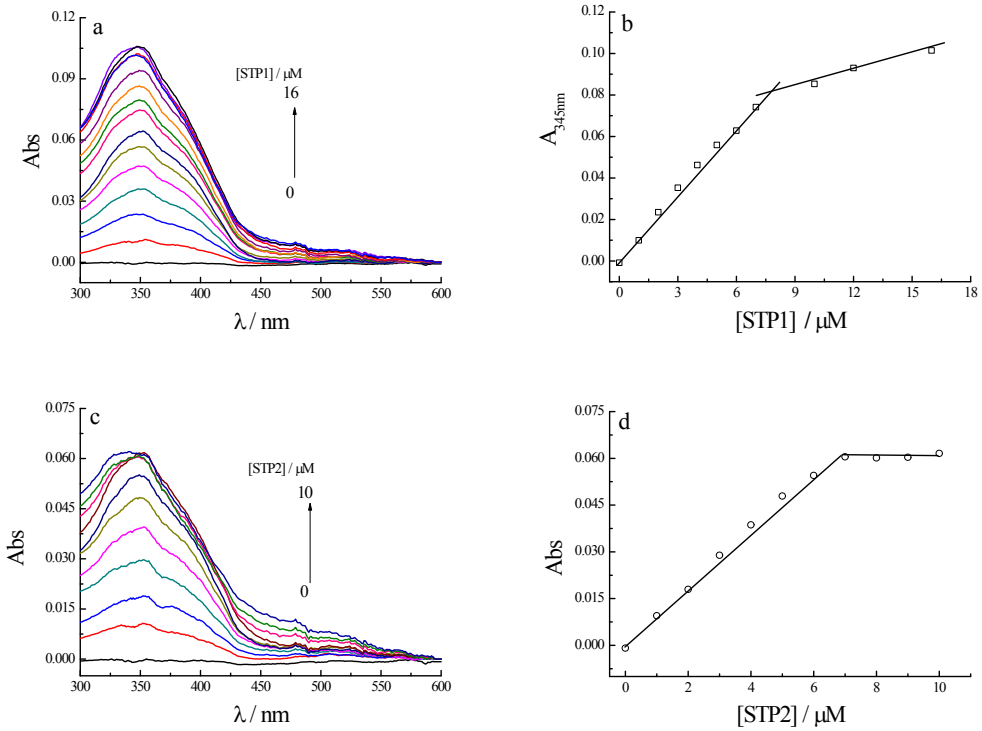


Fig. S4 The concentration-dependent absorption spectra of **STP1** (a) and **STP2** (c) and the absorbance of **STP1** (b) /**STP2** (d) as a function of the concentration in PBS (20 mM, pH 7.4), λ_{ab} = 345 nm, 25 °C.

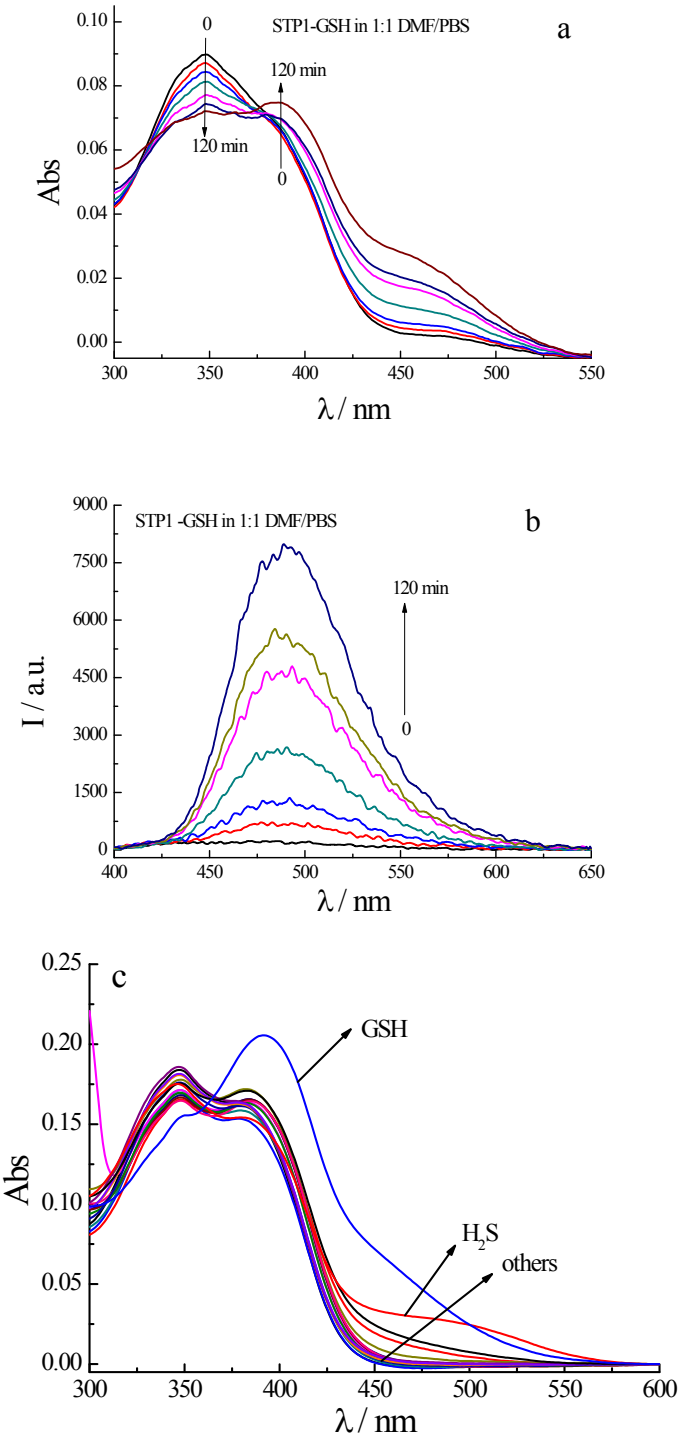


Fig. S5 Time-dependent UV-vis (a) and fluorescence (b, λ_{ex} = 375 nm) spectra of **STP1** (15 μM) in the presence of 5 mM GSH in 1:1 DMF/PBS (20 mM, pH 7.4), 37°C. (c) The absorption spectrum of **STP1** in the presence of different additives in 1 mM CTAB-PBS (20 mM, pH 7.4), 37°C.

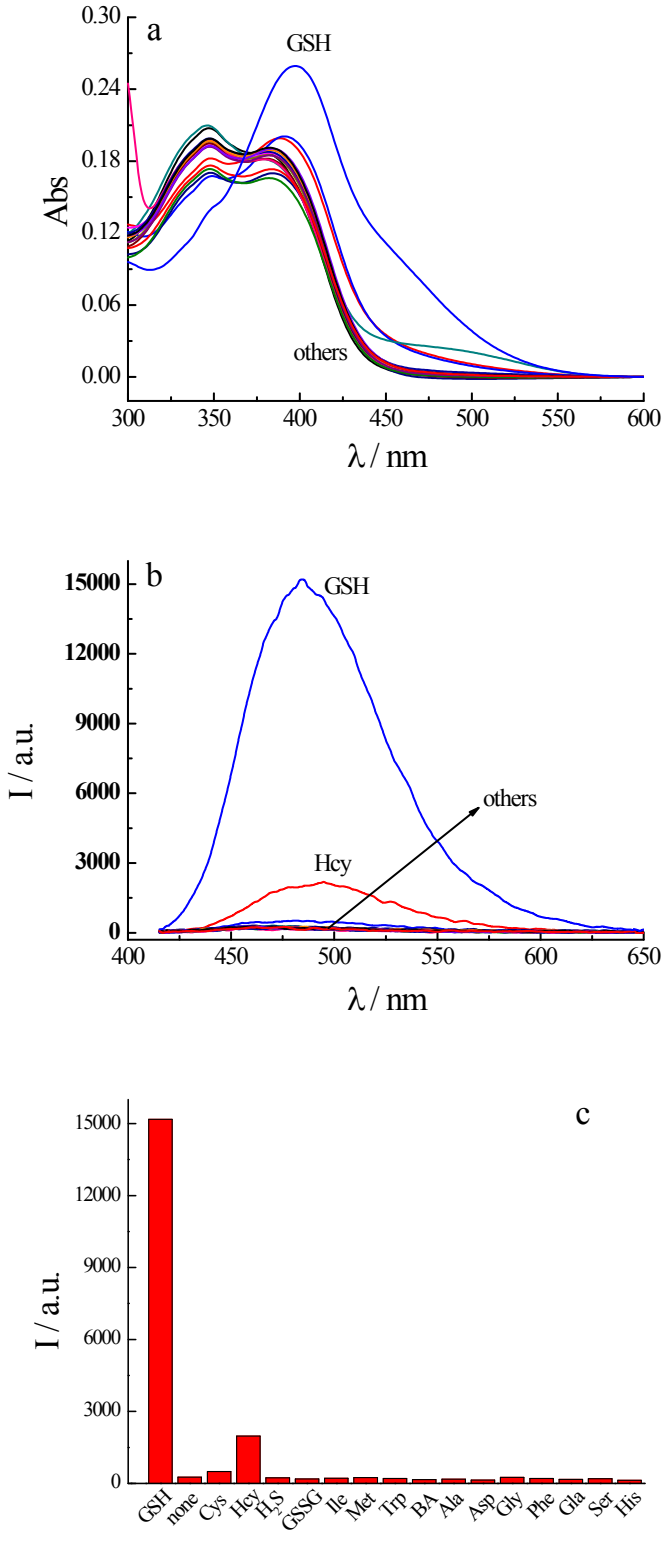


Fig. S6 The absorption (a) and emission (b, λ_{ex} = 390 nm) spectra and the fluorescence intensity at 490 nm (c) of **STP2** (15 μM) in the presence of different additives including GSH, Ile, Cys, n-butylamine, Hcy, Gly, Ser, Met, GSSG, Trp, Ala, Asp, Phe, Gla, His, H_2S. [analyte] = 300 μM, recorded 90 min after the addition of the reagent.

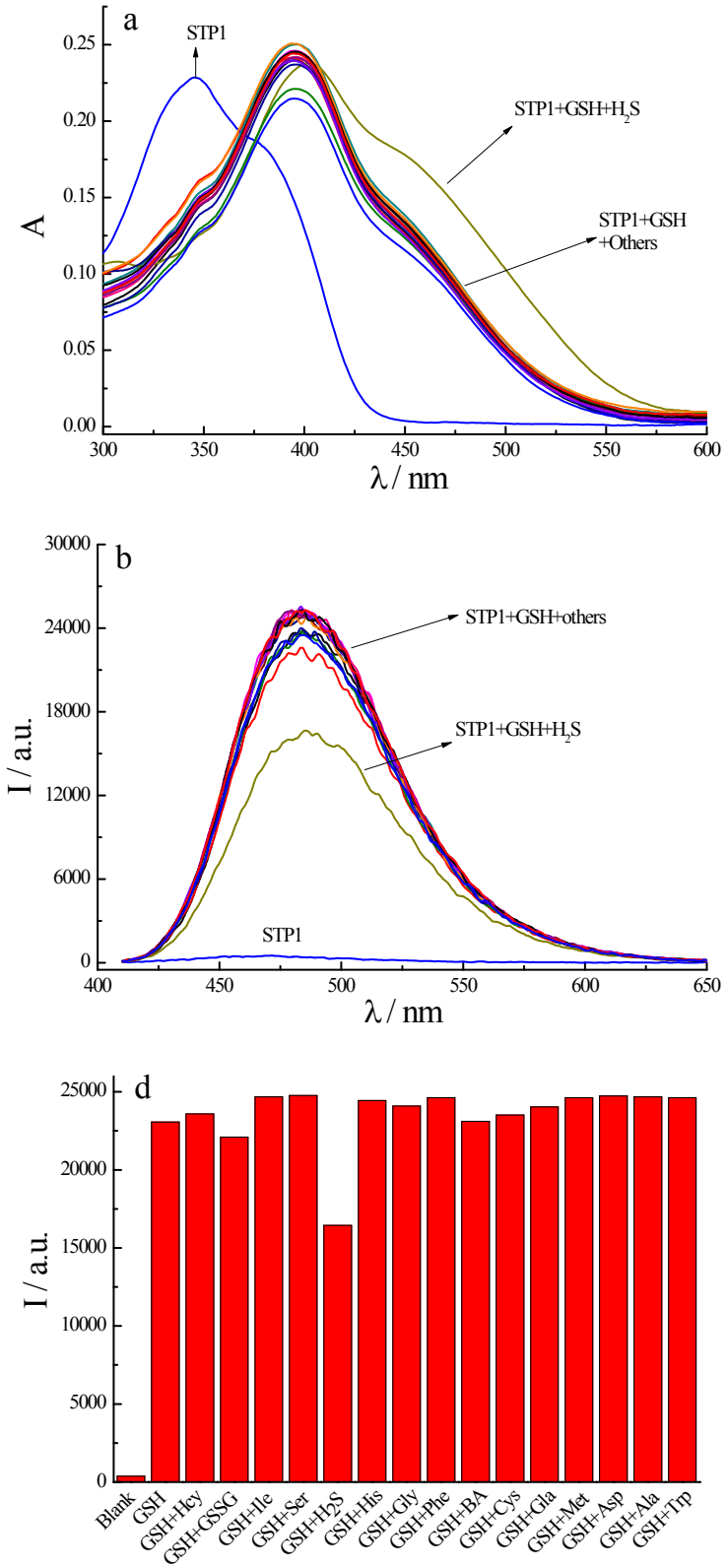


Fig. S7 The absorption (a) and emission (b) spectra of **STP1**-GSH in the presence of 300 μM different additives in 1 mM CTAB-PBS (20 mM, pH 7.4). [**STP1**] = 15 μM, [GSH] = 300 μM, additives: Hcy, GSSG, Ile, Ser, H2S, His, Gly, Phe, BA, Cys, Gla, Met, Asp, Ala, Trp; λ_{ex} = 390 nm.

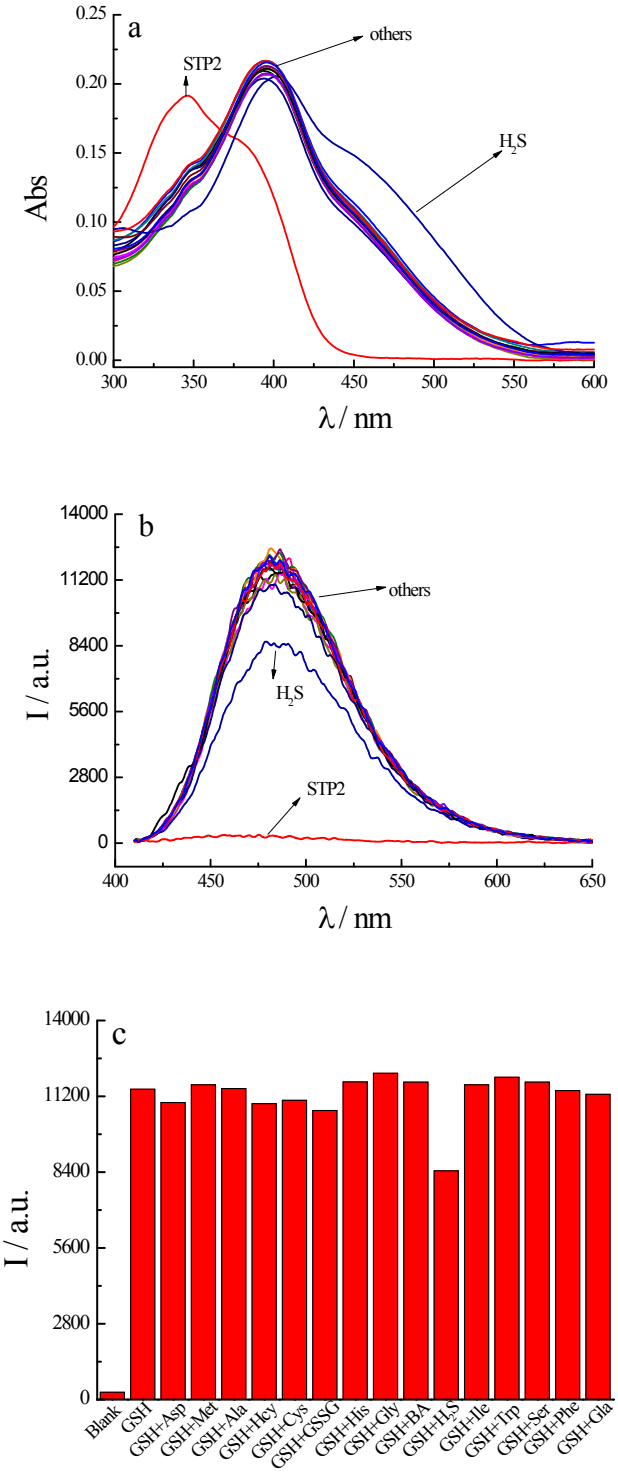


Fig. S8 The absorption (a), emission (b) spectra and the fluorescence intensity at 490 nm (c) of STP2-GSH in the presence of 300 μM different additives in 1 mM CTAB-PBS (20 mM, pH 7.4). [STP1] = 15 μM, [GSH] = 300 μM, additives: Hcy, GSSG, Ile, Ser, H2S, His, Gly, Phe, BA, Cys, Gla, Met, Asp, Ala, Trp; λ_{ex} = 390 nm, 37°C.

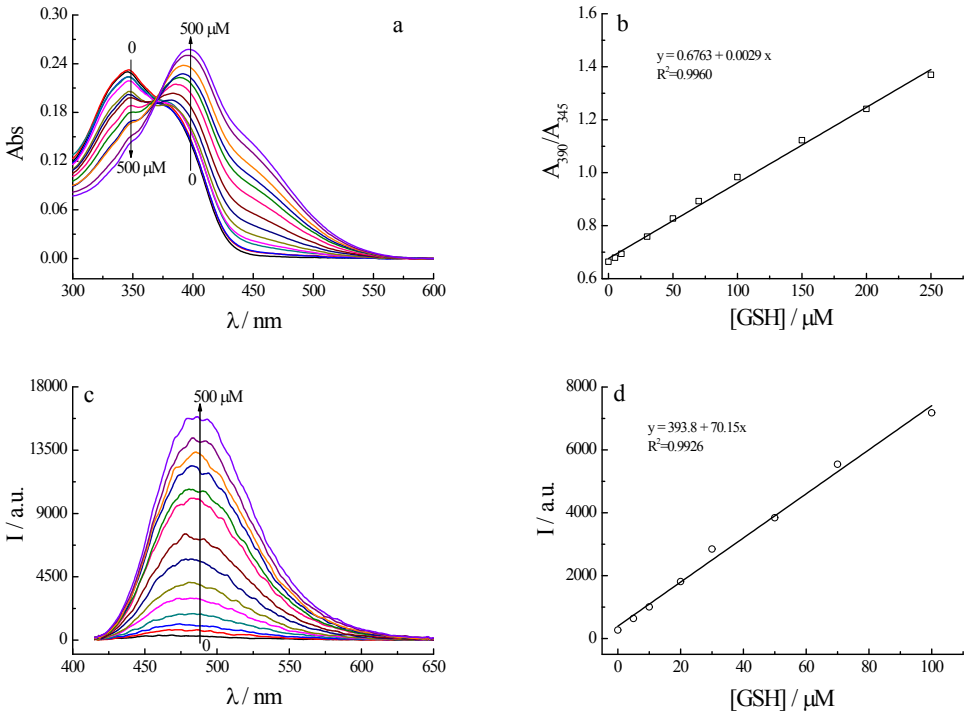


Fig. S9 The absorption (a) and the fluorescence (c) spectra of **STP2** with different concentrations of GSH, and the ratio of A_{390} and A_{345} (b) and fluorescence intensity I_{490} (d) of **STP2** as a function of GSH concentration. 1 mM CTAB-PBS (20 mM, pH 7.4), equilibrated at 37 °C for 90 min, [**STP2**] = 15 μM, λ_{ex} = 390 nm.

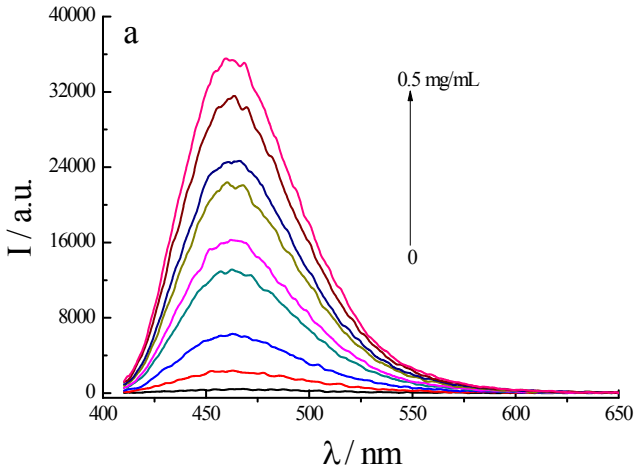


Fig. S10 The fluorescence spectra (a) of **STP1** with different concentrations of OVA in 1 mM CTAB-PBS (20 mM, pH 7.4), λ_{ex} = 390 nm, **[STP1]** = 15 μM, 37°C, each time recorded 90 min after the addition of the OVA.

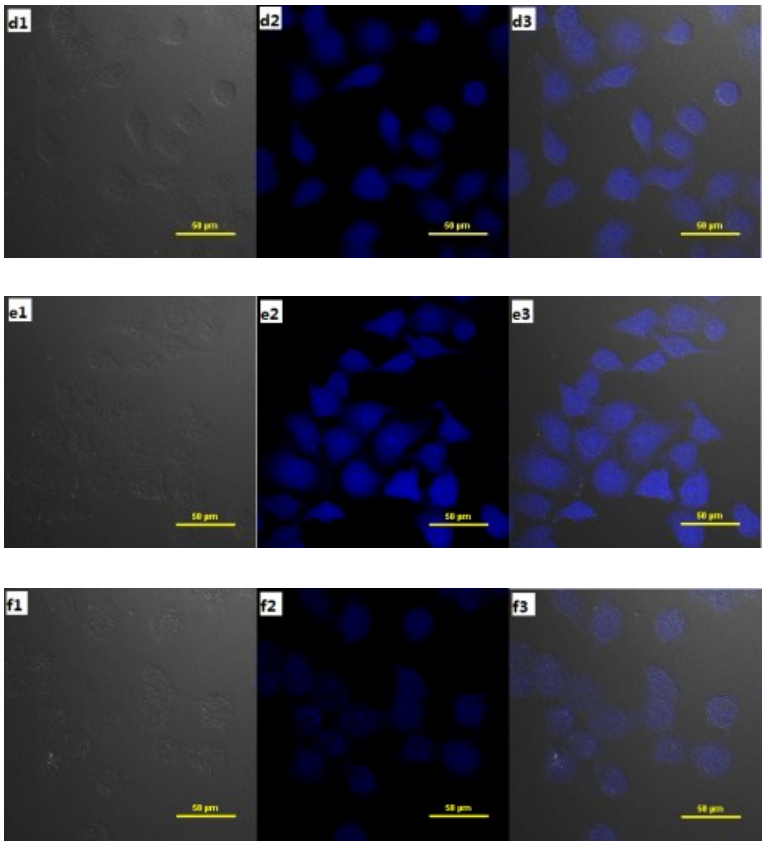


Fig. S11 Fluorescence images of Hela cells incubated with 15 μM **STP2** at 37 °C. **1**: bright field images; **2**: fluorescence images at the blue channel; **3**: the merged images of **1** and **2**. **d**: Hela cells incubated with **STP2** for 90 min; **e**: Hela cells pretreated with 0.5 mM GSH for 30 min followed by incubated with **STP2** for 90 min； **f**: Hela cells pretreated with 0.5 mM NME for 30 min, then incubated with **STP2** for 90 min. λ_{ex} = 404 nm, 1 mM CTAB-PBS (20 mM, pH 7.4).