Multi-Channel Colorimetric and Fluorescent Probes for Differentiating between Cysteine and Glutathione/ Homocysteine

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Supporting Information

Contents

Experimental

Reagents

Unless otherwise specified, all the commercial reagents were of analytical grade and used without further purification. All the chemicals were purchased from Aladdin Corporation. Fetal bovine serum was purchased from the JONLN industrial Co., LTD, Shanghai. Milk was from Mengniu Dairy Company. Chromatographic grade acetonitrile was purchased from Merck Chemicals Co., Ltd. Ultra-pure water was prepared through Sartorius Arium 611DI system.

Characterization and measurement

NMR spectra were performed with a Bruker AV-400 spectrometer (400 M Hz). Mass spectra were recorded on a MA 1212 Instrument on standard condition (ESI, 70 eV). Absorption spectra were measured with an Evolution 220 UV–vis spectrophotometer (Thermo Scientific). Fluorescence spectra were carried out on a Lumina Fluorescence Spectrometer (Thermo Scientific), all the fluorescence spectra were uncorrected. Melting points were tested with a WRS-1B digital melting point Apparatus (Shanghai instrument and electrical physical optical instrument Co., Ltd.). The experiments were performed at 25 °C using nondegassed samples.

Absorbance and Fluorescence titration

Accurately weighted amount of compounds **TP1**-**3** were dissolved in DMF to obtain 1×10^{-3} M stock solutions. Thiols and other analytes were dissolved in phosphate buffer solution (PBS) to obtain stock solutions with appropriate concentrations. The stock solution was diluted with a mixture of DMF:PBS = 4:1 to acquired 20 μM dye solutions.

In the kinetic measurements, 30 μL of Cys/ or GSH/ or Hcy stock solution was added to 3 mL of 2×10^{-5} M dye aqueous solution; while in the titration experiments, 0~50 μL of Cys or GSH stock solutions were added into 3 mL of 2×10^{-5} M **TP3** aqueous solution.

Fluorescent detecting proteins and fetal bovine serum (FBS)

An accurately weighted amount of BSA was dissolved in PBS (20 mM, pH 7.4) to obtain 3.0 mg/mL stock solution. 4.0 mL BSA stock solution was added into 16.0 mL DMF to keep [BSA] = 0.6 mg/mL. 60 μL of the **TP3** stock solution were added into 3 mL of the above solution to keep [**TP3**] = 20 μM. The sample was equilibrated at 25 °C for 30 min and then collected the fluorescence spectrum. The same procedure was

used for other proteins, the concentration of OVA was 0.3 mg/mL, and those of the other proteins were 1.0 mg/mL. Fetal bovine serum was 50 times diluted in 4:1 DMF:PBS, and milk was 25 times diluted in 4:1 DMF:PBS.

HPLC traces

High-performance liquid chromatography (HPLC) spectra were carried out on an Agilent Technologies 1260 Infinity LC system and a SinoChrom ODS-BP 5 μm (4.6 mm×50 mm). The mobile phases were degassed with an ultrasonic apparatus for 10 min. 20 μM of **TP3** and 400 μM of Cys/ or GSH/ or Hcy were dissolved in DMF and equilibrated at 25°C. 20 μL of the above sample at different time intervals was injected into the HPLC system and determined with a UV detector at a wavelength of 380 nm or 420 nm. The column heater was set at 30°C. The separation was performed at a flow rate of 1.5 mL/min, with 0.05% TFA-Water (eluent A) and 0.05% TFA-acetonitrile (eluent B) as mobile phase. The gradient elution: 1 ~ 5.5 min, 5% ~ 95% B; 6.0 ~ 6.5 min, 95 ~ 5% B; isocratic elution: 0 ~ 1 min and 6.5 ~ 7 min, 5% B; 5.5 ~ 6.0 min, 95% B.

Determination of the detection limit

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

Living cell culture and fluorescence imaging

L929 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37 ^{o}C and under 5% CO_2 in a CO_2 incubator. The cells were washed with phosphate buffered solution (PBS) and then incubated with **TP3** (20 μM) in DMEM for 30 min at 37 °C and washed 3 times with PBS. For the control experiment, the cells were pretreated with 0.5 mM maleimide (or Cys) for 30 min at 37 °C followed by incubated with 20 μM of **TP3** for 30 min. Cell imaging was carried out after washing cells with PBS. Emission was collected at green channel.

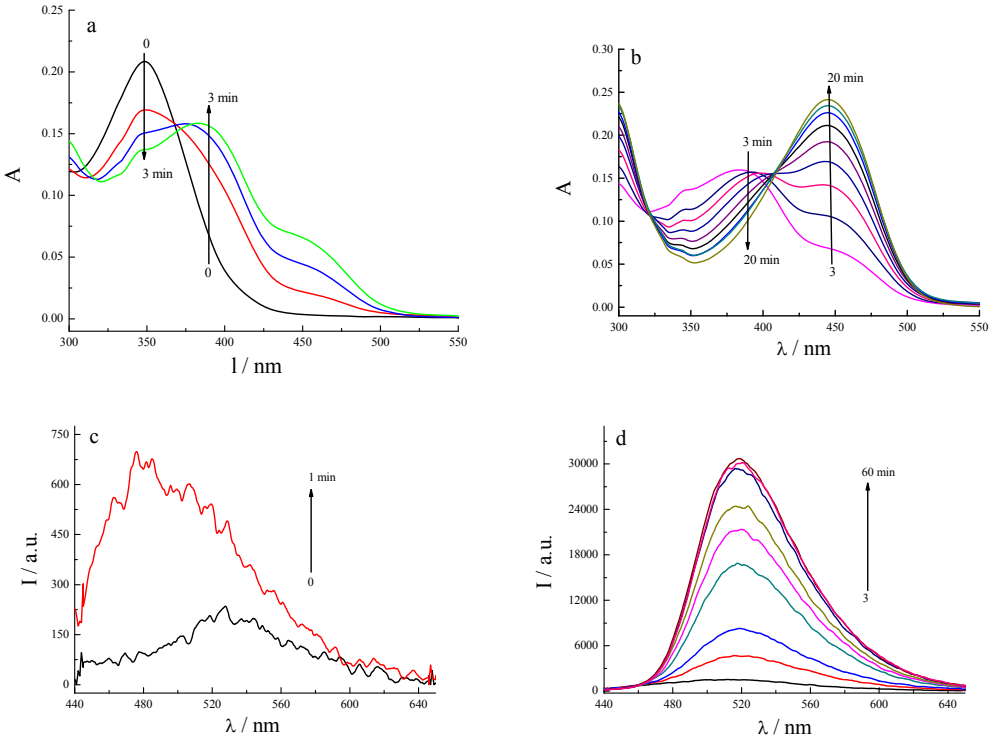


Fig. S1 The time-dependent UV-vis (a, b) and fluorescence (c, λ_{ex} = 380 nm; d, λ_{ex} = 420 nm) spectra of **TP2** (20 μM) in the presence of 400 μM Cys in DMF at 25 °C.

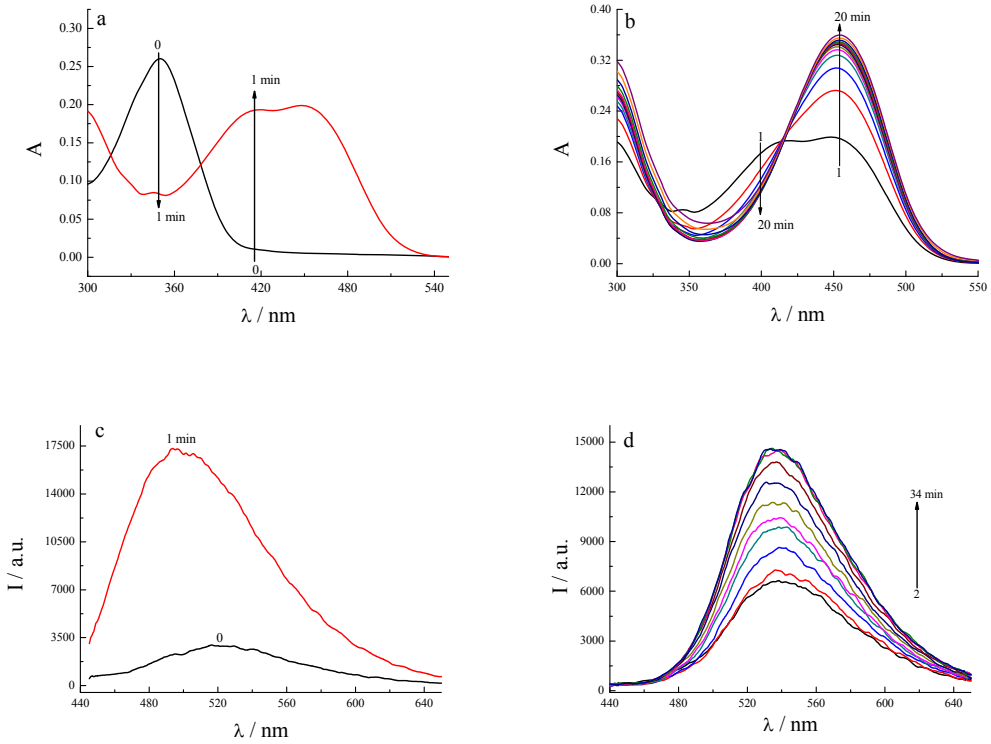


Fig. S2 The time-dependent UV-vis (a, b) and fluorescence (c, λ_{ex} = 380 nm; d, λ_{ex} = 420 nm) spectra of **TP3** (20 μM) in the presence of 400 μM Cys in DMF at 25 °C.

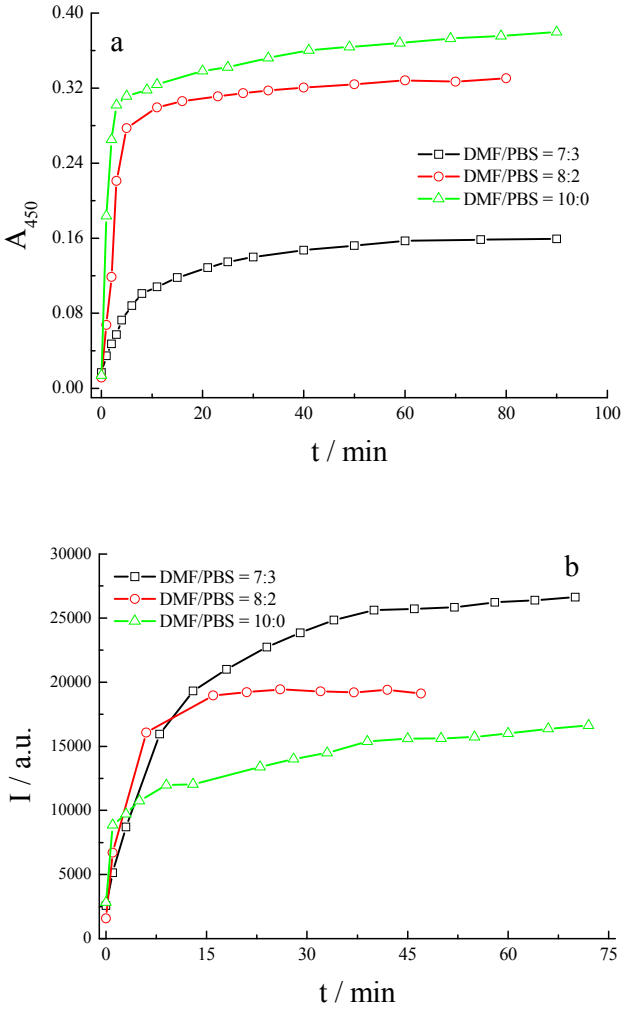


Fig. S3 Time-dependent absorbance (a, λ_{ab} = 455 nm) and emission intensity (b, λ_{em} = 543 nm) of **TP3**-Cys solution in different solvents. [**TP3**] = 20 μM, [Cys] = 400 μM, pH 7.4 PBS (20 mM), λ_{ex} = 420 nm, 25°C.

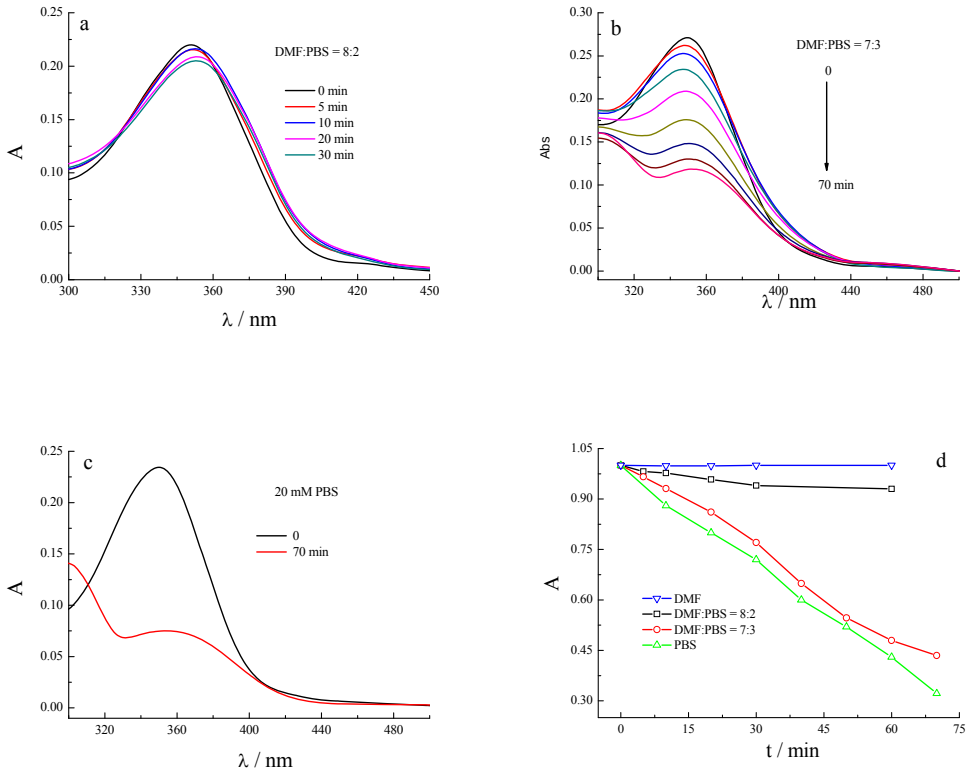


Fig. S4 The time-dependent UV-vis spectra of **TP3** in different media. [**TP3**] = 20 μM, 20 mM PBS, pH 7.4.

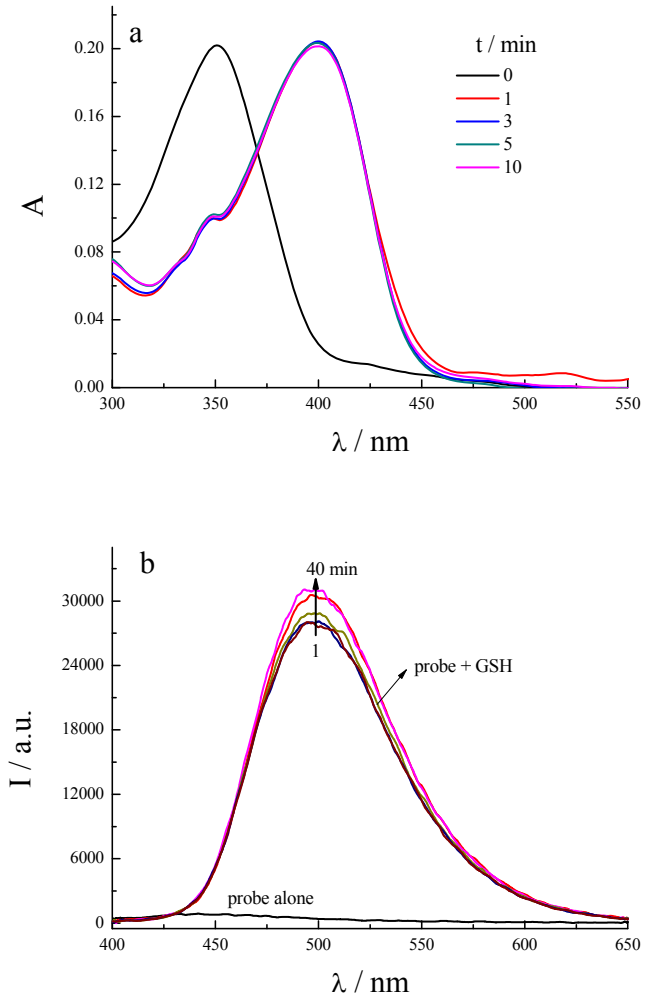


Fig. S5 time-dependent UV-vis (a) and emission (b) spectra of **TP3** (20 μM) in the presence of 400 μM GSH in 4:1 DMF/PBS, 20 mM PBS, pH 7.4, λ_{ex} = 380 nm.

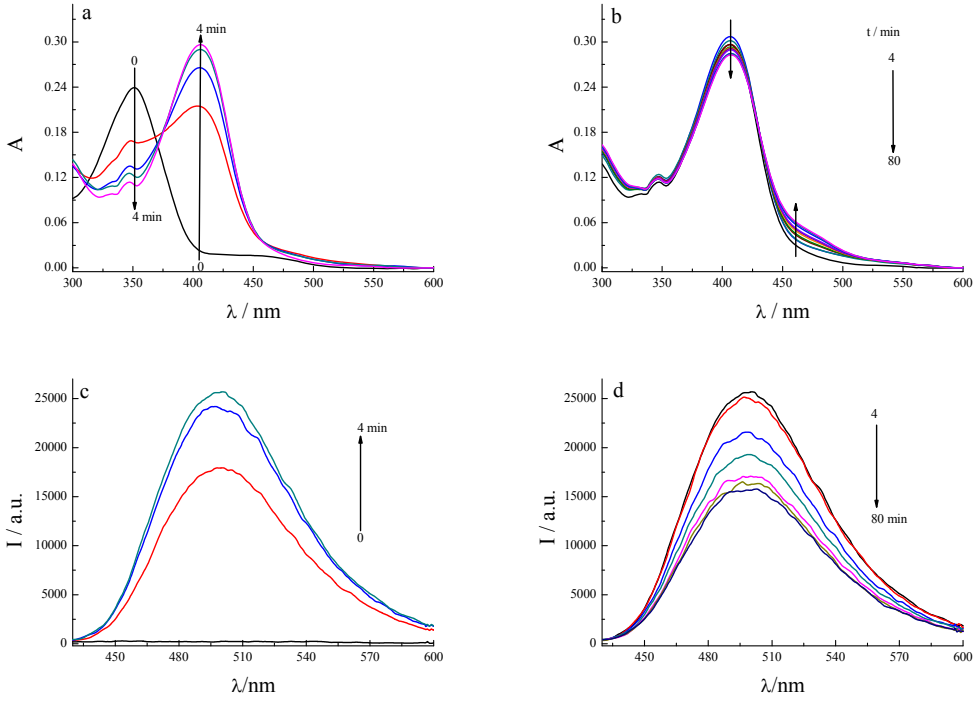


Fig. S6 The time-dependent UV-vis (a-b) and emission (c-d) spectra of **TP3** (20 μM) in the presence of 400 μM Hcy in 4:1 DMF/PBS, 20 mM PBS, pH 7.4, λ_{ex} = 380 nm .

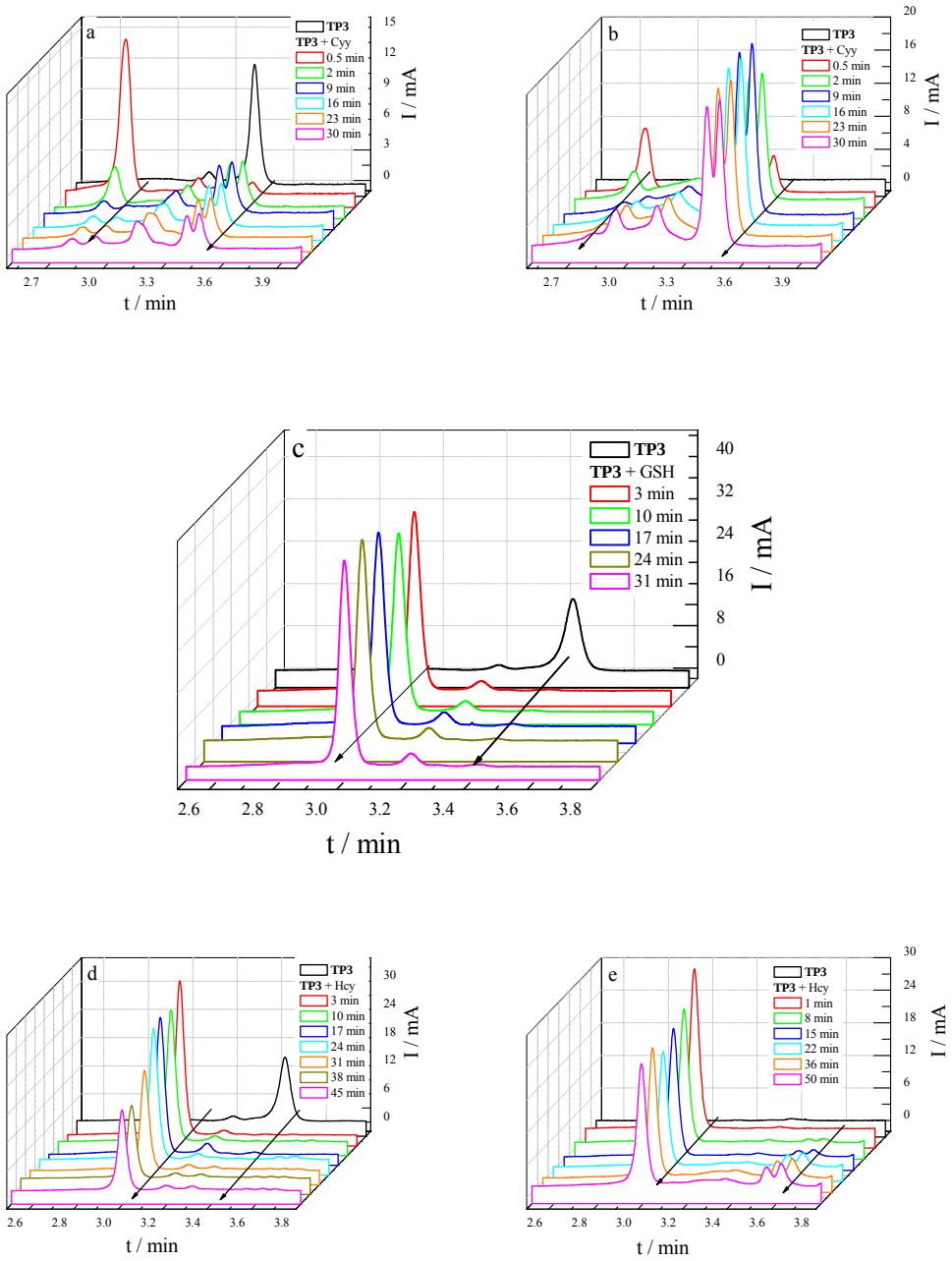


Fig. S7 time-dependent HPLC spectra of the mixture of **TP3**-biothiols. (a, b) Cys, (c) GSH, (d, e) Hcy in DMF at 25°C. Injection volume: 20 µL; mobile phase: A – 0.05% TFA/water, B –0.05% TFA/acetonitrile; gradient elution: 1-5.5 min, 5-95%B; 6 – 6.5 min, 95-5% B; Isocratic elution: 0-1 and 6.5-7 min, 5%B; 5.5-6 min, 95%B; flow rate: 1.5 mL min^{-1}; detection wavelength: (a, c, d) 380 nm, (b, e) 420 nm. [**TP3**] = 20 µM, [thiol] = 400 µM.

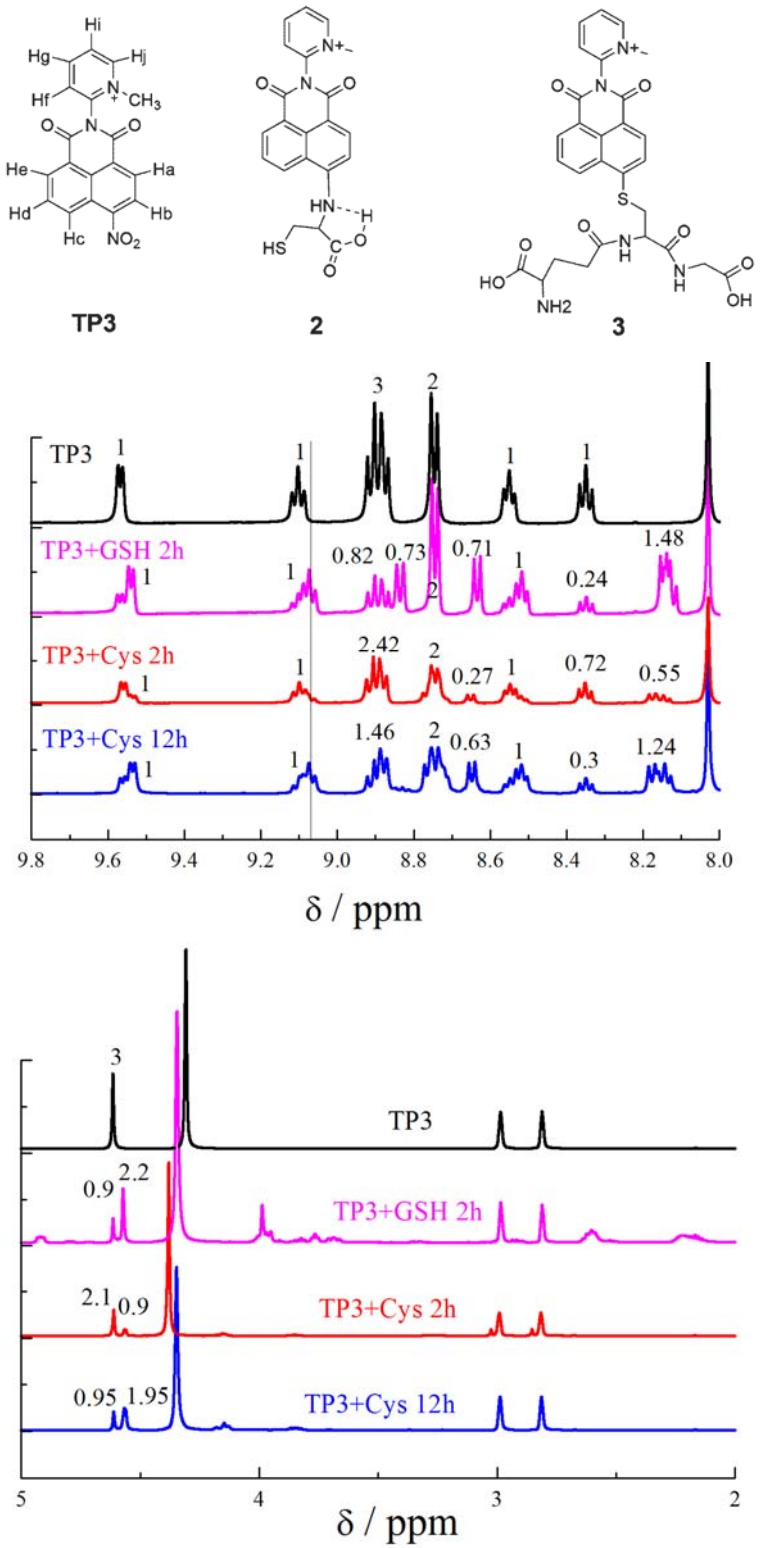


Fig. S8 Partial ^{1}H NMR spectra of **TP3** (20 mM) in the presence of 1.2 equiv Cys / GSH in 4:1 DMF-d6/D_2O (v/v) at room temperature (the number above the peak is the integration of the peak).

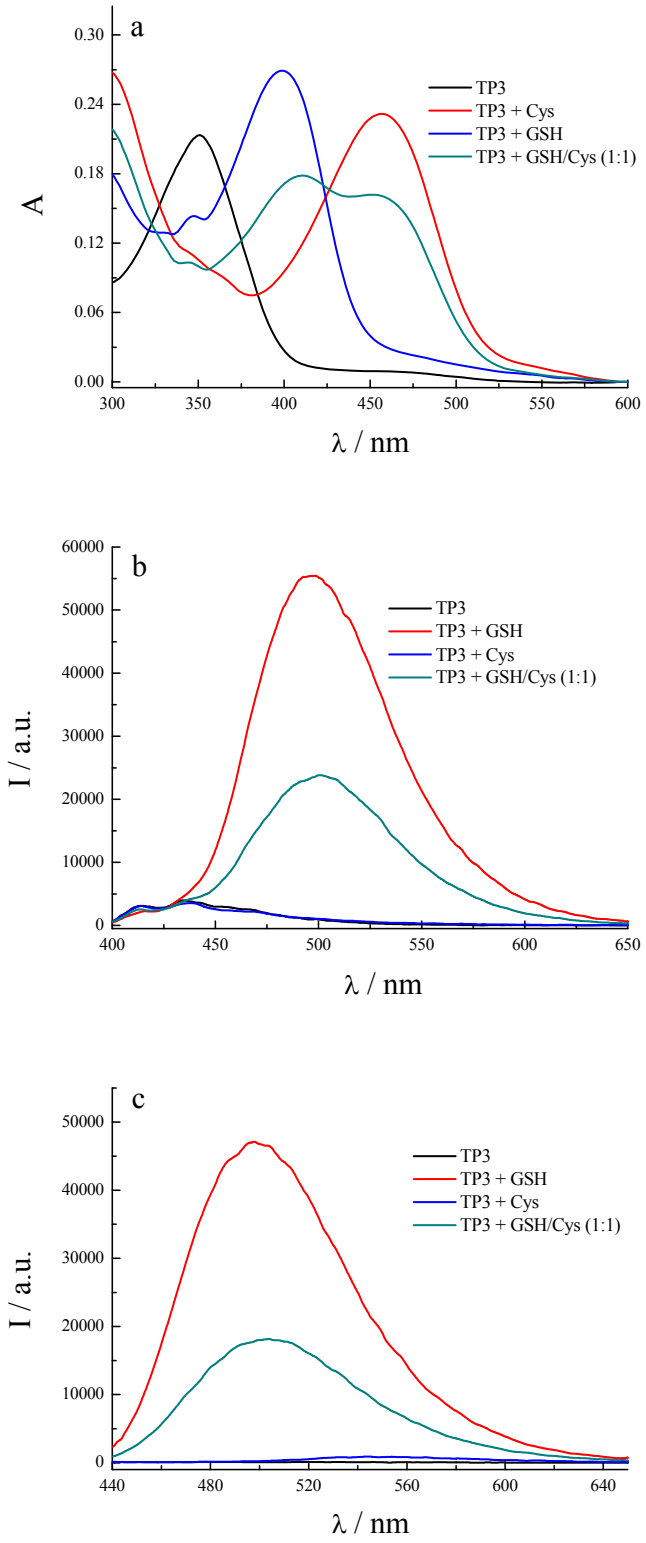


Fig. S9 The UV-vis (a) and emission (b, c) spectra of **TP3** in the presence of different additives (b, λ_{ex} = 380 nm; c, λ_{ex} = 420 nm), [**TP3**] = 20 μM, [GSH] = [Cys] = 50 μM, recorded 30 min after addition of the reagent.

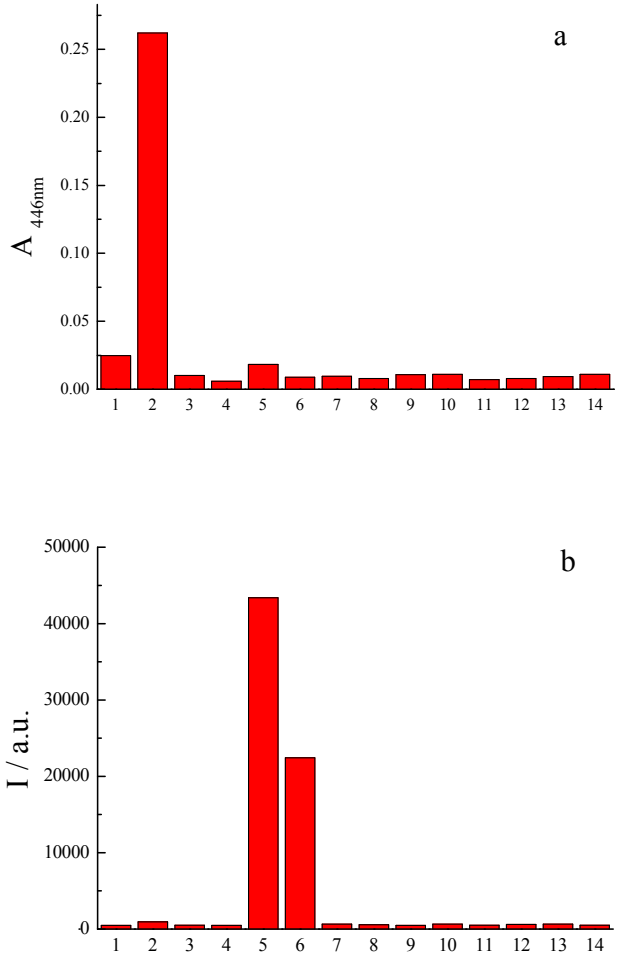


Fig. S10 The absorbance at 446 nm (a) and fluorescence intensity at 498 nm (b, λ_{ex} = 380 nm) of **TP3** in the presence of different additives: (1) none; (2) Cys; (3) Pro; (4) n-butylamine; (5) GSH; (6) Hcy; (7) Gly; (8) Ala; (9) Arg; (10) Ser; (11) ASP; (12) Gly; (13) His; (14) Ile; recorded 30 min after addition of the reagent.

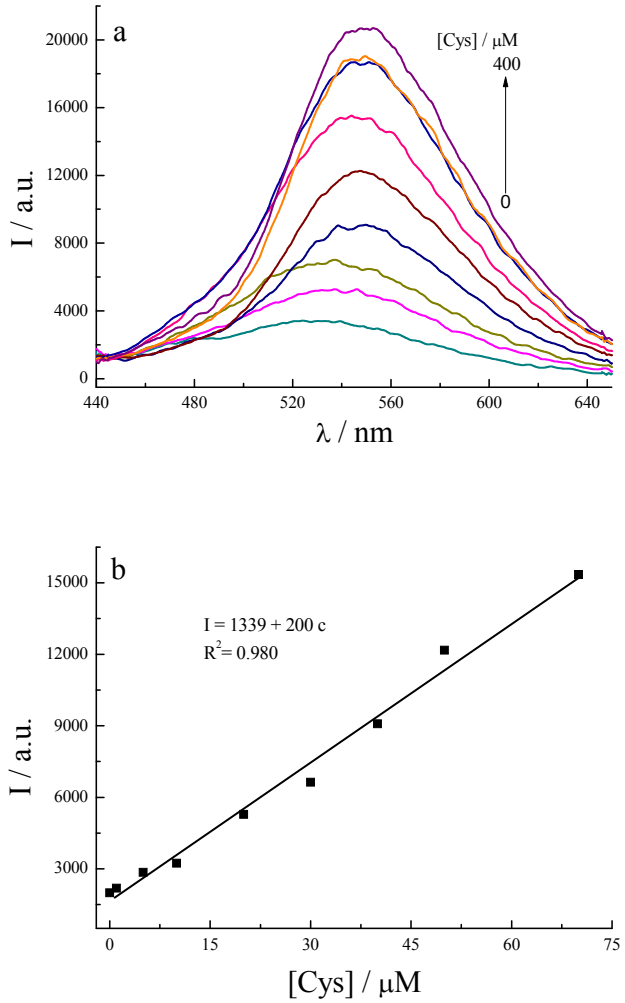


Fig. S11 The fluorescence spectra of **TP3** with different concentrations of Cys (a), and the fluorescence intensity at 543 nm as a function of Cys concentration (b). 4:1 DMF/PBS, 20 mM PBS, pH 7.4, 25 °C, [**TP3**] = 20 μM, λ_{ex} = 420 nm; recorded 30 min after addition of the reagent.

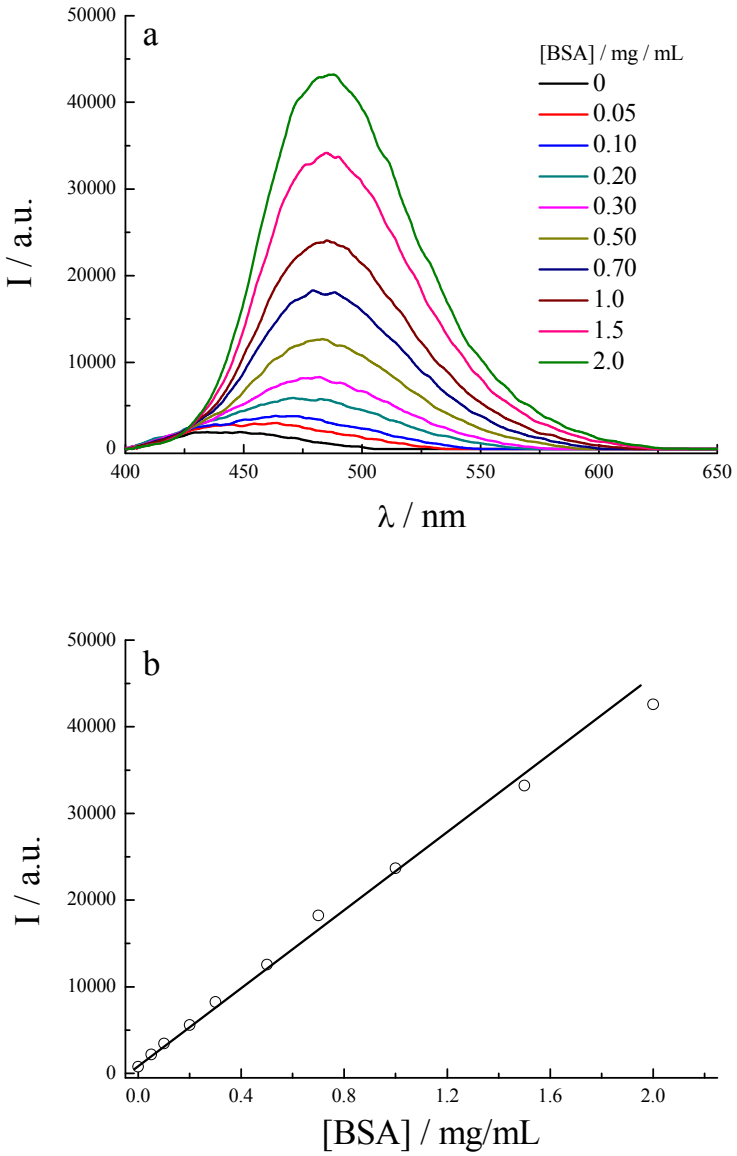
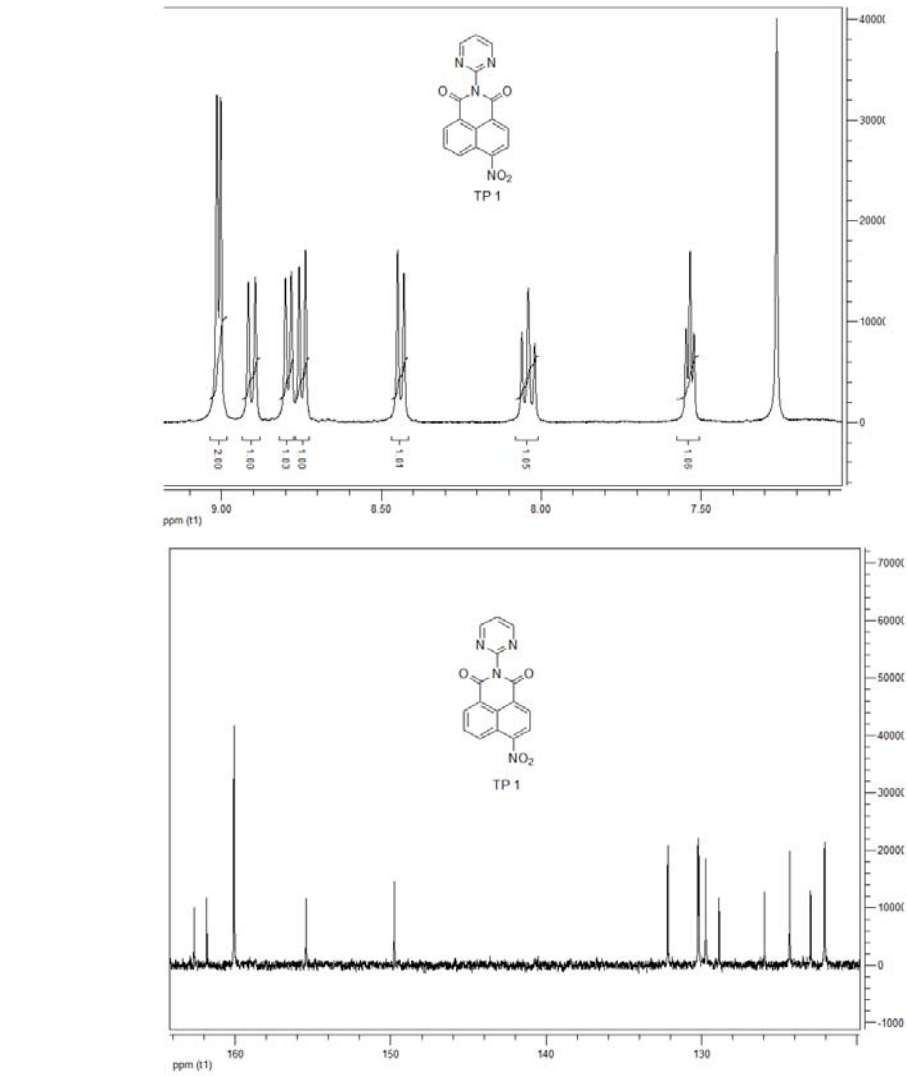


Fig. S12 The fluorescence spectra of **TP3** with different concentrations of BSA (a), and the fluorescence intensity at 488 nm as a function of BSA concentration (b). 4:1 DMF/PBS, 20 mM PBS, pH 7.4, 25 °C, [**TP3**] = 20 μM, λ_{ex} = 380 nm; recorded 30 min after addition of the reagent.



Monoisotopic Mass, Even Electron Ions
317 formula(e) evaluated with 28 results within limits (up to 1 closest results for each mass)
Elements Used:
C: 0-40 H: 0-60 N: 0-7 O: 0-10

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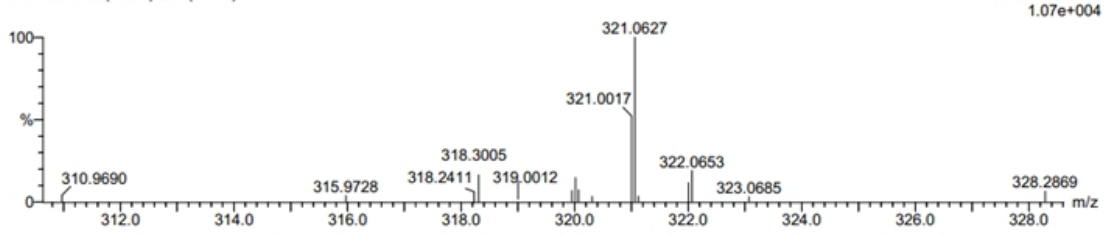
ZWB-SL-6 83 (0.608) Cm (64:91) 1: TOF MS ES+ 1.07e+004



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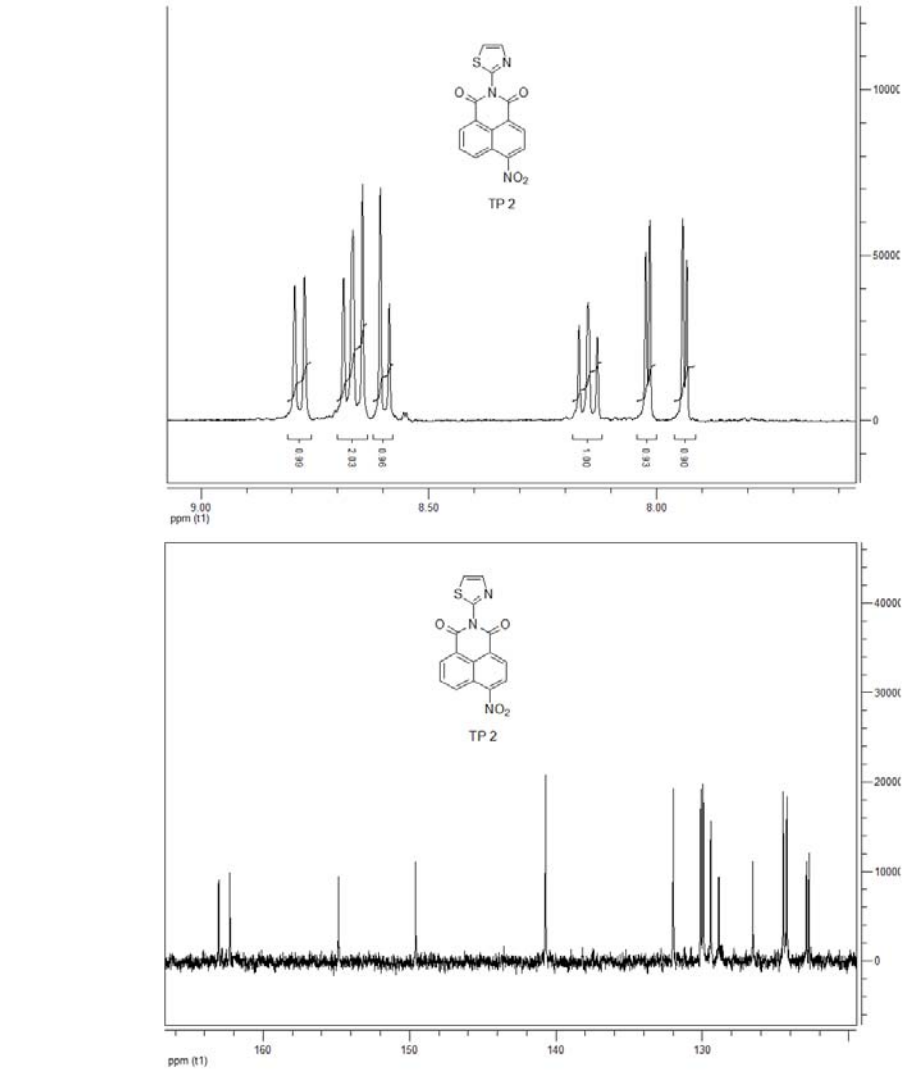
Maximum:		30.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
321.0627	321.0624	0.3	0.9	14.5	32.7	0.0	C16 H9 N4 O4

Fig. S13 ^{1}H NMR, ^{13}C NMR and ESI spectra of **TP1**.



Monoisotopic Mass, Even Electron Ions
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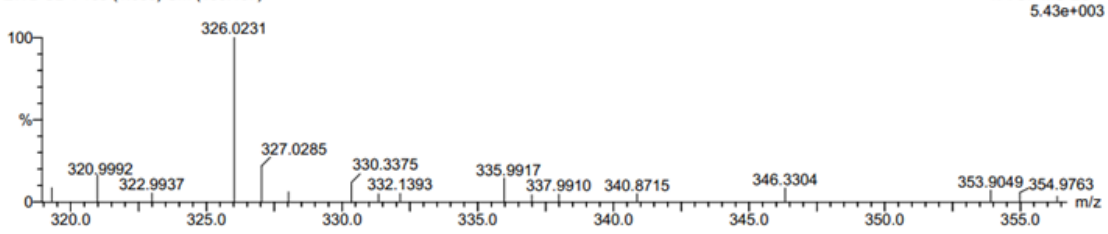
ZWB-SL-4 155 (1.053) Cm (150:157) 1: TOF MS ES+ 5.43e+003



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Maximum:		30.0	50.0	100.0			
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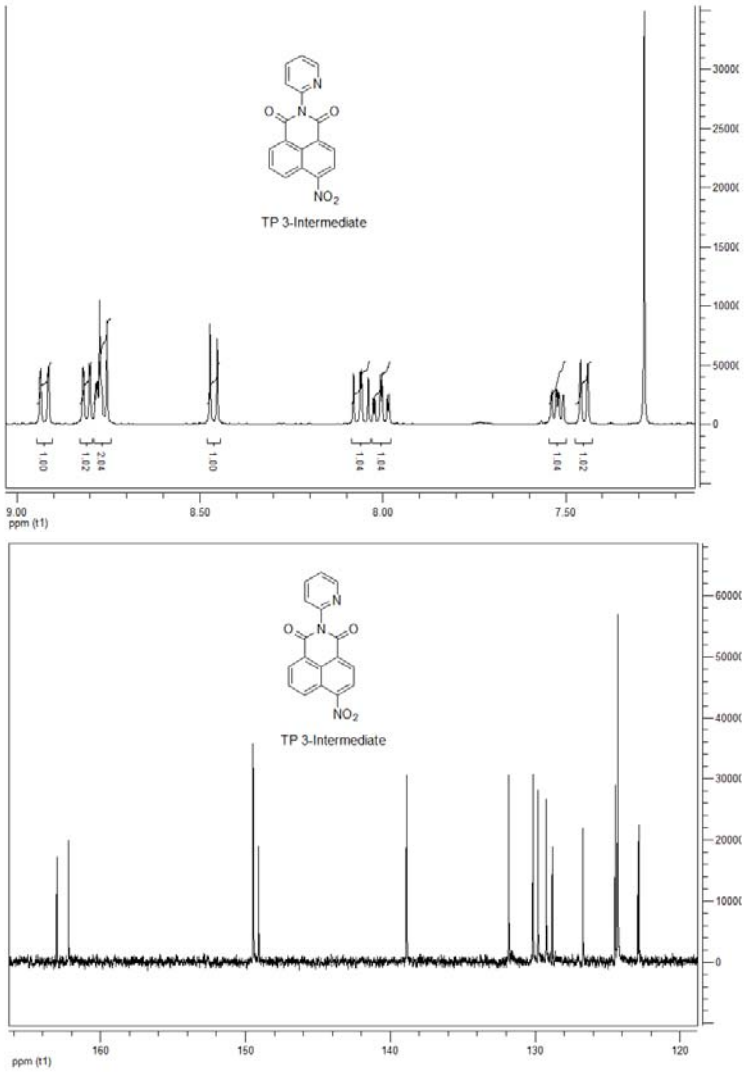
326.0231	326.0236	-0.5	-1.5	13.5	12.6	0.0	C15 H8 N3 O4 S

Fig. S14 ^{1}H NMR, ^{13}C NMR and ESI spectra of **TP2**.



Monoisotopic Mass, Even Electron Ions
308 formula(e) evaluated with 28 results within limits (up to 1 closest results for each mass)
Elements Used:
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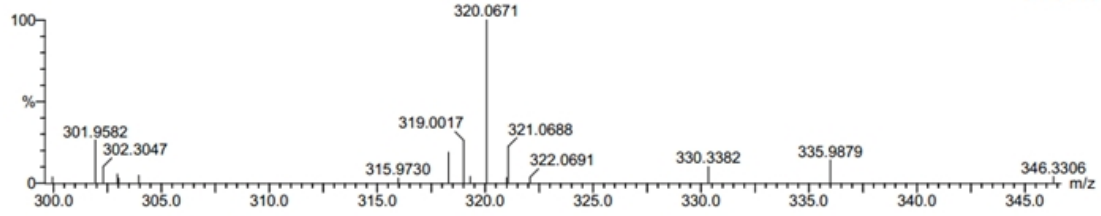

Minimum:				-1.5			
Maximum:		30.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
320.0671	320.0671	0.0	0.0	14.5	20.2	0.0	C17 H10 N3 O4

Fig. S15 [1] H NMR, [13]C NMR and ESI spectra of **TP3-IM**.

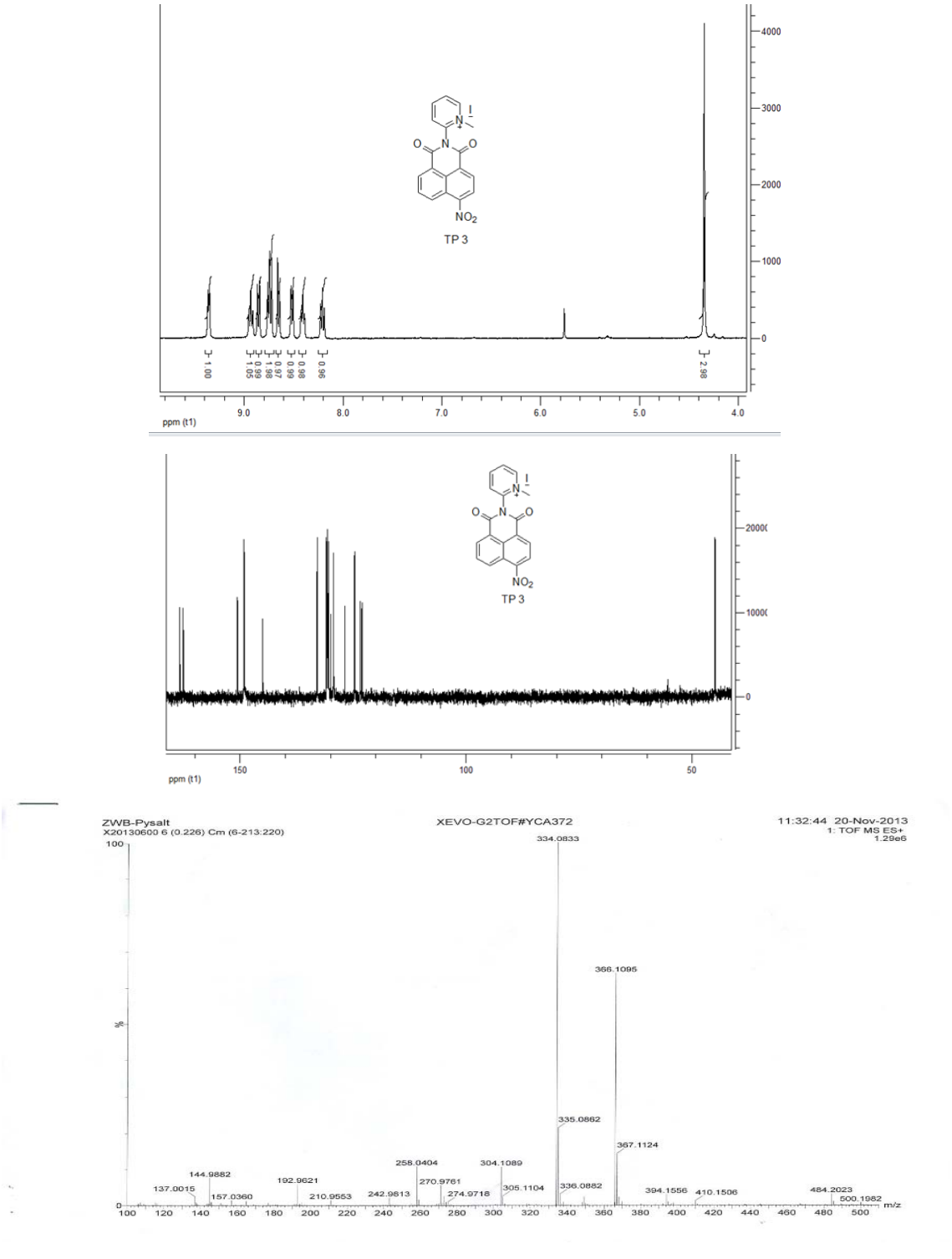

Fig. S16 ^{1}H NMR, ^{13}C NMR and ESI spectra of **TP3**.

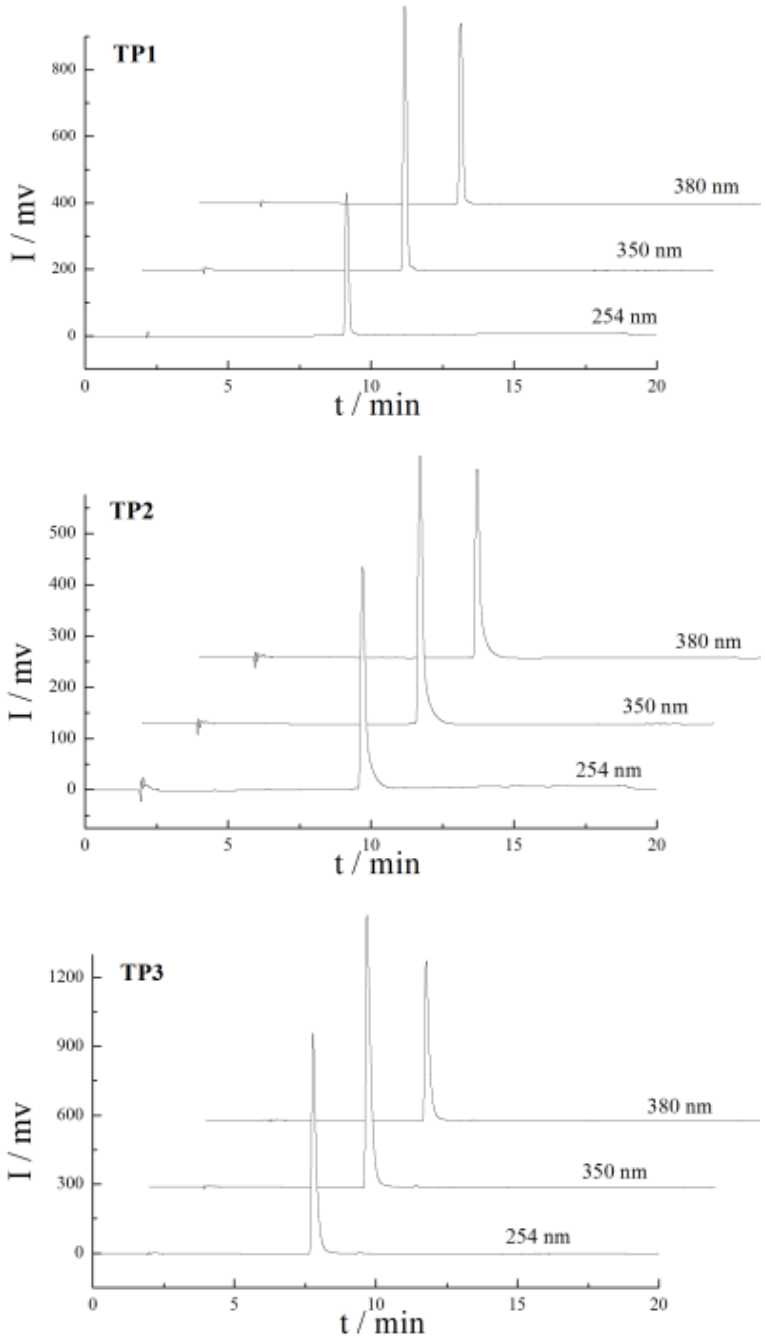

Fig. S17 HPLC spectra of the **TP1-3**. Elite 230 Series chromatographic instrument (Elite HPLC, Dalian, China) and a Hypersil BDS-BP 5 μm (4.6 mm×150 mm) were used. Injection volume: 20 μL; mobile phase: A – 0.05% TFA/water, B –0.05% TFA/ACN; gradient elution: 0-10 min, 5-95%B; 15 – 17 min, 95-5% B; Isocratic elution: 10-15 min, 95%B; 17-20 min, 5%B; flow rate: 1.0 mL min^{-1}; detection wavelength: 254 nm, 350 nm and 380 nm.

Table S1 Determination of BSA concentrations in fetal bovine serum (FBS)

	[BSA] spiked mg/ml	[BSA] found mg/mL	Recovery %
FBS*	0	0.454	---
FBS* + BSA1	0.05	0.498	88.0
FBS* + BSA2	0.1	0.543	89.0

*FBA was 75 times diluted, and the BSA concentration in FBS was estimated to be 34.1 mg/mL.