Supporting Information

Fluorescent "AND" logic gates for simultaneous detection of thiols and proton: photophysical properties, mechanism and bioimaging of living cells

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Experimental Section

Calculation of fluorescence quantum yield

The fluorescence quantum yield of each probe was calculated according to the following equation with 4-amino-N-(*n*-butyl) naphthalimide as the reference (Φ = 0.21 in DMSO).

$$\Phi^S = \Phi^R \times (S^S/S^R) \times (n^S/n^R)^2 \times (A^R/A^S)$$

Where Φ is the fluorescence quantum yield; A represents the absorbance at the excited wavelength; S is the integral area of the emission peak, and n is the refractive index of the solvent. The superscript "S" and "R" represent the sample and the reference, respectively.

High-performance liquid chromatography (HPLC) traces

HPLC spectra were obtained on an iChrom 5100 LC system and a Sinopak C18 reversed-phase colum (4.6 mm × 25 cm). The mobile phase was degassed with an ultrasonic device for 10 minutes. Mobile phase: A-water, B-acetonitrile; injection volume: 20 μL; flow rate: 1.0 mL/min; detection wavelength: 450 nm; elution condition: gradient elution, 0-20 min 10-70% B, 20-21 min 70-10% B; Isocratic elution, 21-30 min 10% B.

Computational methods

The density functional theory (DFT) was employed to optimize the structure of the probes through Gaussian 16. All structure optimizations were performed using DFT-M062X functionals and Def2SVP basis set for the ground states. Solvation effects were taken into account using the SMD mode and the solvent was the same as in the experiment (water). Frequency calculations were performed to confirm that we obtained stable structures without imaginary vibrational frequencies.

Confocal fluorescence imaging

HeLa, RH30 and L929 cell lines (National Collection of Authenticated Cell Cultures, Shanghai, China) were cultured in Dulbecco's Modified Eagle Medium (High glucose) containing 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C in an atmosphere of 5% CO_2. The cells were transferred to confocal dishes and incubated for 24 h and followed by washed with PBS (20 mmol/L, pH 7.4) for three times. Then the cells were cultured with serum-free DEME medium containing probes (1 μmol/L) for 30 min and washed with PBS for three times again.

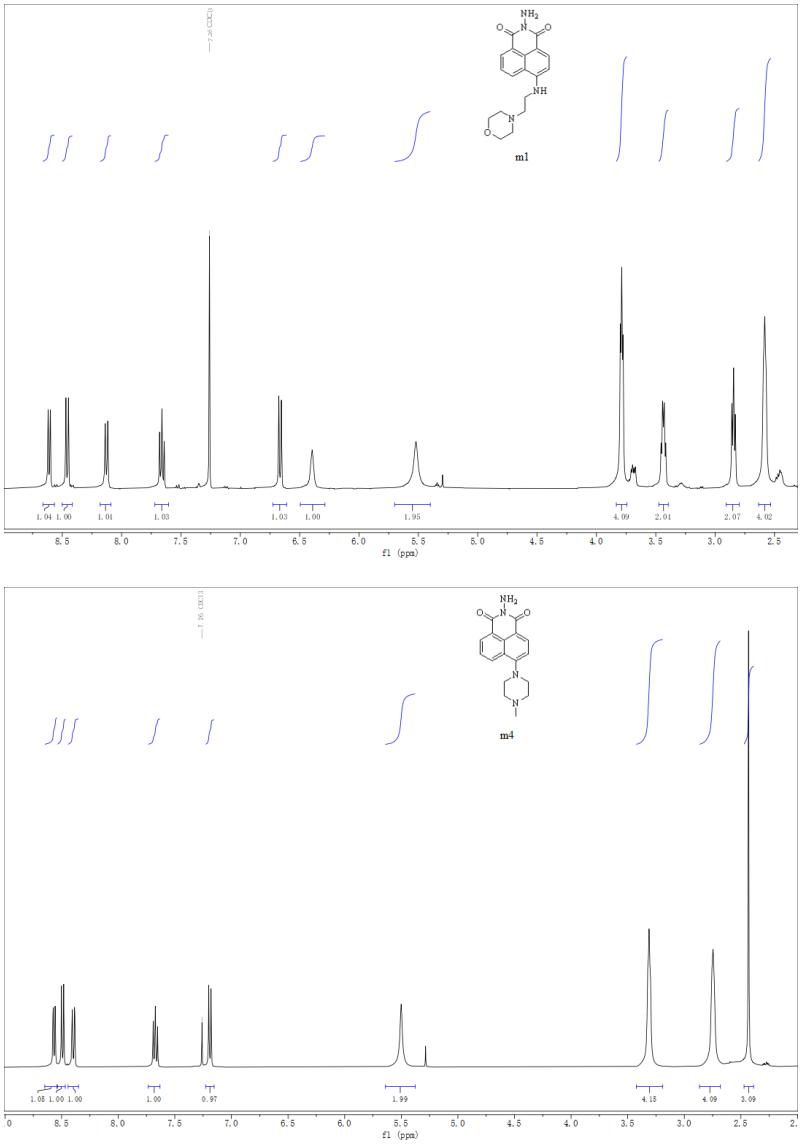
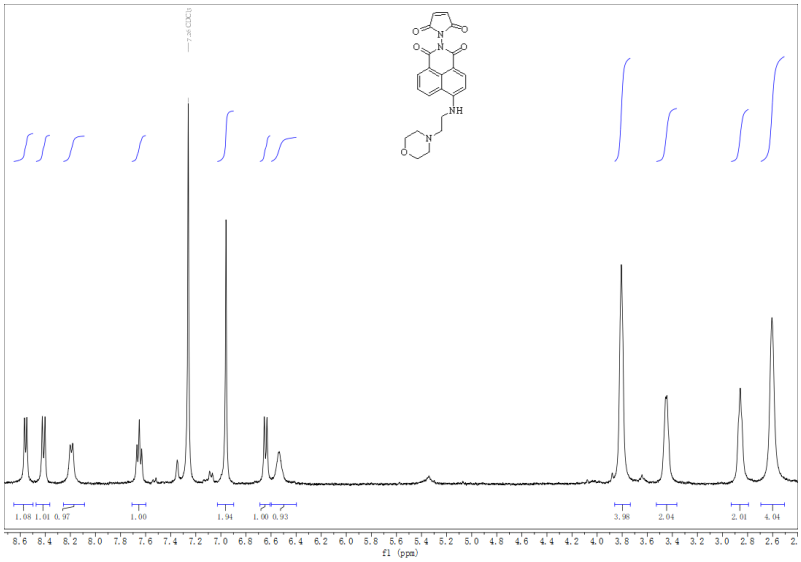
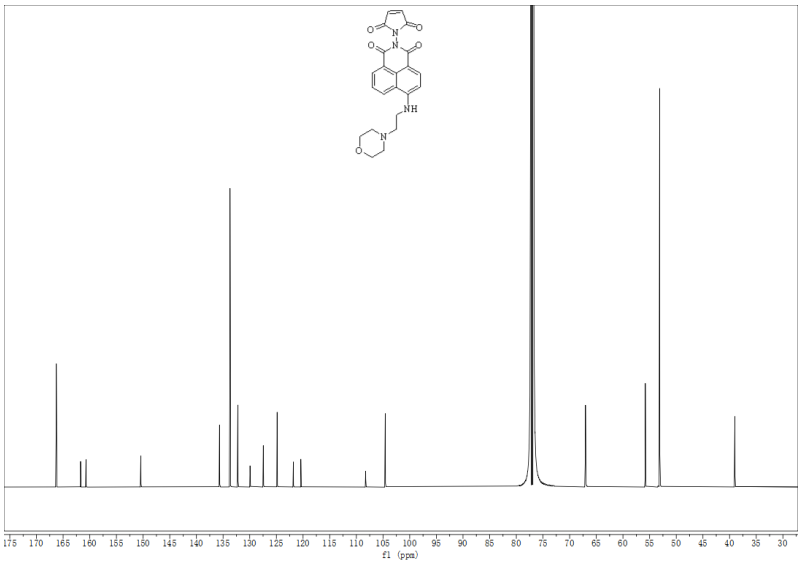


Fig. S1 ^{1}H-NMR spectra of **m1** and **m4**.



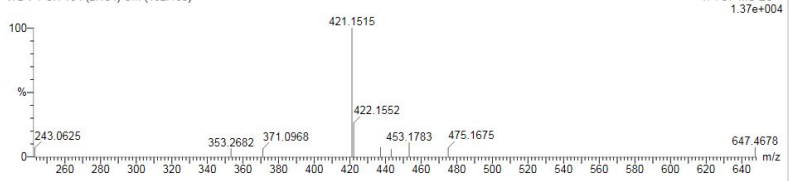


Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

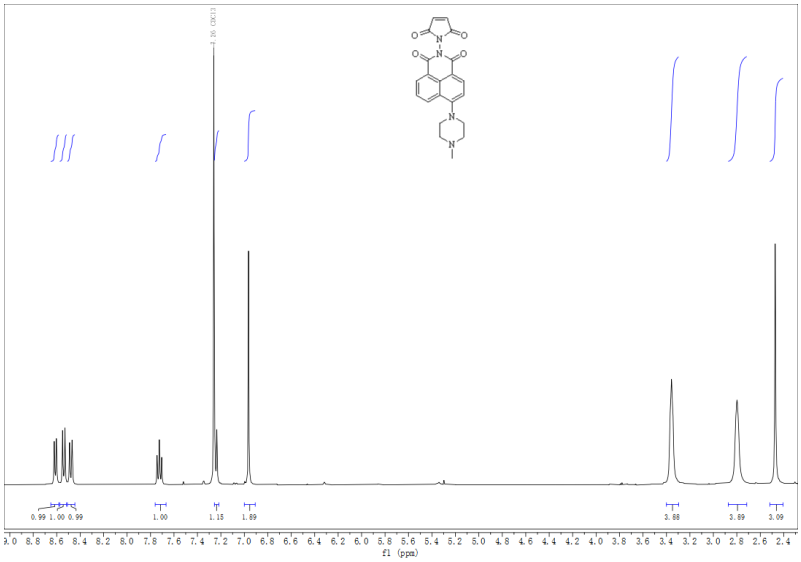
Monoisotopic Mass, Even Electron Ions
25 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-22 H: 0-21 N: 0-4 O: 0-5
WB-ZHANG
WB-P1-SH 184 (2.104) Cm (182:185)

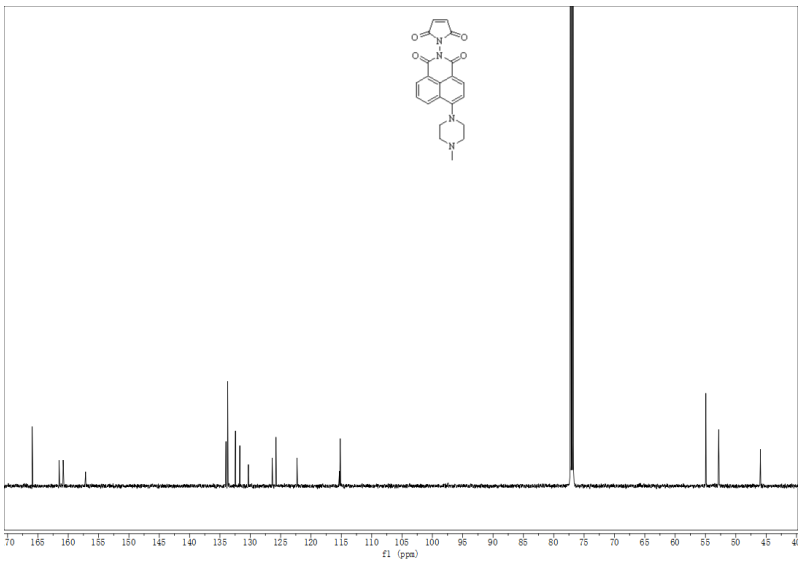
1: TOF MS ES+
1.37e+004



Minimum:				-1.5			
Maximum:		5.0	10.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
421.1515	421.1512	0.3	0.7	14.5	27.6	0.0	C22 H21 N4 O5

Fig. S2 ^{1}H-NMR, ^{13}C-NMR and ESI-Mass spectra of **TP1**.





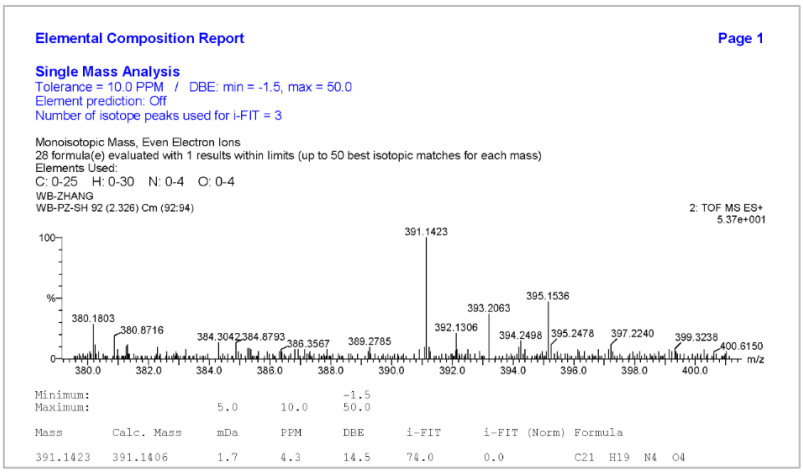


Fig. S3 ^{1}H-NMR, ^{13}C-NMR and ESI-Mass spectra of **TP4**.

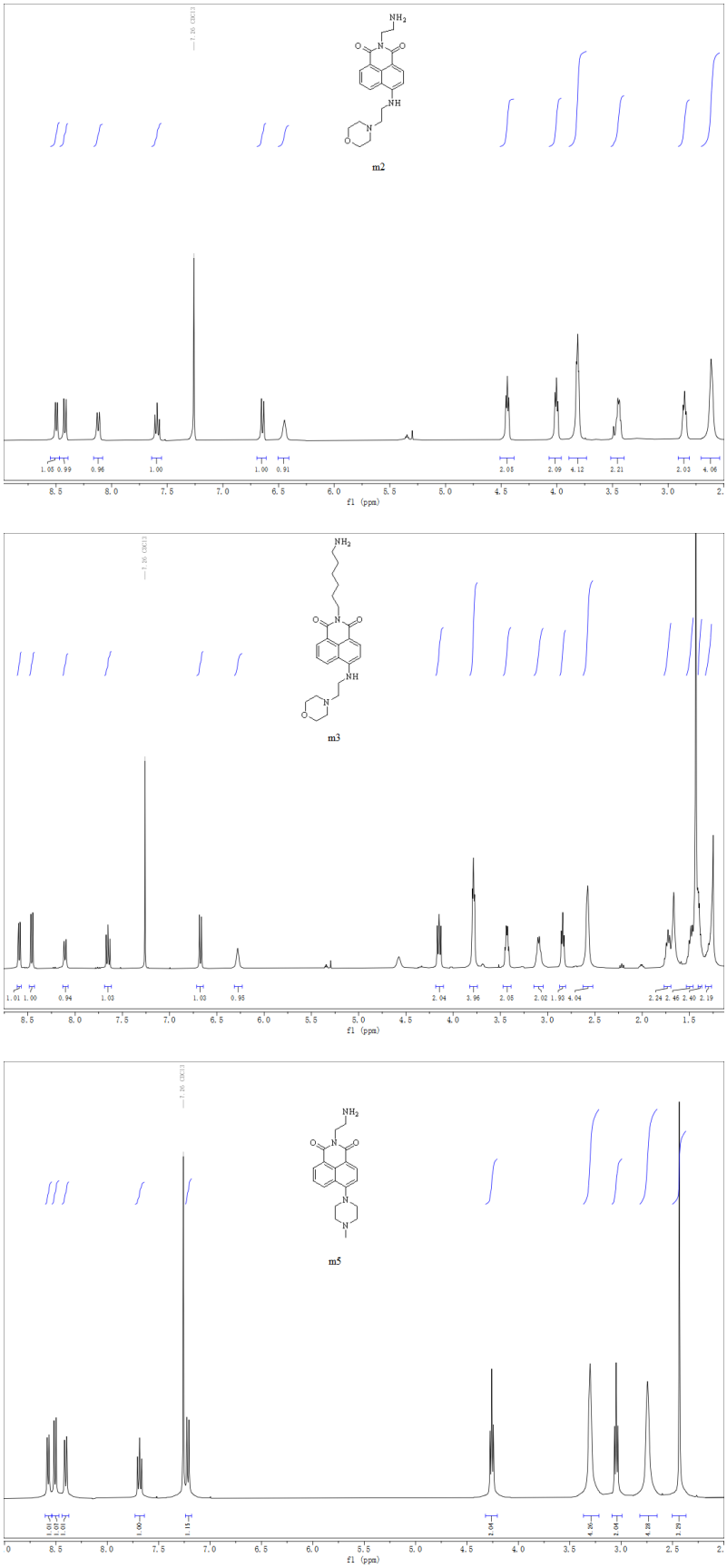
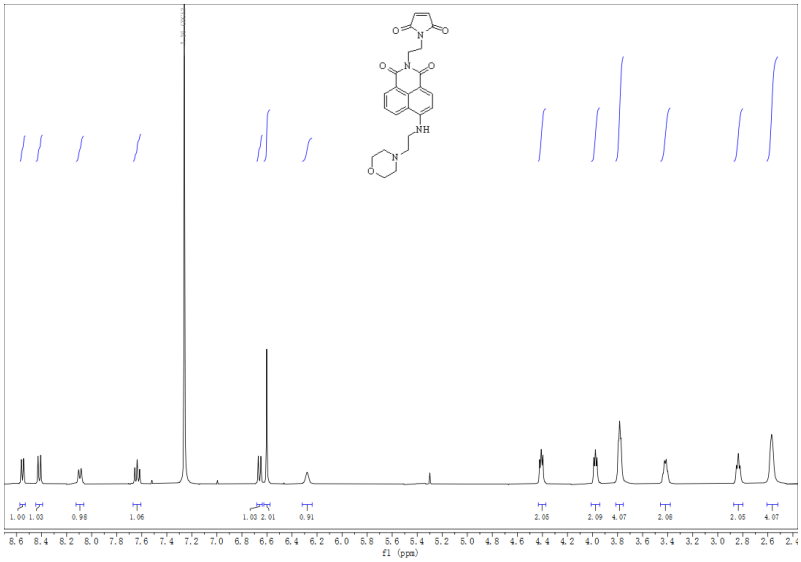
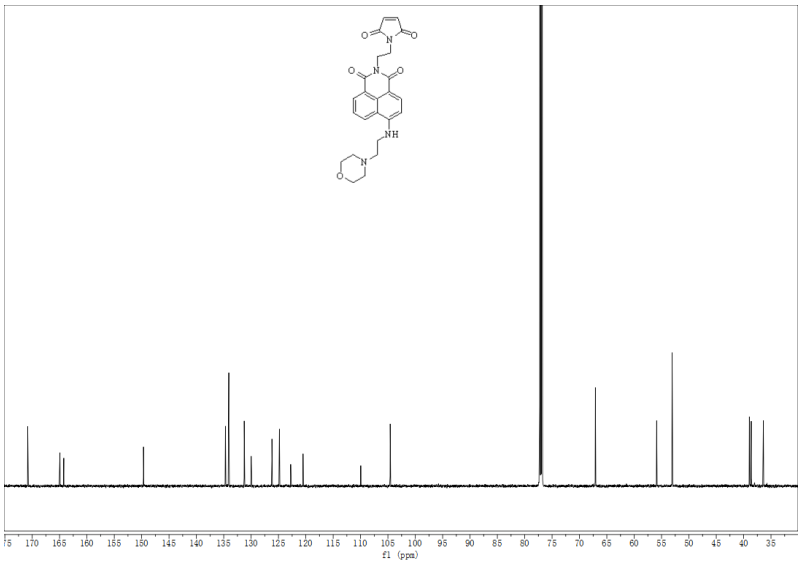


Fig. S4 ^{1}H-NMR spectra of **m2**, **m3** and **m5**.



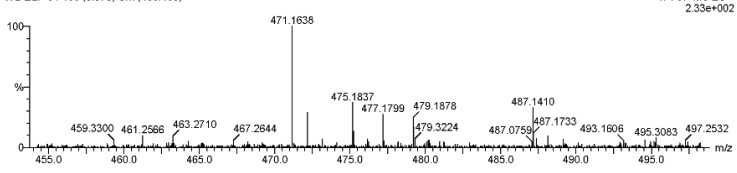


Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

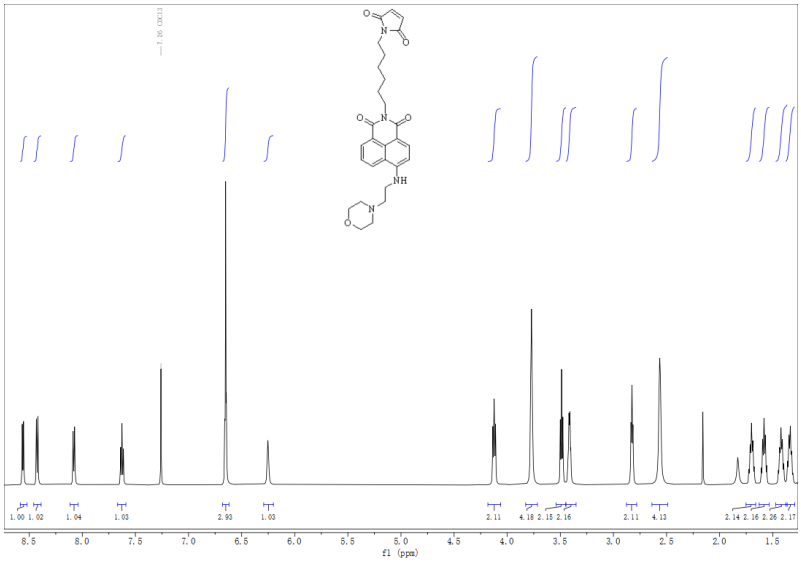
Monoisotopic Mass, Even Electron Ions
52 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-24 H: 0-25 N: 0-4 O: 0-5 Na: 0-1
WB-ZHANG
WB-ZLP-64 468 (5.379) Cm (466:469)

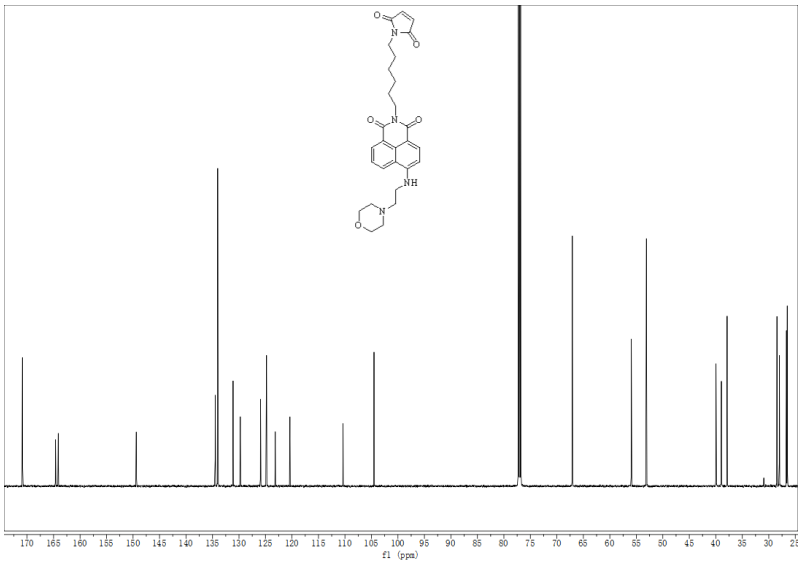
1: TOF MS ES+
2.33e+002



Minimum:				-1.5			
Maximum:		5.0	10.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
471.1638	471.1644	-0.6	-1.3	14.5	95.6	0.0	C24 H24 N4 O5 Na

Fig. S5 ^{1}H-NMR, ^{13}C-NMR and ESI-Mass spectra of **TP2**.



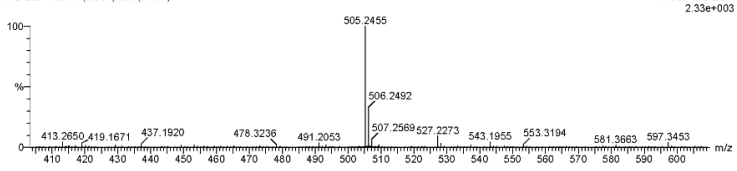


Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

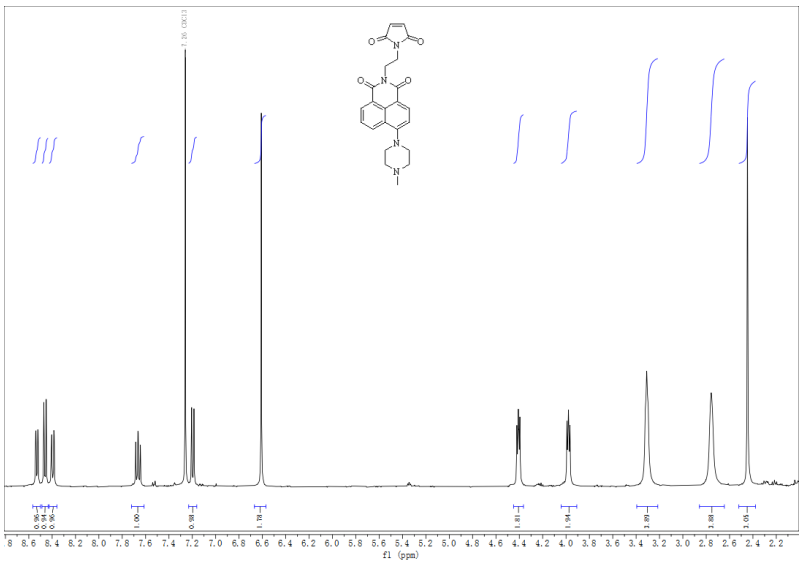
Monoisotopic Mass, Even Electron Ions
47 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-28 H: 0-33 N: 0-4 O: 0-5 Na: 0-1
WB-ZHANG
WB-ZLP-640 77 (0.867) Cm (76:77)

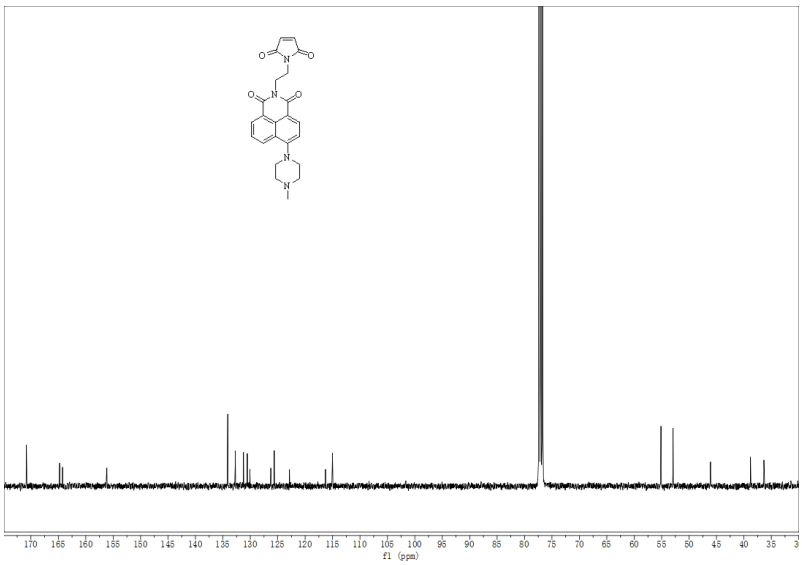
1: TOF MS ES+
2.33e+003



Minimum:				-1.5			
Maximum:		5.0	10.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
505.2455	505.2451	0.4	0.8	14.5	125.3	0.0	C28 H33 N4 O5

Fig. S6 ^{1}H-NMR, ^{13}C-NMR and ESI-Mass spectra of **TP3**.





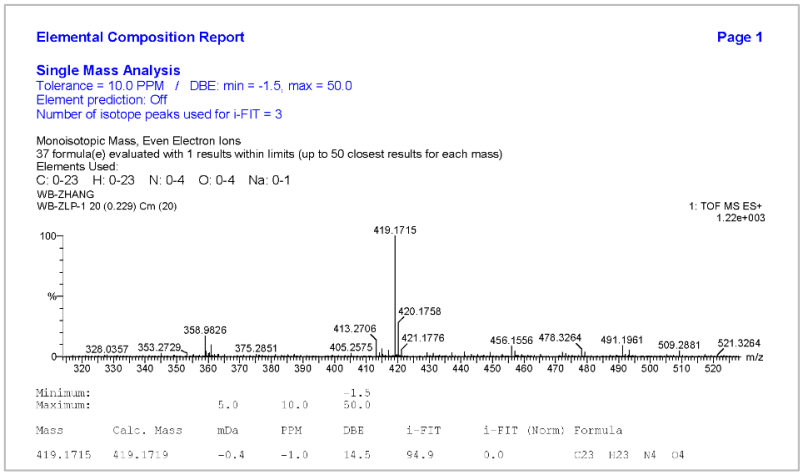


Fig. S7 ^{1}H-NMR, ^{13}C-NMR and ESI-Mass spectra of **TP5**.

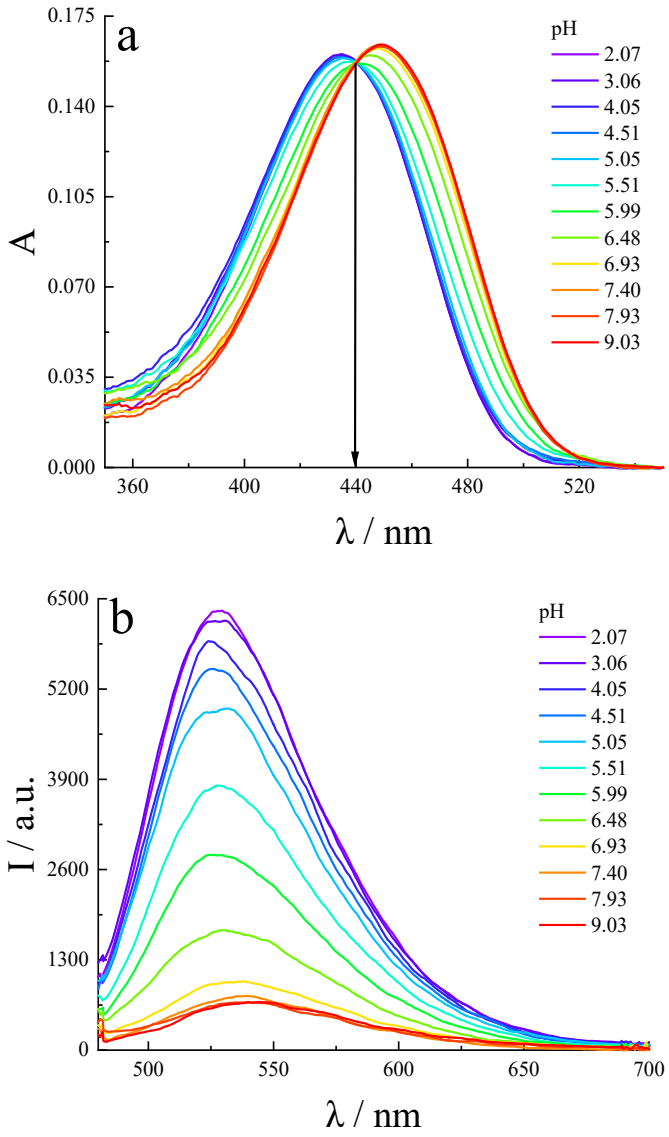


Fig. S8 pH-dependent absorption (a) and emission (b) spectra of **TP2** in 20 mM PBS. [**TP2**] = 10 μM, λ_{ex} = 450 nm.

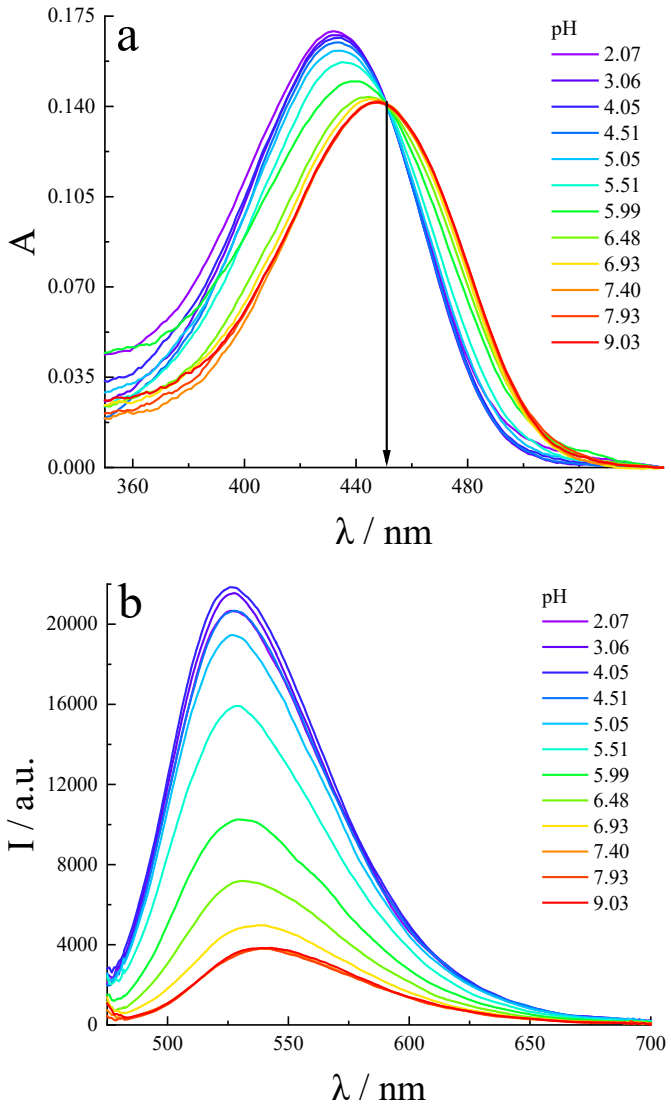


Fig. S9 pH-dependent absorption (a) and emission (b) spectra of **TP3** in 20 mM PBS. [**TP3**] = 10 μM, λ_{ex} = 450 nm.

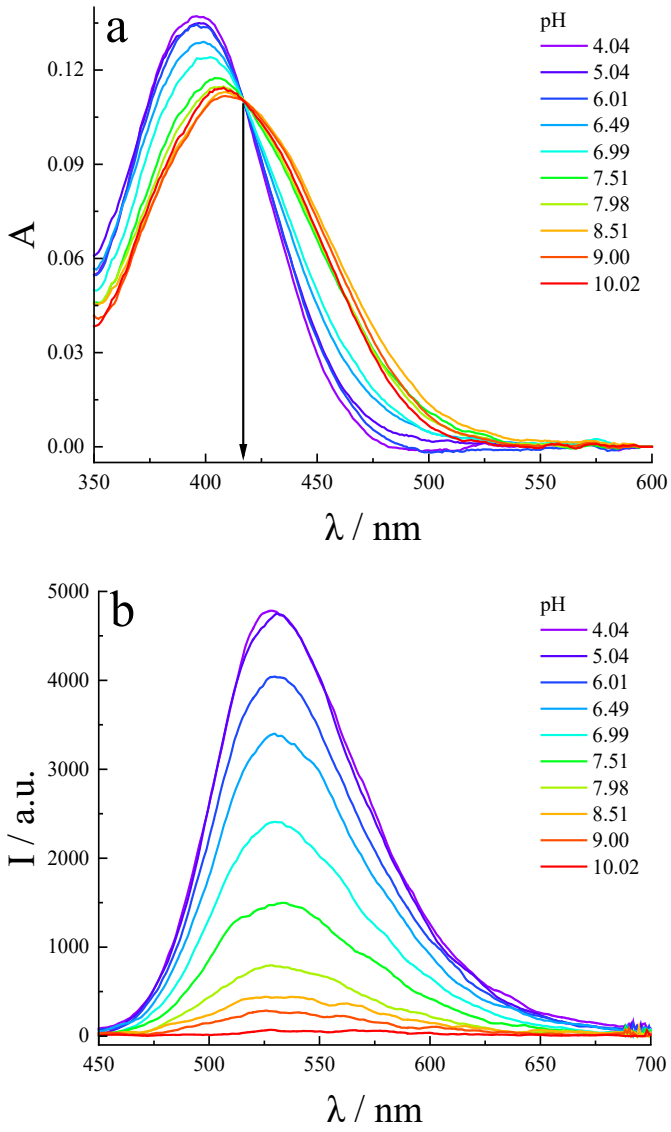


Fig. S10 pH-dependent absorption (a) and emission (b) spectra of **TP4** in 20 mM PBS. [**TP4**] = 10 μM, λ_{ex} = 400 nm.

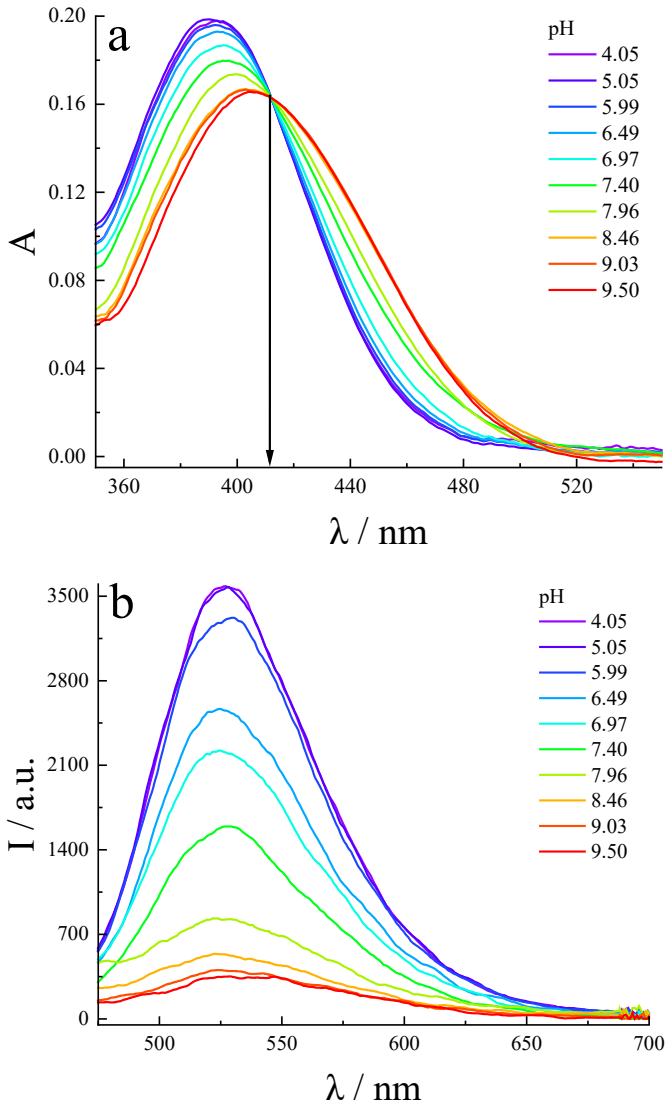


Fig. S11 pH-dependent absorption (a) and emission (b) spectra of **TP5** in 20 mM PBS. [**TP5**] = 10 μM, λ_{ex} = 400 nm.

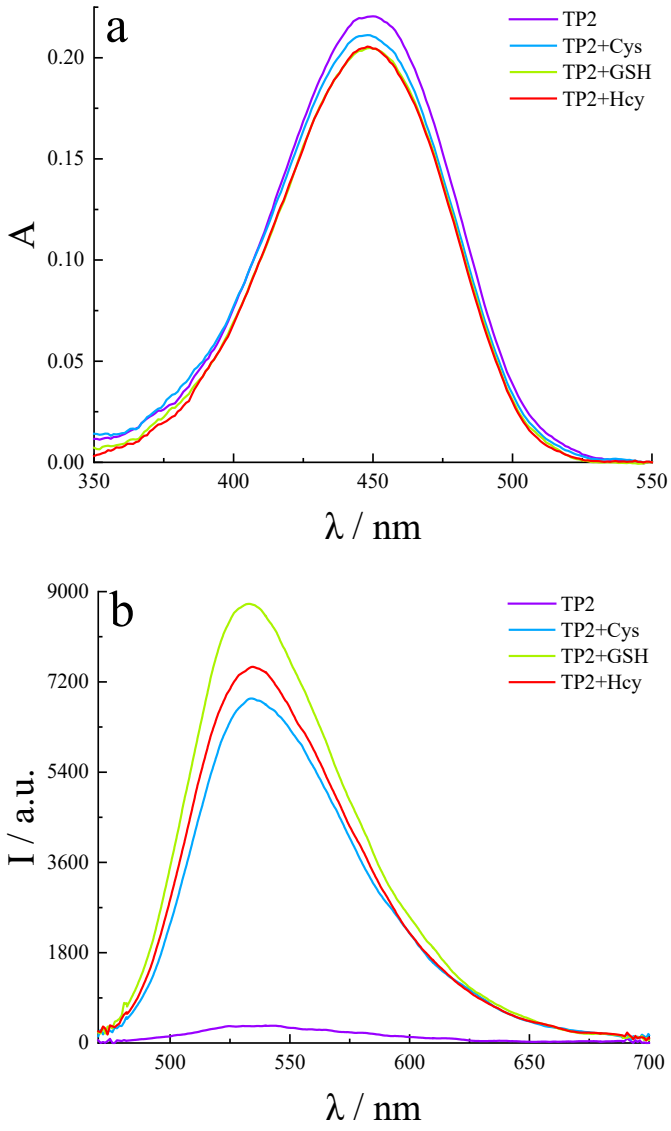


Fig. S12 Absorption (a) and emission spectra (b) of **TP2** in the presence of 10 equiv. thiol. [**TP2**] = 10 μM, [Cys] = 100 μM, λ_{ex} = 450 nm.

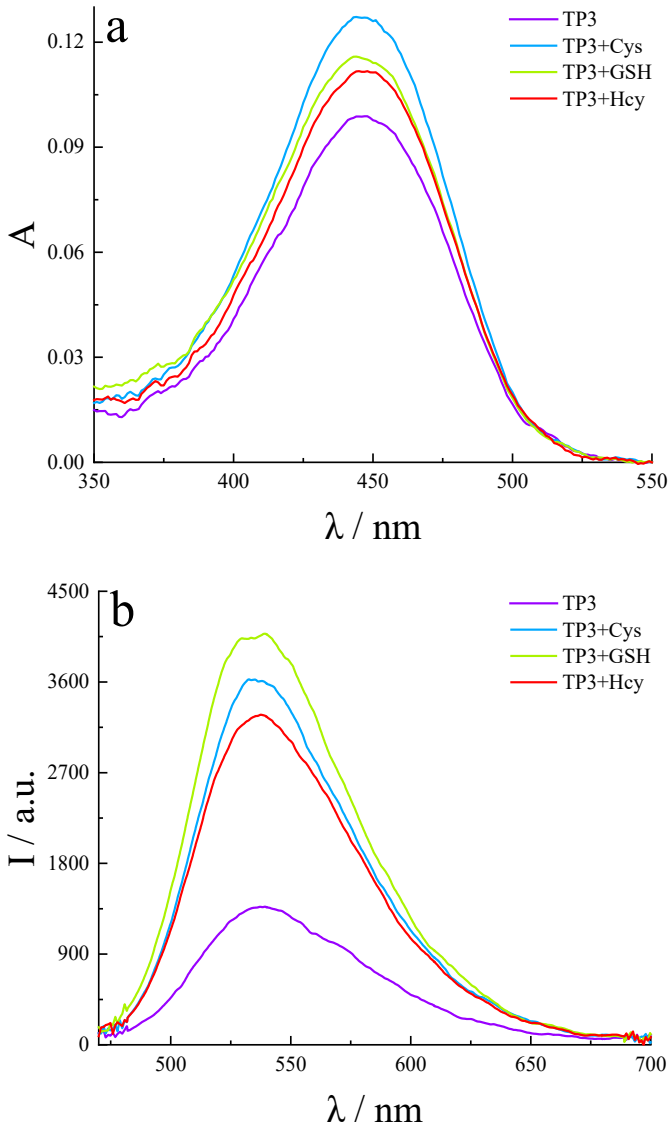


Fig. S13 Absorption (a) and emission spectra (b) of **TP3** in the presence of 10 equiv. thiol. [**TP3**] = 10 μM, [Cys] = 100 μM, λ_{ex} = 450 nm.

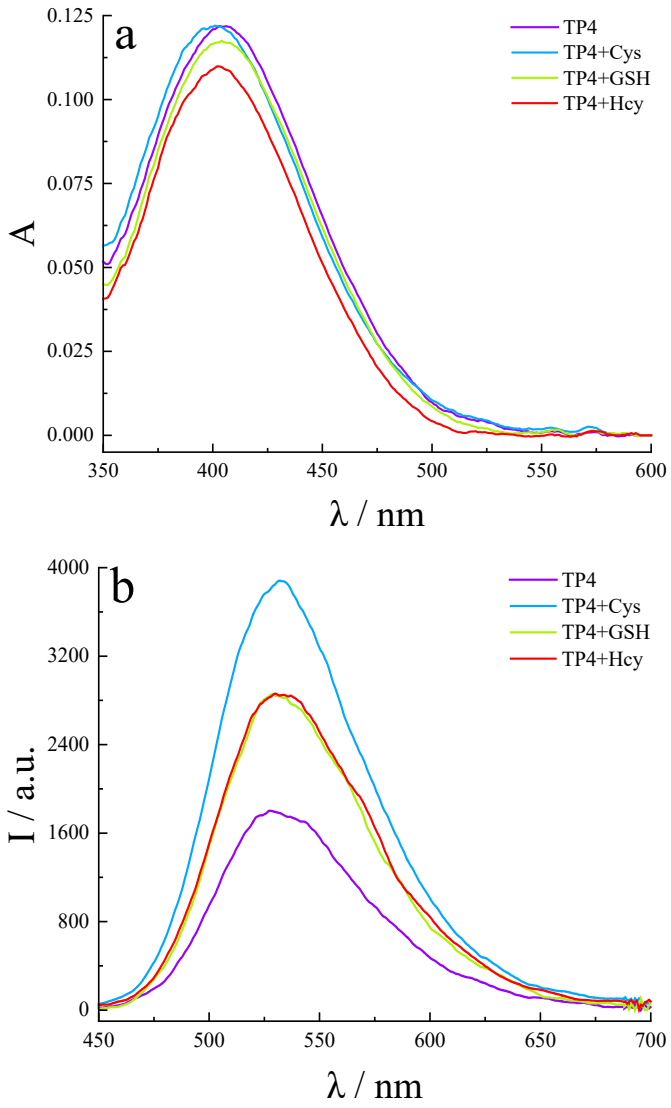


Fig. S14 Absorption (a) and emission spectra (b) of **TP4** in the presence of 10 equiv. thiol. [**TP4**] = 10 μM, [Cys] = 100 μM, λ_{ex} = 400 nm.

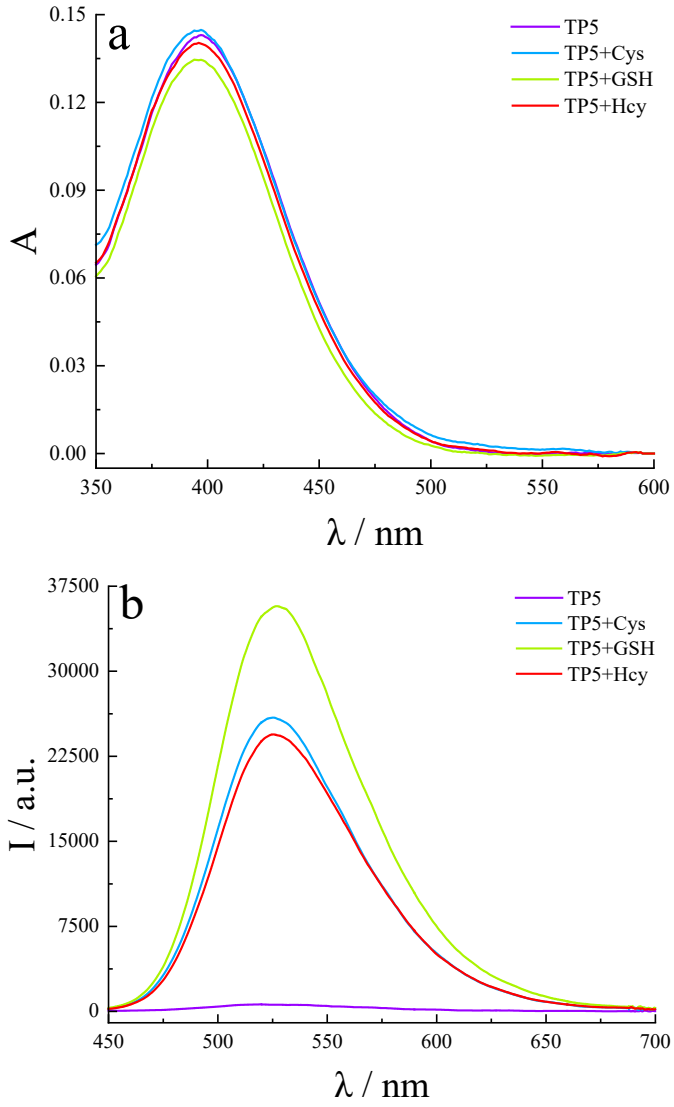


Fig. S15 Absorption (a) and emission spectra (b) of **TP5** in the presence of 10 equiv. thiol. [**TP5**] = 10 μM, [cys] = 100 μM, λ_{ex} = 400 nm.

Table S1 The thiol detection abilities of some reported fluorescent probes.

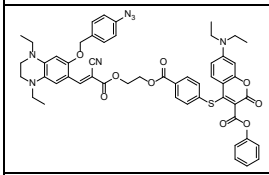
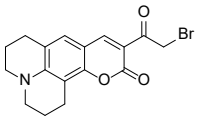
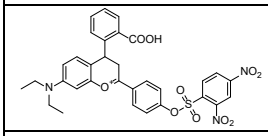
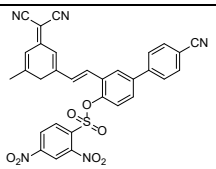
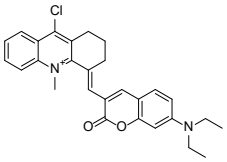
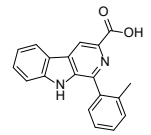
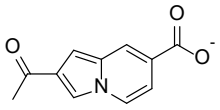
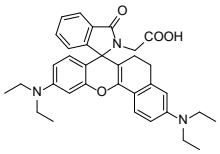
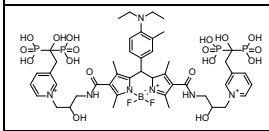
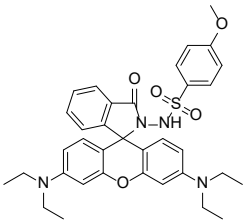
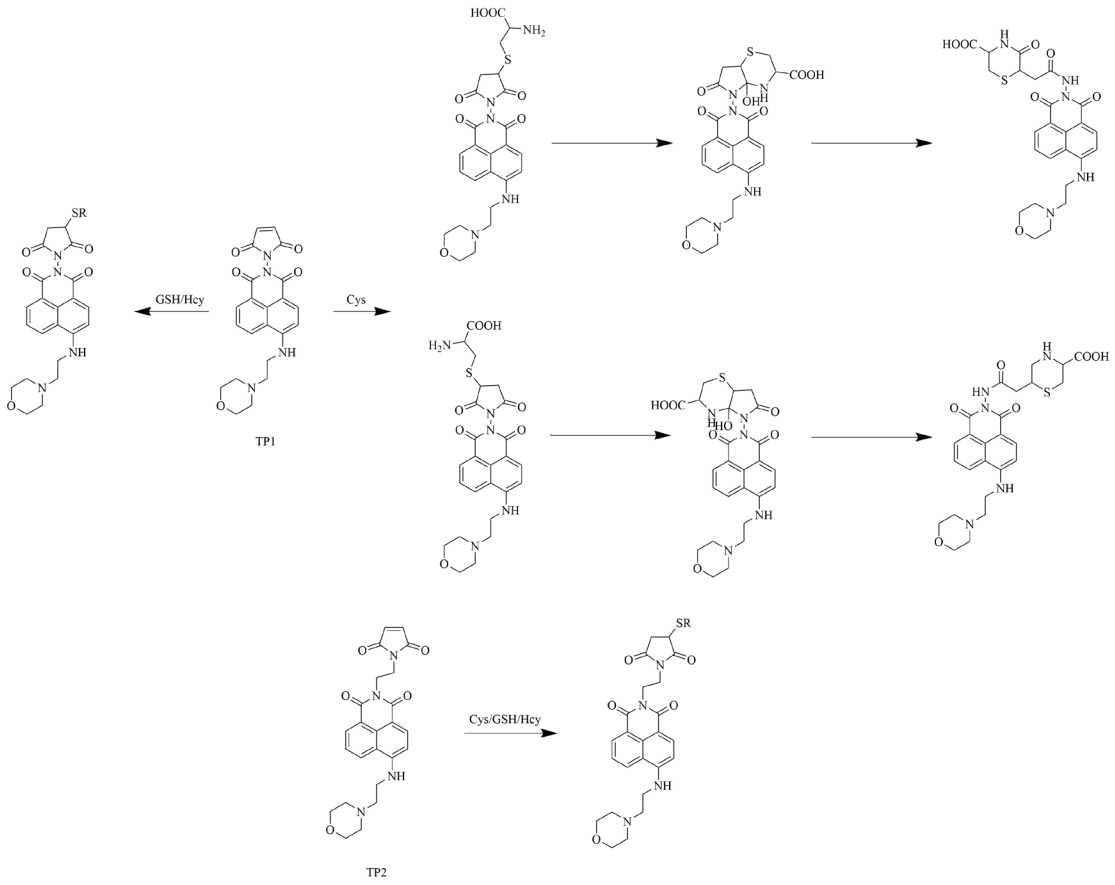
Probe	Analyte	Detect Time	Fluorescence Enhancement	Ref.
	Cys Hcy GSH	8 min 1 h 12 min	36-fold 29-fold 26-fold	Sens. Actuator B-Chem., 2021, **331**, 129394
	GSH	5 min	~15-fold	ACS Sens., 2020, **5**, 242-249
	Cys Hcy GSH	60 min 40 min 60 min	~55-fold	J. Photochem. Photobiol. A-Chem. 2022, **425**, 113654
	Cys Hcy GSH	5 min	26-fold 22-fold 24-fold	Spectroc. Acta Pt. A-Molec. Biomolec. Spectr., 2020, **241**, 118655
	Cys Hcy	3 min 15 min	20-fold 15-fold	Sens. Actuator B-Chem., 2023, **374**, 132799
TP2	Thiol Proton Thiols and proton	1 min	~20-fold ~9-fold ~160-fold	This work

Table S2 The proton detection abilities of some reported fluorescent probe.

Probe	Analyte	pK_a	Fluorescence Enhancement	Ref.
	Proton	6.42	120-fold	Chem. Biodivers., 2021, **18**, e2000829
	Proton	3.85	8.3-fold	Sens. Actuator B-Chem., 2017, **247**, 46-52
	Proton	6.54	~12-fold	Spectroc. Acta Pt. A-Molec. Biomolec. Spectr., 2022, **280**, 121496
	Proton	6.8	15-fold	Angew. Chem.-Int. Edit., 2020, **59**, 20996-21000
	Proton	3.62	~425-fold	Sens. Actuator B-Chem., 2022, **366**, 131963
TP2	Thiol Proton Thiols and proton	5.71	~20-fold ~9-fold ~160-fold	This work



Scheme S1 The proposed reaction diagram between probe **TP1**/**TP2** and thiols.

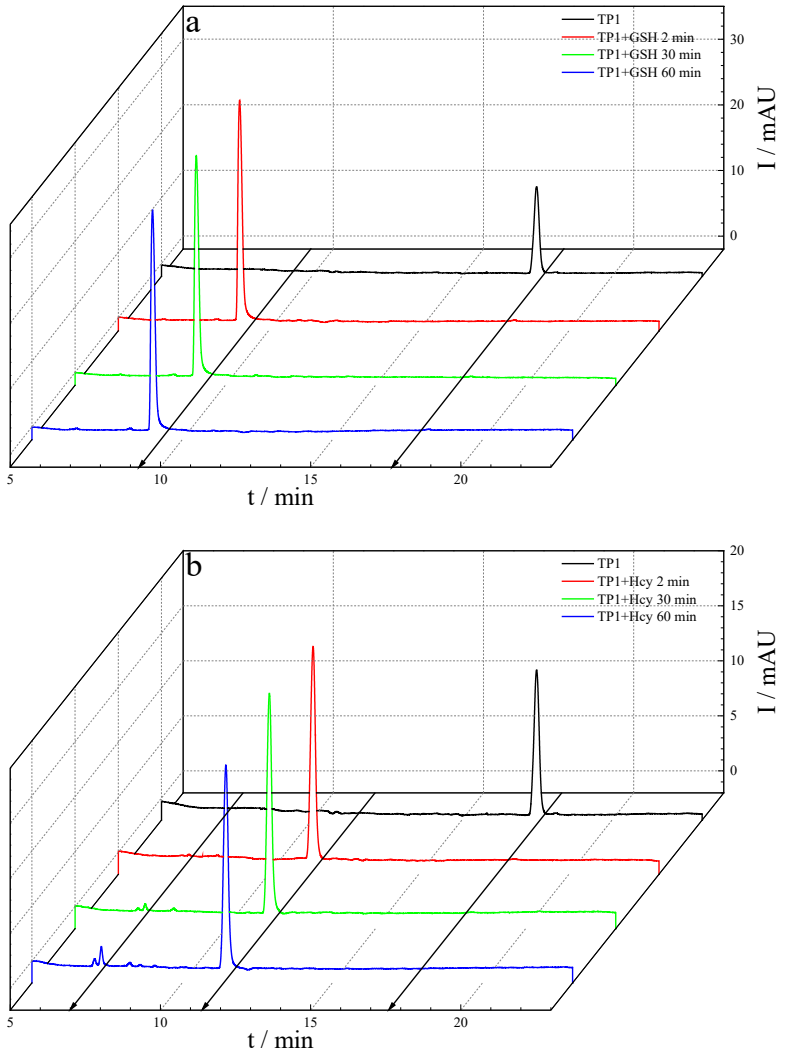


Fig. S16 HPLC spectra of GSH-**TP1** (a)/Hcy-**TP1** (b) system at different reaction time. [**TP1**] = 20 μM, [GSH] = [Hcy] = 500 μM, 20 mM PBS (pH 7.4), the detection wavelength was 450 nm.

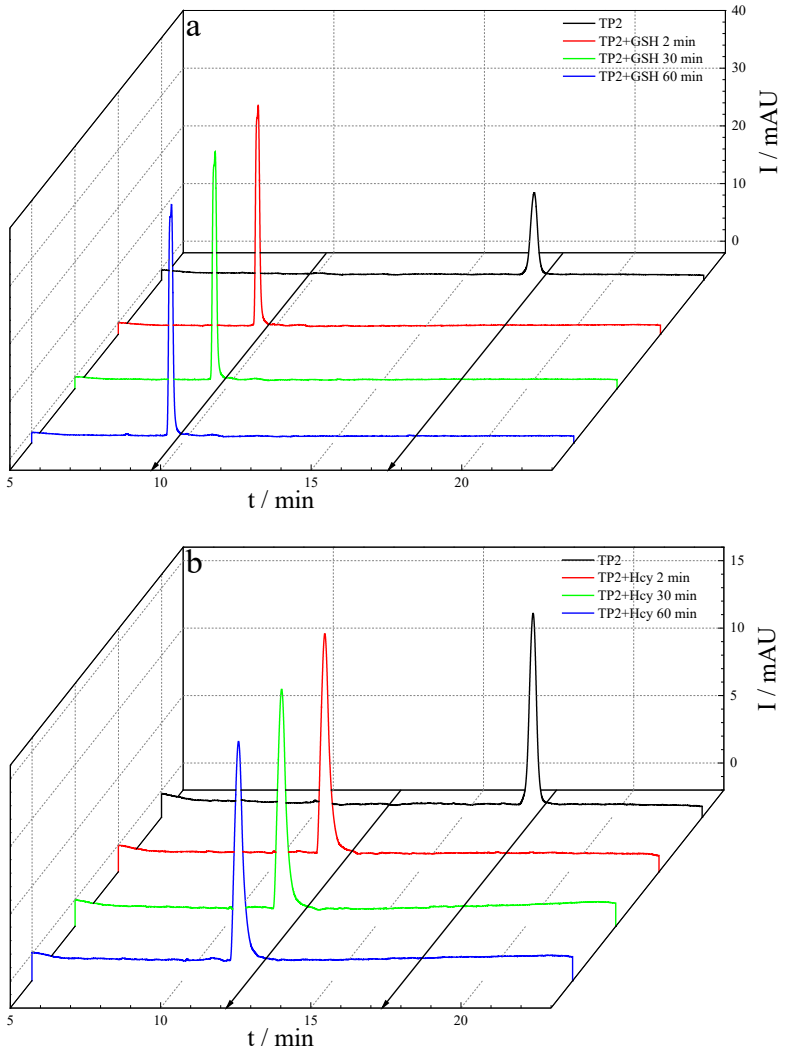


Fig. S17 HPLC spectra of GSH-**TP2** (a)/Hcy-**TP2** (b) system at different reaction time. [**TP2**] = 20 μM, [GSH] = [Hcy] = 500 μM, 20 mM PBS (pH 7.4), the detection wavelength was 450 nm.

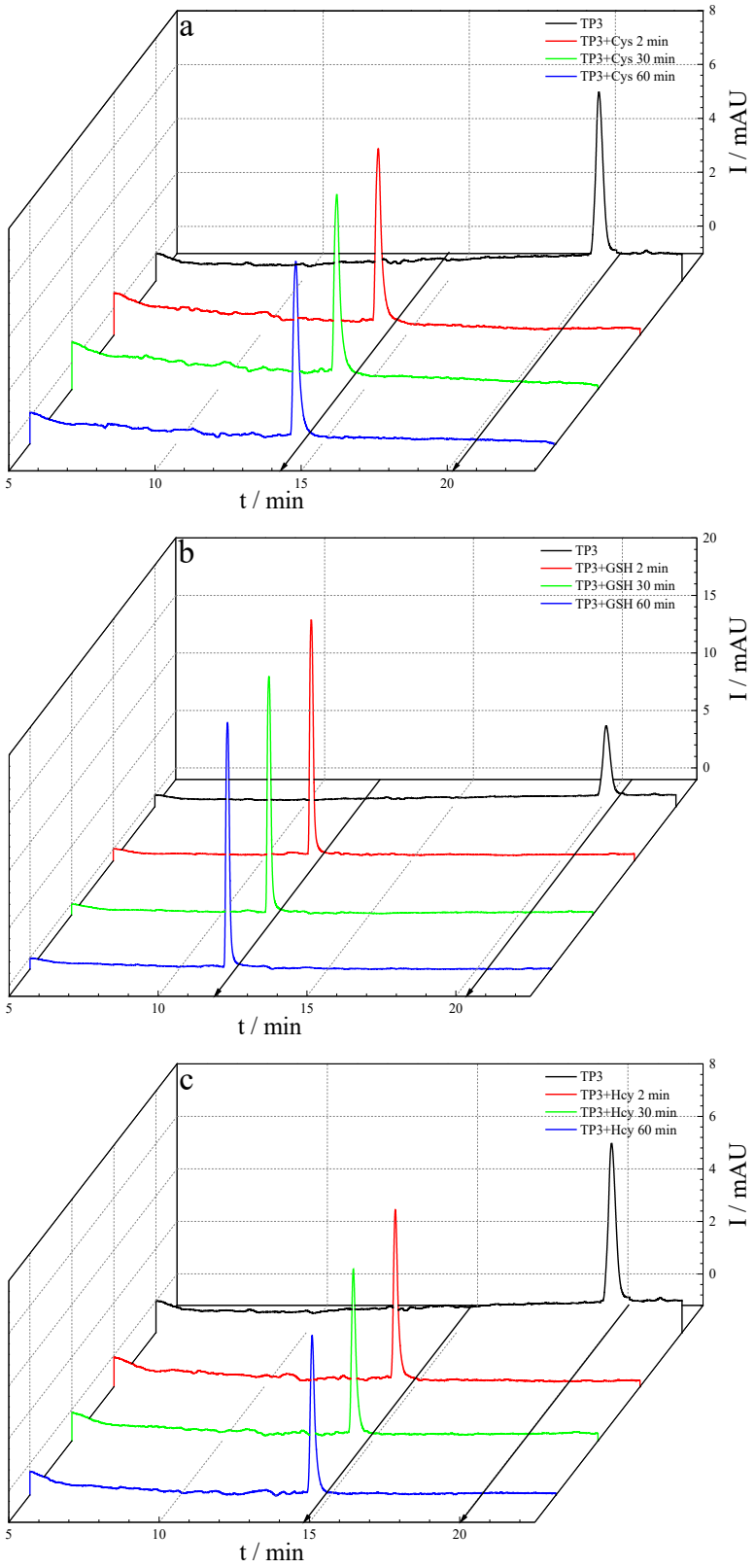


Fig. S18 HPLC spectra of Cys-**TP3** (a)/GSH-**TP3** (b)/Hcy-**TP3** (c) system at different reaction time. [**TP3**] = 20 μM, [Cys] = [GSH] = [Hcy] = 500 μM, 20 mM PBS (pH 7.4), the detection wavelength: 450 nm.

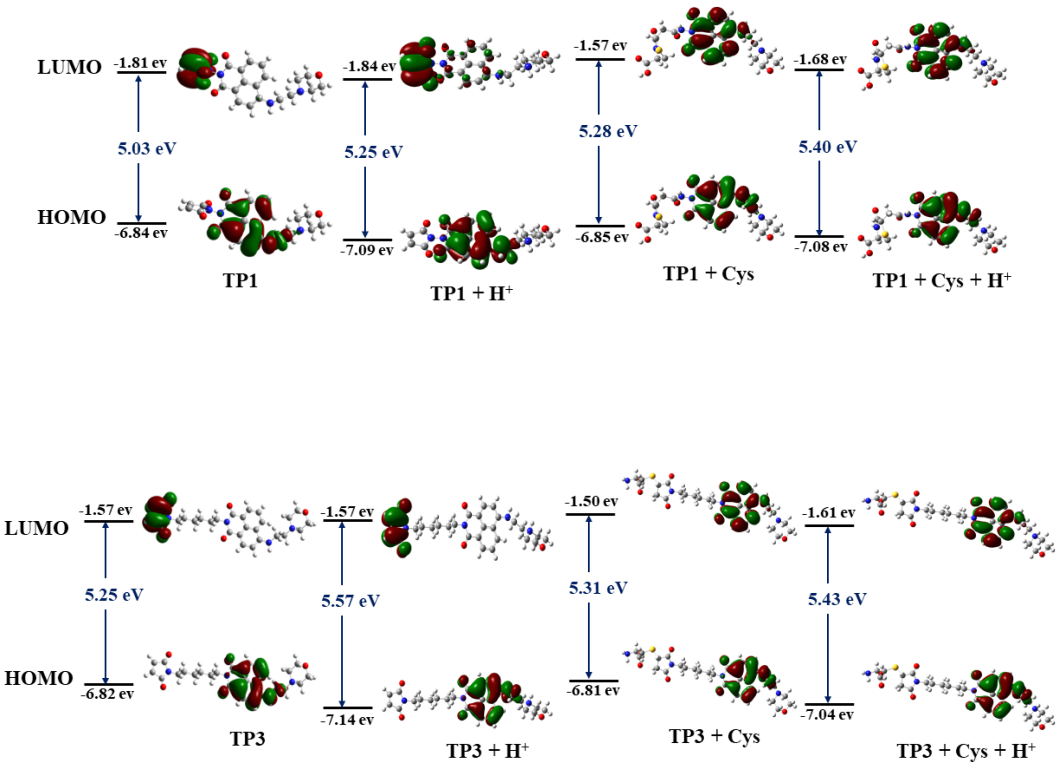


Fig. S19 The molecular orbitals of **TP1, TP3**, and their corresponding reaction products with Cys/proton.

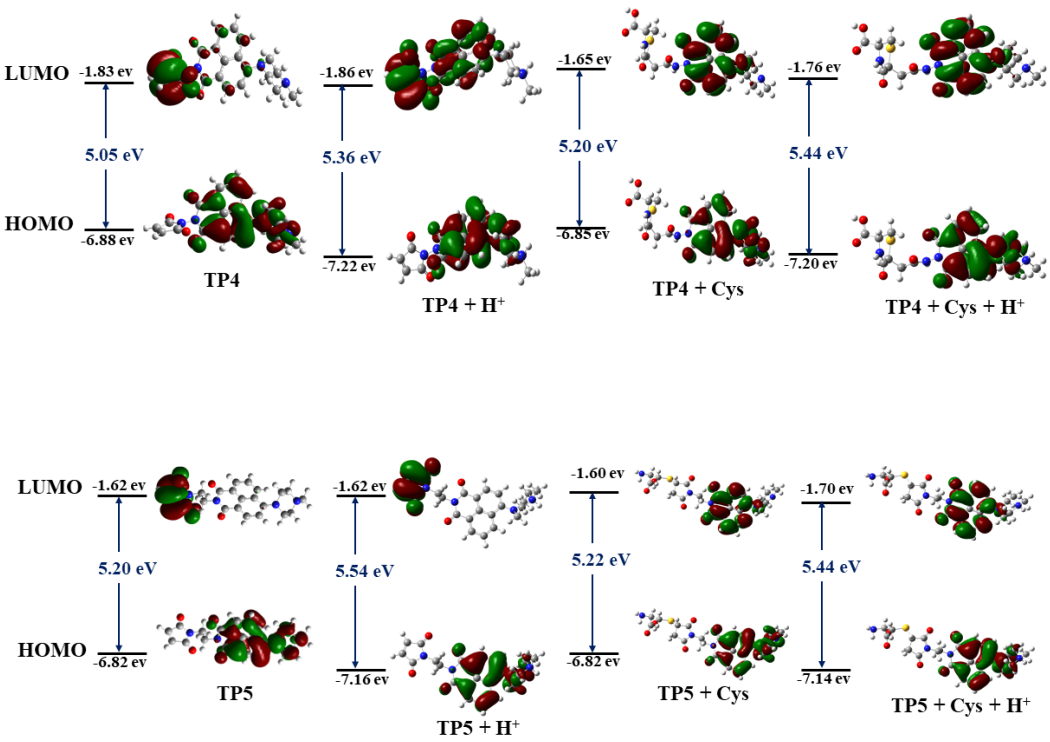


Fig. S20 The molecular orbitals of **TP4**, **TP5**, and their corresponding reaction products with Cys/proton.

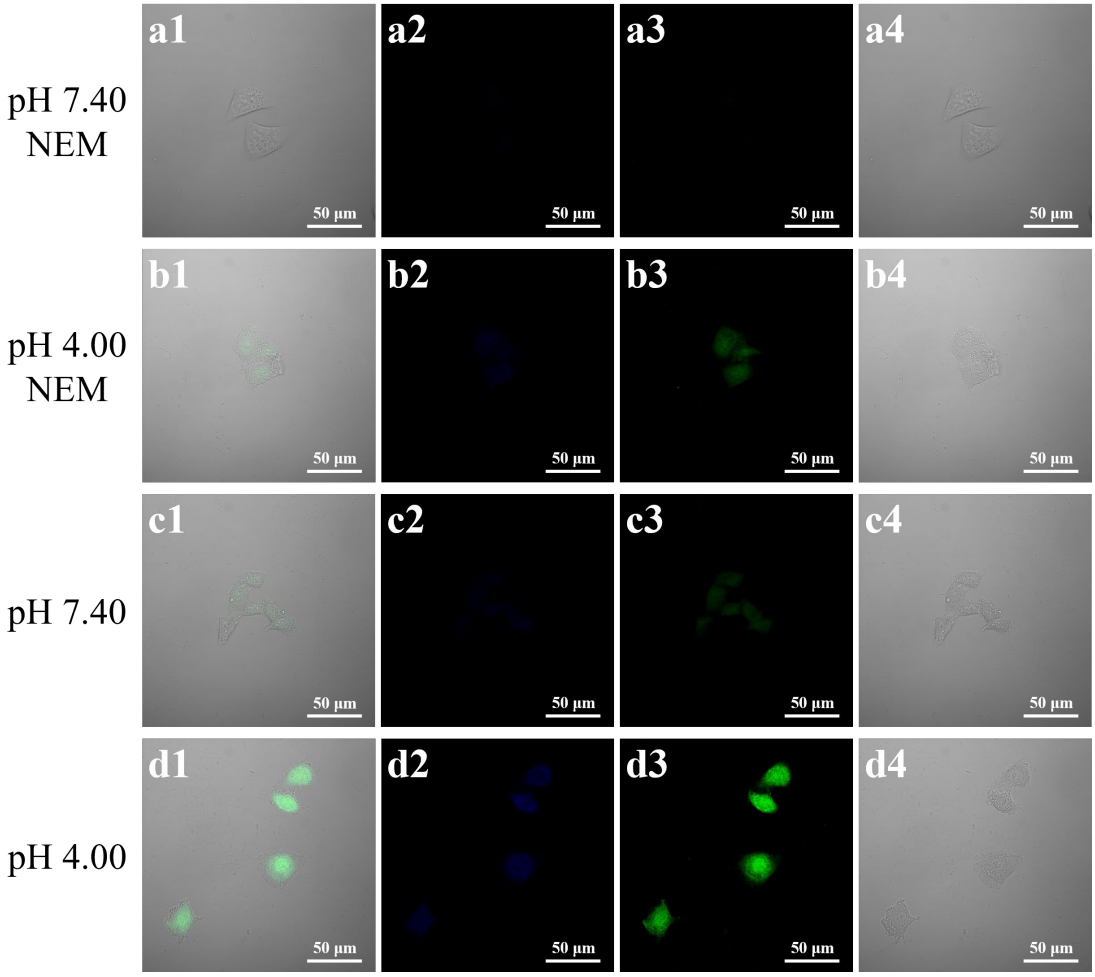


Fig. S21 Confocal fluorescence imaging of HeLa cells stained with 1 μM **TP1**. (a, b) pre-treated with NEM; and the pH of the high K^+ buffer for imaging was 7.40 (a, c) and 4.00 (b, d). (1) Merged of (2), (3) and (4); (2) blue channel; (3) green channel; (4) bright field. For blue and green channel, collecting 425-475 nm under excited with a 405 nm laser, and collecting 500-550 nm as excited with a 488 nm laser, respectively.

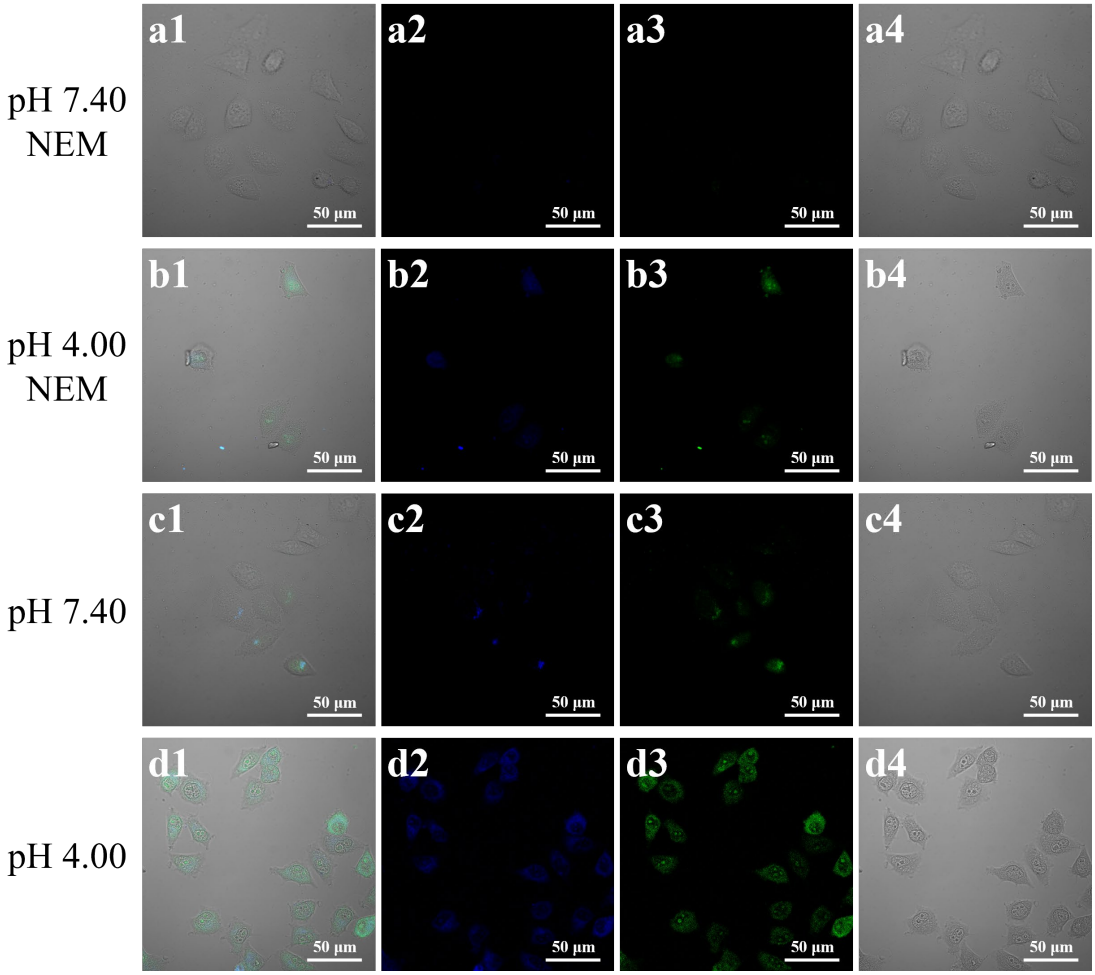


Fig. S22 Confocal fluorescence imaging of HeLa cells stained with 1 μM **TP3**. (a, b) pre-treated with NEM; and the pH of the high K^+ buffer for imaging was 7.40 (a, c) and 4.00 (b, d). (1) Merged of (2), (3) and (4); (2) blue channel; (3) green channel; (4) bright field. For blue and green channel, collecting 425-475 nm under excited with a 405 nm laser, and collecting 500-550 nm as excited with a 488 nm laser, respectively.

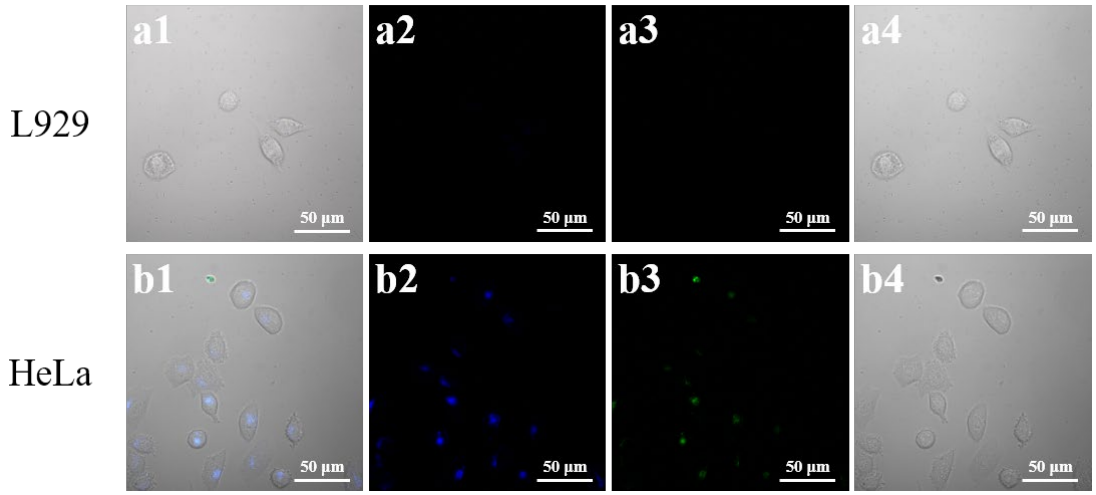


Fig. S23 Confocal fluorescence imaging of L929 (a) and HeLa (b) cells stained with 1 μM **TP4**. (1) Merged of (2), (3) and (4); (2) blue channel; (3) green channel; (4) bright field.