Supporting Information for

A PET-based turn-on fluorescent probe for sensitive detection of thiols and H_2S and its bioimaging application in living cells, tissues and zebrafish

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Instruments

Mass spectra were performed using an LCQ Advantage ion trap mass spectrometer from Thermo Finnigan; High resolution mass spectrometric (HRMS) analyses were measured on a Finnigan MAT 95 XP spectrometer; NMR spectra were recorded on an INOVA-400 spectrometer (400 MHz), using TMS as an internal standard; Electronic absorption spectra were obtained on a LabTech UV Power spectrometer; Photoluminescent spectra were recorded with a HITACHI F4600 fluorescence spectrophotometer; The optical density was measured by a Thermo Scientific Multiskan FC microplate reader in cytotoxicity assay; The fluorescence imaging of cells was performed with Nikon A1MP confocal microscope; The pH measurements were carried out on a Mettler-Toledo Delta 320 pH meter; The melting point was measured by **X-5** micro melting point apparatus. TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300), both of which were obtained from the Qingdao Ocean Chemicals.

Determination of the detection limit

The detection limit was determined from the fluorescence titration data based on a reported method. **NP-S** (10.0 μM) was titrated with different concentrations of Cys/Hcy/GSH/H_2S, the linear relationship between the values of emission intensity at 551 nm and the concentration of Cys/Hcy/GSH/H_2S was fitted based on the fluorescence titration.

$$\text{Detection limit} = 3\sigma/k$$

Where σ is the standard deviation of the blank sample and 'k' is the slope of the linear regression equation.

Fluorescence quantum yields measurement

The relative fluorescence quantum yields were determined with Rhodamine B (F = 0.89) in water as a standard and calculated using the following equation:

$$\Phi_x = \Phi_s \left(\frac{F_x}{F_s}\right)\left(\frac{A_s}{A_x}\right)\left(\frac{\lambda_s}{\lambda_x}\right)\left(\frac{n_x}{n_s}\right)^2$$

Where Φ stands for quantum yield; F represents integrated area under the appropriate emission spectrum; A stands for absorbance at the excitation wavelength; λ is the excitation wavelength; n is the refractive index of the solution (because of the low concentrations of the solutions, the refractive indices of the difference solutions are similar, which can be omitted); and the subscripts x and s refer to test sample and reference substance, respectively.

Fluorescence lifetime measurement

The solutions of **NP-S** (10 μM) and **NP-S** + GSH (10 μM) were prepared in MeCN. The solutions were sonicated for 5 min to eliminate air bubbles. After standing for 1 h at room temperature, the solutions were measured in a fluorescence lifetime measuring equipment (Edinburgh Instruments) with the S4 excitation wavelength at 455 nm and detection at 551nm.

Kinetic Studies. The reaction of probe **NP-S** (5 μM) with Cys/Hcy/GSH/H_2S (300 μM) in aqueous solution (25 mM PBS buffer, pH 7.4, mixed with 20% EtOH) was monitored using the fluorescence intensity at 551 nm. The *pseudo*-first-order rate constant for the reaction was determined by fitting the fluorescence intensities of the samples to the *pseudo* first-order equation:

$$\mathrm{Ln}\,[(F_{max} - F_{t)} / F_{max}] = - kt$$

Where F_t and F_{max} are the fluorescence intensities at 551 nm at time t and the maximum value obtained after the reaction was complete. k is the *pseudo*-first-order rate constant.

Two-photon absorption (TPA) cross sections. Two-photon absorption (TPA) cross sections were measured using the two-photon induced fluorescence method, and the cross section can be calculated by means of equation: [1-3]

$$\delta_x=\delta_s(\Phi_s/\Phi_x)(n_s/n_x)(c_s/c_x)(F_x/F_s)$$

Where the subscripts x and s refer to the test sample and the standard substance, respectively. The terms c and n are the concentration and refractive index of the solution, respectively. F is two-photon excited fluorescence integral intensity. Φ is the fluorescence quantum yield. s is the TPA cross-section of Rhodamine B in EtOH (δ = 95 GM) at 780nm.[4]

Cells culture. HeLa cells were cultured in Dulbecco's Modified Eagle Medium media (DMEM, Hyclone) supplemented with 10 % heat-inactivated fetal bovine serum (FBS, Sijiqing) at 37 °C and 5 % CO_2. Before the imaging experiments, 1 mL of HeLa cells were subcultured and seeded in the glass bottom culture dishes at a density of 1×10^5. About 36 hours later, the cells reached about 75 % confluence for the further

experiments.

Cytotoxicity assay. The cytotoxicity of the **NP-S** was evaluated by the standard MTT assay. The cells were inoculated into a 96-well plate, each well containing approximately 10000 cells. Increasing volumes of **NP-S** in 99 % MEM and 1 % DMSO were added to the cells after one day of culture, and the final concentrations of the probe were 5, 10，25, and 50 μM (five parallel tests). After incubation at a constant temperature for 24 hours, 10 mL of MTT (5 mg/mL in PBS) was added to the cells and incubated for another 4 hours. Then, the culture medium was removed and 100 mL of DMSO was added into each well. The absorbance of each well at 570 nm was measured by a microplate reader (five parallel tests). The cell viability was measured by calculating (OD - OD_{blank})/ ($OD_{control}$ - OD_{blank}) wherein OD, $OD_{control}$ and OD_{blank} denote the optical density of cells in the presence or absence of the probe and the optical density of the culture medium, respectively.

Fluorescence imaging of Cys/Hcy in living cells

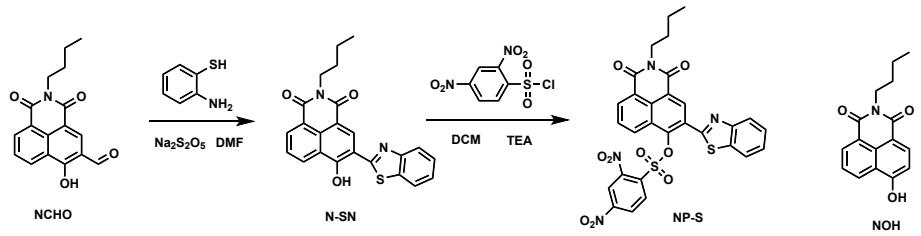
About the cell imaging experiments, as the control group, the one is HeLa cells were incubated with **NP-S** (5 μM) for 40 min, then washed by PBS buffer before imaging. The other one is HeLa cells were preincubated with 500 μM N-Ethylmaleimide (NEM) for 40 min, and incubated with **NP-S** (5 μM) for 40 min, then washed by PBS buffer before imaging. As the experimental groups, HeLa cells were preincubated with 500 μM N-Ethylmaleimide (NEM) for 40 min, followed by treatment with 500 μM Cys or Hcy for 40 min, respectively, and incubated with **NP-S** (5 μM) for 40 min, and then washed by PBS buffer before imaging. The confocal microscopic imaging uses Nikon A1MP confocal microscope with an excitation filter of 405 nm and the collection wavelength range is from 500-550 nm (green channel). The images were obtained by the images of green channel.

Fluorescence imaging of Cys/Hcy/H_2S in zebrafish

All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Shandong University and approved by the Animal Ethics Committee of Shandong University. About the zebrafish imaging experiments, as the control group, the one is zebrafish was incubated with **NP-S** (10 μM) for 40 min, then washed by PBS buffer before imaging. The other one is zebrafish was preincubated with 500 μM N-Ethylmaleimide (NEM) for 40 min, and incubated with **NP-S** (10 μM) for 40 min, then washed by PBS buffer before imaging. As the experimental groups, zebrafish was preincubated with 500 μM N-Ethylmaleimide (NEM) for 40 min, followed by treatment with 500 μM Cys, Hcy or H_2S for 40 min, respectively, and incubated with **NP-S** (10 μM) for 40 min, and then washed by PBS buffer before

imaging. The confocal microscopic imaging uses Nikon A1MP confocal microscope with an excitation filter of 405 nm and the collection wavelength range is from 500-550 nm (green channel). The images were obtained by the images of green channel.

Synthesis. The chemosensor **NP-S** was synthesized as following scheme S1. The compound **NOH** and **NCHO** were prepared via previous methods.[14]



Scheme S1. Synthesis of the chemosensor **N-PS** and the structure of compounds **NOH** and **NCHO** and **N-SN**.

Synthesis of N-SN. The compound **NCHO** (297 mg, 1 mmol), 2-aminothiophenol (150 mg, 1.2 mmol) and $Na_2S_2O_5$ (380 mg, 2 mmol) were added to a 100 mL round-bottom flask. The mixture was dissolved in 10 mL DMF. Then, the mixture was refluxed at 140 ºC for 2 h. The mixture was cooled to rt, and addition with water then filtered to remove solvent. And product **N-SN** (118 mg) was obtained as a yellow solid in 46 % yield.

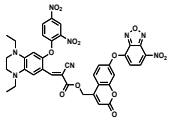
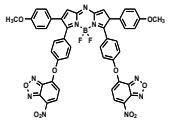
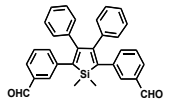
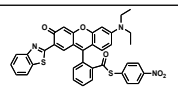
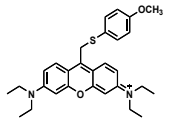
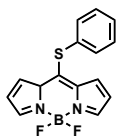
Compound **NP-S** was also prepared by the reaction between probe **NPS** and GSH. The compound **NP-S** (63.2 mg, 0.1 mmol), glutathione (92.1 mg, 0.3 mmol) were added to a 25 mL round-bottom flask. The mixture was dissolved in 3 mL ethanol. Then, the mixture was stirred at rt for 6 h. After solvent evaporation, the crude product was purified by silica gel column chromatography using CH_2Cl_2/PE (v/v 3:1) and product **N-SN** (26 mg) was obtained as a yellow solid in 64 % yield.

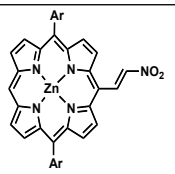
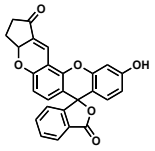
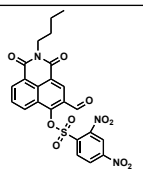
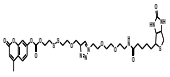
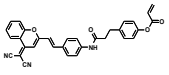
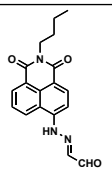
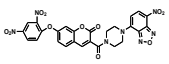
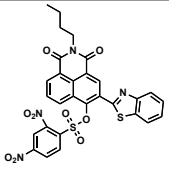
Melting point: 206℃. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 1.02 (t, J = 7.2 Hz, 3H), 1.49 (q, J = 7.6 Hz, 2H), 1.75 (m, 2H), 4.19 (t, J = 8.0 Hz, 2H), 7.47 (t, J = 8.0 Hz, 1H), 7.55 (t, J = 8.4 Hz, 1H), 7.76 (t, J = 7.6 Hz, 1H), 7.98 (dd, J_1 = 14.0 Hz, J_2 = 8.4 Hz, 2H), 8.63 (d, J = 8.4 Hz, 1H), 8.75 (d, J = 8.4 Hz, 1H), 8.78 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 13.87, 20.42, 29.70, 30.26, 40.19, 111.49, 114.01, 120.82, 121.84, 122.53, 123.35, 125.96, 126.39, 127.11, 130.05, 130.89, 132.28, 132.83, 150.88, 160.81, 163.37, 163.91, 168.54. HRMS (ESI) m/z calcd for $C_{23}H_{18}N_2O_3S$ $[M + H]^+$: 403.1111. Found 403.1107.

Synthesis of NP-S. The compound **N-SN** (100.5 mg, 0.25 mmol), 2, 4-

dinitrobenzenesulfonyl chloride (73 mg, 0.275 mmol) and triethylamine (28 mg, 0.275 mmol) were added to a 50 mL round-bottom flask. The mixture was dissolved in 5 mL dichloromethane. Then, the mixture was stirred at rt for 6h. After solvent evaporation, the crude product was purified by silica gel column chromatography using CH_2Cl_2/PE (v/v 3:1) and product **NP-S** (71 mg) was obtained as a faint yellow solid in 68 % yield. Melting point: 184 ℃. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 1.02 (t, *J* = 7.2 Hz, 3H), 1.49 (q, *J* = 7.6 Hz, 2H), 1.76 (m, 2H), 4.24 (t, *J* = 7.6 Hz, 2H), 7.40 (t, *J* = 8.4 Hz, 1H), 7.48 (t, *J* = 7.6 Hz, 1H), 7.74 (d, *J* = 8.4 Hz, 1H), 7.82 (m, 3H), 8.05 (t, *J* = 7.6 Hz, 1H), 8.40 (d, *J* = 2.4 Hz, 1H), 8.79 (dd, J_1 = 13.6 Hz, J_2 = 7.2 Hz, 2H), 8.91 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 6.53, 15.88, 16.34, 26.77, 106.07, 107.74, 19.13, 109.25, 109.92, 111.88, 112.27, 112.97, 113.26, 113.71, 115.58, 115.65, 115.89, 118.07, 118.54, 119.48, 120.72, 122.01, 133.46, 135.64, 139.07, 147.33, 148.66, 149.48. HRMS (ESI) m/z calcd for $C_{29}H_{21}N_4O_9S_2$ [M + H] $^+$: 633.0705; Found 633.0750.

Table S1. Summary of the properties of representative fluorescent probes for detecting thiols and H_2S.

Probes	Chemical structures	Emission wavelength/nm	Enhancement of fluorescence signal	Limit of detection/M	Response time/min	Action TP absorption cross section (GM)	Ref
NCQ		490/544 for Cys Hcy 490 for GSH H_2S	Cys/Hcy /GSH: ~8-fold; H_2S: 10-fold	Cys: 5.7×10^{-7} Hcy: 6.5×10^{-7} GSH: 4.9×10^{-7} H_2S: 5.2×10^{-7}	~10-15	Not mentioned	5
1		540 for Cys/Hcy 730 for GSH	Cys/Hcy: ~30-fold GSH: 28-fold	Cys: 8×10^{-8} Hcy: 1.7×10^{-7} GSH: 5×10^{-8}	~15-25	Not mentioned	6
DMBFDPS		424 for Cys/Hcy 484 for GSH	Cys: 13-fold Hcy:2.4-fold	Not mentioned	~120	Not mentioned	7
1		454 for Cys/Hcy 587 for GSH	Cys:222-fold Hcy:215-fold	Cys: 4.4×10^{-8} Hcy: 5.2×10^{-8} GSH: 9.6×10^{-8}	~10-20	Not mentioned	8
4F-2CN		500 for Cys 450 for Hcy/GSH	Cys:400-fold Hcy:29-fold GSH:26-fold	Cys: 2×10^{-8} Hcy: 2.27×10^{-6} GSH: 2.5×10^{-5}	~120	Not mentioned	9
1		546 for Cys/Hcy 622 for GSH	Cys: 90-fold Hcy:135-fold GSH: 63-fold	Not mentioned	~2-10	Not mentioned	10
1		467 for Cys/Hcy 535 for GSH/H2S	GSH: 500-fold; H_2S: 5-fold	GSH: 1×10^{-8}	~10-30	Not mentioned	11

XQ1		631 for Cys/Hcy /GSH	Cys:23-fold Hcy:23-fold GSH:18-fold	Cys: 6.5× 10^{-7} Hcy: 6.8× 10^{-7} GSH: 1.1× 10^{-6}	~5-10	Not mentioned	12
1		520 for Cys/Hcy /GSH	Cys:85-fold Hcy:44-fold GSH:61-fold	Cys: 5× 10^{-8} Hcy: 5.3× 10^{-8} GSH: 1× 10^{-7}	~10	Not mentioned	13
NQNO		525 for Cys/Hcy /GSH	Cys:5.5-fold Hcy:6.3-fold GSH:10-fold	GSH: 8.59× 10^{-8}	~25	Not mentioned	14
6		450 for Cys/Hcy /GSH	Cys: 5-fold Hcy: 5-fold GSH: 5-fold	Not mentioned	~15	Not mentioned	15
DCM-Cys		640 for Cys	Cys: 20-fold	Cys: 2.1× 10^{-8}	~15	Not mentioned	16
1		524 for Cys/Hcy	Cys: 52-fold Hcy: 65-fold	Not mentioned	60	Not mentioned	17
1		455 for H_2S	H_2S: 2000-fold	2.8 × 10^{-5}	60	Not mentioned	18
NP-S		551 for Cys/Hcy /GSH/H_2S	Cys:13-fold Hcy:8.3-fold GSH:23-fold H_2S:21-fold	Cys: 4.2× 10^{-7} Hcy: 5.2× 10^{-7} GSH:3.7× 10^{-7} H_2S:2.2×10^{-7}	2.5	80.3	This work

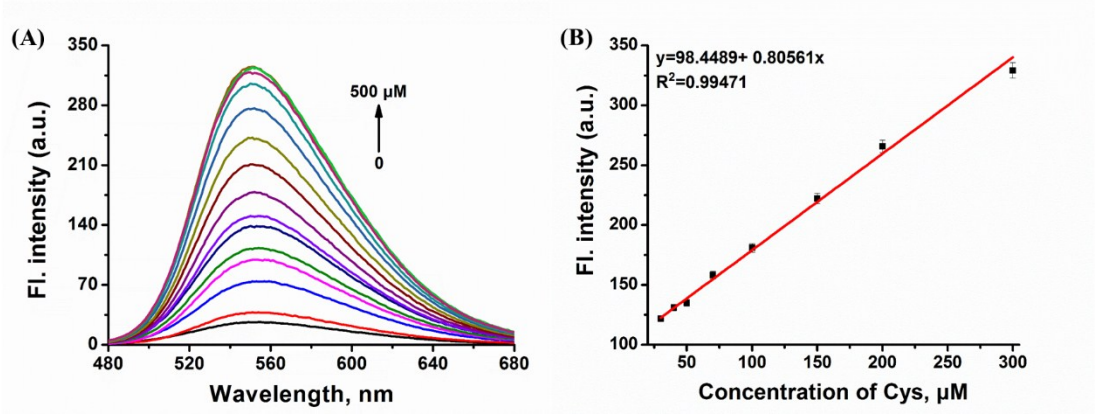


Fig. S1 (A) Emission spectra of **NP-S** (10 μM) with Cys (0-500μM) in aqueous solution (25 mM PBS buffer, pH 7.4, mixed with 20 % EtOH). (B) The linear relationship between the values of fluorescence intensity (I_{551}) and the concentration of Cys (30-300 μM).

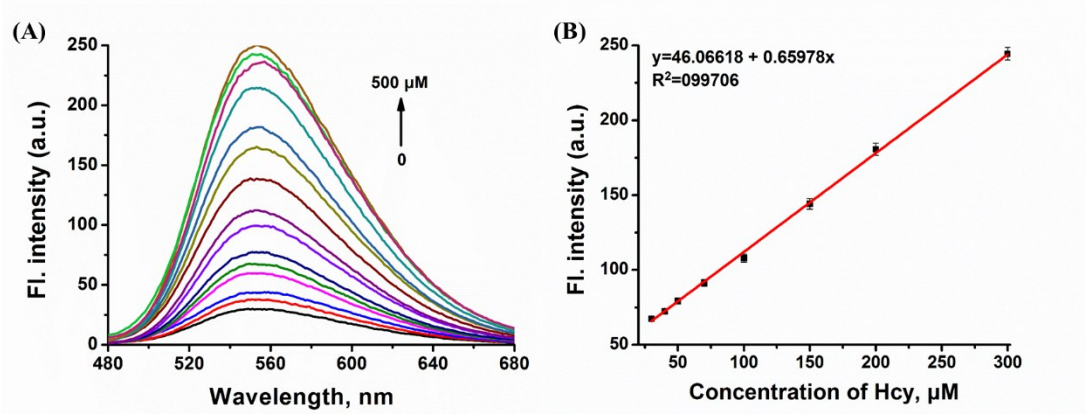


Fig. S2 (A) Emission spectra of NP-S (10 μM) with Hcy (0-500 μM) in aqueous solution (25 mM PBS buffer, pH 7.4, mixed with 20 % EtOH). (B) The linear relationship between the values of fluorescence intensity (I_{551}) and the concentration of Hcy (30-300 μM).

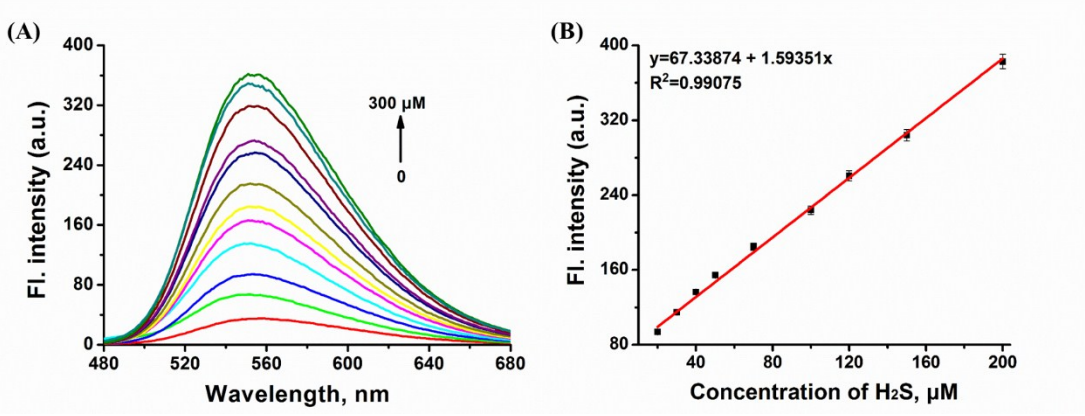


Fig. S3 (A) Emission spectra of **NP-S** (10 μM) with H_2S (0-300μM) in aqueous solution (25 mM PBS buffer, pH 7.4, mixed with 20 % EtOH). (B) The linear relationship between the values of fluorescence intensity (I_{551}) and the concentration of H_2S (20-200 μM).

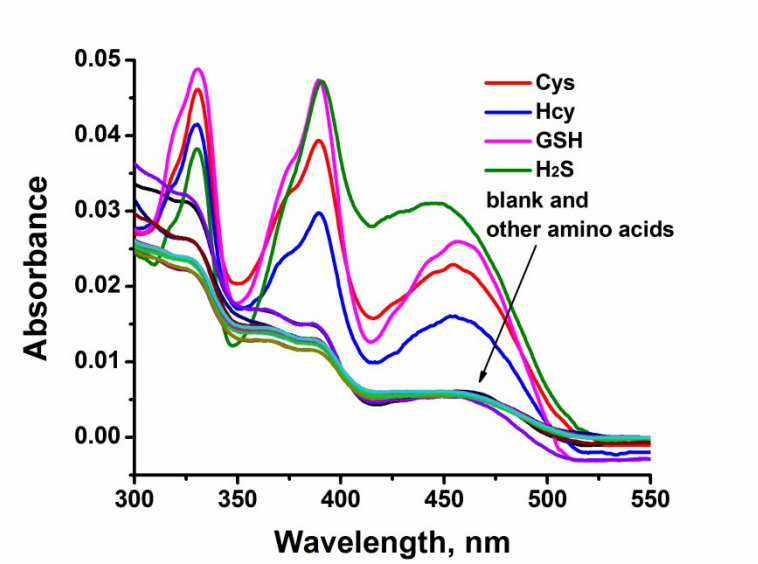


Fig. S4 UV/Vis absorption spectra of **NP-S** (10 mM) in PBS buffer (25 mM, pH 7.4, 20 % EtOH) in the presence of 100 mM analytes including Cys, Hcy, GSH, H_2S, Ala, Arg, Asp, Glu, His, Ser, Thr, Val, Ile, Phe and Trp.

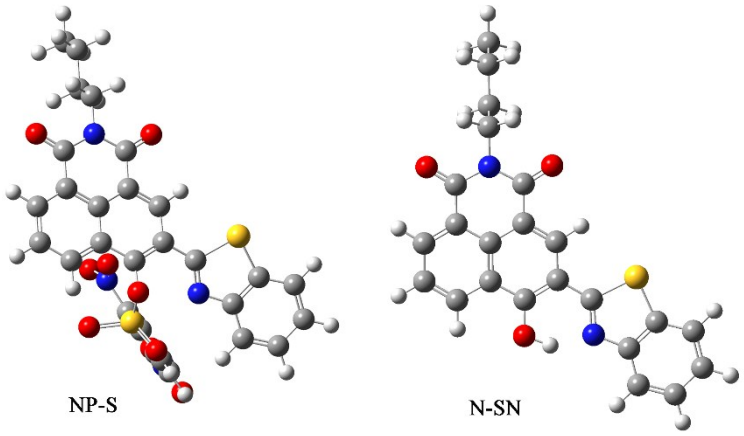


Fig. S5 The optimized chemical structures of probe NP-S and compound N-SN by DFT method (B3LYP/6-31G(d)).

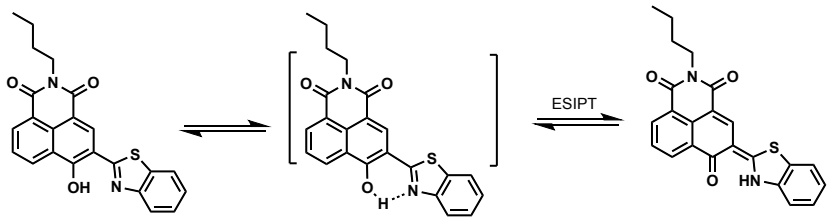


Fig. S6 The convertible enol and ketone form of compound **N-SN** regulated by excited-state intramolecular proton transfer (ESIPT) process.

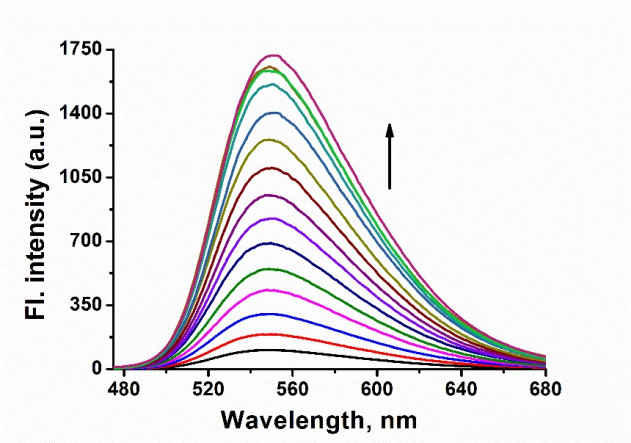


Fig. S7 Emission spectra of **NP-S** (10 μM) with GSH (0-300 μM) in MeCN solvent. Excited at 455 nm.

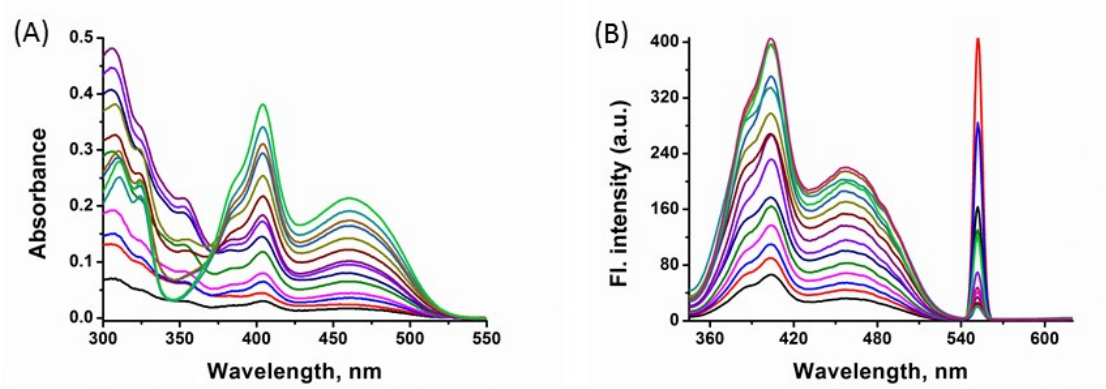


Fig. S8 (A) Absorption spectra of **NP-S** (10 μM) with GSH (0-300 μM) in MeCN solvent. (B) Excitation spectra of **NP-S** (10 μM) with GSH (0-300 μM) in MeCN solvent. Excited at 551 nm.

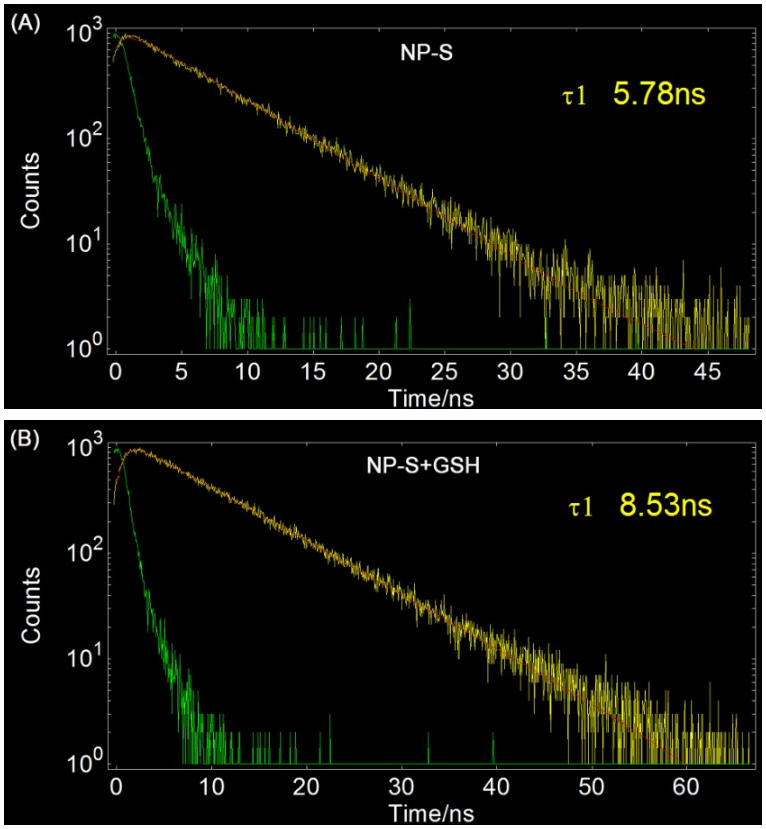


Fig S9 The fluorescence decay of the probes (A) **NP-S** (10 μM) and (B) **NP-S** + GSH (10 μM) in the pure MeCN.

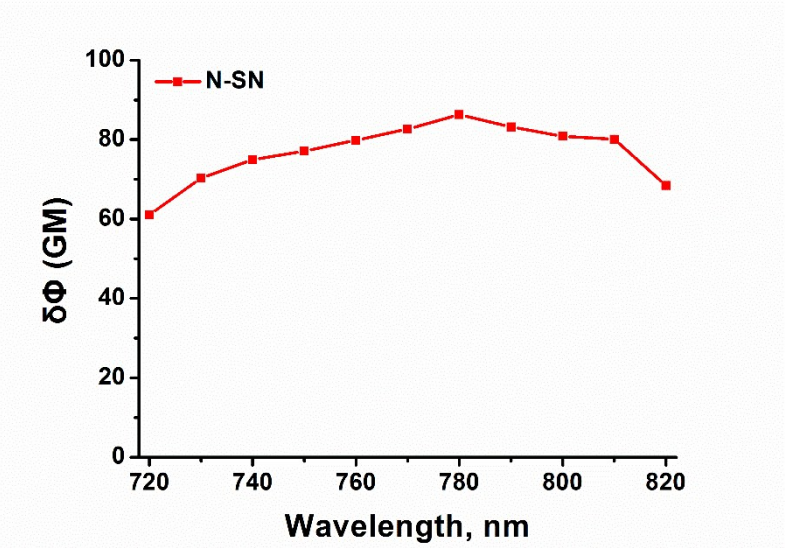


Fig. S10 TP absorption cross-section of **N-SN** (10 μM) in buffered (25 mM PBS, pH = 7.4) 20% EtOH aqueous solution (v/v); Rhodamine B as the references standard, the excitation wavelength was 780 nm.

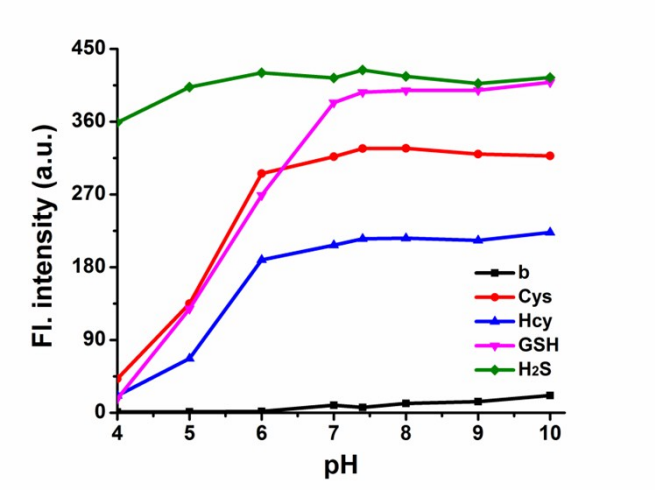


Fig. S11 The pH influence on the fluorescence intensity (I_{551nm}) of **NP-S** (10 μM) in the absence or presence of thiols (300 μM) and H_2S (200 μM).

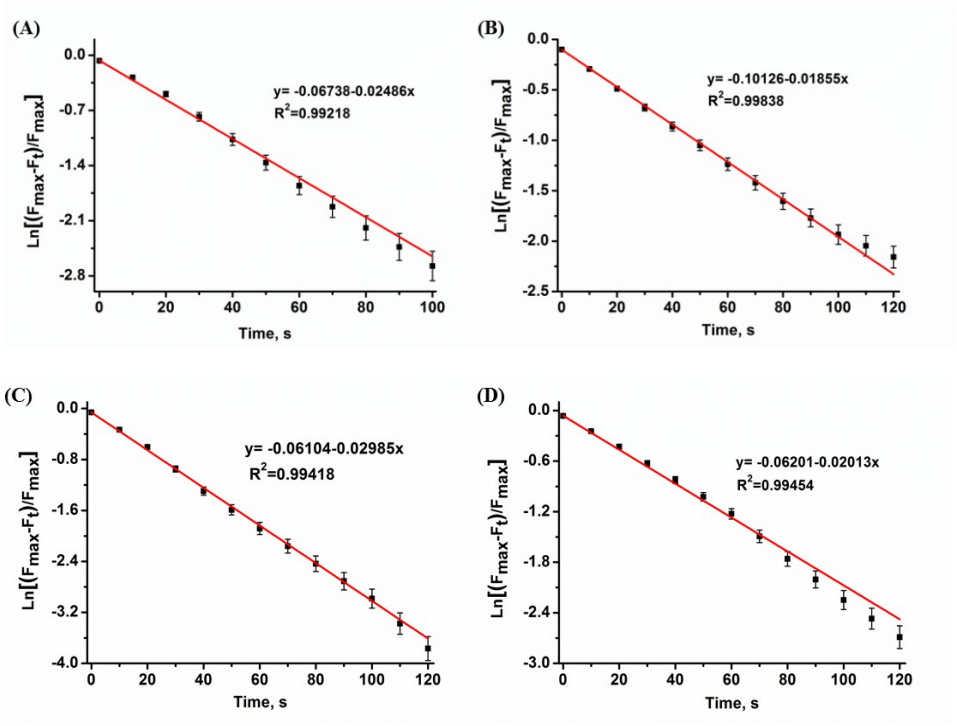


Fig. S12 *Pseudo* first-order kinetic plot of the reaction of **NP-S** (10 μM) with (A) Cys (300 μM), (B) Hcy (300 μM), (C) GSH (300 μM) and (D) H_2S (200 μM) in aqueous solution (25 mM PBS buffer, pH 7.4, mixed with 20% EtOH). Slope = 0.02486 s^{-1} (Cys), 0.01855 s^{-1} (Hcy), 0.02985 s^{-1} (GSH) and 0.02013 s^{-1} (H_2S).

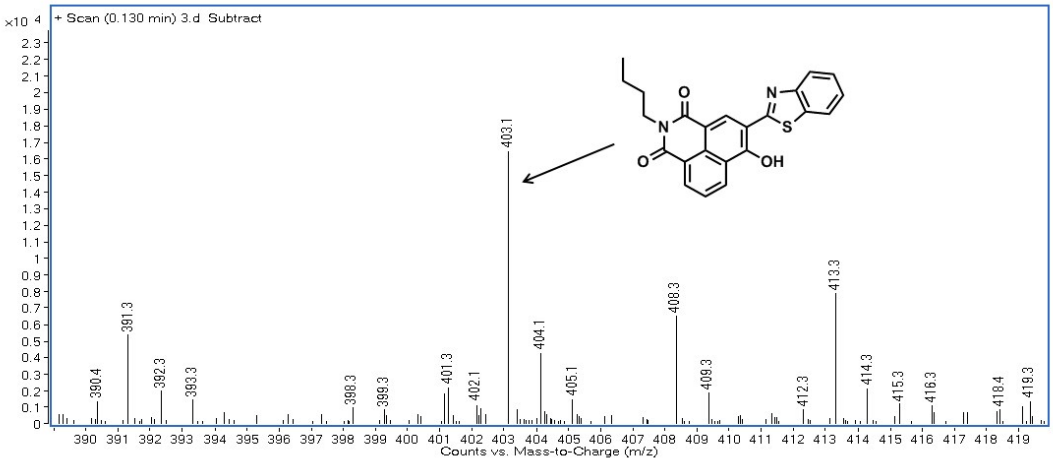


Fig. S13 Mass spectra (ESI) of **NP-S** in the presence of GSH in aqueous solution. Compound **N-SN**: $C_{23}H_{18}N_2O_3S$; HRMS (ESI) m/z calcd for $C_{23}H_{18}N_2O_3S$ $[M + H]^+$: 403.1. Found 403.1.

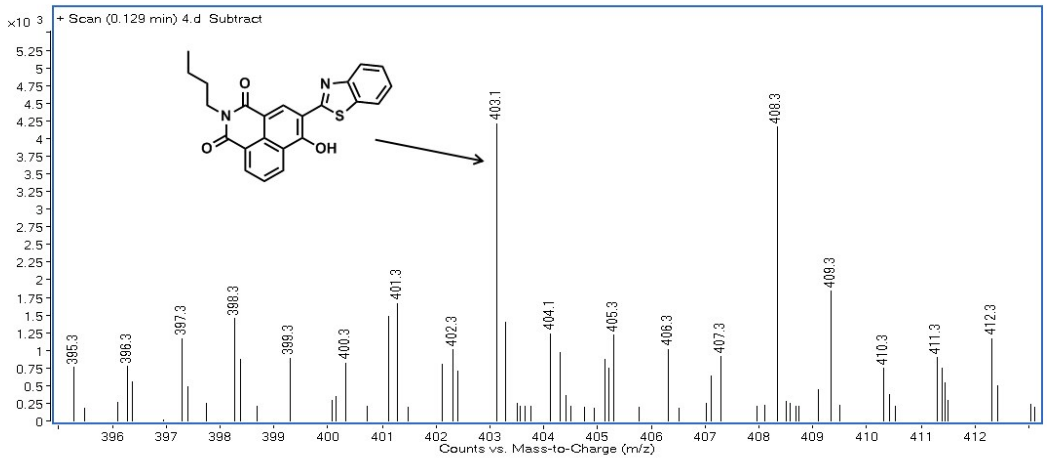


Fig. S14 Mass spectra (ESI) of **NP-S** in the presence of H_2S in aqueous solution. Compound **N-SN**: $C_{23}H_{18}N_2O_3S$; HRMS (ESI) m/z calcd for $C_{23}H_{18}N_2O_3S$ $[M + H]^+$: 403.1. Found 403.1.

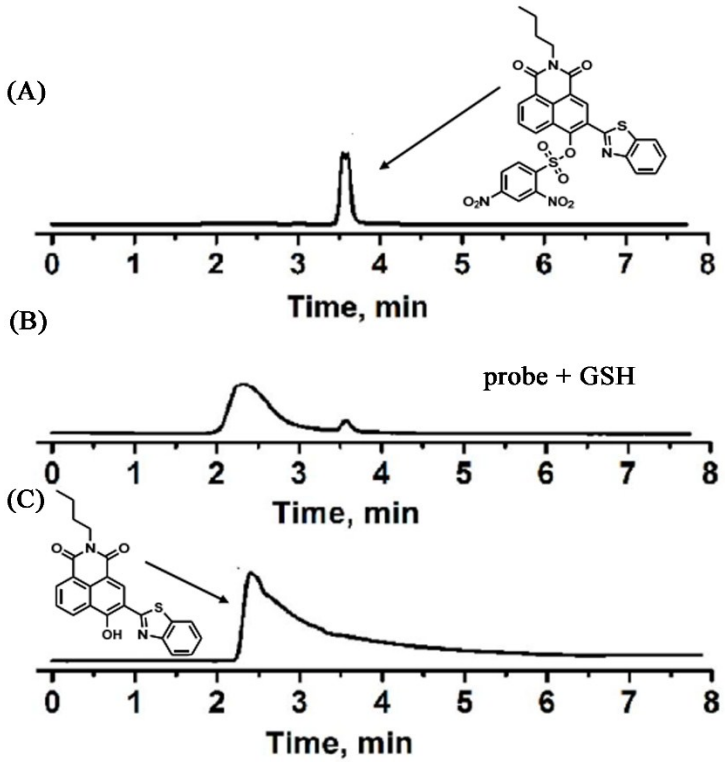


Fig. S15 Reversed-phase HPLC with absorption (455 nm) detection. (A) Reversed-phase HPLC chromatograms of 10 μM probe **NP-S**; (B) Reversed-phase HPLC chromatograms of 10 μM probe **NP-S** in the presence of 1.5 mM GSH; (C) Reversed-phase HPLC chromatograms of 10 μM compound **N-SN**. Eluant conditions: ethanol.

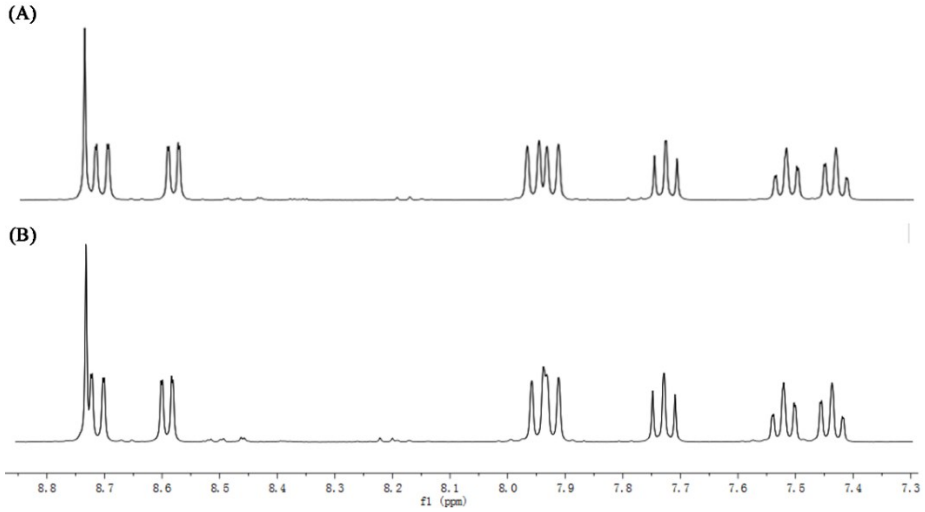


Fig. S16 The partial ^{1}H NMR spectra of compound **N-SN** synthesized by compound **NCHO** (A) and isolated from reaction between probe **NP-S** and GSH (B) in $CDCl_3$.

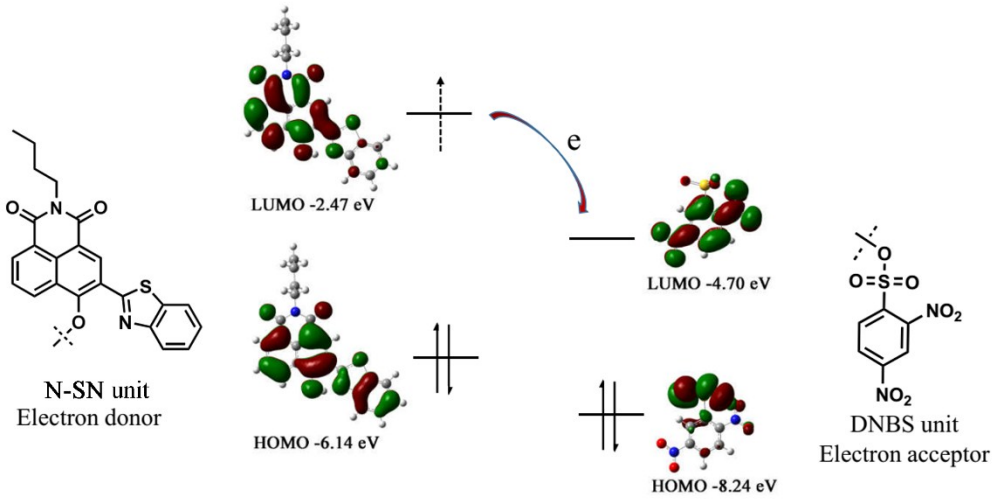


Figure S17 Frontier orbital energy representation of the PET processes in probe **NP-S**. Left: N-SN unit as electron donor; Right: DNBS unit as electron acceptor. Calculations were performed with the DFT method [B3LYP/6-31G(d)] using Gaussian 09 program.

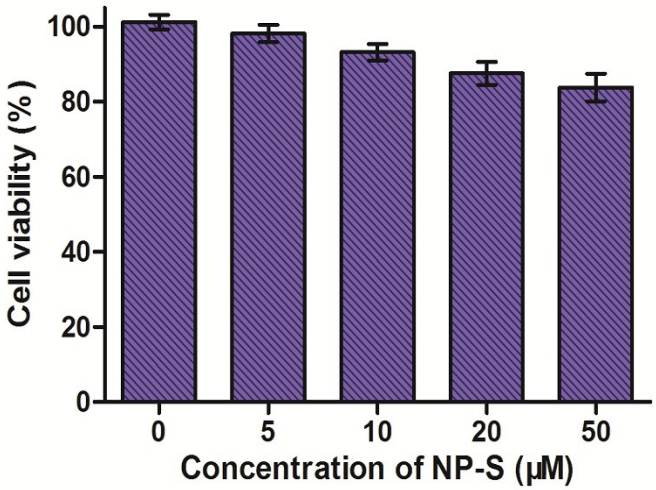


Fig. S18 Cell viability of HeLa cells incubated with probe **NP-S** of different concentration (0, 5, 10, 20, or 50 μM) for 24 h.

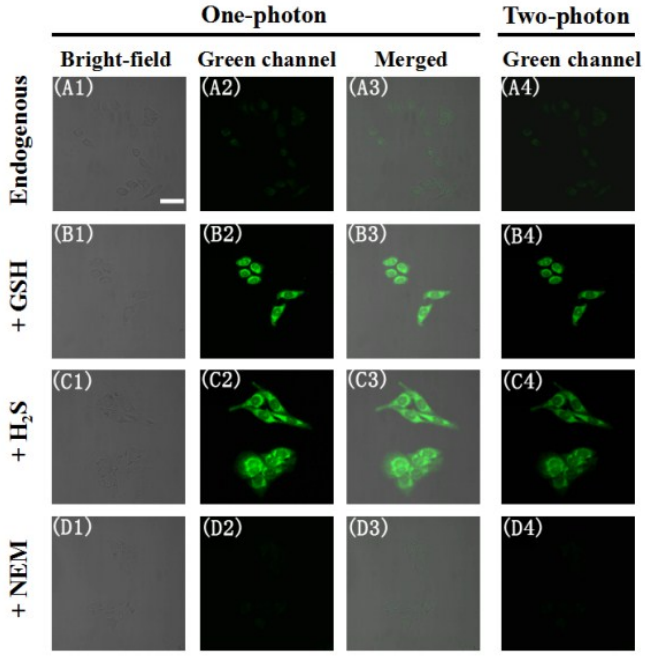


Fig. 19 Confocal fluorescence images of probe **NP-S** responding to GSH and H_2S in living cells under one or two photon conditions. (A) HeLa cells incubated with 5 μM probe **NP-S** for 40 min, (B) HeLa cells preincubated with 500 μM NEM for 40 min and followed by treatment with 500 μM GSH for 40 min, then incubated with 5 μM probe **NP-S** for another 40 min, (C) HeLa cells preincubated with 500 μM NEM for 40 min and followed by treatment with 500 μM H_2S for 40 min, then incubated with 5 μM probe **NP-S** for another 40 min, (D) HeLa cells preincubated with 500 μM NEM for 40 min and then treatment with 5 μM probe **NP-S** for 40 min. Excitation: 405 nm. Scale bar: 25 μm.

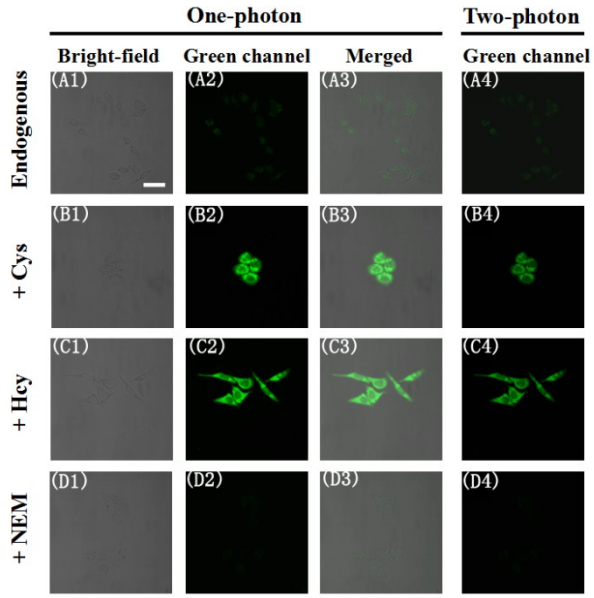


Fig. S20 Confocal fluorescence images of probe **NP-S** responding to endogenous or exogenous thiols in living cells under one or two photon. (A) HeLa cells incubated with probe **NP-S** (5 μM) for 30 min, (B) HeLa cells preincubated with NEM (500 μM) for 30 min and followed by treatment with Cys (500 μM) for 40 min, then incubated with probe **NP-S** (5 μM) for another 30 min, (C) HeLa cells preincubated with NEM (500 μM) for 30 min and followed by treatment with Hcy (500 μM) for 40 min, then incubated with probe **NP-S** (5 μM) for another 30 min, (D) HeLa cells preincubated with NEM (500 μM) for 30 min and then treatment with probe **NP-S** (5 μM) for 30 min. Excitation: 405 nm. Scale bar: 25 μm.

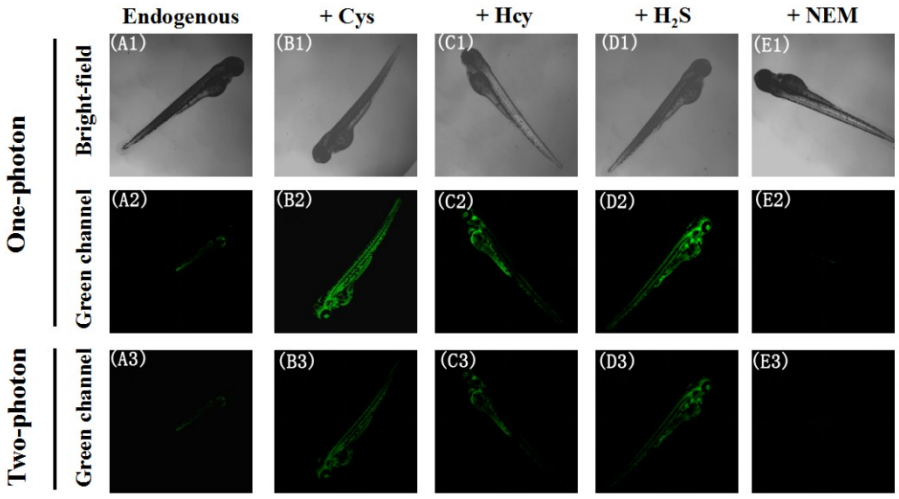


Fig. S21 Confocal fluorescence images of probe **NP-S** responding to thiols or H_2S in zebrafish under one or two photon. (A) zebrafish incubated with probe **NP-S** (10 μM) for 30 min, (B) zebrafish preincubated with NEM (500 μM) for 30 min and followed by treatment with Cys (500 μM) for 40 min, then incubated with probe **NP-S** (10 μM) for another 30 min, (C) zebrafish preincubated with NEM (500 μM) for 30 min and followed by treatment with Hcy (500 μM) for 40 min, then incubated with probe **NP-S** (10 μM) for another 30 min, (D) zebrafish preincubated with NEM (500 μM) for 30 min and followed by treatment with H_2S (500 μM) for 40 min, then incubated with probe **NP-S** (10 μM) for another 30 min, (E) zebrafish preincubated with NEM (500 μM) for 30 min and then treatment with probe **NP-S** (10 μM) for 30 min. Excitation: 405 nm. Scale bar: 25 μm.

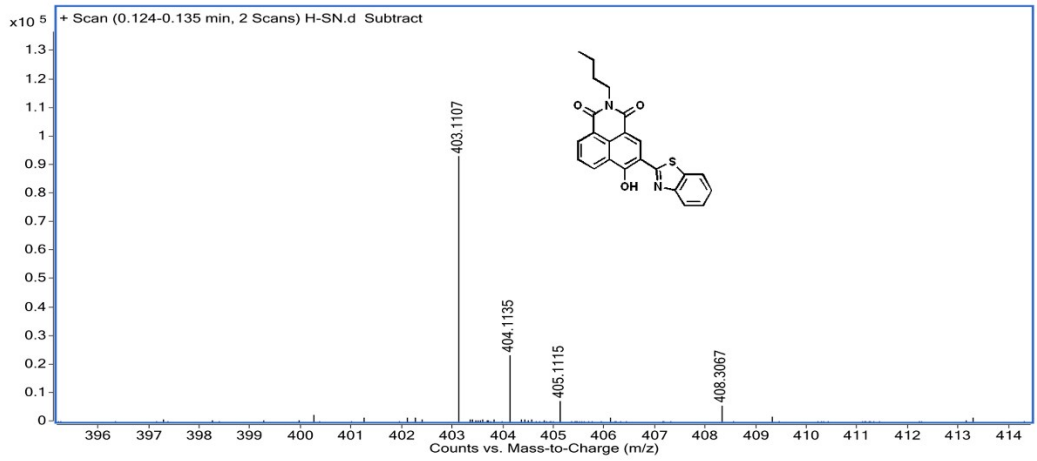


Fig. S22 Mass spectra (ESI) of compound **N-SN**. Compound **NP-S**: $C_{23}H_{18}N_2O_3S$; HRMS (ESI) m/z calcd for $C_{29}H_{21}N_4O_9S_2$ $[M + H]^+$: 403.1111. Found 403.1107.

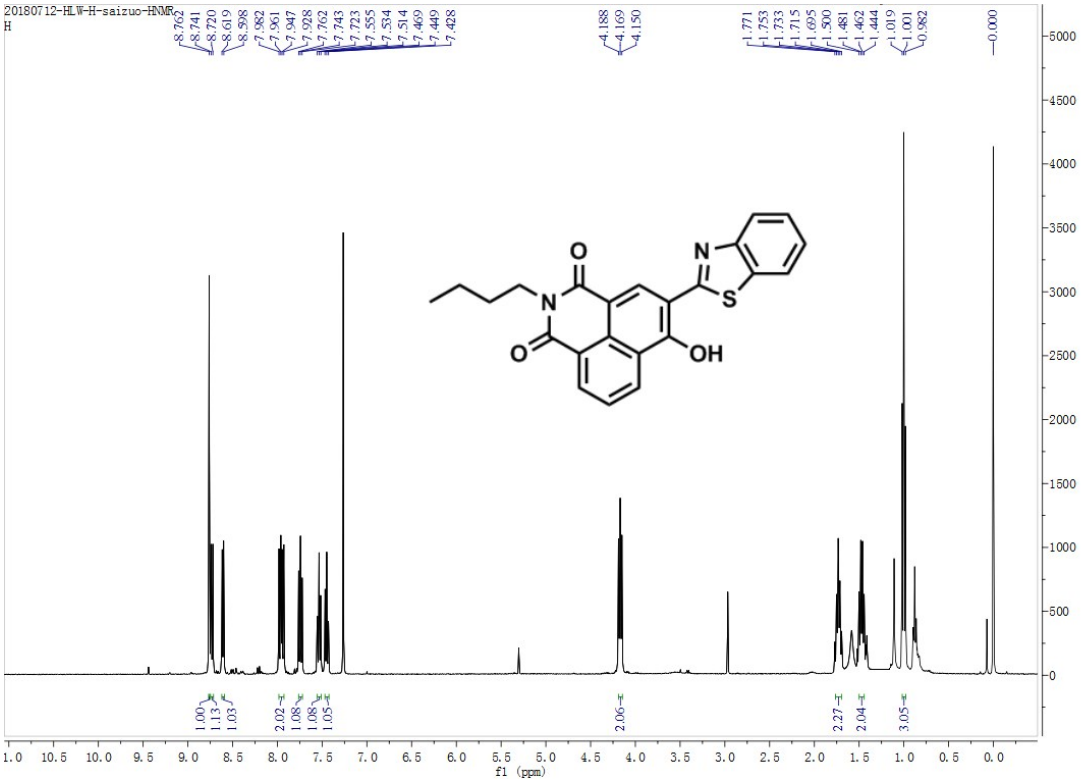


Fig. S23 ^{1}H NMR spectrum of compound **N-SN** in $CDCl_3$.

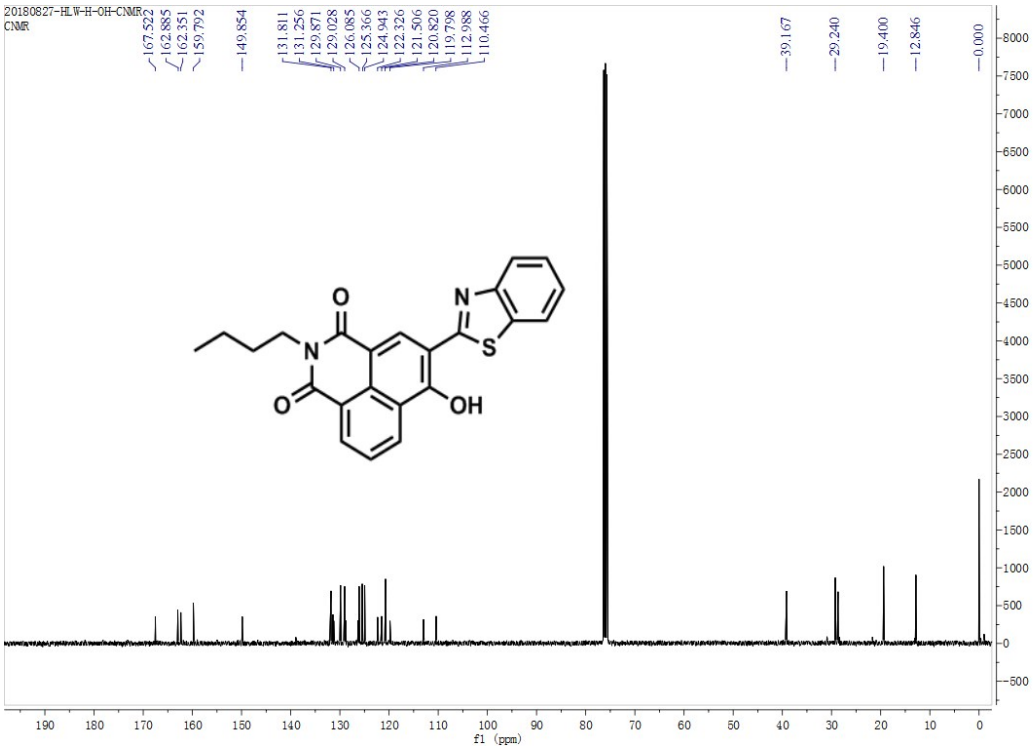


Fig. S24 ^{13}C NMR spectrum of compound **N-SN** in $CDCl_3$.

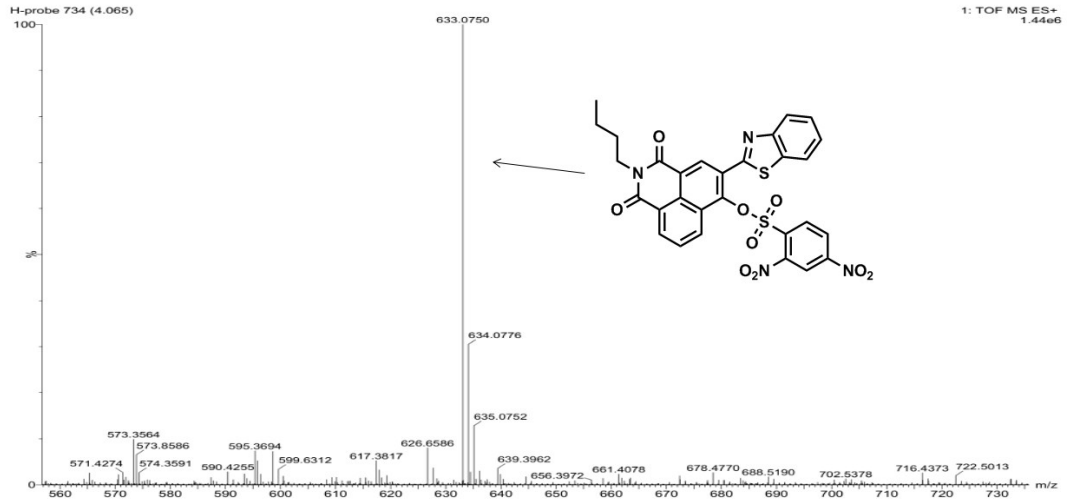


Fig. S25 Mass spectra (ESI) of **NP-S**. Compound **NP-S**: $C_{29}H_{21}N_4O_9S_2$; HRMS (ESI) m/z calcd for $C_{29}H_{21}N_4O_9S_2$ $[M + H]^+$: 633.0705. Found 633.0750.

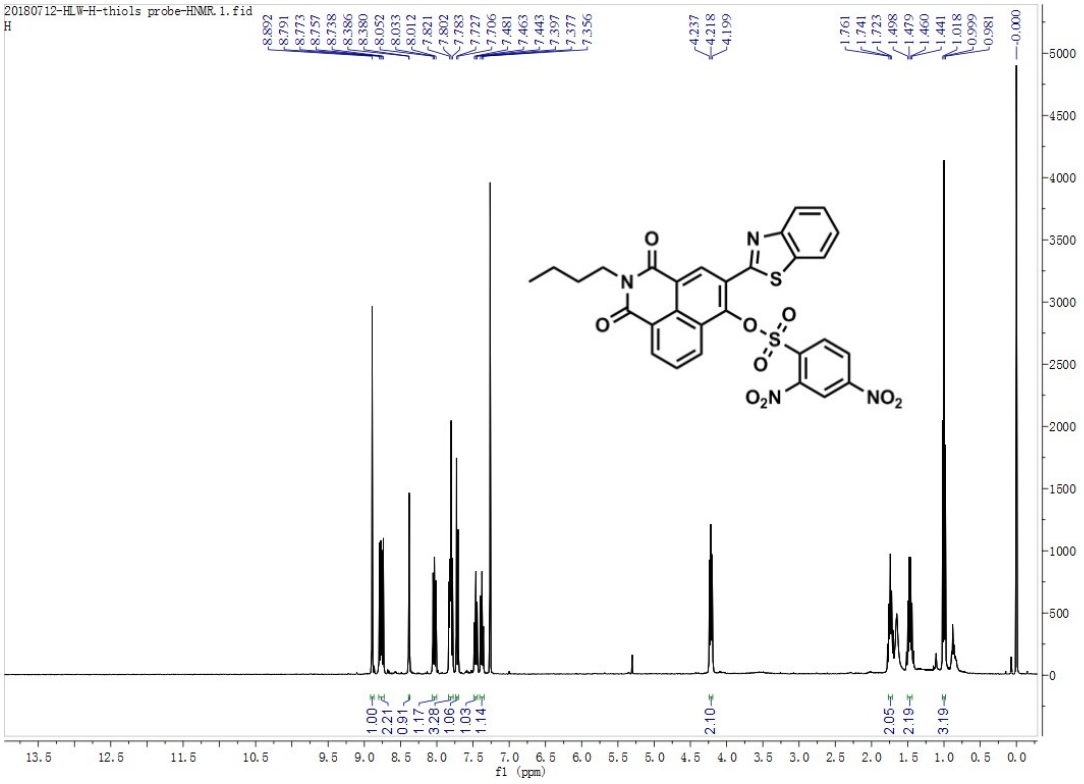


Fig. S26 ^{1}H NMR spectrum of compound **NP-S** in $CDCl_3$.

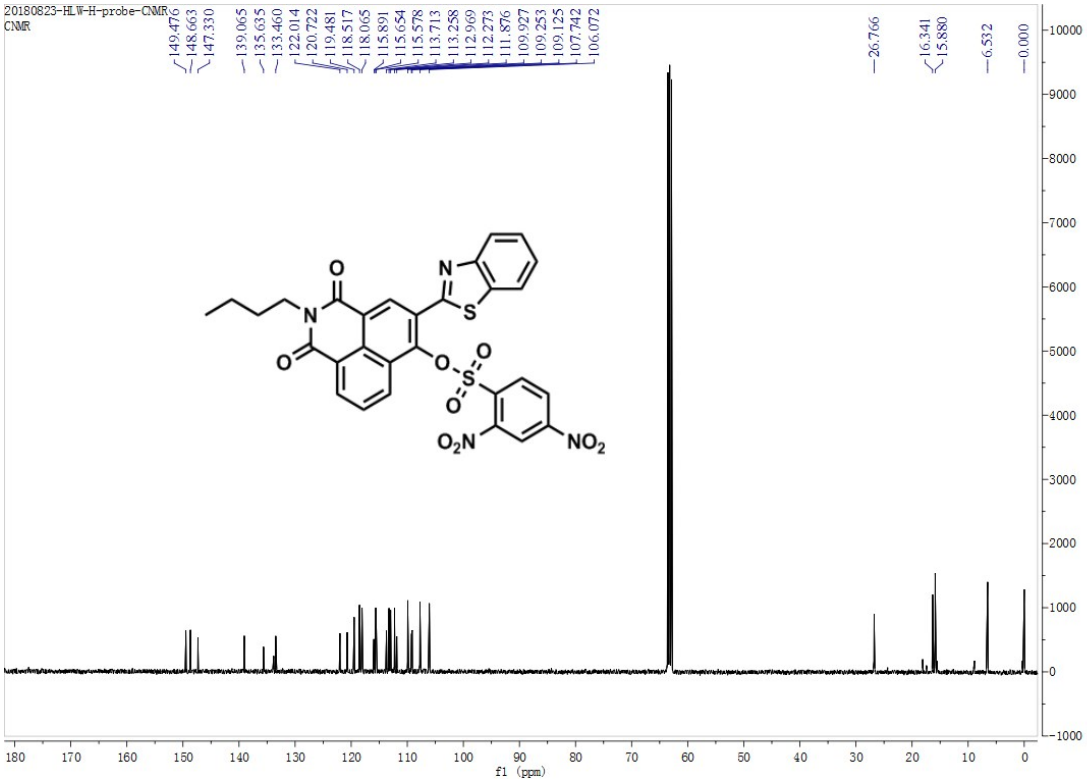


Fig. S27 ^{13}C NMR spectrum of compound **NP-S** in $CDCl_3$.

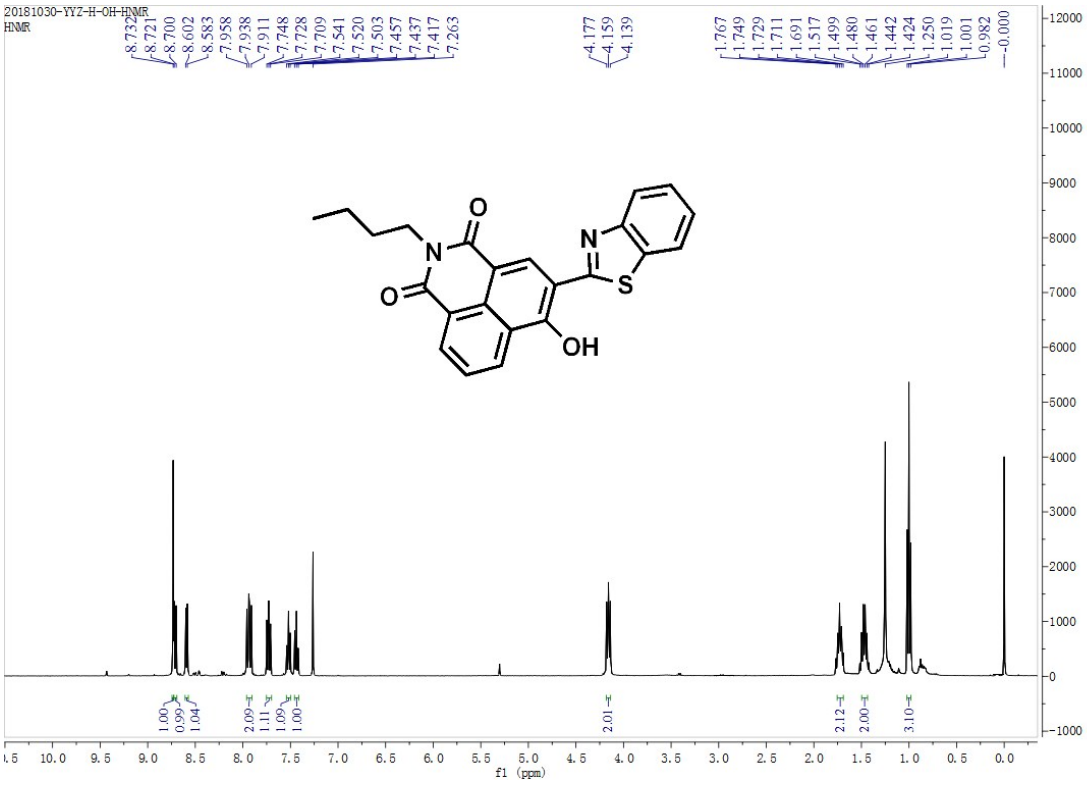


Fig. S28 ^{1}H NMR spectrum of the nucleophilic substitution product **N-SN** in $CDCl_3$.

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