Supporting Information

A multifunctional logic gate based on a triple-chromophore fluorescent biothiols probe with diverse fluorescence signal patterns

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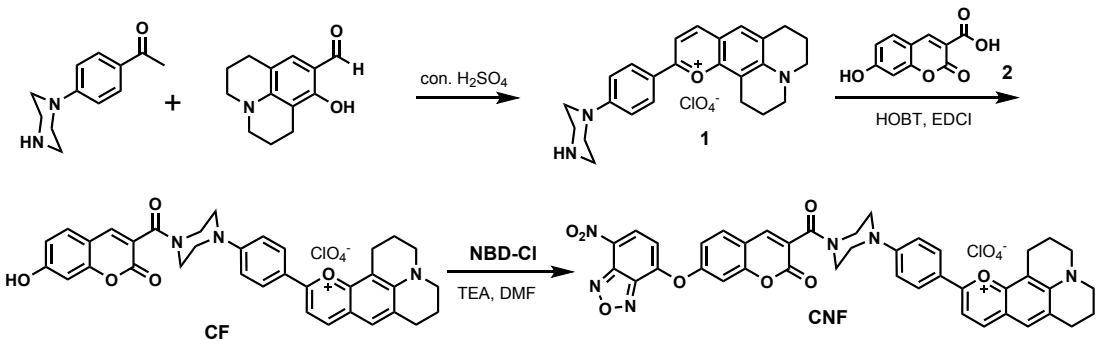
Table of contents

Page

Materials and instruments: Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. Twice-distilled water was used throughout all experiments; Mass spectrometric analyses were measured on a Finnigan MAT 95 XP spectrometer; High resolution mass spectrometric (HRMS) analyses were measured on an Agilent 1100 HPLC/MSD spectrometer; NMR spectra were recorded on an AVANCE III 400 MHz Digital NMR Spectrometer, using TMS as an internal standard; Electronic absorption spectra were obtained on a Shimadzu UV-2700 power spectrometer; Photoluminescent spectra were recorded with a HITACHI F4600 fluorescence spectrophotometer with a 1 cm standard quartz cell; The fluorescent images of solution and filter paper strip were excited by a 365 nm lighting of ZF-1 UV analyzer; The pH measurements were carried out on a Mettler-Toledo Delta 320 pH meter; 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.



Scheme S1. The synthetic route of probe **CNF**.

Synthesis of compound 1. In a 100 ml round bottomed flask were added 1-(4-(piperazin-1-yl)phenyl)ethan-1-one (408 mg, 2 mmol), 8-Hydroxyjulolidine-9-carboxaldehyde (434 mg, 2 mmol), were dissolved in conc. H_2SO_4 (5ml), and stirred at 90 ^oC for 4 h. After cooling to room temperature, the solution was poured into trash ice, then 70% perchloric acid (6ml) added. The reaction mixture was filtered under reduced pressure, and washed with water to afford crude product. The crude product was purified by column chromatography on silica gel using CH_2Cl_2/MeOH (v/v 20:1) as eluent to afford a dark solid (737 mg, yield 76 %). ^1H NMR (400 MHz, CD_3OD), δ (ppm): 2.04-2.08 (2H), 2.14-2.17 (2H), 2.94-2.95 (t, J = 6.4 Hz, 2H), 3.13-3.16 (t, J = 6.4 Hz, 2H), 3.61-3.67 (4H), 3.71-3.74 (4H), 7.22-7.24 (d, J = 8.8 Hz, 2H), 7.48 (s, 1H), 7.67-7.69 (d, J = 8.0 Hz, 2H), 8.16-8.18 (d, J = 9.2 Hz, 2H), 8.31-8.33 (d, J = 8.4 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD containing 30% $CDCl_3$): δ (ppm): 29.49, 43.48, 44.62, 50.60, 51.05, 105.15, 106.77, 114.96, 118.09, 127.31, 128.01, 129.62, 145.80, 152.54, 153.80. HRMS (ESI) *m/z* calcd for $C_{25}H_{28}N_3O^+$ (M^+): 386.2249. Found 386.2247.

Synthesis of compound CF. Compound **1** (485 mg, 1 mmol), EDCI (287 mg, 1.5 mmol), HOBt (338 mg, 2.5 mmol), 7-hydroxycoumarin carboxylic acid **2** (206 mg, 1 mmol) were dissolved in DCM (3ml) and stirred for overnight at room temperature. The solvent was concentrated under reduced pressure to afford crude product. The crude product was purified by column chromatography on silica gel using

CH_2Cl_2/MeOH (v/v 25:1) as eluent to afford a purplish dark blue solid (431 mg, yield 64 %). ^{1}H NMR (400 MHz, CD_3OD), δ (ppm): 2.03-2.09 (2H), 2.12-2.17 (2H), 2.93-2.94 (t, *J* = 6.0 Hz, 2H), 3.11-3.14 (t, *J* = 6.4 Hz, 2H), 3.60-3.70 (10H), 3.91-3.94 (2H), 6.79 (1H), 6.86-6.89 (dd, *J* = 8.4 and 2.4 Hz, 1H), 7.15-7.18 (d, *J* = 9.2 Hz, 2H), 7.58-7.60 (d, *J* = 8.4 Hz, 1H), 7.64-7.66 (d, *J* = 8.0 Hz, 1H), 7.84-7.86 (d, *J* = 8.4 Hz, 1H), 8.10-8.12 (2H), 8.14 (s, 1H), 8.28-8.30 (d, *J* = 8.0 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD containing 30% $CDCl_3$): δ (ppm): 19.21, 19.54, 20.19, 27.52, 41.81, 45.98, 46.23, 46.75, 50.49, 50.96, 105.09, 106.66, 108.75, 111.19, 114.32, 116.78, 117.53, 117.67, 118.53, 123.70, 127.22, 127.69, 129.73, 131.29, 131.93, 133.42, 143.35, 144.32, 145.20, 145.77, 151.68, 152.29, 153.63, 154.26, 155.40, 157.06, 157.90, 164.06, 165.93. HRMS (ESI) *m/z* calcd for $C_{35}H_{32}N_3O_5^+$ (M^+): 574.2336. Found 574.2341.

Synthesis of compound CNF. The mixture of compound **CF** (24 mg, 0.05 mmol) and NBD-Cl (15 mg, 0.075 mmol) was dissolved absolutely in dry DMF, followed by addition of a drop triethylamine. Under the protection of nitrogen, the mixture was stirred at room temperature for 1.5 h. After complete reaction, about 50 mL water was added into the flask and the reaction mixture was extracted with dichloromethane (100 mL × 3). The organic phase was collected and dried using $MgSO_4$. Then the solvent was removed under reduced pressure affording the crude product, which was purified by flash chromatography column using CH_2Cl_2/MeOH (v/v 30:1) to afford dark blue solid as compound **CNF** (27 mg, yield 65 %). ^{1}H NMR (400 MHz, CD_3OD containing 30% $CDCl_3$), δ (ppm): 2.04-2.10 (2H), 2.12-2.17 (2H), 2.94-2.97 (t, *J* = 5.6 Hz, 2H), 3.13-3.16 (t, *J* = 6.4 Hz, 2H), 3.37-3.39 (t, *J* = 5.0 Hz, 2H), 3.61-3.75 (10H), 3.94-3.95 (2H), 3.97 (2H), 7.02-7.04 (d, *J* = 8.0 Hz, 1H), 7.17-7.21 (2H), 7.42-7.45 (dd, *J* = 8.4 and 2.4 Hz, 1H), 7.47 (1), 7.51-7.52 (1), 7.67-7.69 (d, *J* = 8.4 Hz, 1H), 8.15-8.17 (d, *J* = 9.2 Hz, 1H), 8.26 (1H), 8.30-8.32 (d, *J* = 8.4 Hz, 1H), 8.63-8.65 (d, *J* = 8.0 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD/$CDCl_3$ (v/v 1:1)): δ (ppm): 19.21, 19.56, 20.19, 27.55, 29.49, 41.85, 45.98, 46.74, 50.51, 50.99, 105.09, 106.65, 108.80, 111.14, 114,34 116.75, 117.57, 117.66, 118.52, 123.71, 127.22, 127.68, 129.75,

131.28, 133.35, 143.48, 144.31, 145.17, 145.73, 151.67, 152.29, 153.63, 154.26, 155.41, 157.07, 157.88, 164.01, 179.14. HRMS (ESI) *m/z* calcd for $C_{41}H_{33}N_6O_8^+$ (M^+): 737.2354. Found 737.2357.

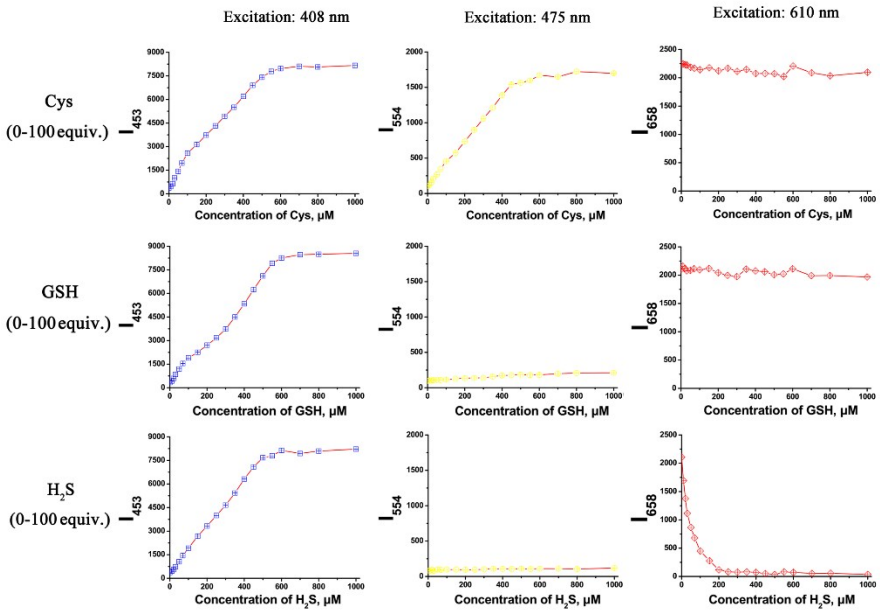


Fig. S1. The fluorescence intensity of **CNF** (10 μM) at 453 nm (the first column, excitation at 408 nm), 554 nm (the second column, excitation at 475 nm), and 658 nm (the third column, excitation at 610 nm) upon addition of 0-100 equiv. of Cys (the first row), GSH (the second row), H_2S (the third row) in PBS buffer (25 mM, pH 7.4, containing 20% DMSO), respectively.

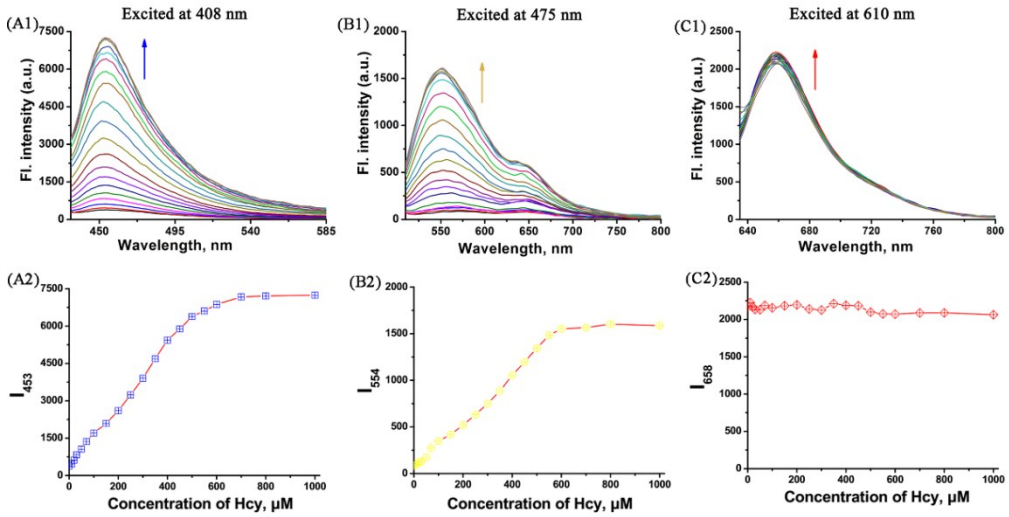


Fig. S2. The titration fluorescence spectra of **CNF** (10 μM) and the fluorescence intensity of **CNF** (10 μM) at 453 nm (A2, excitation at 408 nm), 554 nm (B2, excitation at 475 nm), and 658 nm (C2, excitation at 610 nm) upon addition of 0-100 equiv. of Hcy in PBS buffer (25 mM, pH 7.4,

containing 20% DMSO), respectively.

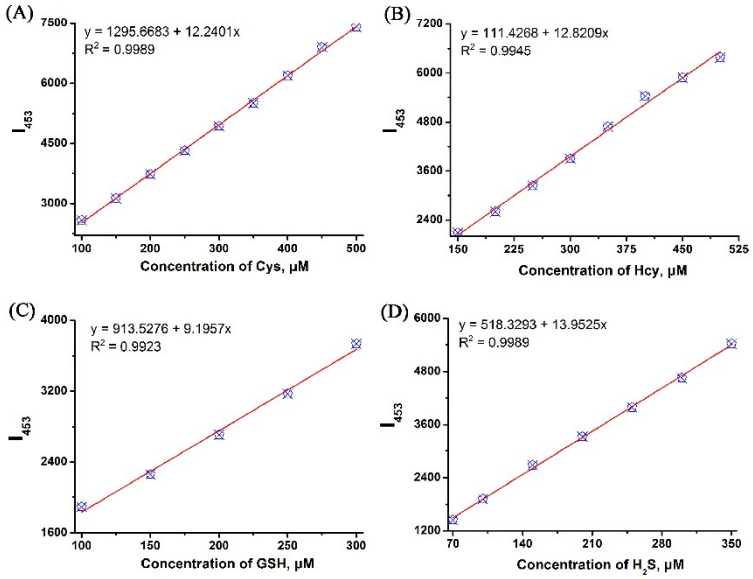


Fig. S3. Linear correlation between the fluorescence emission intensity at 453 nm of **CNF** (10 μM) and (A) Cys (100–500 μM), (B) Hcy (150–500 μM), (C) GSH (100–30 μM), and (D) H_2S (70–350 μM) in PBS buffer (25 mM, pH 7.4, containing 20% DMSO). Excitation at 408 nm.

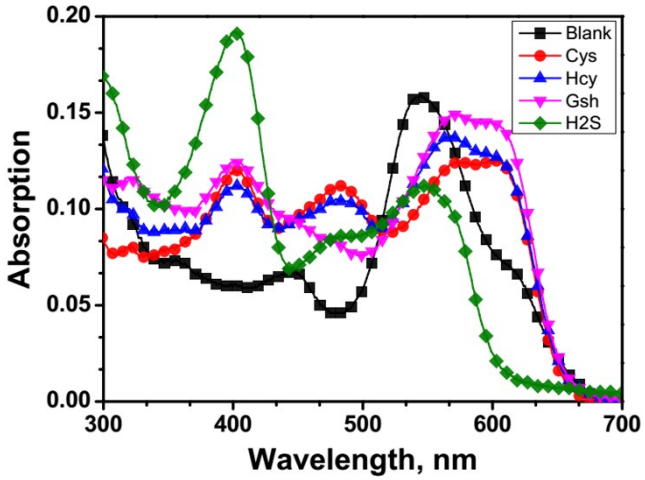


Fig. S4. The absorption spectra of **CNF** (10 μM) in the absence or presence of 100 equiv. of Cys, Hcy, GSH and H_2S in PBS buffer (25 mM, pH 7.4, containing 20% DMSO), respectively.

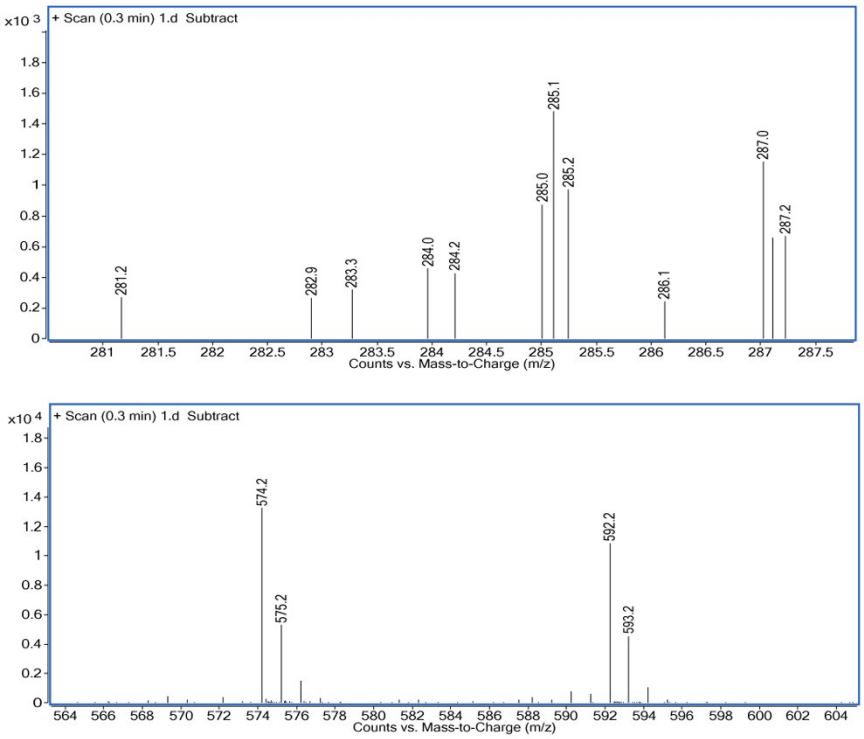


Fig. S5. The mass spectra (ESI) of **CNF** in the presence of Cys in aqueous solution. **NBD-Cys** *m/z* ($[M+H]^+$) = 285.1; **CF** ($[M]^+$) *m/z* = 574.2.

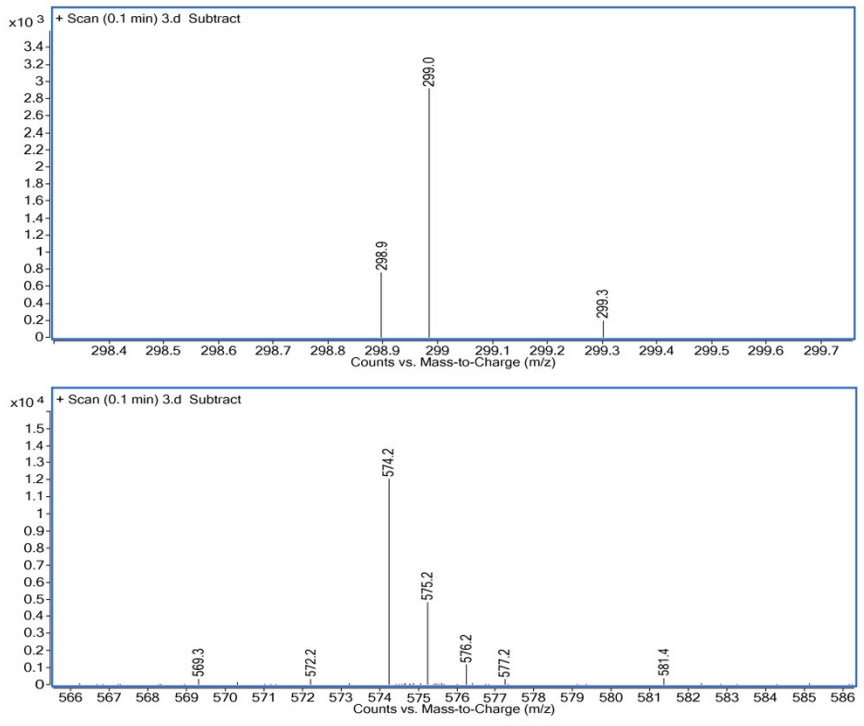


Fig. S6. The mass spectra (ESI) of **CNF** in the presence of Hcy in aqueous solution. **NBD-Hcy** *m/z* ($[M+H]^+$) = 299.0; **CF** ($[M]^+$) *m/z* = 574.2.

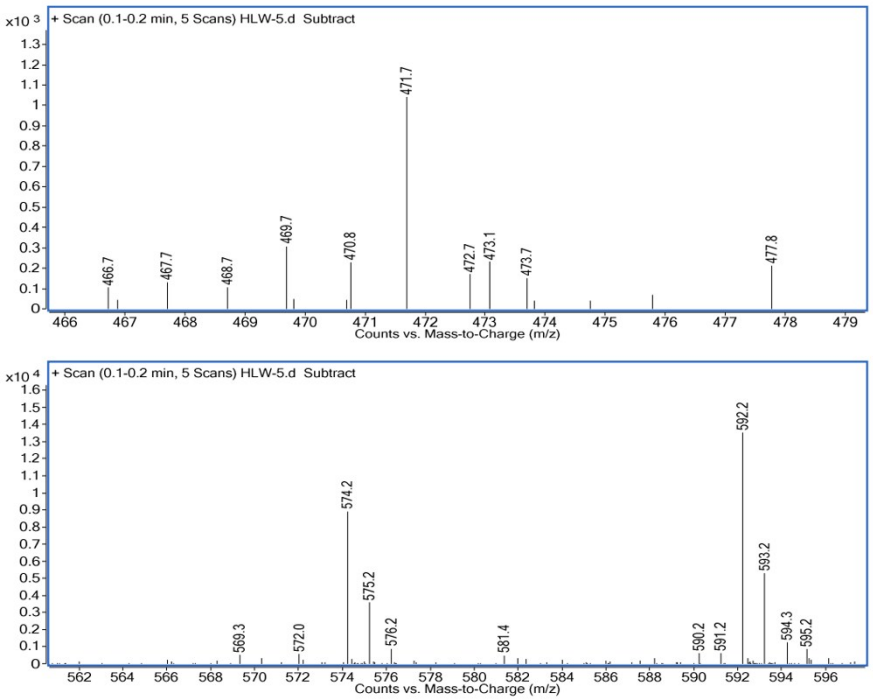


Fig. S7. The mass spectra (ESI) of **CNF** in the presence of GSH in aqueous solution. **NBD-GSH** *m/z* ($[M+H]^+$) = 471.7; **CF** ($[M]^+$) *m/z* = 574.2.

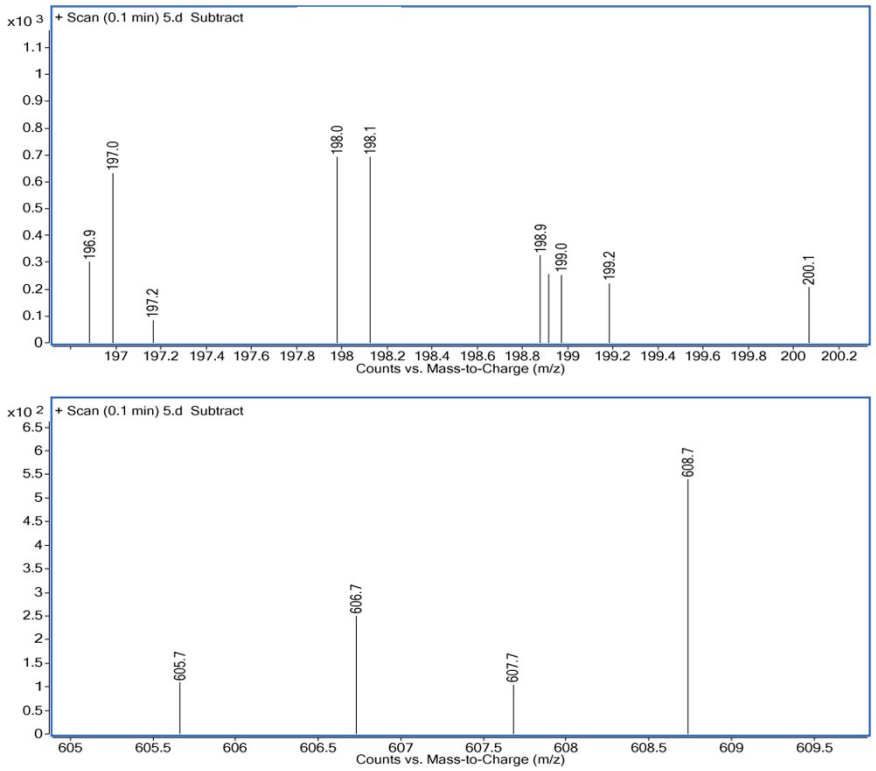


Fig. S8. The mass spectra (ESI) of **CNF** in the presence of H_2S in aqueous solution. **NBD-SH** *m/z* ($[M+H]^+$) = 198.0; **CF-SH** ($[M+H]^+$) *m/z* = 608.7.

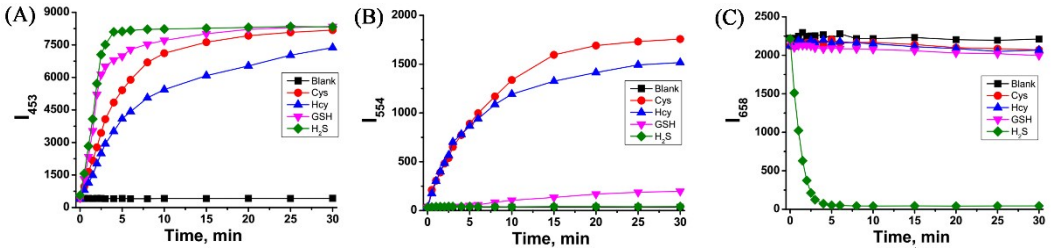


Fig. S9. Reaction-time profiles of the probe **CNF** in the presence or absence of 100 equiv. of Cys, Hcy, GSH and H_2S in PBS buffer (25 mM, pH 7.4, containing 20% DMSO), respectively. (A) Fluorescence intensity at 453 nm, excitation at 408 nm; (B) Fluorescence intensity at 554 nm, excitation at 475 nm; (C) Fluorescence intensity at 658 nm, excitation at 610 nm.

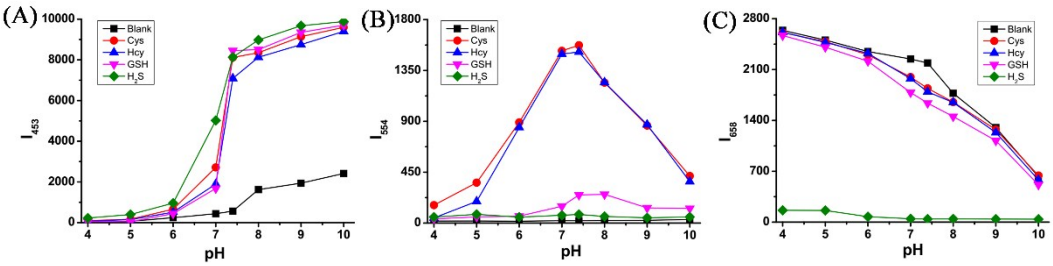


Fig. S10. The fluorescence intensity of CNF (10 μM) at (A) 453 nm (excitation at 408 nm), (B) 554 nm (excitation at 475 nm), and (C) 658 nm (excitation at 610 nm) in the presence or absence of biothiols (1 mM) in different pH ranging 4.0-10.0 PBS buffer (25 mM, containing 20% DMSO), respectively.

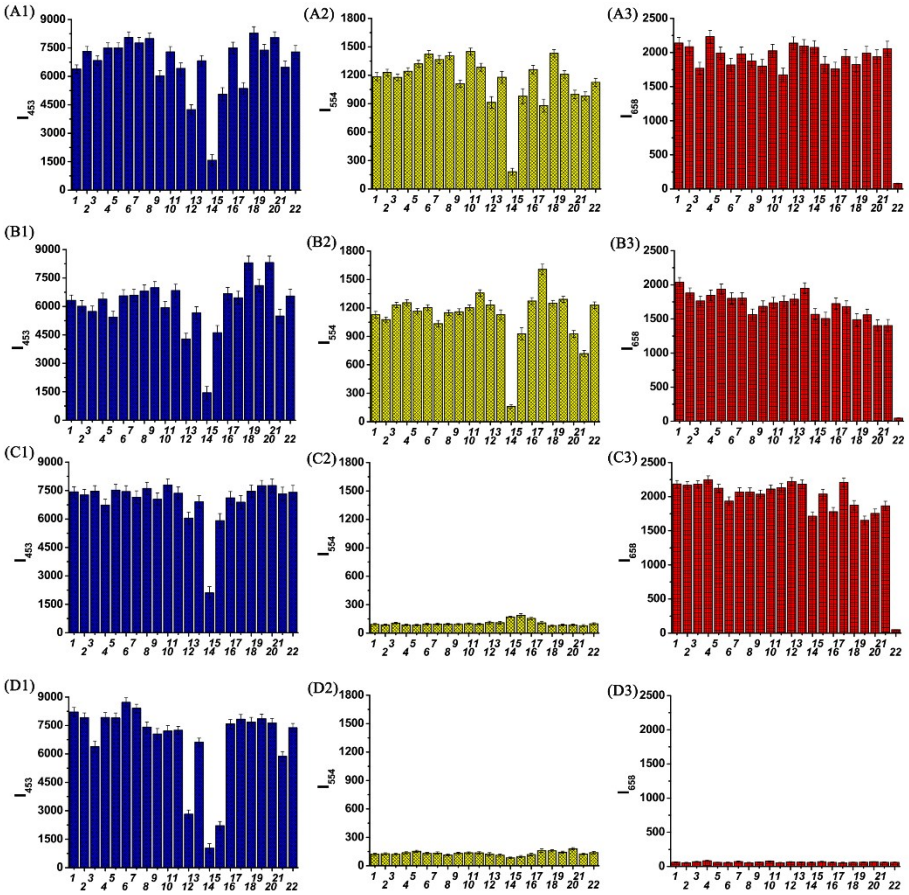


Fig. S11 The fluorescence intensity of **CNF** (10 μM) at (A1-D1) 453 nm (excited at 408 nm), (A2-D2) 554 nm (excited at 475 nm), and (A3-D3) 658 nm (excited at 610 nm) in the presence of 1 mM various analytes (1-22) mixed with 1 mM each biothiol (A1-A3 for Cys, B1-B3 for Hcy, C1-C3 for GSH, and D1-D3 for H_2S, respectively). (1) Ala, (2) Arg, (3) Glu, (4) Phe, (5) Thr, (6) Trp, (7) Asp, (8) His, (9) Ile, (10) Ser, (11) Vai, (12) Al^{3+}, (13) Ca^{2+}, (14) Cu^{2+}, (15) Fe^{2+}, (16) K^{+}, (17) Zn^{2+}, (18) ·OH, (19) H_2O_2, (20) HBHP, (21) $S_2O_2^{2-}$, (22) SO_3^{2-} in PBS buffer (25 mM, pH 7.4, containing 20% DMSO), respectively.

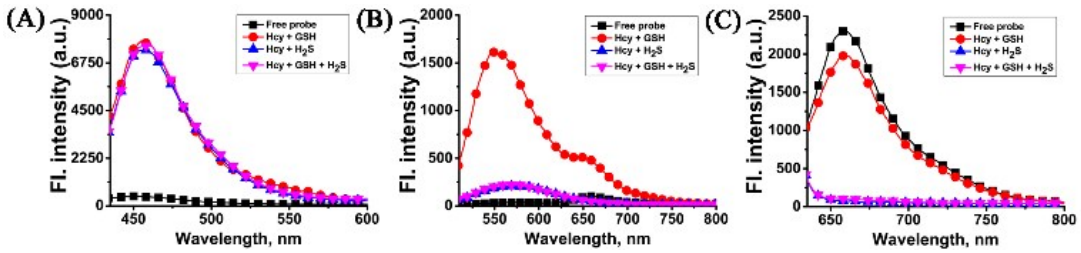


Fig. S12. The fluorescence spectra of **CNF** (10 μM) in the presence of random pairwise or three biothiols (Hcy, GSH and H_2S, 1 mM) excited at (A) 408nm, (B) 475 nm, and (C) 610 nm in PBS buffer (25 mM, pH 7.4, containing 20% DMSO), respectively.

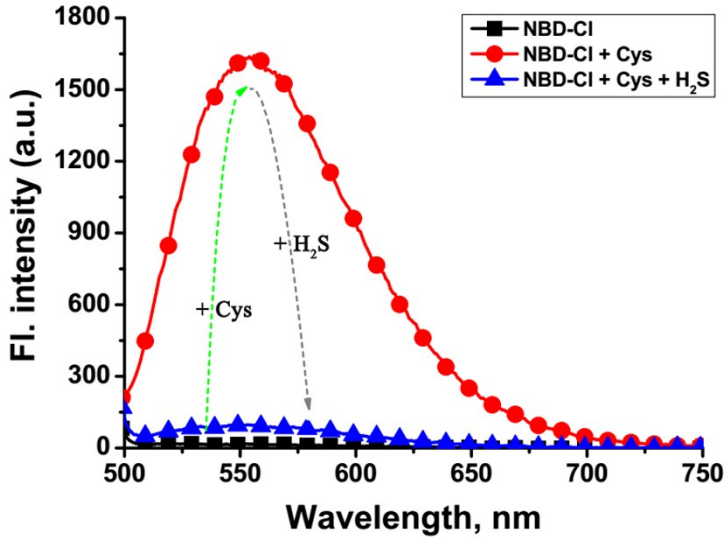


Fig. S13. The fluorescence spectra of **NBD-Cl** (10 μM) treated with Cys (1 mM) and H_2S (1 mM) in turn with an excitation at 475 nm in PBS buffer (25 mM, pH 7.4, containing 20% DMSO).

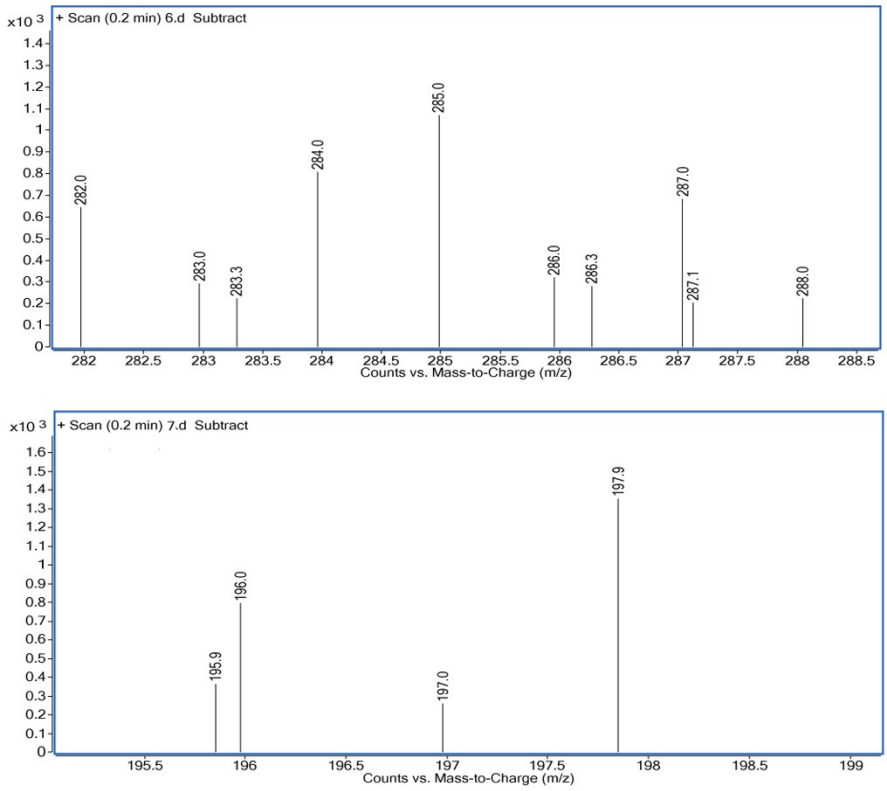


Fig. S14. The mass spectra (ESI) of **NBD-Cl** treated with Cys (top) and followed by addition of H_2S (bottom) in aqueous solution.

Table S1. The truth table for logic operations based on probe **CNF**. The absence of inputs and the negative signal of outputs were considered as 0, and the presence of inputs and the positive signal of outputs were considered as 1. Em_{453}, Em_{554} and Em_{658} denote the fluorescence emission at 453, 554 and 658 nm, respectively.

(A) OR gate

Inputs			Outputs
Cys/Hcy	GSH	H_2S	Em_{453}
0	0	0	0
1	0	0	1
0	1	0	1
0	0	1	1
1	1	0	1
1	0	1	1
0	1	1	1
1	1	1	1

(B) TANSFER gate

Inputs		Outputs
Cys/Hcy	GSH	Em_{554}
0	0	0
0	1	0
1	0	1
1	1	1

(C) INH gate

Inputs		Outputs
Cys/Hcy	H_2S	Em_{554}
0	0	0
0	1	0
1	0	1
1	1	0

(D) NOT gate

Input	Outputs
H_2S	Em_{658}
0	1
1	0

(E) YES gate

Input	Outputs
Cys/Hcy/GSH/H_2S	Em_{453}
0	0
1	1

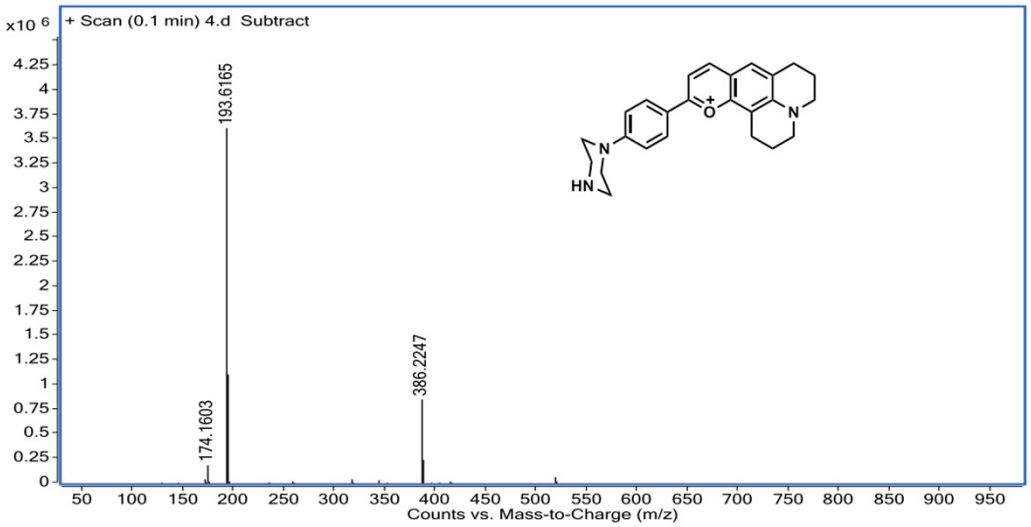


Fig. S15 HRMS (ESI) of compound **1**. *m/z* calcd for $C_{25}H_{28}N_3O^+$ (M^+): 386.2249. Found 386.2247.

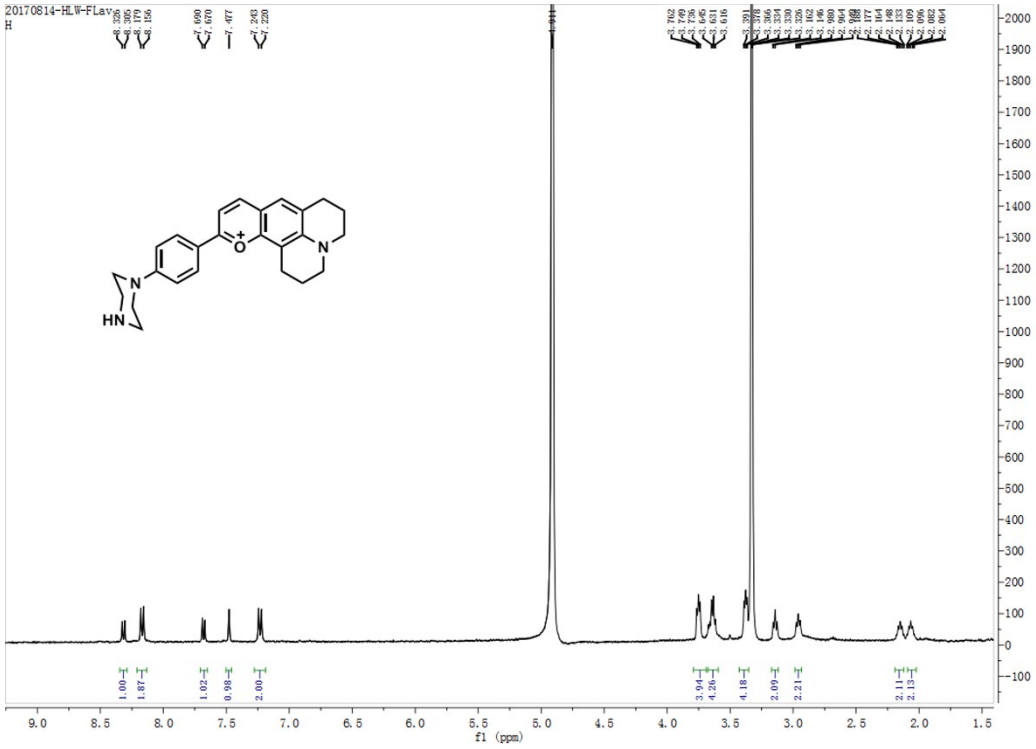


Fig. S16 ^{1}H NMR of compound **1** in CD_3OD.

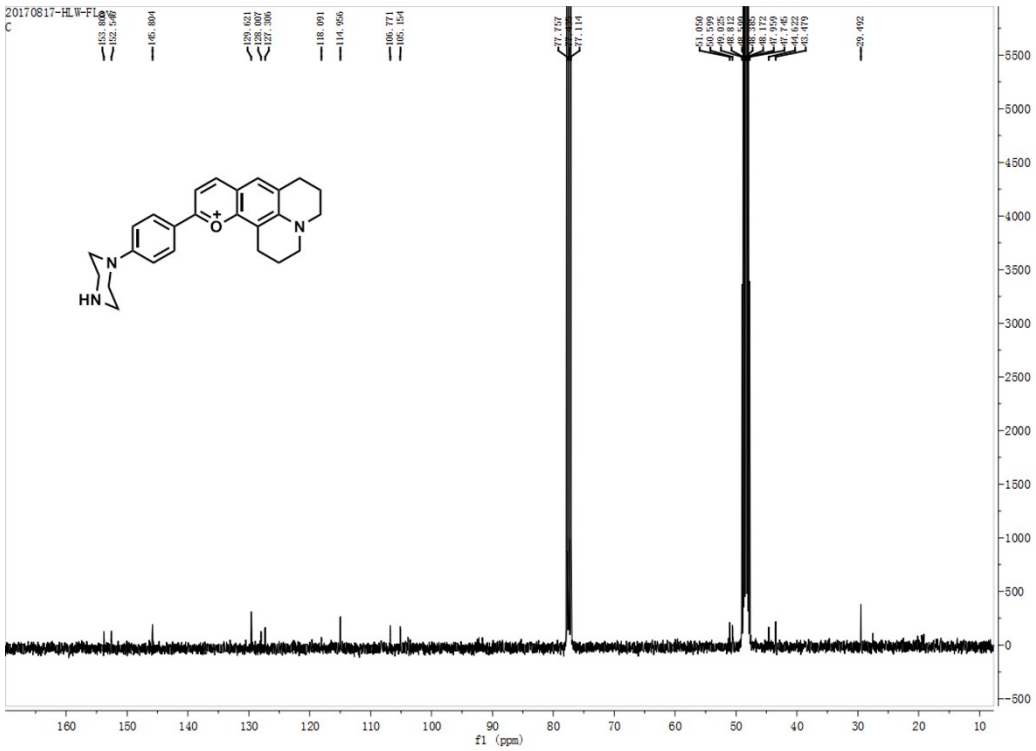


Fig. S17 ^{13}C NMR of compound **1** in CD_3OD containing 30 % $CDCl_3$.

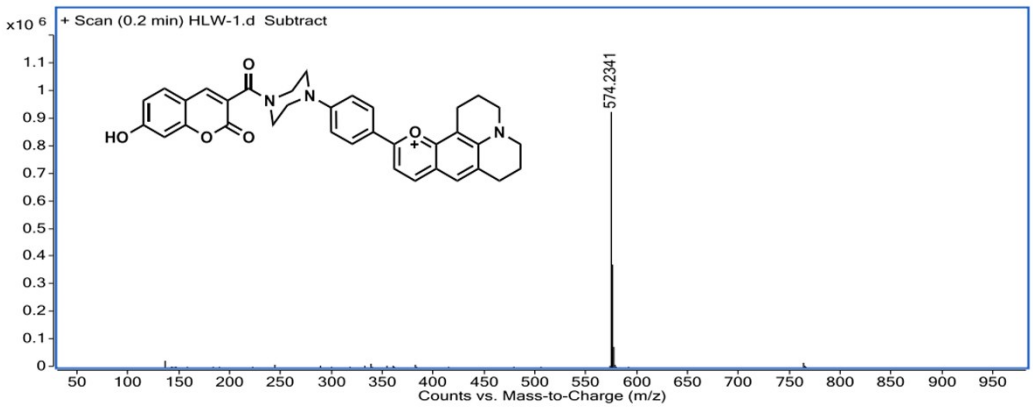


Fig. S18 HRMS (ESI) of compound **CF**. *m/z* calcd for $C_{35}H_{32}N_3O_5^+$ (M^+): 574.2336. Found 574.2341.

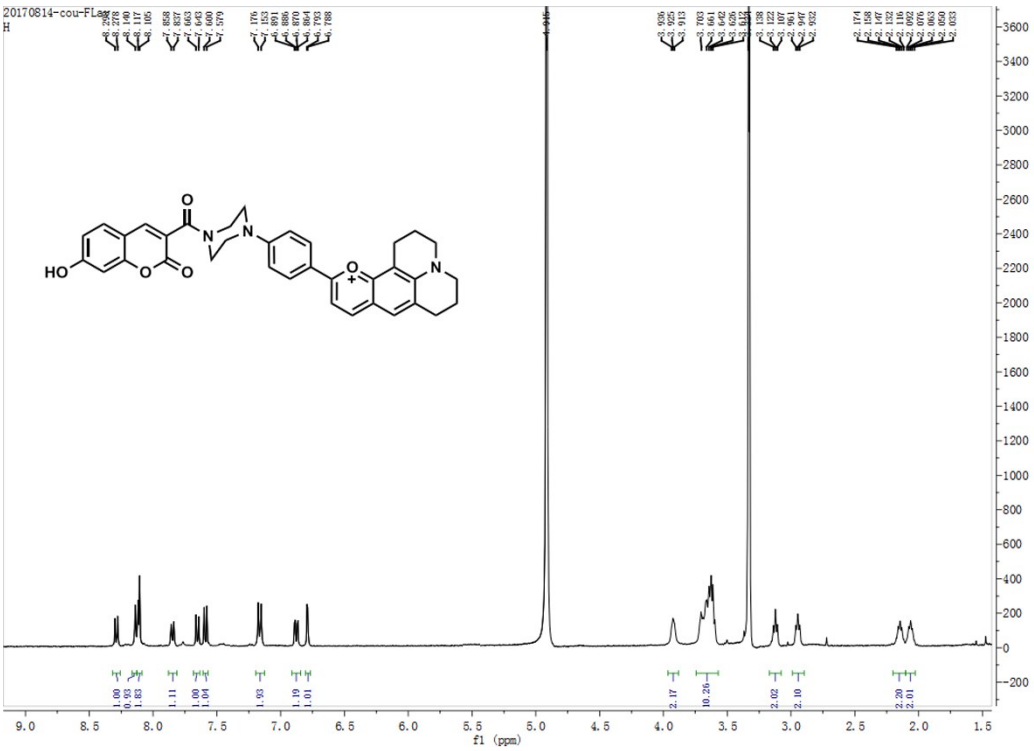


Fig. S19 ^{1}H NMR of compound **CF** in CD_3OD.

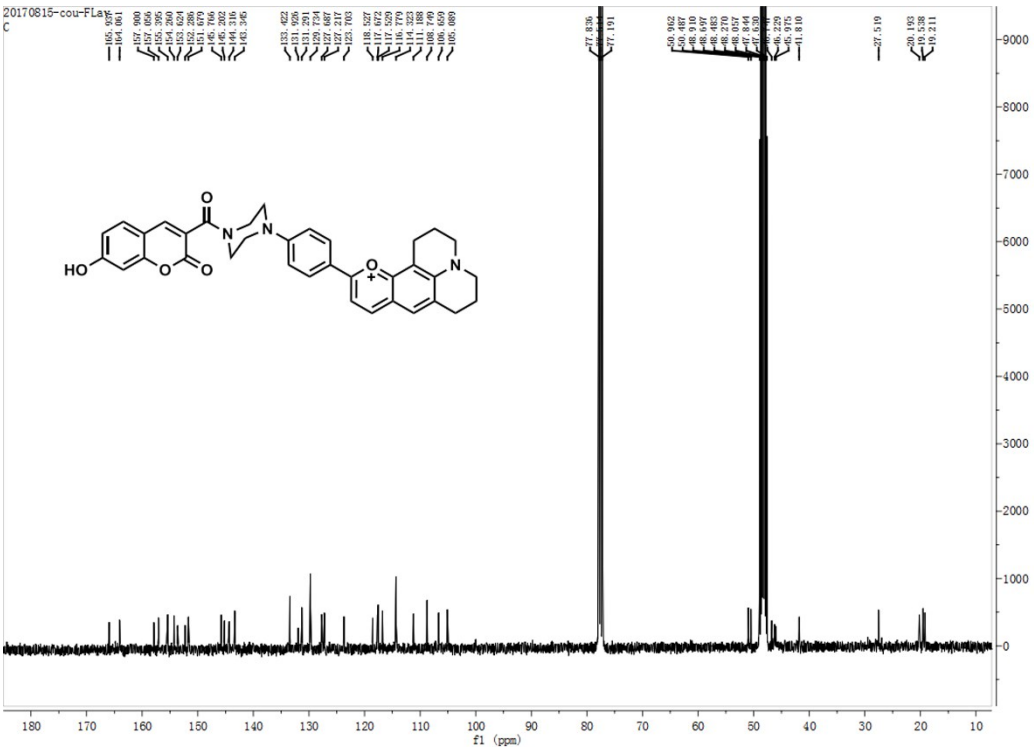


Fig. S20 ^{13}C NMR of compound **CF** in CD_3OD containing 30 % $CDCl_3$.

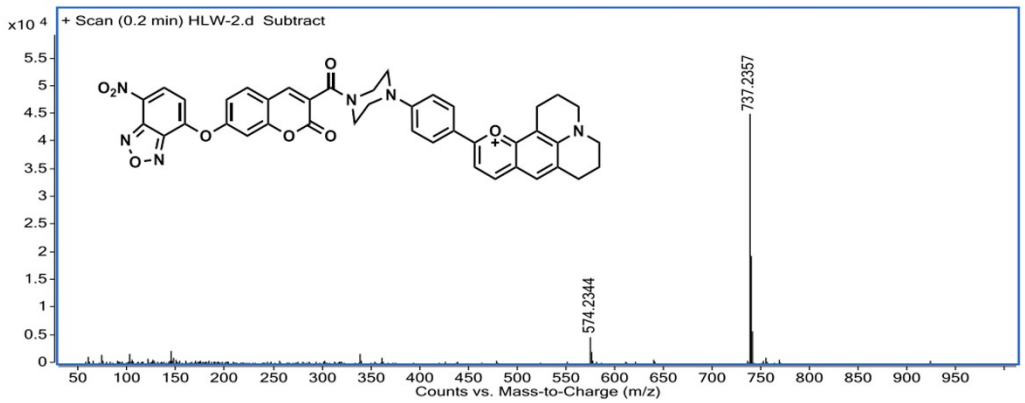


Fig. S21 HRMS (ESI) of compound **CNF**. *m/z* calcd for $C_{41}H_{33}N_6O_8^+$ (M^+): 737.2354. Found 737.2357.

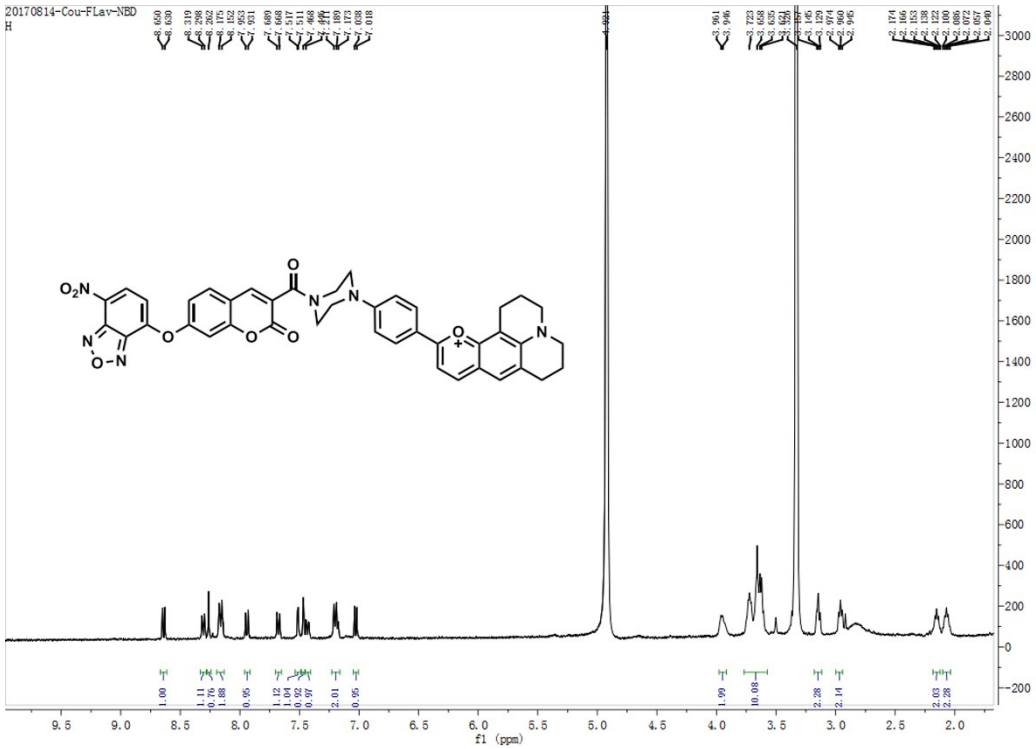


Fig. S22 ^{1}H NMR of compound **CNF** in CD_3OD.

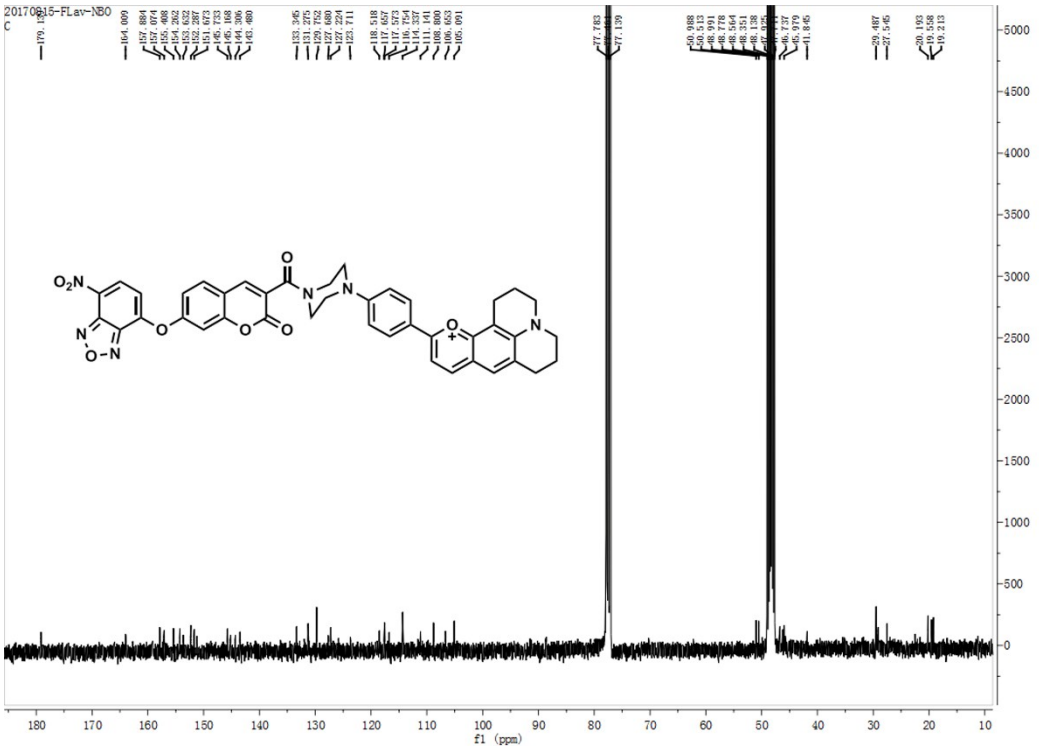


Fig. S23 ^{13}C NMR of compound **CNF** in CD_3OD containing 30 % $CDCl_3$.