Supporting Information

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1. General Information

1.1 Materials

All chemicals were purchased from commercial suppliers and used without further purification. All solvents were purified prior to use. Distilled water was used after passing through a water ultra-purification system. PBS buffer solution was obtained by mixing of 0.05mol/L Na_2HPO_4 water solution and 0.05mol/L KH_2PO_4 water solution with the volume ratio 4:1. Cysteine and Na_2SO_3 were used as a source of Cys and SO_2 in this study. Cysteine, Na_2SO_3 and various analytes were purchased from Shanghai Experiment Reagent Co., Ltd (Shanghai, China). All chemicals and solvents used were of analytical grade. All solution samples were made by dissolving their each solid in water or DMSO.

1.2 Instruments

TLC analysis was performed using precoated silica plates. Ultraviolet–visible (UV–vis) spectra were recorded on U-3900 UV-Visible spectrophotometer. Hitachi F-7000 fluorescence spectrophotometer was employed to measure fluorescence spectra. Shanghai Huamei Experiment Instrument Plants, China provided a PO-120 quartz cuvette (10 mm). ^{1}H NMR and ^{13}C NMR experiments were performed with a BRUKER AVANCE III HD 600 MHz and 151 MHz NMR spectrometer, respectively (Bruker, Billerica, MA). Coupling constants (J values) are reported in hertz. ESI-MS was measured with a Thermo Scientific Q Exactive. The cells imaging experiments were measured by a Zeiss LSM880 Airyscan confocal laser scanning microscope. The imaging assays of living body were performed in Bruker In-Vivo FX Pro small animal optical imaging system.

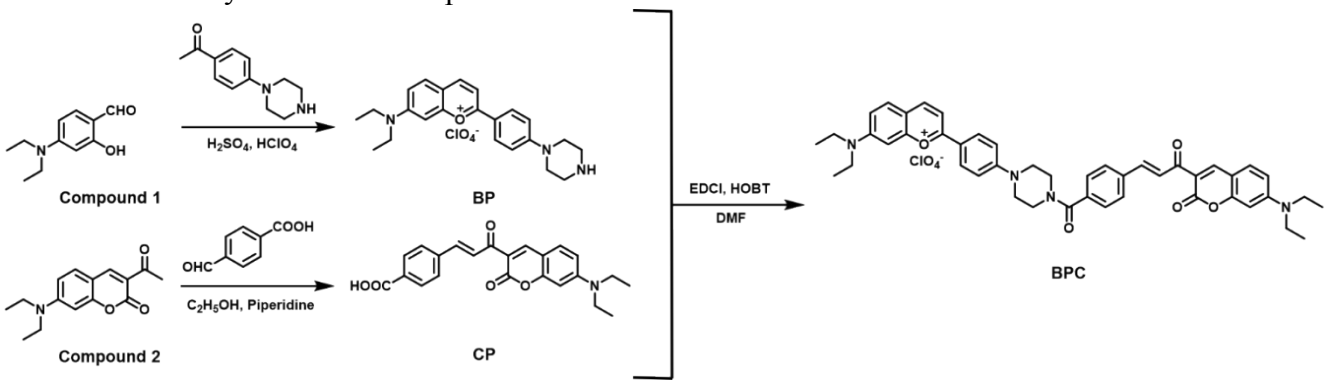
1.3 Bio-imaging

Cell Culture and Imaging. The HeLa cells were grown in Dulbecco's Modified Eagle's medium supplemented with 10% Fetal Bovine Serum and 1% antibiotics at 37 °C in humidified environment of 5% CO_2. Cells were plated on 6-well plate and allowed to adhere for 24 h. Before the experiments, cells were washed with PBS 3 times.

Mice and tumor model. All the animal experiments were performed by following the protocols approved by Radiation Protection Institute of Drug Safety Evaluation Center in China (Production license: SYXK (Jin) 2018 0005). Balb/c type mouse (6-8 weeks, male) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. Experiments were conducted according to the National Institute of Health Guide for the Care and Use of Laboratory Animals. Tumor-bearing mice were obtained by subcutaneous injection of HeLa cells. The images were recorded in a dual emission mode, Red channel: λ_{em} = 610-670 nm and Green channel: λ_{em} = 470-530 nm (λex = 430 nm).

2. Experimental Section

2.1 **Scheme S1**. Synthesis route of probe **BPC**.



2.2 Synthesis and characterization

Compound **2** was prepared according to the previous reported literature[1].

Synthesis of Compound **2**

Compound **1** (1.93 g, 10 mmol), ethyl acetoacetate (1.95 g, 15 mmol), and piperidine (0.2 mL) were dissolved in EtOH (40 mL). After the mixture solution was refluxed at 85 °C for 10 h. Then the reaction mixture was cooled to room temperature, the result precipitate was filtered and washed with cool EtOH to obtain Compound **2** (2.16 g, 83.4 % yield).

Compound **BP** was prepared according to the previous reported literature[2].

Synthesis of **BP**

The mixture of compound **1** (0.97 g, 5 mmol) and 4-piperazinoacetophenone (1.53 g, 7.5 mmol) was dissolved in concentrated H_2SO_4 (30 mL), followed by stirred at 92 °C for 6 h. Cooling the solution to room temperature, then was added slowly to the ice water (100 mL) and 70% perchloric acid (2.0 mL) were successively added. After stirred for 40 min to forming the precipitate. The crude product was filtered, washed with 10 mL H_2O and dried in vacuum. The product was purified by column chromatograph with dichloromethane and methanol (v:v = 10:1) to give compound **BP** as a purple black powder (0.74 g, 41.2 % yield).

Synthesis of **CP**

Compound **2** (1.29 g, 5 mmol), 4-formylbenzoic acid (0.90 g, 6 mmol) and piperidine (3 mL) were dissolved in EtOH (40 mL), followed by refluxed at 85 °C for 72 h. Then the solution was cooled to room temperature and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel by using (CH_2Cl_2 : CH_3OH = 30:1) to obtain **CP** (0.83 g, 42.5 % yield). ^{1}H NMR (600 MHz, DMSO) δ 13.15 (s, 1H), 8.63 (s, 1H), 8.07 (d, *J* = 15.8 Hz, 1H), 8.00 (d, *J* = 8.3 Hz, 2H), 7.83 (d, *J* = 8.3 Hz, 2H), 7.72 (d, *J* = 4.9 Hz, 1H), 7.70 (s, 1H), 6.83 (d, *J* = 9.1 Hz, 1H), 6.63 (s, 1H), 3.51 (q, *J* = 7.0 Hz, 4H), 1.16 (t, *J* = 7.0 Hz, 6H). ^{13}C NMR (151 MHz, DMSO) δ 185.87, 167.31, 160.44, 158.79, 149.13, 140.80, 139.47, 132.98, 132.36, 130.37, 128.87, 127.64, 110.80, 108.43, 96.39, 44.96, 12.85.

Synthesis of probe **BPC**.

To a solution of **CP** (0.39 g, 1 mmol) were added 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCl, 0.38 g, 2 mmol) and 1-Hydroxybenzotriazole (HOBt, 0.28 g, 2 mmol), and the resulting mixture was stirred in dried DMF at 0 °C under N_2 for 30 min. Then, compound **BP** (0.29 g,

0.8 mmol) was sequentially added. The resulting mixture was stirred at room temperature for 24 h, and then poured into water and filtered. This crude product was purified by column chromatography on silica gel (CH_2Cl_2 : CH_3OH = 20:1) to afford probe **BPC**. (0.27 g, 46.2 % yield). ^{1}H NMR (600 MHz, DMSO) δ 8.62 (d, *J* = 8.8 Hz, 2H), 8.27 (d, *J* = 9.0 Hz, 2H), 8.05 (d, *J* = 15.8 Hz, 1H), 7.97 (d, *J* = 8.5 Hz, 1H), 7.90 (d, *J* = 9.3 Hz, 1H), 7.83 (d, *J* = 8.0 Hz, 2H), 7.74 (s, 1H), 7.71 (s, 1H), 7.57 (d, *J* = 8.0 Hz, 2H), 7.35 (d, *J* = 9.3 Hz, 1H), 7.30 (s, 1H), 7.16 (d, *J* = 9.1 Hz, 2H), 6.83 (d, *J* = 9.0 Hz, 1H), 6.62 (s, 1H), 3.79 (d, *J* = 30.4 Hz, 4H), 3.67 (d, *J* = 7.0 Hz, 6H), 3.52 (d, *J* = 7.0 Hz, 6H), 1.24 (t, *J* = 7.0 Hz, 6H), 1.16 (t, *J* = 7.0 Hz, 6H). ^{13}C NMR (151 MHz, DMSO) δ 185.31 , 168.53 , 166.88 , 159.89 , 158.19 , 158.06 , 154.84 , 154.22 , 153.00 , 148.57 , 136.87 , 136.12 , 132.46 , 132.30 , 131.70 , 131.56 , 130.74 , 130.60 , 128.33 , 128.19 , 127.80 , 127.67 , 126.19 , 116.68 , 116.23 , 115.12 , 113.60 , 110.22 , 107.84 , 95.78 , 45.06 , 44.38 , 39.91 , 12.27. ESI-MS m/z: $[M]^+$ Calcd. for 735.3541, Found 735.3543.

3. Spectrometric Studies

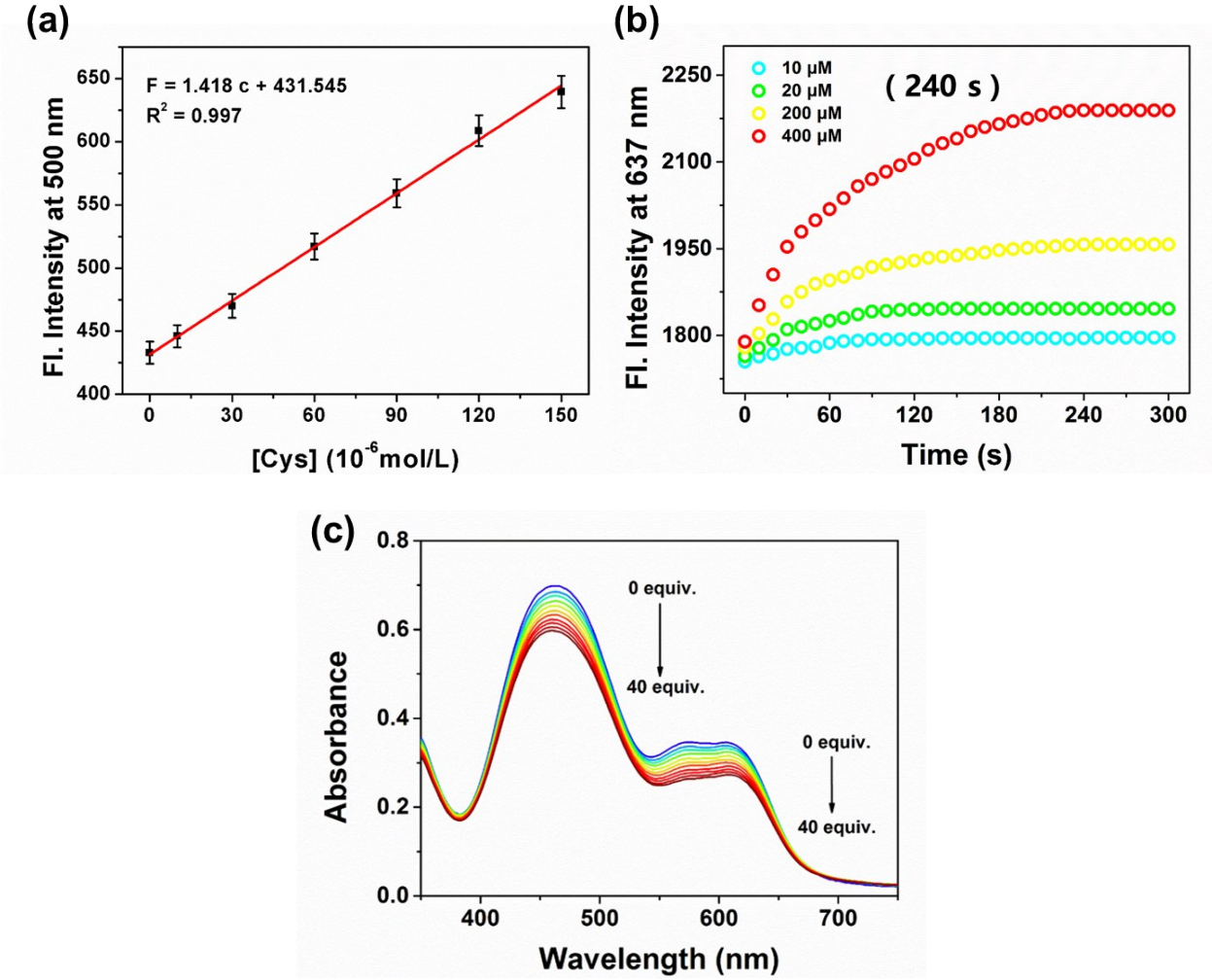


Figure S1: (a) The linear curve obtained by the fluorescence intensity of probe **BPC** (10 μM) with Cys 0-150 μM. (λ_{ex} = 430 nm, λ_{em} = 500 nm, slit: 5 nm/10 nm) (b) Time-dependent of probe **BPC** (10 μM) for different concentrations of Cys (10 μM, 20 μM, 200 μM, 400 μM). (λ_{ex} = 585 nm, λ_{em} = 637 nm, slit:

5 nm/10 nm) (c) The Absorption spectra of probe **BPC** (10 μM) toward Cys (0-40 equiv.). Condition: DMSO/PBS solution (10.0 mM, pH = 7.4, 1:9, v/v).

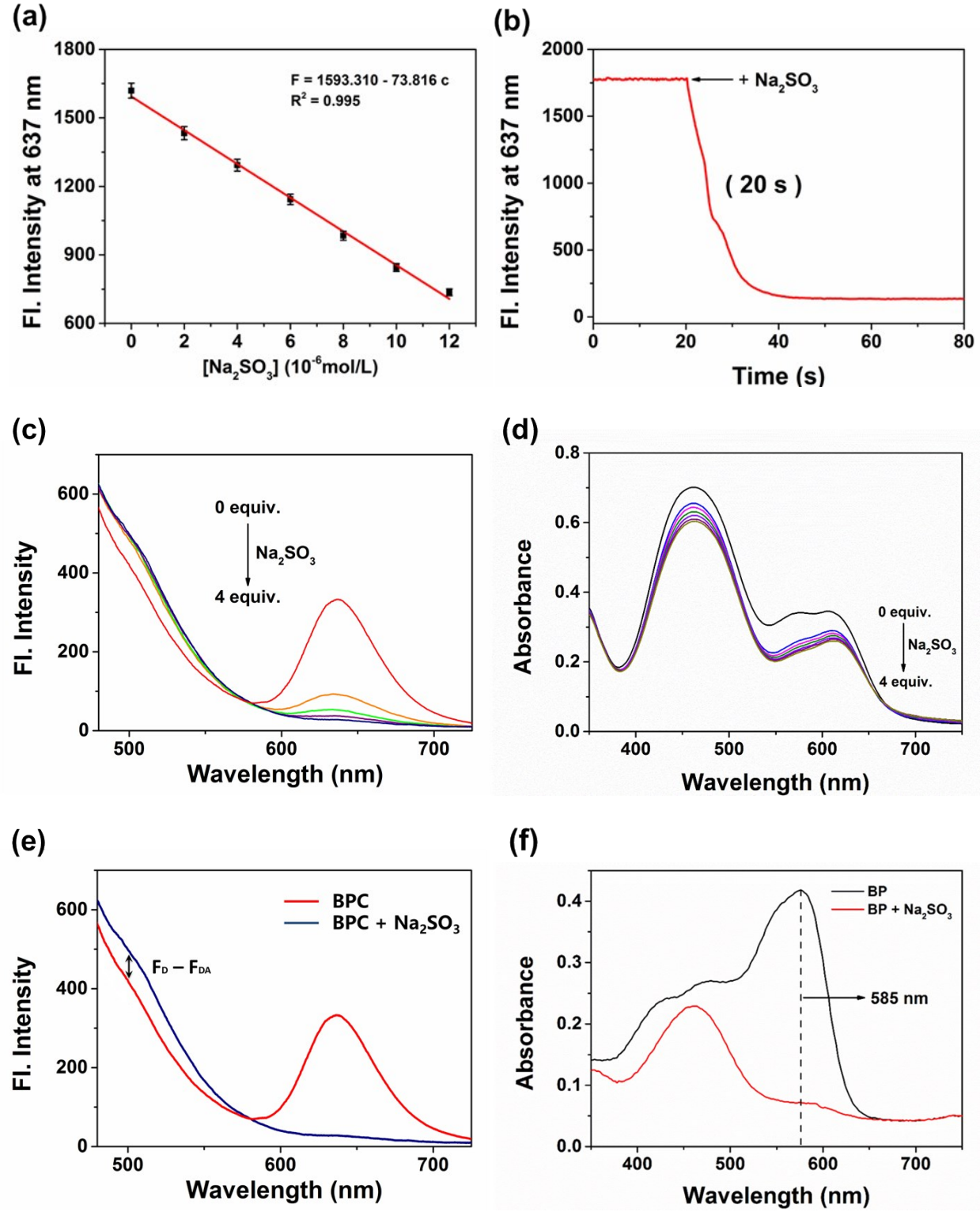


Figure S2: (a) The linear curve obtained by the fluorescence intensity of probe **BPC** (10 μM) with Na_2SO_3 0-12 μM. (b) Time-dependent of probe **BPC** (10 μM) for Na_2SO_3 (40 μM). (λ_{ex} = 585 nm, λ_{em} =

637 nm, slit: 5 nm/10 nm). (c) The fluorescent spectra of probe **BPC** (10 μM) toward Na_2SO_3 (0-4 equiv.), (λ_{ex} = 430 nm, λ_{em} = 500 nm and 637 nm, slit: 5 nm/10 nm). (d) The Absorption spectra of probe **BPC** (10 μM) toward Na_2SO_3 (0-4 equiv.). (e) The fluorescence intensity of **CP** in the absence (F_D: blue line) and presence (F_{DA}: red line) of the **BP** with excitation wavelength at 430 nm. (f) The Absorption spectra of **BP** (10 μM) toward Na_2SO_3 (40 μM). Condition: DMSO/PBS solution (10.0 mM, pH = 7.4, 1:9, v/v).

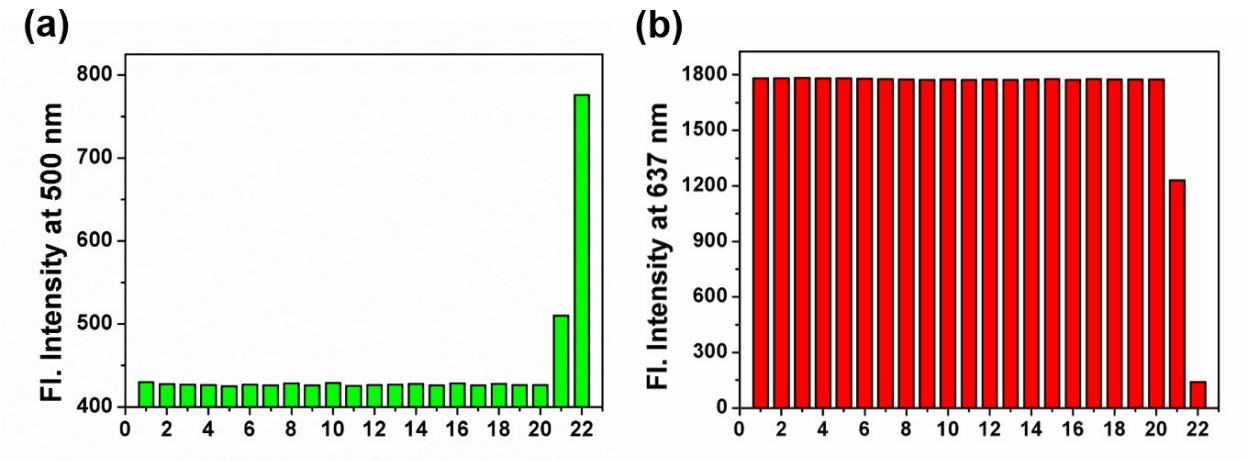


Figure S3: (a) Fluorescence response of probe **BPC** (10 μM) towards various biologically relevant species (400 μM) at 500 nm, (1) Blank; (2) SO_4^{2-}; (3) S^{2-}; (4) Gly; (5) Glu; (6) Cystine; (7) Cysteamine; (8) Ca^{2+}; (9) Cu^{2+}; (10) Mg^{2+}; (11) Na^{+}; (12) K^{+}; (13) F^{-}; (14) Cl^{-}; (15) I^{-}; (16) NO_3^{-}; (17) NO_2^{-}; (18) ClO^{-}; (19) $S_2O_3^{2-}$; (20) GSH; (21) Hcy ; (22) Cys. (λ_{ex} = 430 nm. Slit: 5 nm/10 nm) (b) Fluorescence response of probe **BPC** (10 μM) towards various biologically relevant species (400 μM) at 637 nm, (1) Blank; (2) GSH; (3) Hcy; (4) Cys; (5) Gly; (6) Glu; (7) Cystine; (8) Cysteamine; (9) Ca^{2+}; (10) Mg^{2+}; (11) Na^{+}; (12) K^{+}; (13) F^{-}; (14) Cl^{-}; (15) I^{-}; (16) NO_3^{-}; (17) NO_2^{-}; (18) ClO^{-}; (19) $S_2O_3^{2-}$; (20) SO_4^{2-}; (21) S^{2-}; (22) SO_3^{2-} (40 μM). (λ_{ex} = 585 nm. Slit: 5 nm/10 nm). Condition: DMSO/PBS solution (10.0 mM, pH = 7.4, 1:9, v/v).

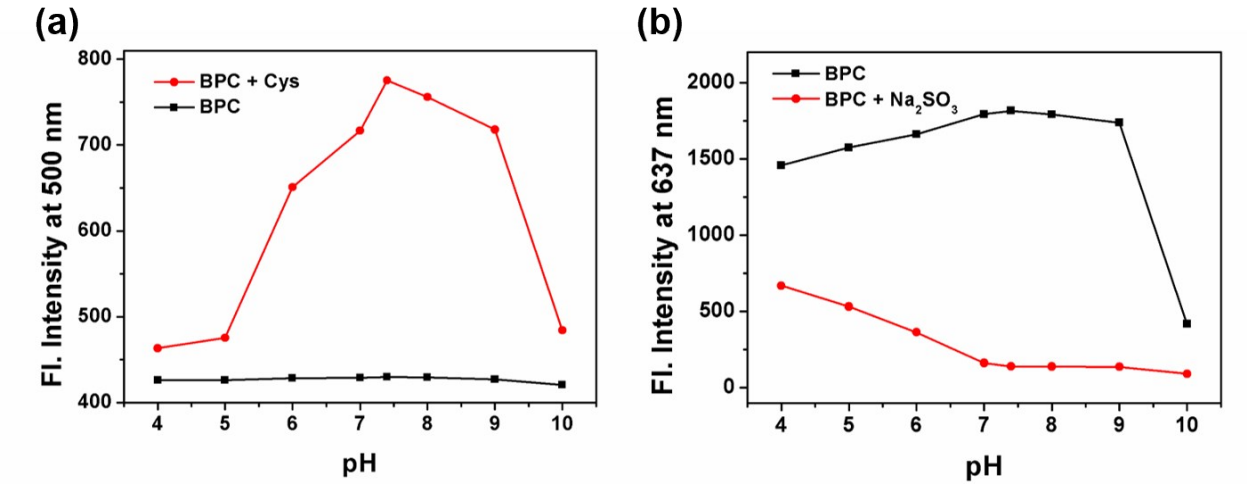


Figure S4: (a) Fluorescence response of probe **BPC** (10 μM) before or after adding Cys (400 μM) with

different pH values. (λ_{ex} = 430 nm. Slit: 5 nm/10 nm) (b) Fluorescence response of probe **BPC** (10 μM) before or after adding Na_2SO_3 (40 μM) with different pH values. (λ_{ex} = 585 nm. Slit: 5 nm/10 nm).

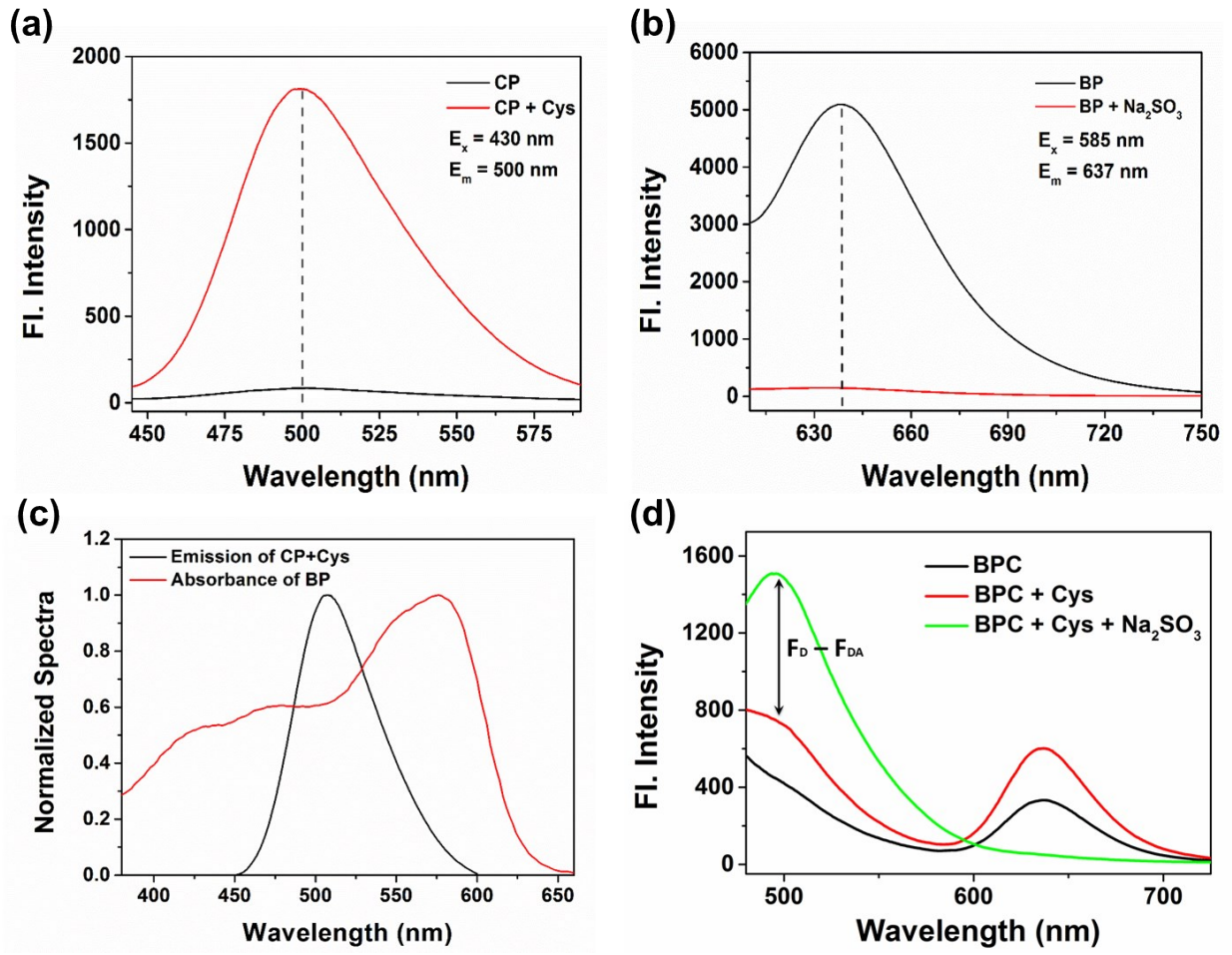
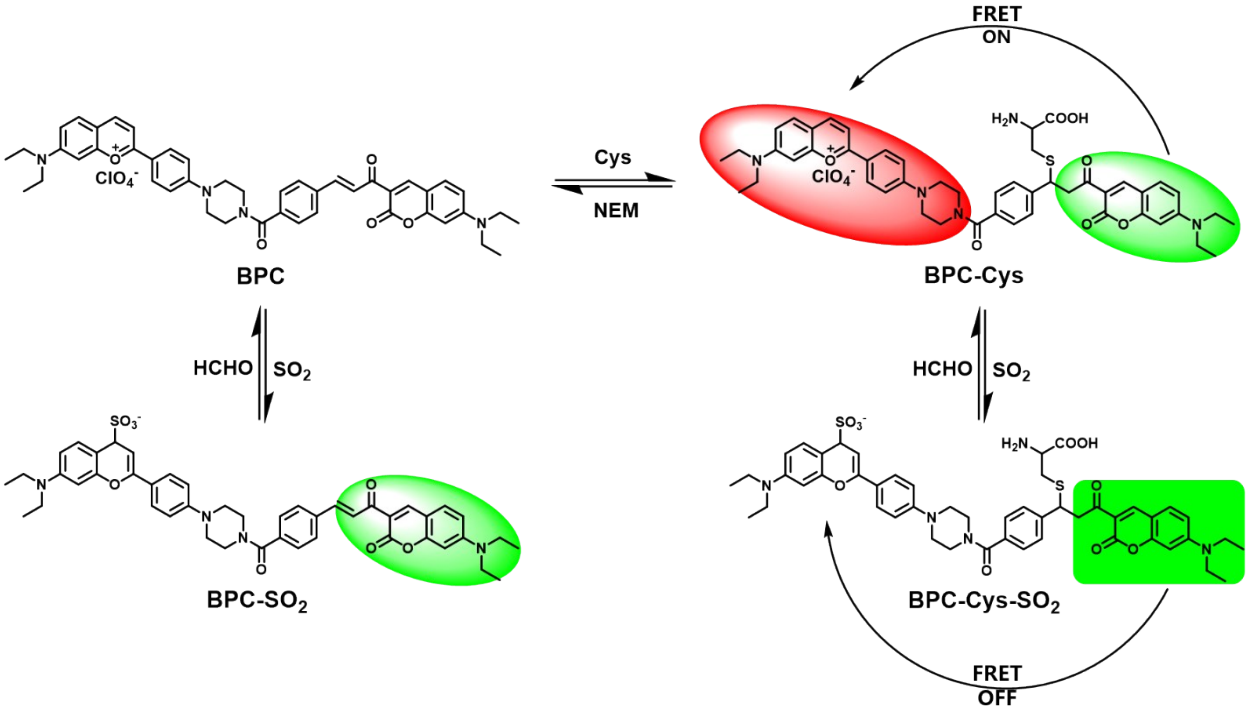


Figure S5: (a) The fluorescent spectra of **CP** (10 μM) toward Cys (40 equiv.) or (b) of **BP** toward Na_2SO_3 (4 equiv.), λ_{ex} = 430 nm, λ_{em} = 500 nm, slit: 5 nm/5 nm; and λ_{ex} = 585 nm, λ_{em} = 637 nm, slit: 5 nm/10 nm. (c) Normalized fluorescence emission spectrum of the "**CP + Cys**" and normalized UV-vis absorption spectrum of the **BP**. (d) The fluorescence intensity of **CP-Cys** in the absence (F_D: green line) and presence (F_{DA}: red line) of the **BP** with excitation wavelength at 430 nm. Condition: DMSO/PBS solution (10.0 mM, pH = 7.4, 1:9, v/v).



Scheme S2. Sensing mechanism of probe **BPC** toward Cys and SO_2.

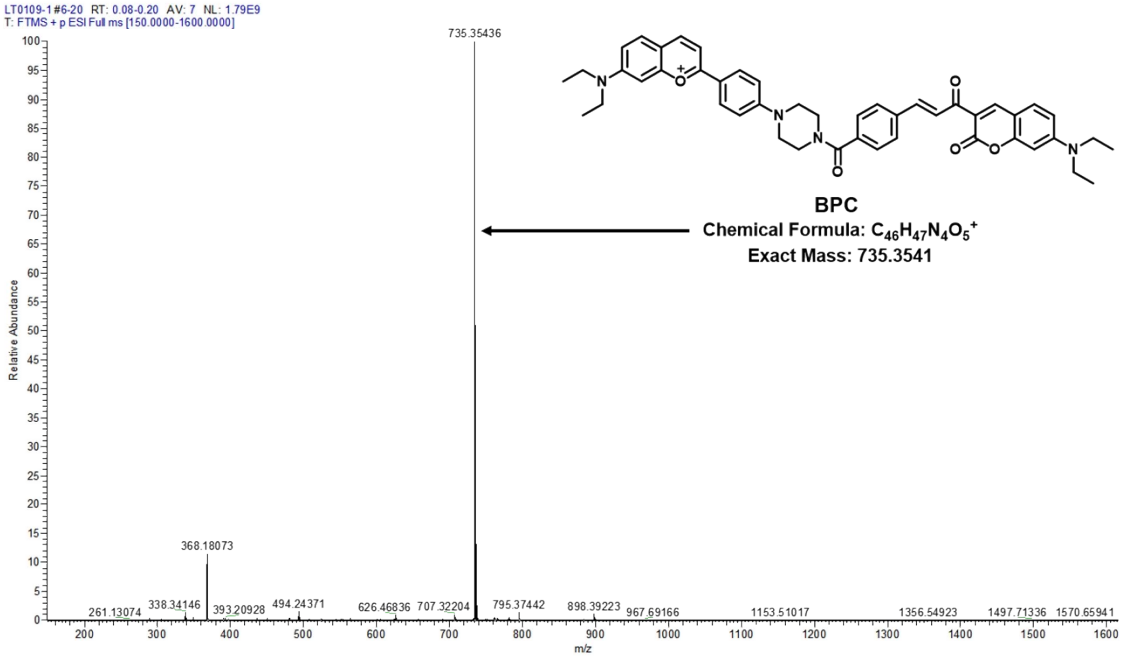


Figure S6: ESI-MS of probe **BPC**.

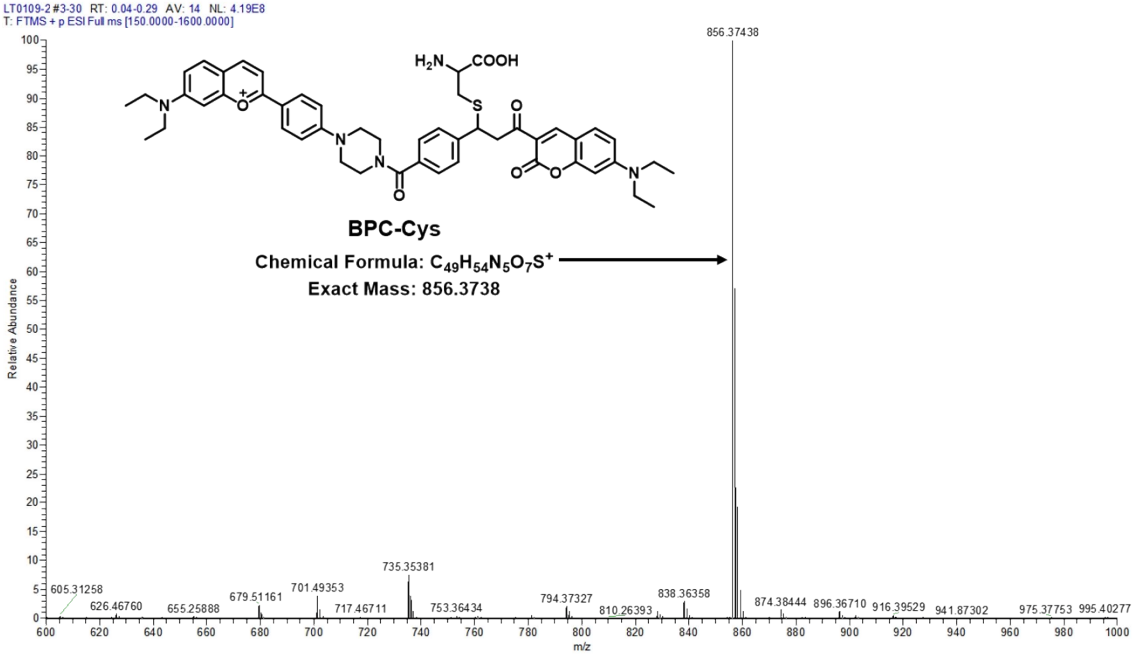


Figure S7: ESI-MS of probe **BPC** with Cys.

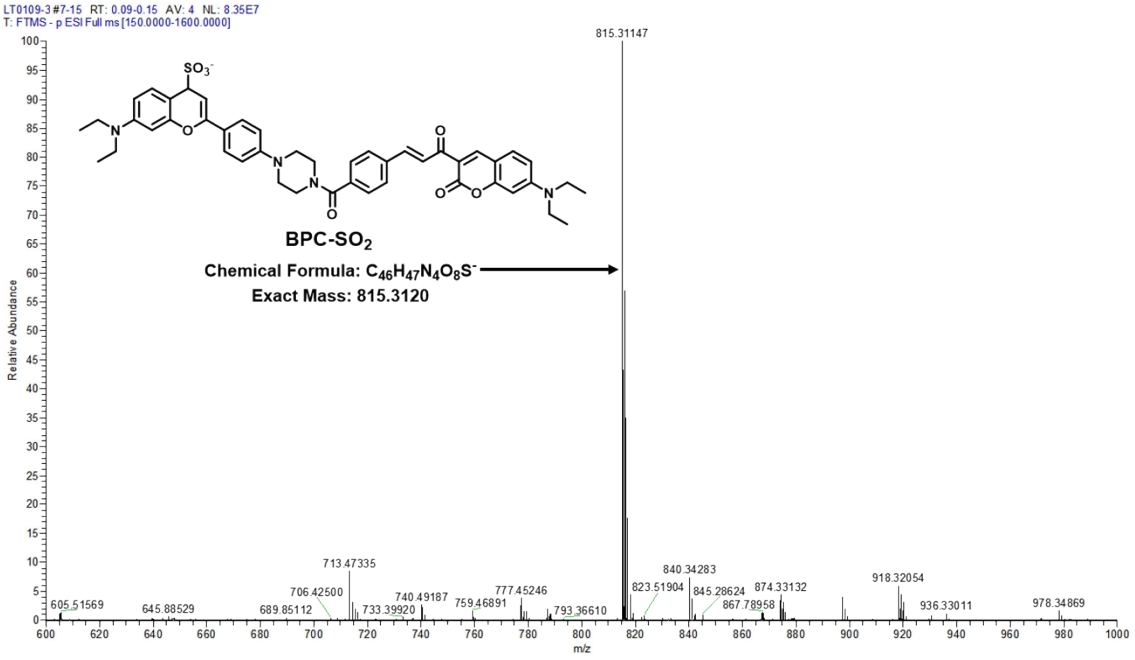


Figure S8: ESI-MS of probe **BPC** with SO_2.

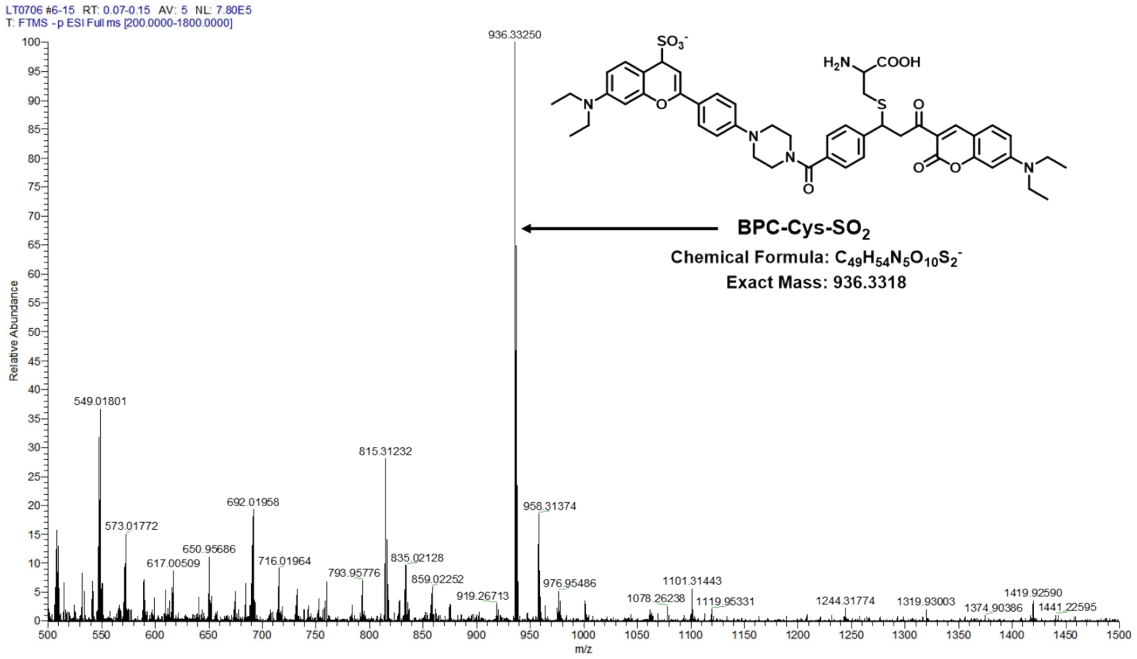


Figure S9: ESI-MS of probe **BPC** with Cys and SO_2.

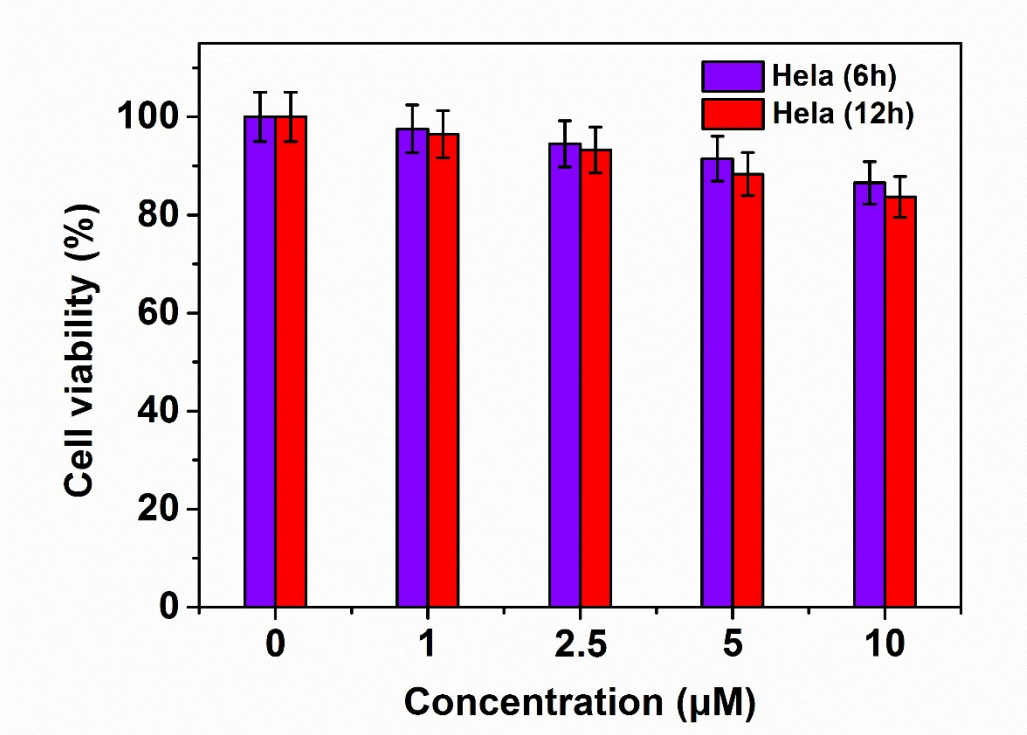


Figure S10: The cytotoxicity test.

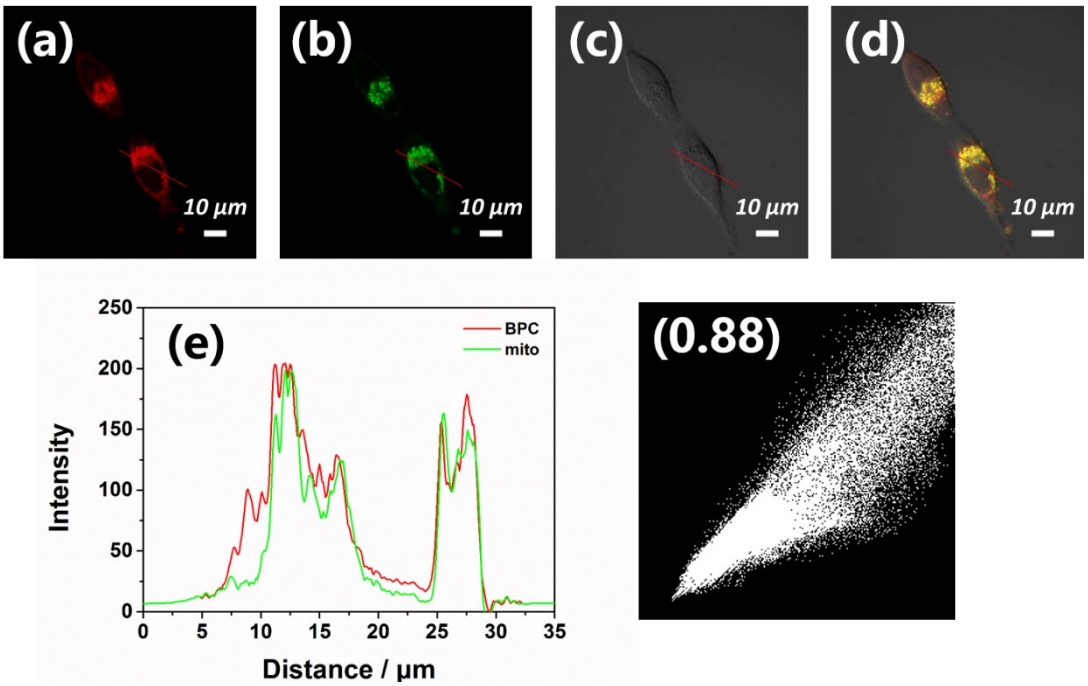
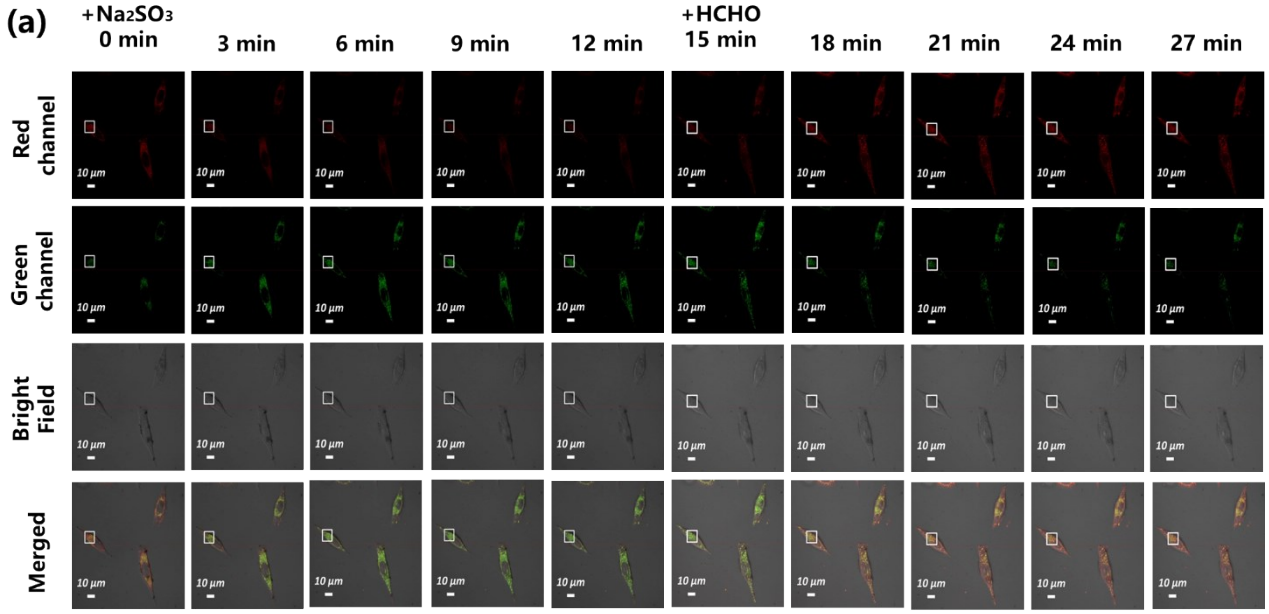


Figure S11: The Confocal fluorescence image of HeLa cells stained with **BPC** and MitoTracker Green. (a) Red channel (λ_{em} = 610-670 nm. λ_{ex} = 561 nm). (b) Green channel (λ_{em} = 490-530 nm. λ_{ex} = 458 nm). (c) Bright field pattern of HeLa cells. (d) Merged image of (a), (b) and (c). (e) Intensity profile of ROI in two channels.



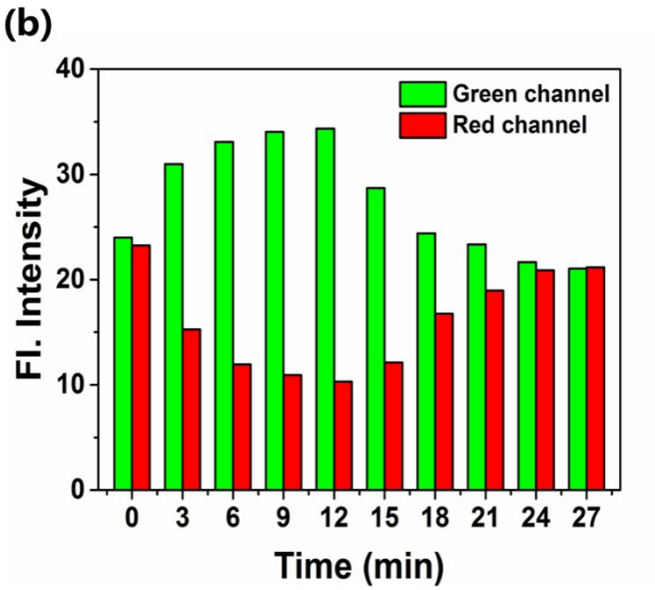


Figure S12: (a) Reversible fluorescence images of **BPC** (10 μM) with exogenous SO_2 (40 μM) and HCHO (40 μM) in livng cells; (b) Corresponding average fluorescence intensities of red and green channel in every 3 min (from 0 to 27 min). Red channel, λ_{em} = 610-670 nm; and green channel, λ_{em} = 470-530 nm (λ_{ex} = 458 nm). Scale bar: 10 μm.

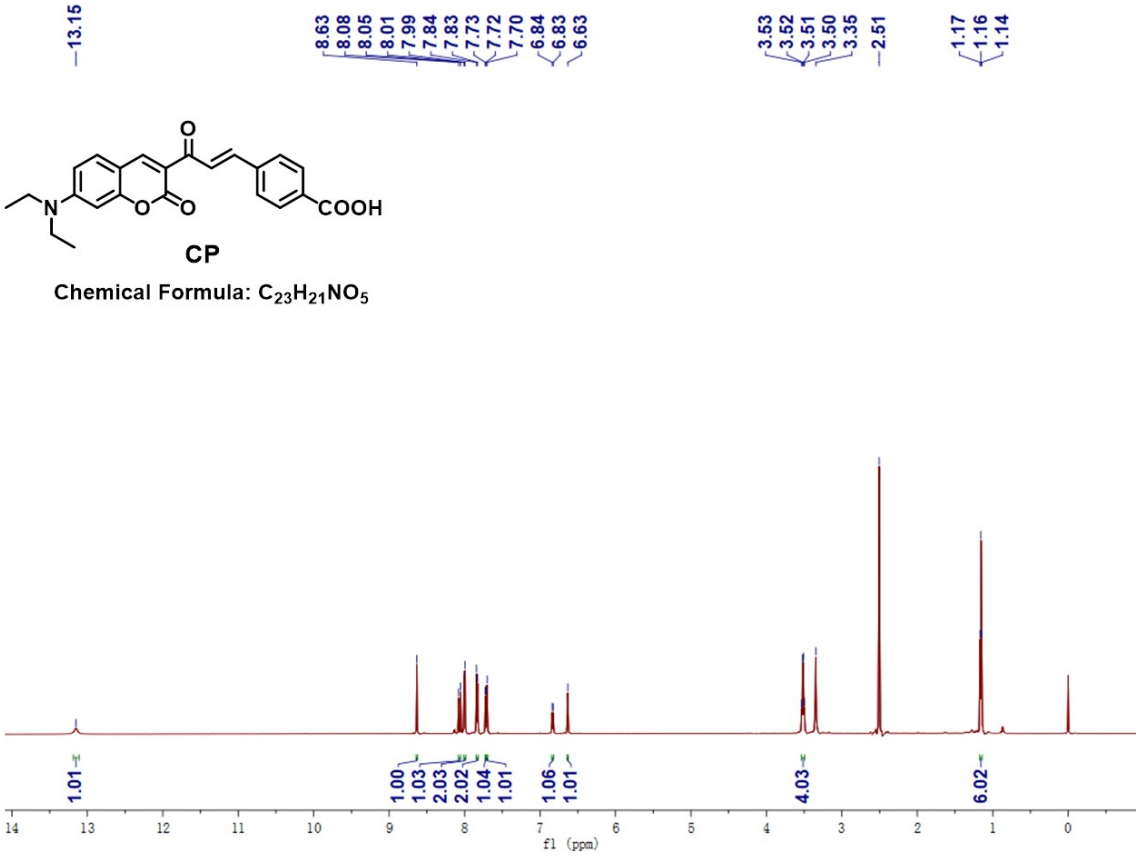


Figure S13: ^{1}H-NMR spectrum of **CP** in DMSO-d_6.

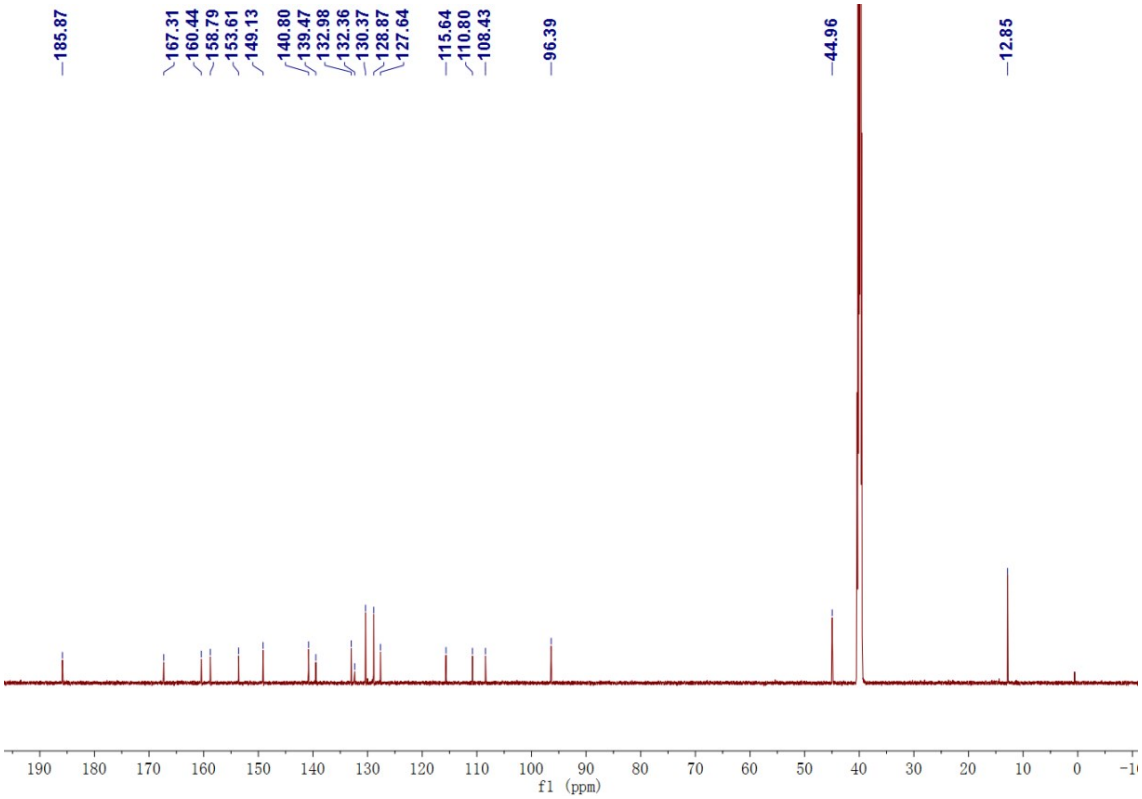


Figure S14: ^{13}C-NMR spectrum of **CP** in DMSO-d_6.

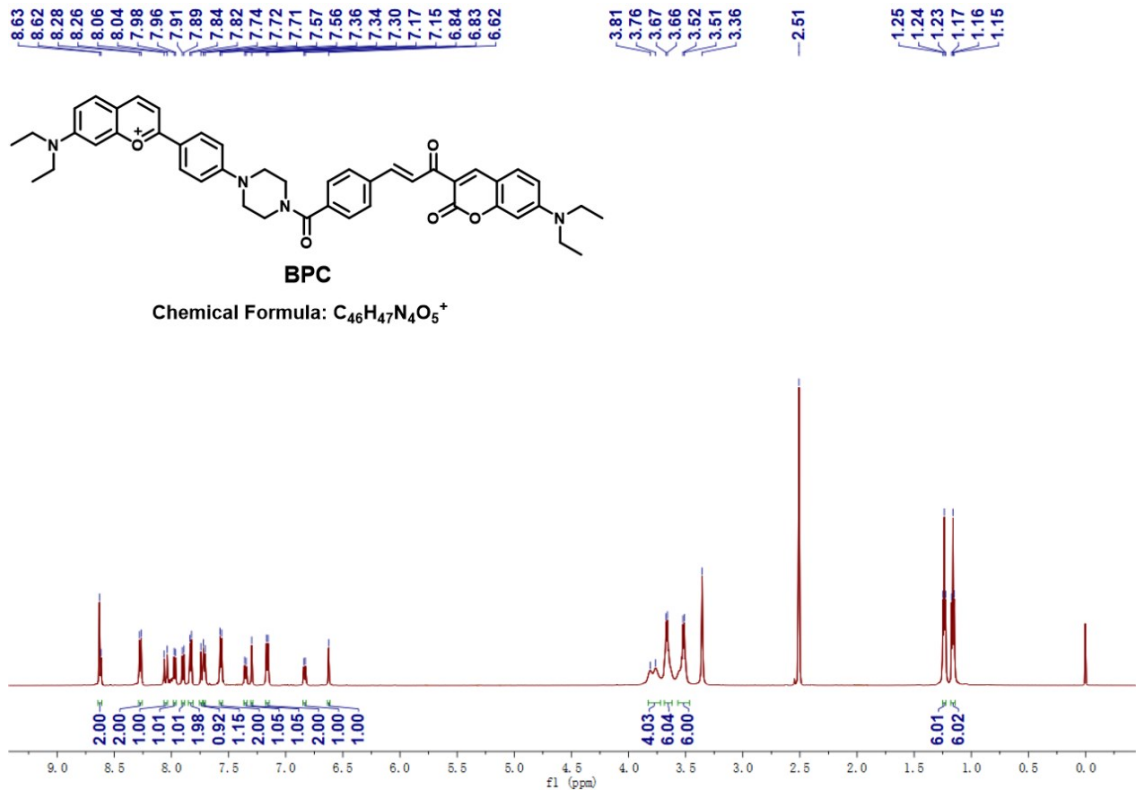


Figure S15: ^{1}H-NMR spectrum of **BPC** in DMSO-d_6.

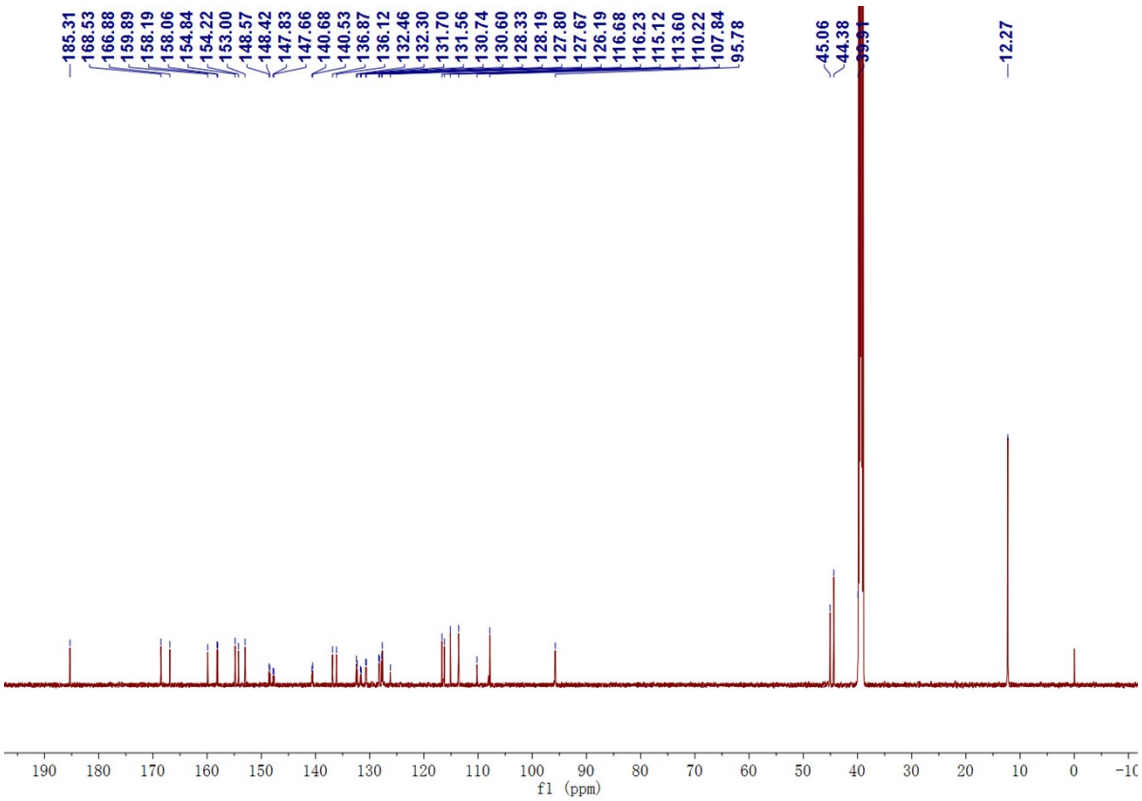


Figure S16: ^{13}C-NMR spectrum of **BPC** in DMSO-d_6.

REFERENCES

(1) Yin, C.X.; Li, X.Q.; Yue, Y.K.; Chao, J.B.; Zhang, Y.B.; Huo, F.J. *Sensors and Actuators B*., 2017, **246**, 615-622.

(2) Ma, Y.Y.; Gao, W.J.; Zhu, L.L.; Zhao. Y.P.; Lin, W.Y. *Chem. Commun.*, 2019, ***55***, 11263-11266.