Supporting Information for

Development of a unique reversible fluorescent probe for tracking endogenous sulfur dioxide and formaldehyde fluctuation *in vivo*

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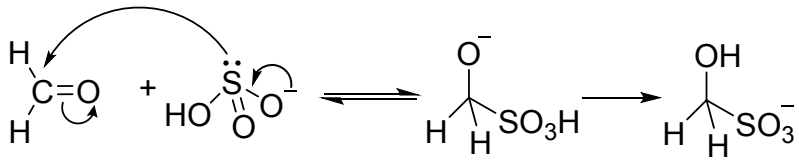
1. The reaction of FA and HSO_3^-



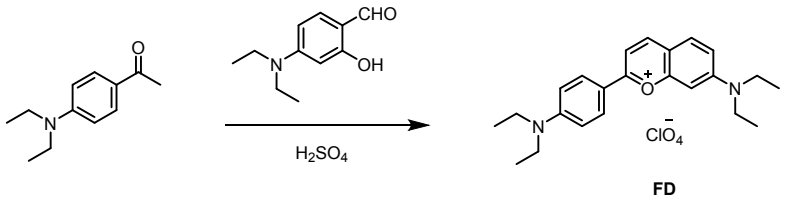
Scheme S1 The reaction of FA and HSO_3^-.[S1]

2. Materials and instruments

Unless otherwise stated, ultrapure water was used in all experiments and all reagents were obtained from commercial suppliers without further purification. Solvents were purified by standard methods prior to use. TLC analysis carried out on silica gel plates and column chromatography was conducted over silica gel (mesh 200−300), both of them were purchased from the Qingdao Ocean Chemicals. ^1H and ^{13}C NMR spectra were recorded on an AVANCE III 400 Nanobay (Bruker, 400 MHz for ^1H, 100 MHz for ^{13}C) at room temperature, using DMSO-d_6 as solvent and tetramethylsilane (TMS) as internal reference. High-resolution mass spectrometric (HRMS) analyses were measured on a Bruker apex-Ultra mass spectrometer (Bruker Daltonics Corp., USA) in electrospray ionization (ESI) mode. HRMS for response mechanism was collected using Agilent 6510 Q-TOF LC/MS for studying response mechanism.

3. Synthesis of compound

3.1 Synthesis route of the acceptor FD



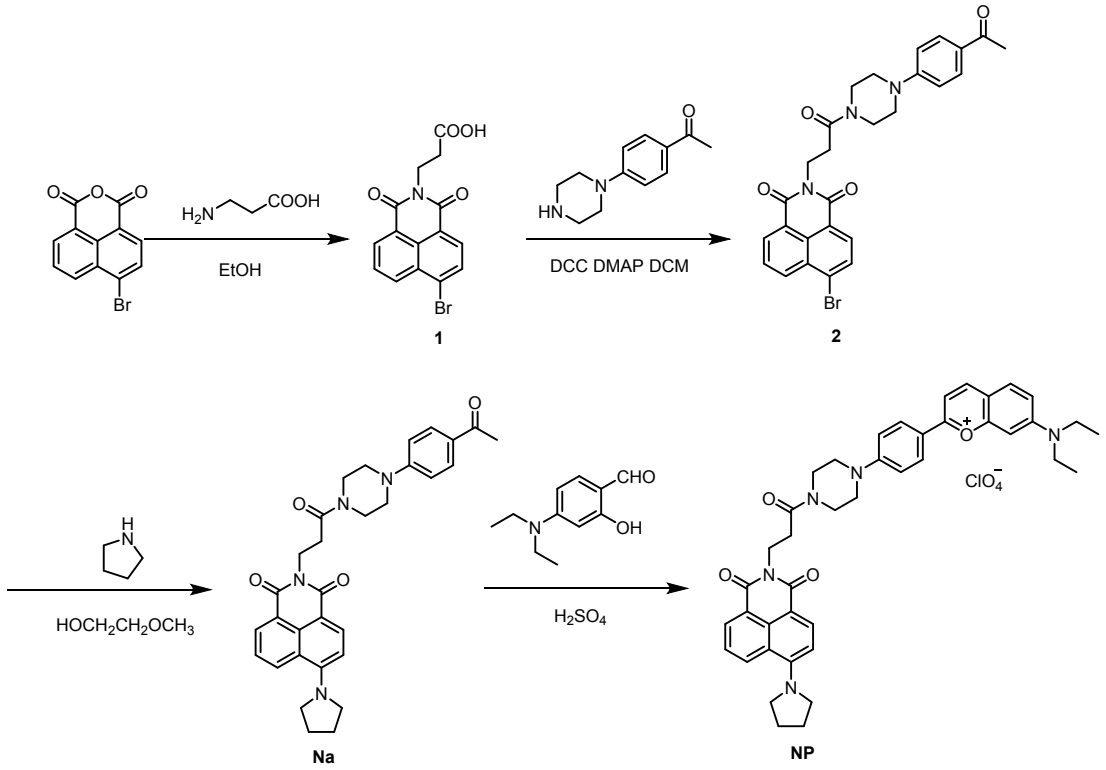
Scheme S2 Synthesis route of the acceptor **FD.**

3.1.1 Synthesis of compound FD.

4-diethylaMinoacetophenone (191 mg, 1 mmol) and 4-diethylamino-salicylaldehyde (386 mg, 2 mmol) were dissolved in conc. H_2SO_4 (10 mL) and stirred at 90 °C for 5 h. After cooling to room temperature, the solution was added 70% perchloric acid (1mL) and ice water (100 mL). Then the precipitated solid was filtered, and washed with water to afford crude product. The crude product was

purified by silica gel flash chromatography (CH_2Cl_2/CH_3OH, 15:1) to afford bluish violet solid.

3.2 Synthesis route of the fluorescent probe NP



Scheme S3 Synthesis route of **NP**.

3.2.1 Synthesis of compound 1

4-bromo-1,8-naphthalic anhydride(554 mg g, 2 mmol) and 3-aminopropanoic acid (356 mg, 4 mmol) were added into 10 mL ethanol and then refluxed for 3 h. After cooling to room temperature, the precipitated solid was filtered and washed with ethanol and dried under a vacuum to afford an gray solid of compound **1** (625 mg, 90%).

3.2.2 Synthesis of compound 2

A mixture of compound **1** (870 mg, 2.5 mmol), 4-piperazinoacetophenone (650 mg, 3 mmol), DCC (750 mg, 3.75 mmol) and DMAP (15 mg, 0.125mmol) in anhydrous dichloromethane (10 mL) was stirred for 20 h at room temperature. Then the mixture was evaporated under reduced pressure. The residue was purified by column chromatography (CH_2Cl_2: MeOH=30:1), and gave the yellow solid (735 mg, yield 55%).^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.59 (t, J =8.0 Hz, 2H), 8.36 (d, J = 8.0 Hz, 1H), 8.25 (d, J = 8.0 Hz, 1H), 8.02 (m, 1H), 7.82 (d, J = 8.0 Hz, 2H), 6.99 (d, J = 12 Hz, 2H), 4.27 (t, J = 8.0 Hz 2H), 3.61 (m, 4H), 3.42 (m, 4H), 2.77 (t, J = 8.0 Hz, 2H), 2.46 (s, 3H).

3.2.3 Synthesis of compound Na

Compound **2** (1.6 g, 3 mmol) and pyrrolidine (1 g, 15 mmol) in 10 mL 2-methoxyethanol were refluxed at 130 °C for 5 h. Then 5 mL ice-water was added into the mixture and precipitated solid was filtered. Purification by column chromatography (CH_2Cl_2: MeOH=25:2) afford compound **3** (1.4 g, 88%) as light yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.76 (t, *J* =8.0 Hz, 1H), 8.45 (d, *J* = 8.0 Hz, 1H), 8.26 (d, *J* = 8.0 Hz, 1H), 7.82 (d, *J* = 8.0 Hz, 2H), 7.63 (m, 1H), 6.98 (d, *J* = 8.0 Hz, 2H), 6.89 (t, *J* = 12.0 Hz, 1H), 4.25 (m, 2H), 3.78 (t, *J* = 6.0 Hz, 4H), 3.62 (m, 4H), 3.39 (m, *4*H), 2.73 (t, *J* = 8.0 Hz, 2H), 2.46 (s, 3H), 2.03 (m, 4H).

3.2.4 Synthesis of compound NP.

Compound **3** (800 mg, 0.5 mmol) and 4-diethylamino-salicylaldehyde (193 mg, 1 mmol) were dissolved in conc. H_2SO_4 (5 mL) and stirred at 90 °C for 5 h. After cooling to room temperature, the solution was added 70% perchloric acid (0.5 mL) and ice water (100 mL). Then the precipitated solid was filtered, and washed with water to afford crude product. The crude product was purified by silica gel flash chromatography (CH_2Cl_2/CH_3OH, 20:1) to afford bluish violet solid (204 mg, 60%). ^{1}H NMR (400 MHz, DMSO-*d6*) δ = 8.77 (d, *J* =8.0 Hz, 1H), 8.61 (d, *J* = 8.0 Hz, 1H), 8.46 (d, *J* = 4.0 Hz, 1H), 8.26 (dd, , J_1= 10 Hz, J_2= 2 Hz 3H), 7.96 (d, *J* = 8.0 Hz, 1H), 7.89 (d, *J* = 8.0 Hz, 1H), 7.64 (m, 1H), 7.34 (dd, , J_1= 8 Hz, J_2= 4 Hz 2H), 7.16 (d, *J* = 8.0 Hz, 2H), 6.90 (t, *J* = 8.0 Hz, 1H), 4.27 (t, *J* = 8.0 Hz, 2H), 3.78 (s, 4H), 3.68 (m,12H), 2.75 (t, , *J* = 6.0 Hz, 2H), 2.03 (m, 4H) 1.24 (t, *J* = 8.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO) δ 169.51, 167.34, 164.17, 163.12, 158.43, 155.34, 154.76, 152.50, 148.00, 133.26, 133.05, 132.09, 131.14, 130.95, 123.53, 122.06, 121.82, 116.97, 116.76, 116.61, 114.00, 109.05, 108.80, 108.53, 96.36, 53.28, 46.04, 45.64, 44.49, 36.41, 31.73, 26.02, 12.92. HRMS (ESI): calcd. for $C_{34}H_{30}N_3OS^+$, $[M]^+$, m/z, 682.3388, found: 682.2386.

4. Measurements

Materials and instruments

The pH measurements were performed with a Mettler-Toledo Delta 320 pH meter. UV−vis absorption spectra were obtained on a Shimadzu UV-2700 spectrophotometer and fluorescence spectra were measured on a HITACHI F4600 fluorescence spectrophotometer. MTT was purchased from J&K Scien-tific Ltd. Fluorescence imaging experiments were performed with Nikon A1MP confocal microscopy. All animal procedures for this study were approved by the Animal Ethical Experimentation

Committee of Shandong University according to the requirements of the National Act on the use of experimental animals (China). In vivo imaging was carried out on a PerkinElmer IVIS Spectrum imaging system equipped with a -90 °C CCD camera.

Spectral measurements of selectivity

The stock solution of **NP** (1 mM) was prepared in CH_3CN. The various testing analytes stock solutions ($NaHSO_3$, NH_4OAc, $NaNO_2$, KCl, KNO_3, KSCN, H_2O_2, Na_2HPO_4, Na_2S, Cys, $BaCl_2$, $AlCl_3$, benzaldehyde, $MgCl_2$, Hcy, $ZnCl_2$, KI, KF, GSH, NaBr, $CaCl_2$, NaClO, TBHP, CH_3CHO, $FeSO_4$, and FA) were prepared at 100 mM in twice distilled water, and the di-*t*-butyl peroxide (DTBP) stock solution (100 mM) was prepared in the DMSO. The test solution contained **NP** (10 μM), PBS buffer (pH 7.4, 10 mM, 20% CH_3CN) and the anions and cations at 1 mM, ROS at 100 μM, representative amino acid at 500 μM, GSH at 2.5 mM, Na_2S at 50 μM and other analytes at 500 μM. For the selectivity of FA, the probe were pre-treated with 50 μM $NaHSO_3$, then the appropriate concentration of each analytes were added. After that, the fluorescence spectra were measured. Unless otherwise noted, for all the measurements, the excitation wavelength was 446 nm, the excitation slit widths were 5 nm, and emission slit widths were 5 nm.

Detection limit of NP for SO_2

The detection limit was based on a reported method. [S2]According to the result of titrating experiment, the fluorescence ratio intensities (I_{540}/I_{645}) of **NP** treated with different $NaHSO_3$ were normalized between the minimum intensity and the maximum intensity. A linear regression curve was then fitted to the normalized fluorescent intensity data and the point at which this line crossed the axis was considered as the detection limit (8.6×10^{-7} M).

Kinetic analysis of the reaction of probe against SO_2 and FA

Kinetic parameters of the probe (10 μM) were determined by fluorescence spectroscopy with a F4600 fluorescence spectrophotometer (Hitachi). The changes of fluorescence intensity upon addition of 50 μM $NaHSO_3$ was recorded at a scan rate of 3 s and upon addition of 200 μM FA was recorded at a scan rate of 30 s.

The reversibility cycle of NP

NP (10 μM) was dissolved in 10 mM PBS buffer containing 20% CH_3CN as a cosolvent. $NaHSO_3$ and FA with mole ratio of 1:4 were added alternately for reversibility.

Cytotoxicity assays

The cytotoxicity of **NP** to HeLa cells were performed by MTT. 2×10^4 cells/mL HeLa cells were seeded in 96-well plates respectively and then incubated with various concentrations of **NP** (0 - 50 μM) for 24 h. After that 10 μL MTT (5 mg/mL) was added to each well. After incubated for 4 h, the supernatants were aspirated and 100 μL DMSO was added. The absorbance of the solution at 570 nm was recorded using microplate reader. The cell viability (%) = $(OD_{sample}-OD_{blank}) / (OD_{control}-OD_{blank}) \times 100$ %.

OD_{sample} denotes the cells cultured with different concentrations of **CaP**, $OD_{control}$ denotes the cells incubated with culture medium, OD_{blank} denotes only the culture medium.

Cell culture and fluorescence imaging experiment

HeLa cells were cultured in Dulbecco's Modified Eagle Medium media (DMEM, Hyclone) supplemented with 10 % fetal bovine serum (FBS, Sijiqing), penicillin (100 U/ml, Hyclone) and streptomycin sulfate (100 U/ml, Hyclone) under an atmosphere of 5% CO_2 and 95% air at 37 °C.

Before the imaging experiments, the HeLa cells were seeded into 35 mm glass bottom dishes for 24 h incubation. Then the cells were treated with 10 μM **NP** for 30 min and washed twice with PBS. The fluorescence images were acquired with Nikon A1R confocal microscope with a 40× objective lens. After that the HeLa cells were further incubated with 100 μM $NaHSO_3$ for another 30 min and prepared for imaging. Experiments to assess reversible variation were performed in the same media supplemented with 400 μM FA for another 30 min. For the reversible cycle experiment, HeLa cells then continuously treated with 100 μM $NaHSO_3$ and 400 μM FA for reversibility, and then imaged. Emission was collected at 500−550 nm (excitation wavelength at 405 nm) for green channel and at 570−620 nm for red channel.

For the endogenous SO_2 experiment, the HeLa cells incubated with 10 μM **NP** in 1 mL medium for 30 min at 37 °C in 5 % CO_2 incubator were washed with PBS and further incubated in the fresh medium, and then imaged. After that, the medium was changed to DMEM containing 200 μM Cys for 1 h and then imaged. For the endogenous reversible variation, the culture medium of the above cells was changed to DMEM containing 400 μM Tet and further incubated for 1 h and imaged. Subsequently, the

same concentration of Cys and Tet were added alternately for imaging.

Flow cytometry analysis of the effect of interaction between SO_2 and FA in living cells

U251 cells treated with 1 mM FA and then 0 mM, 1 mM and 2 mM $NaHSO_3$ was added, respectively, for 24 h incubation. After that the U251 cells were washed with PBS and digested with trypsin. To detect apoptosis, 500 ml PBS, 5 μl Annexin V-FITC and 5 μl PI were added to each tube, and then incubated in the dark at room temperature for 15 min, followed by flow cytometric testing (BD LSRFortessa).

Western blot analysis

U251 cells treated with 1 mM FA and then 0 mM, 1 mM and 2 mM $NaHSO_3$ was added respectively. After 24 h incubation the U251 cells were washed with ice PBS for three times, and then lysed in an ice-cold lysis buffer (radio immunoprecipitation assay (RIPA): phenylmethylsulphonylfluoride (PMSF) =100:1) for 30 min. And then the samples were centrifuged at 14 000 r.p.m. for 25 min at 4 ℃ and the supernatant were obtained. Protein concentration was assessed using a BCA Protein Assay Kit (Beyotime, Shanghai, China). Equivalent amounts of protein for each sample were run on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were then transferred to a PVDF membrane, and blocked with skimmed milk in TBST buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.05% Tween-20) for 1.5 h. The PVDF membranes were incubated with primary antibody diluent (anti- Caspase 3) overnight at 4℃. After washing 3 times with TBST buffer, the membrane was incubated in blocking solution containing secondary antibody for 1 h. Next, the membrane was washed in TBST buffer for 3 times. After removing the residual solution of PVDF membrane, appropriate amount of ECL (electrogenerated chemiluminescence reaction solutions) was added to the protein surface of the membrane and the signal of the immunoblots was visualized using an image analysis system equipped with a software CLINX ChemiScope 6000.

Reversible imaging SO_2 and FA in living mice

The animal studies were approved by the Institutional Animal Care and Use Committee of the Shandong University. 4-week old female balb/c mice were purchased from School of Pharmaceutical

Sciences, Shandong University. Mice were kindly kept in all the experimental process.

The abdominal fur of mice was removed by an electric shaver, then they were anesthetized by 4% chloral hydrate aqueous solution, 100 μL **NP** (50 μM in PBS with 5% CH_3CN) was then injected by intraperitoneal injection. The fluorescence image was collected with an IVIS Lumina XR in vivo imaging system with an excitation filter of 580 nm and an emission filter of 620 nm. After that 100 μL $NaHSO_3$ (500 μM in PBS) was injected into the same location and the fluorescence was collected under the same instrumental condition. Then 100 μL FA (2 mM in PBS) was injected into the same location and imaged under the same instrumental condition. The statistical analysis was performed from three separate biological replicates. A region of interest (ROI) was created around in each image and the mean fluorescence intensity of each sample was measured (via "Measure" function) and averaged across the three fields imaged.

For the imaging of endogenous SO_2 in living mice, the mice was injected 100 μL **NP** (50 μM in PBS with 5% CH_3CN) by intraperitoneal injection after depilation and anesthesia. The fluorescence image was collected with an IVIS Lumina XR in vivo imaging system. After that 100 μL FA (2 mM in PBS) was injected into the same location and the fluorescence was collected under the same instrumental condition. The statistical analysis was performed from three separate biological replicates. A region of interest (ROI) was created around in each image and the mean fluorescence intensity of each sample was measured (via "Measure" function) and averaged across the three fields imaged.

For the imaging of endogenous SO_2 and FA in living mice, the mice was injected 100 μL **NP** (50 μM in PBS with 5% CH_3CN) by intraperitoneal injection after depilation and anesthesia. The fluorescence image was collected with an IVIS Lumina XR in vivo imaging system. After that 100 μL Tet (4 mM in PBS) was injected into the same location and the fluorescence was collected under the same instrumental condition. Then 100 μL $NaHSO_3$ (5 mM in PBS) was injected into the same location and the fluorescence was collected under the same instrumental condition. The statistical analysis was performed from three separate biological replicates. A region of interest (ROI) was created around in each image and the mean fluorescence intensity of each sample was measured (via "Measure" function) and averaged across the three fields imaged.

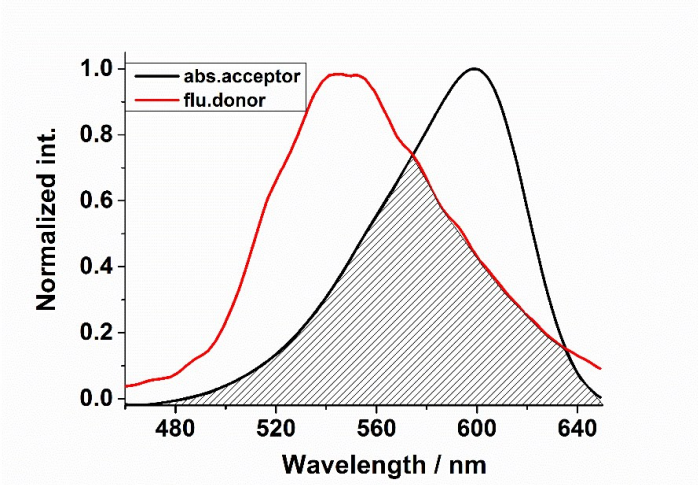


Fig. S1. Normalized absorption spectra (abs) and fluorescence spectra (flu) of acceptor flavylium derivative (**FD**) and donor naphthalimide (**Na**) at pH 7.4 in PBS buffers (20% CH_3CN). The gray area represents the overlap between the fluorescence spectrum of **Na** and the absorption spectrum of **FD**. Fluorescence spectra for **Na**, λ_{ex} = 446 nm.

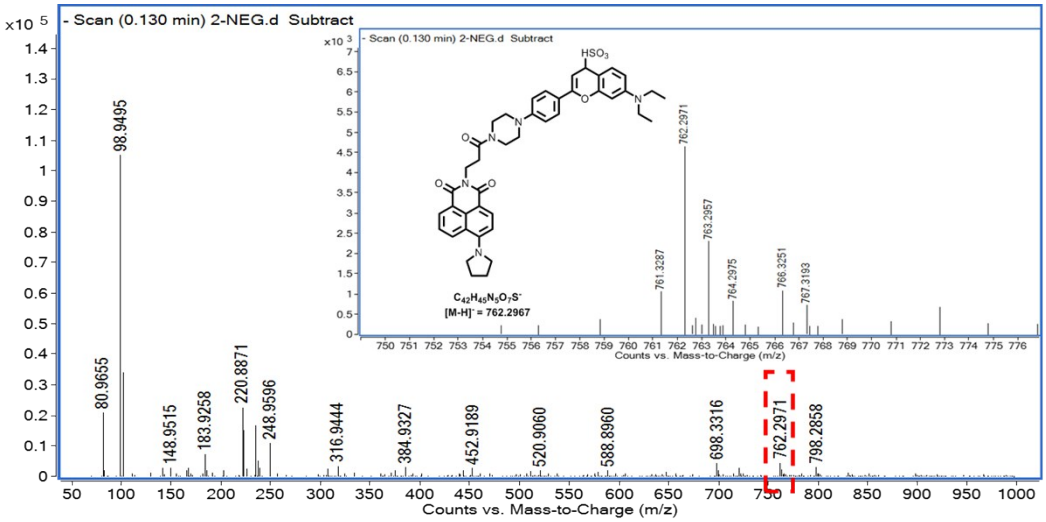


Fig. S2. HR-MS spectrum of the reaction of 20 μM **NP** with 100 μM $NaHSO_3$ in H_2O. Inset: the partial HR-MS spectrum.

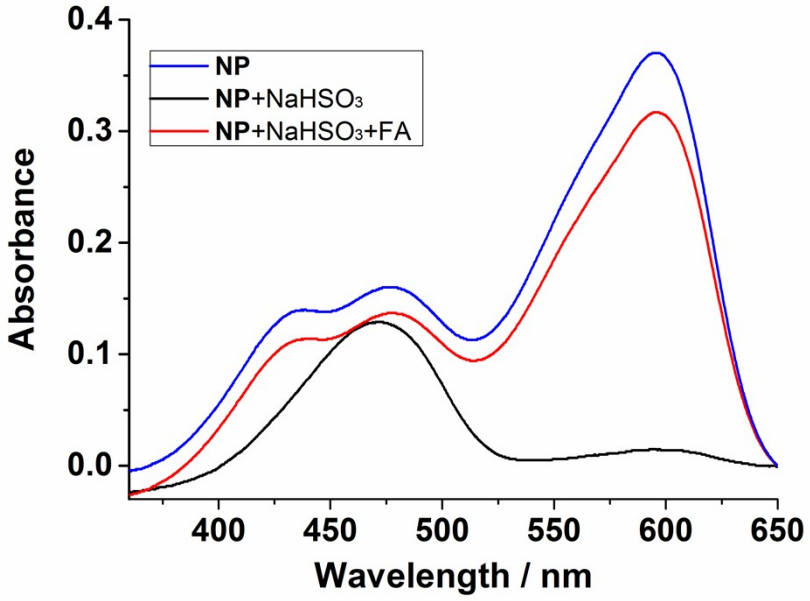


Fig. S3. Absorbance spectral changes of the probe **NP** (10 μM) upon treated with 50 μM $NaSHO_3$ and FA (200 μM).

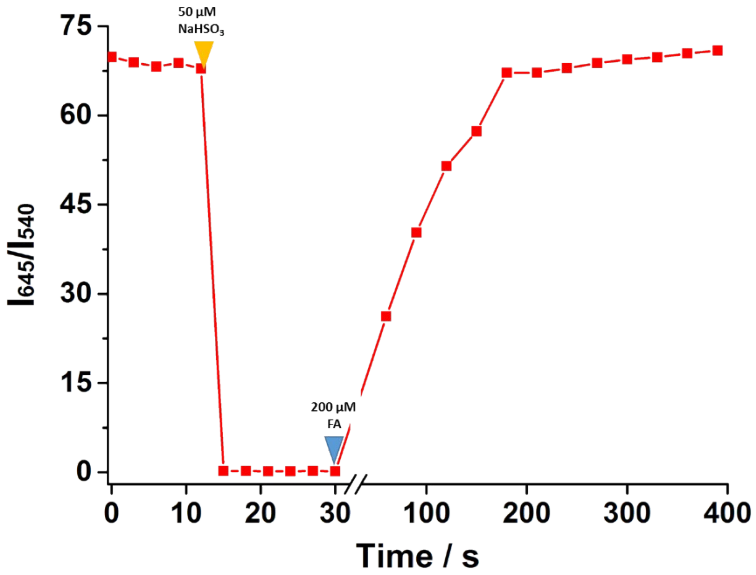


Fig. S4. Time-dependent fluorescence intensity ratios (I_{645}/I_{540}) of **NP** upon addition of $NaHSO_3$ (50 μM) and FA (200 μM).

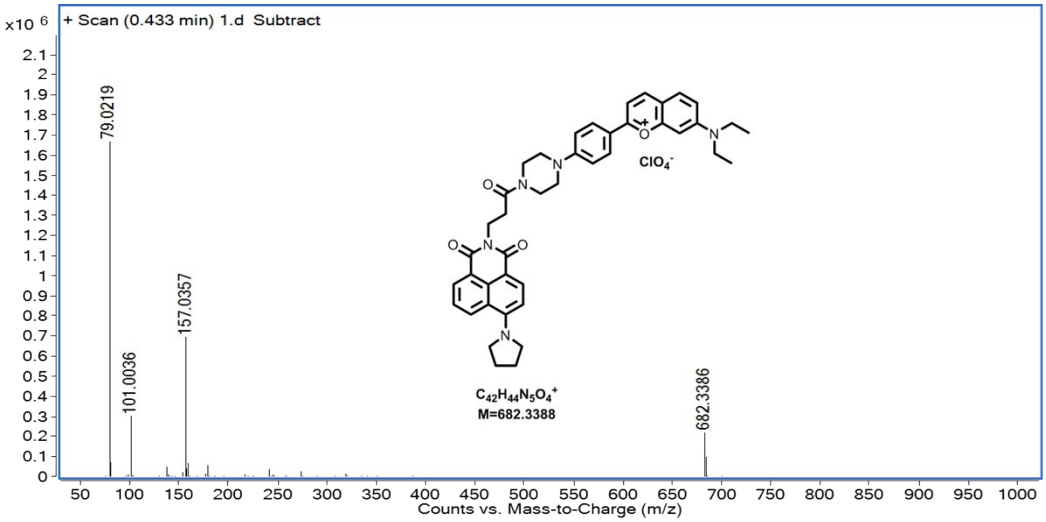


Fig. S5. HR-MS spectrum of the mixture of 20 μM **NP** with 100 μM $NaHSO_3$ upon addition of 400 μM FA.

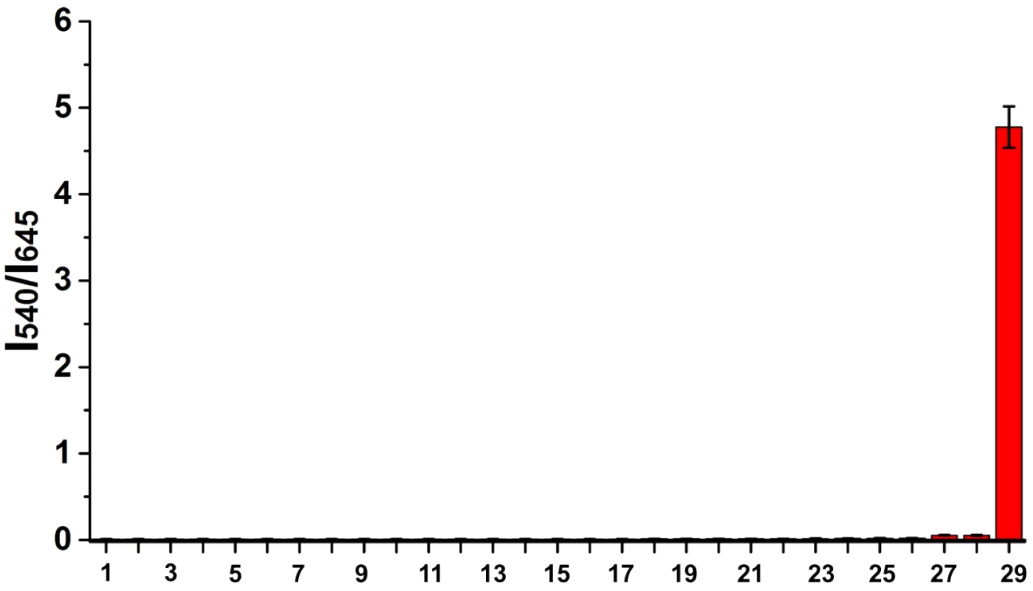


Fig. S6. Fluorescent ratio (I_{540}/I_{645}) of the probe **NP** (10 μM) to various relevant analytes in PBS buffer. 1, only probe **NP** (10 μM); 2, FA (1 mM); 3, KF (1 mM); 4, TBHP (100 μM); 5, KNO_3 (1 mM); 6, KI (1 mM); 7, *ditert*-butyl peroxide (DTBP) (100 μM); 8, $AlCl_3$ (1 mM); 9, $MgCl_2$ (1 mM); 10, KSCN (1 mM); 11, Na_2HPO_4 (1 mM); 12, KCl (1 mM); 13, NaBr (1 mM); 14, $CaCl_2$ (1 mM); 15, H_2O_2 (100 μM); 16, CH_3CHO (500 μM); 17, NH_4OAc (1 mM); 18, $BaCl_2$ (1 mM); 19, H_2O_2 (100 μM); 20, NaClO (100 μM); 21, $ZnCl_2$ (1 mM); 22, $NaNO_2$ (1 mM); 23, $FeSO_4$ (500 μM); 24, Hcy (500 μM); 25, Cys (500 μM); 26, GSH (2.5 mM); 27, Na_2S (50 μM); 28, KO_2 (1 mM); 29, $NaHSO_3$ (50 μM). Error bars represent mean values ± SD. (n = 3)

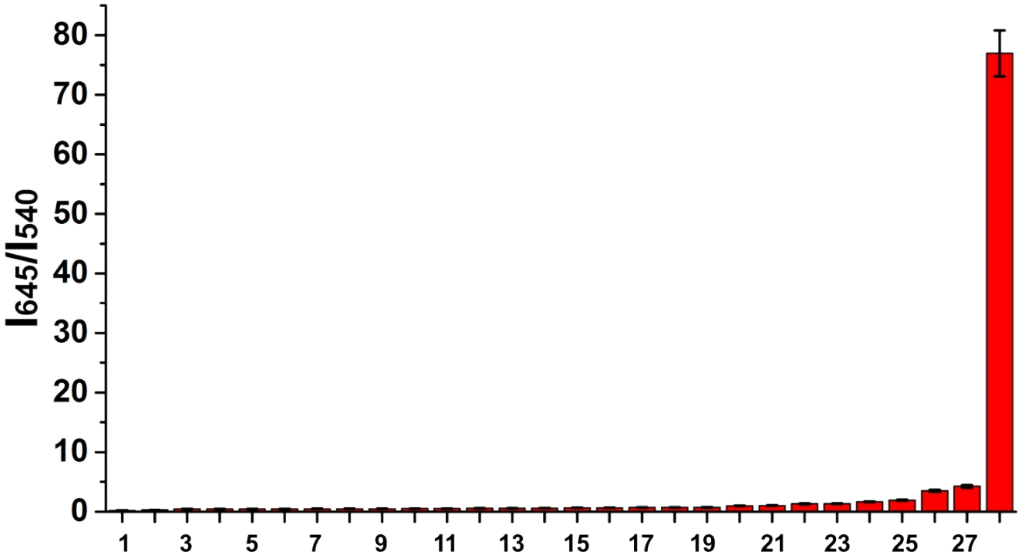


Fig. S7. Fluorescent ratio (I_{645}/I_{540}) of the probe **NP** (10 μM) in the presence of 50 μM $NaHSO_3$ upon addition of various relevant analytes: 1, $NaHSO_3$ (50 μM); 2, KO_2 (1 mM); 3, NH_4OAc (1 mM); 4, $NaNO_2$ (1 mM); 5,KCl (1 mM); 6, KNO_3 (1 mM); 7, KSCN (1 mM); 8, $CaCl_2$ (1 mM); 9, *ditert*-butyl peroxide (DTBP) (100 μM); 10, Na_2HPO_4 (1 mM); 11, Na_2S (50 μM); 12, Cys (500 μM); 13, $BaCl_2$ (1 mM); 14, $AlCl_3$ (1 mM); 15, NaBr (1 mM); 16, $MgCl_2$ (1 mM); 17, Hcy (500 μM); 18, $ZnCl_2$ (1 mM); 19, KI (1 mM); 20, KF (1 mM); 21, GSH (2.5 mM); 22, $FeSO_4$ (500 μM); 23, TBHP (100 μM); 24, H_2O_2 (100 μM); 25, NaClO (100 μM) ; 26, Benzaldehyde (500 μM); 27 CH_3CHO (500 μM); 28, FA (200 μM).

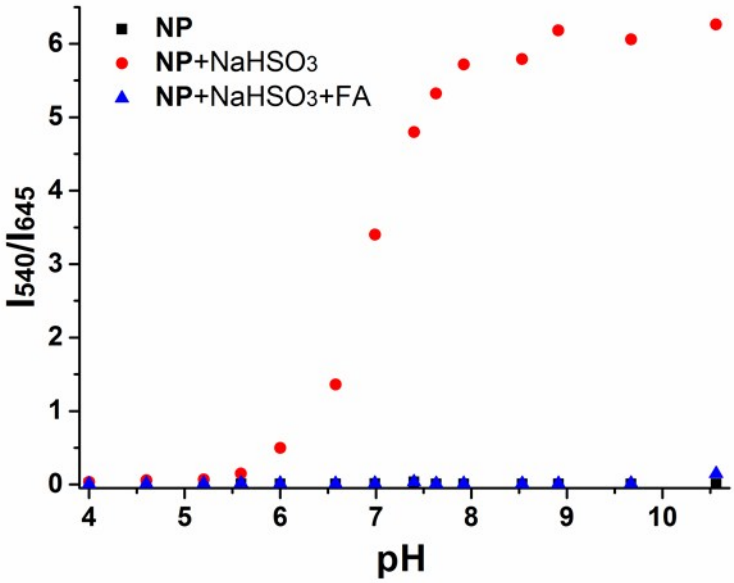


Fig. S8. Fluorescent ratios (I_{540}/I_{645}) of **NP** (10 μM) toward $NaHSO_3$ (50 μM) and FA (200 μM) in PBS

(20 % CH_3CN) at various pH values.

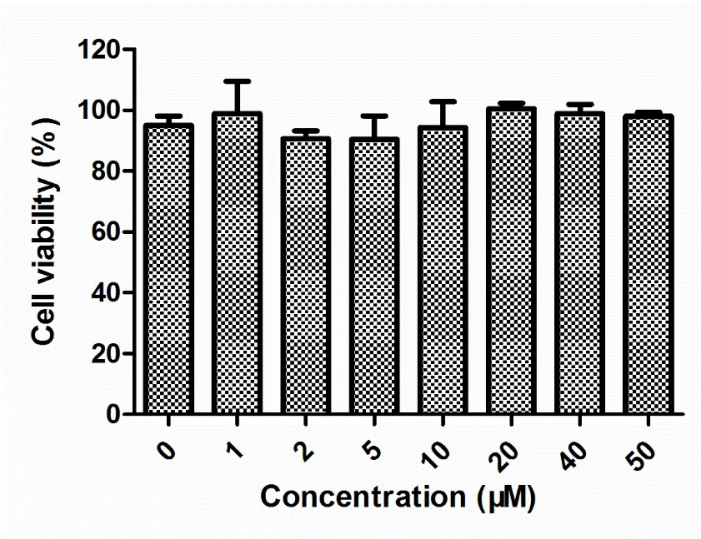


Fig. S9. Viability of HeLa cells treated with various concentrations (0 - 50 μM) of **NP** for 24 h. Error bars represent mean values ± SD. (n = 3).

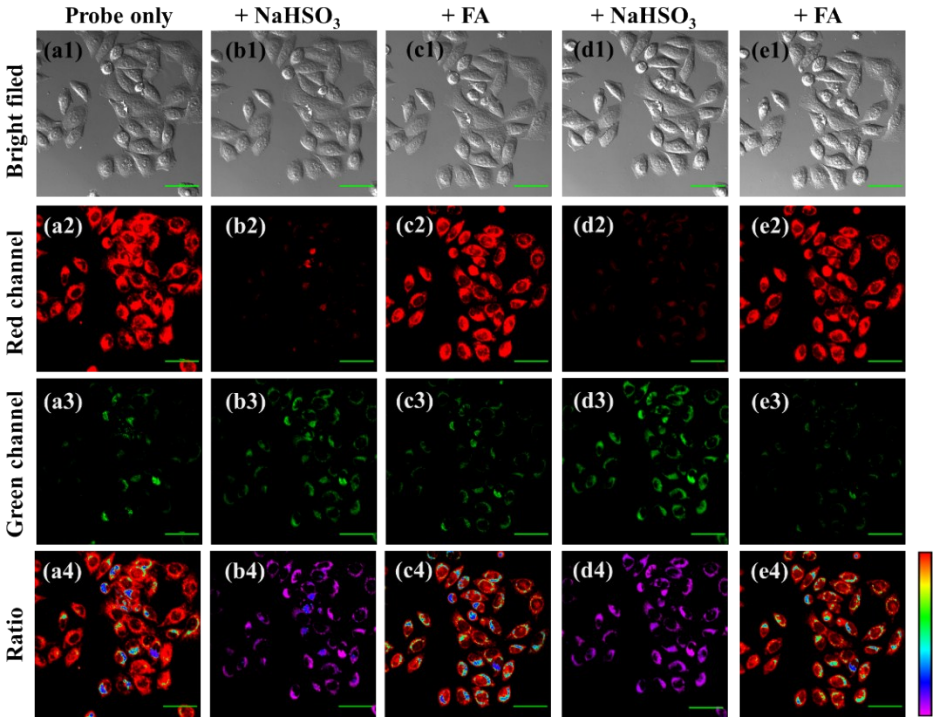


Fig. S10. Reversible cycle of **NP** by addition of exogenous SO_2 and FA in living cells. (a1)-(e1) Bright-filed images of HeLa cells continuously treated with 10 μM **NP** (a1)-(a4), 100 μM $NaHSO_3$ (b1)-(b4), 400 μM FA (c1)-(c4), 100 μM $NaHSO_3$ (d1)-(d4), 400 μM FA (e1)-(e4) for 30 min, respectively. (a2)-(e2) Red channel: λ_{ex} = 405 nm, λ_{em} = 663−738 nm. (a3)-(e3) Green channel: λ_{ex} = 405 nm, λ_{em} =

500−550 nm. (a4)-(e4) Fluorescence ratio images of red channel and green channel. Scale bar: 20 μm.

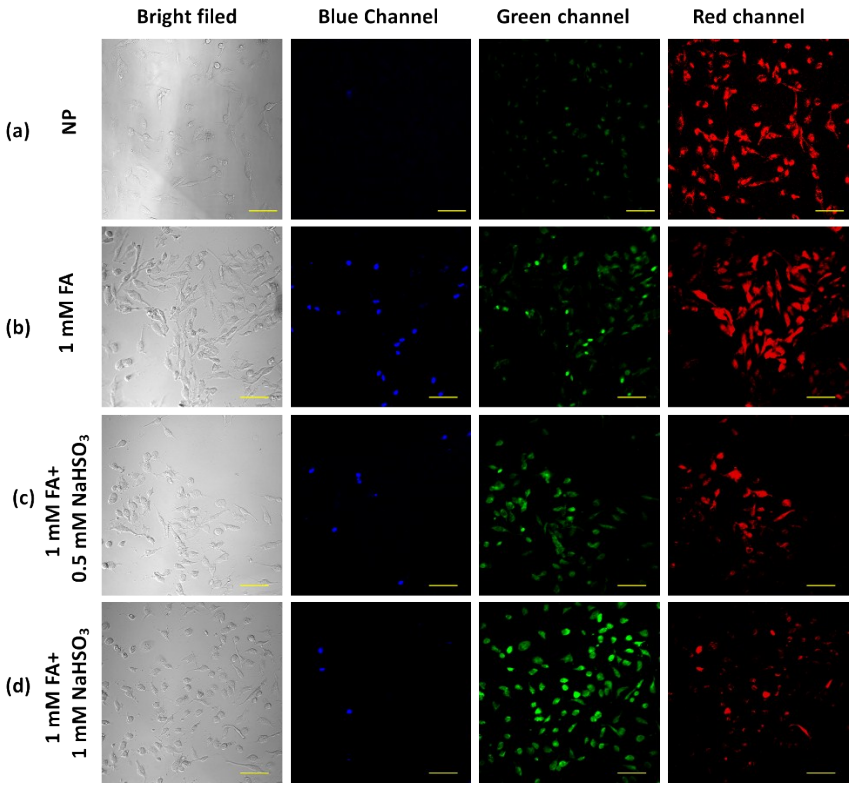


Fig. S11. The apoptosis of U251 cells was visualized by fluorescence imaging. U251 cells were treated with 1 mM of FA in the absence (a) or presence of 0.5 (b) or 1 mM (c) $NaHSO_3$ for 24 h followed by addition of 10 μM **NP** for 30 min and 2.5 μM SYTOX Green Nucleic Acid Stain for 5 min.

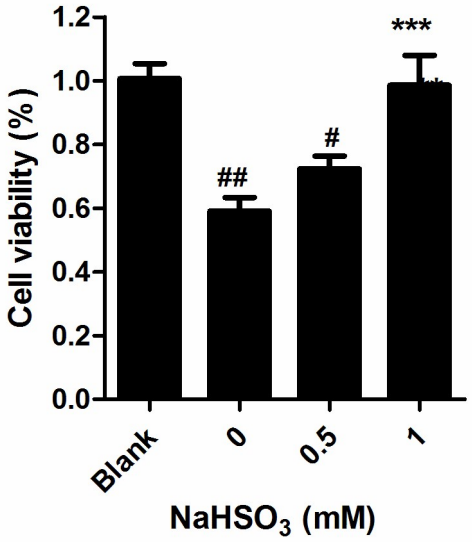


Fig. S12. Effect of SO_2 on formaldehyde-induced cytotoxicity in U251 cells. U251 cells were treated with 1 mM of FA in the absence or presence of 0.5 or 1 mM $NaHSO_3$ for 24 h. Values are the mean ± SEM (n = 3). $^{\#}P < 0.05$, versus non-treated control group (Blank); $^{*}P < 0.05$, versus FA-treated alone

group.

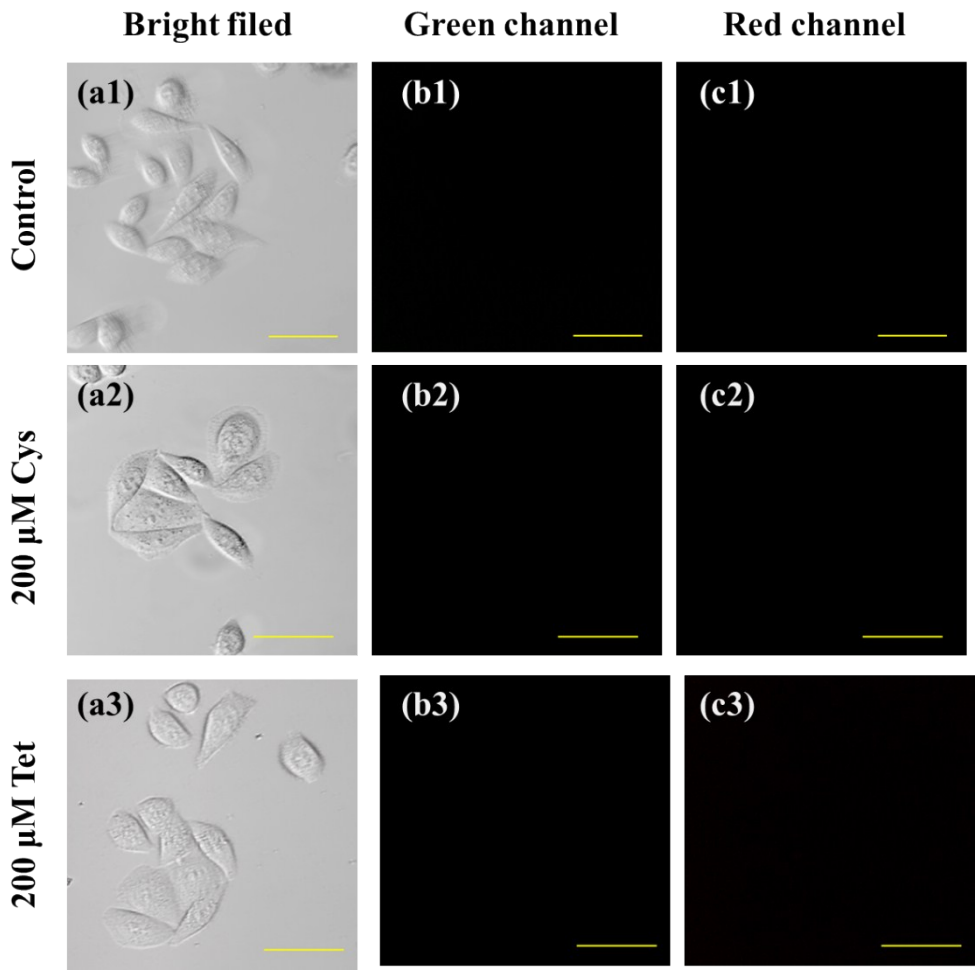


Fig. S13. Fluorescence imaging of HeLa cells (a1)-(c1) treated with 200 μM Cys (a2)-(b2) and 400 μM Tet (a3)-(c3). (a1) Bright-field image of HeLa cells. (b1) and (c1) are the fluorescence image of (a1) in green channel and red channel. (a2) Bright-field image of HeLa cells treated with Cys. (b2) and (c2) are the fluorescence image of (a2) in green channel and red channel. (a3) Bright-field image of HeLa cells treated with Tet. (b3) and (c3) are the fluorescence image of (a3) in green channel and red channel.

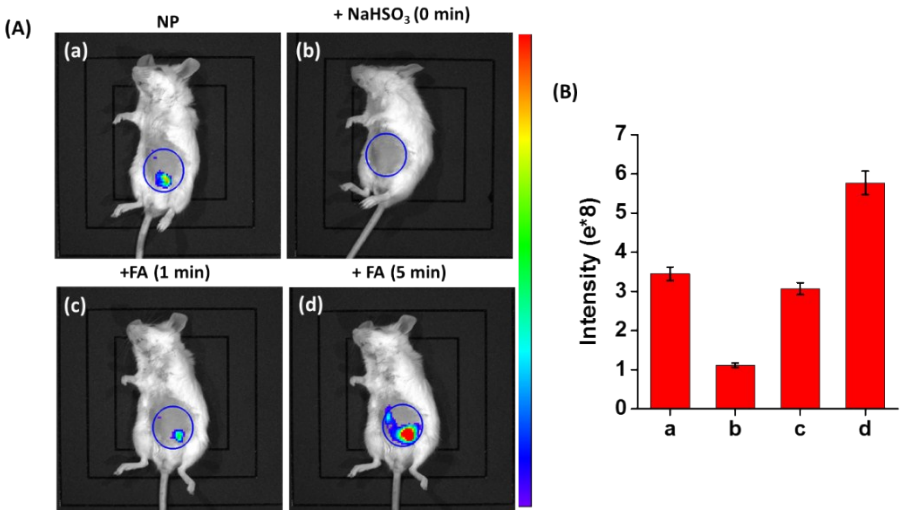


Fig. S14. *In vivo* fluorescence images of exogenous SO_2 and FA in living mice by using **NP**. (A) Time-dependent fluorescence images of exogenous SO_2 and FA. First row: the mice was injected with only 100 μL of 50 μM **NP** (a) and then injected 100 μL of 500 μM $NaHSO_3$ (b). Second row: 100 μL of 50 μM **NP** and 100 μL of 500 μM $NaHSO_3$ were injected, then 100 μL of 2 mM FA was injected and imaged for 1 min (c) and 5 min (d), respectively. (B) The relative average fluorescence intensity change of (A) in the blue circle area. Error bars represent standard deviation (±S.D.). n = 3, the statistical analysis was performed from three separate biological replicates. λ_{ex} = 580 nm, λ_{em} = 620 nm.

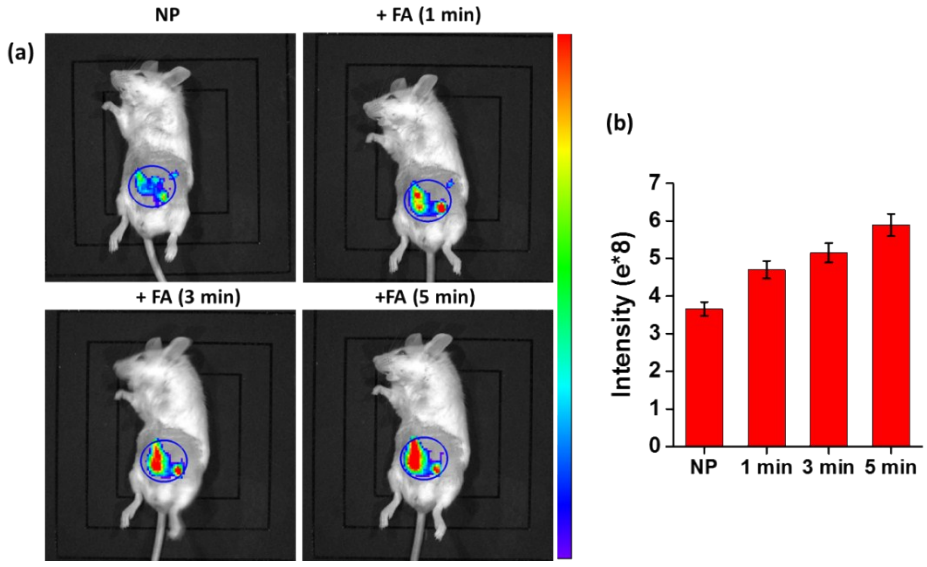


Fig. S15. *In vivo* fluorescence images of reversible detection endogenous SO_2 in living mice by **NP**. (a) Time-dependent fluorescence images of endogenous SO_2. The mice was injected with 100 μL of 50 μM **NP** and then injected 100 μL of 2 mM FA and imaged for 1, 3 min and 5 min, respectively. (b) The relative average fluorescence intensity change of (a) in the blue circle area. Error bars represent standard deviation (±S.D.). n = 3, the statistical analysis was performed from three separate biological replicates. λ_{ex} = 580 nm, λ_{em} = 620 nm.

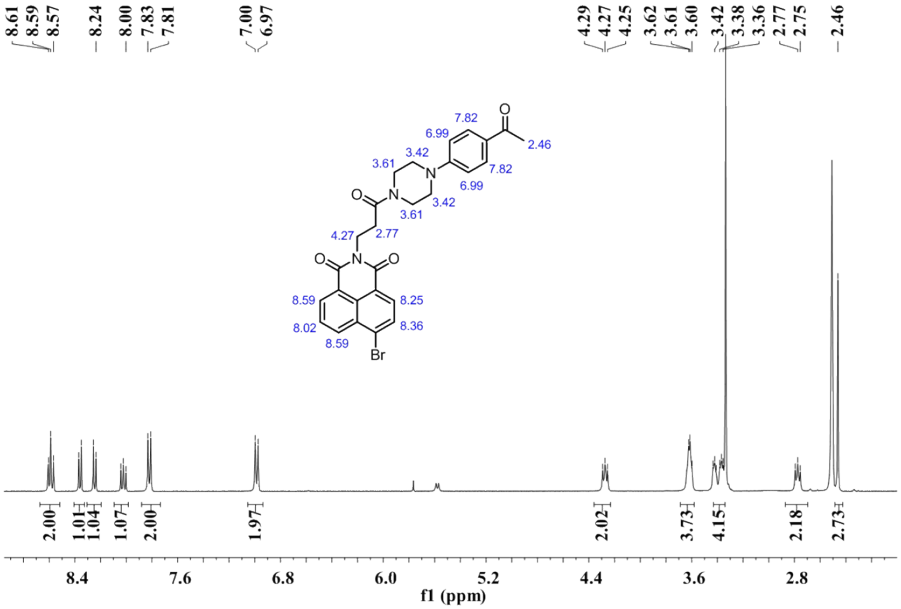


Fig. S16. ^{1}H NMR spectrum of Compound **1** in DMSO-d_6.

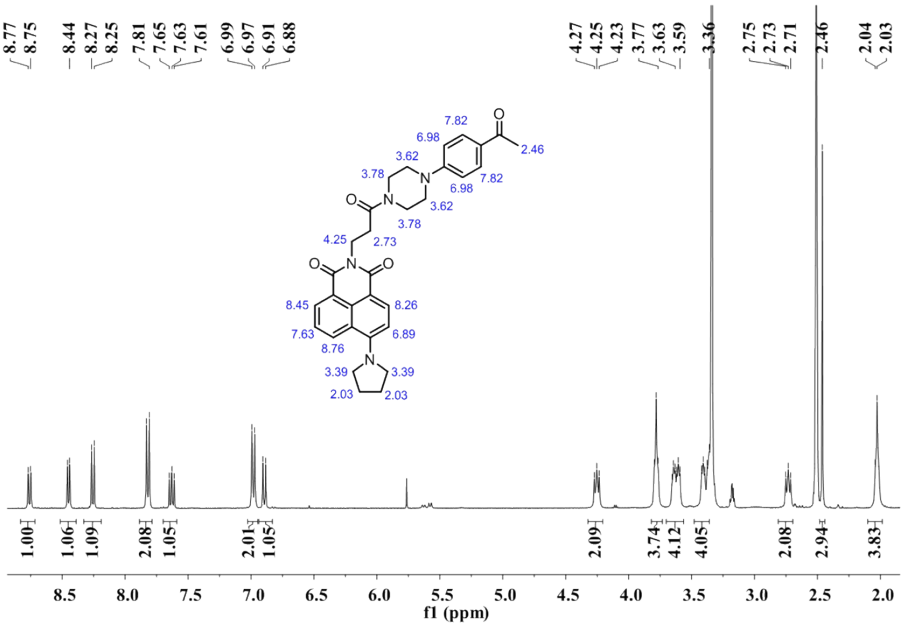


Fig. S17. ^{1}H NMR spectrum of Compound **Na** in DMSO-d_6.

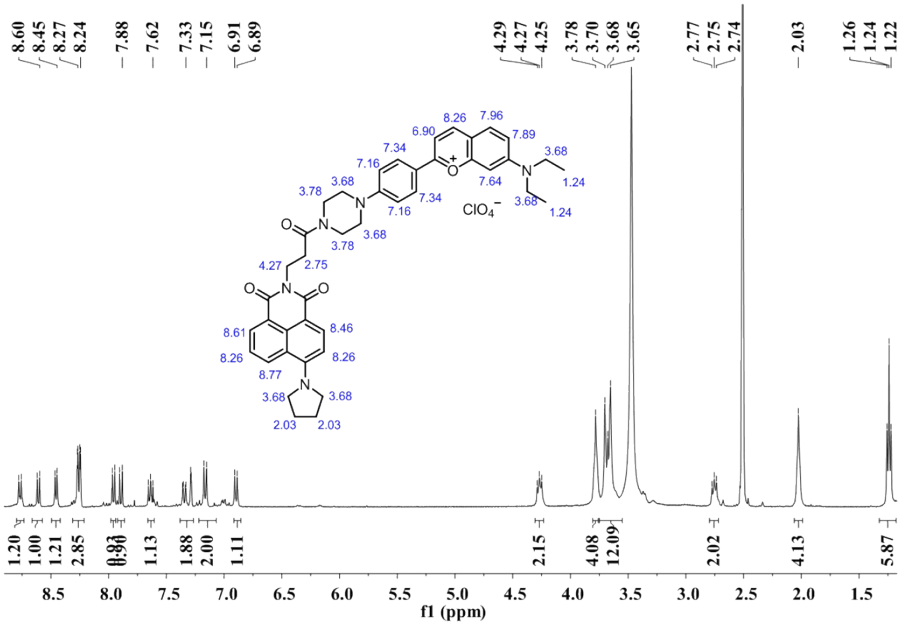


Fig. S18. ^{1}H NMR spectrum of Compound **NP** in DMSO-d_6.

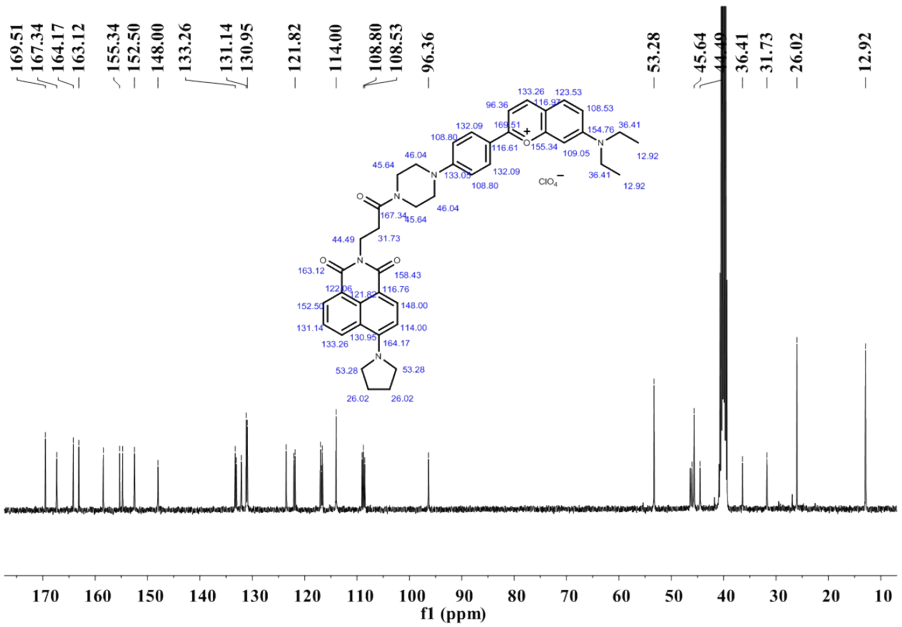


Fig. S19. ^{13}C NMR spectrum of **NP** in DMSO-d_6.

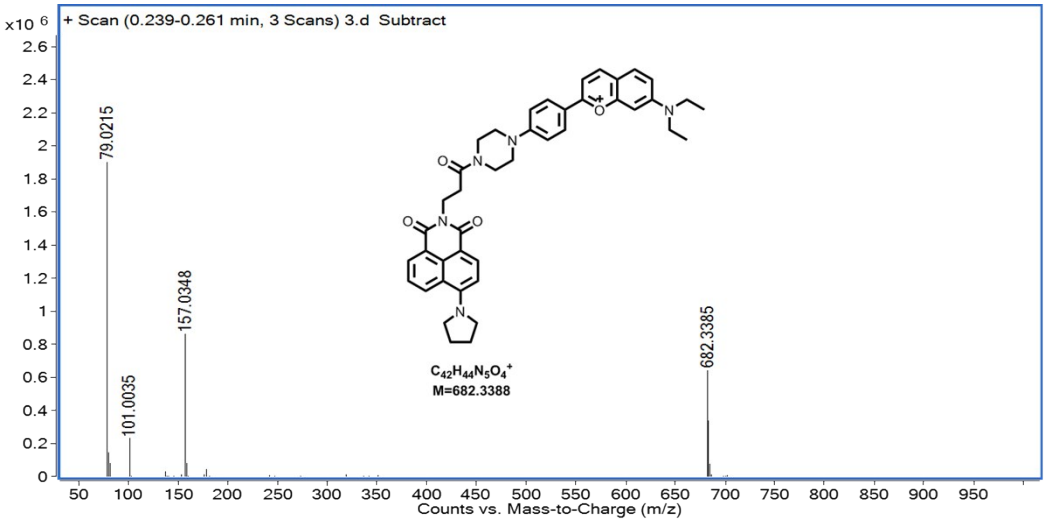


Fig. S20. HR-MS spectrum of **NP** in H_2O (20% CH_3CN).

Supplementary References

[S1] Igawa, M., and Hoffmann, M. R. *Chem. Lett.*, 1988, **17***,* 597-600.

[S2] Shortreed, M., Kopelman, R., Kuhn, M. and Hoyland, B. *Anal. Chem.,* 1996, **68**, 1414-1418.