

## Supporting Information

### A novel fluorescent turn-on probe for imaging of biothiols based on S<sub>N</sub>Ar substitution-skeletal rearrangement strategy

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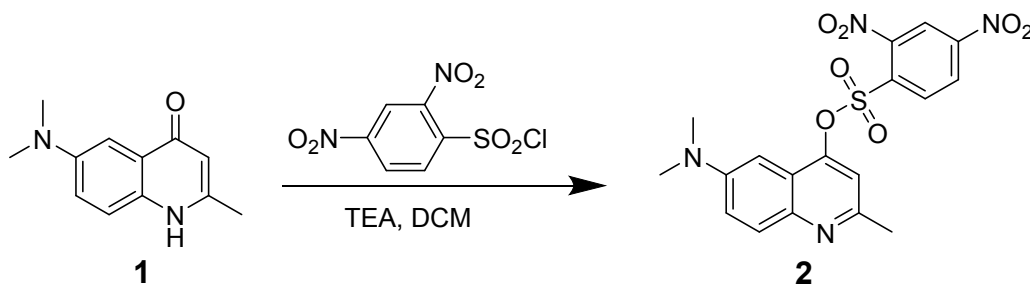
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## 1 Synthesis of compound 2

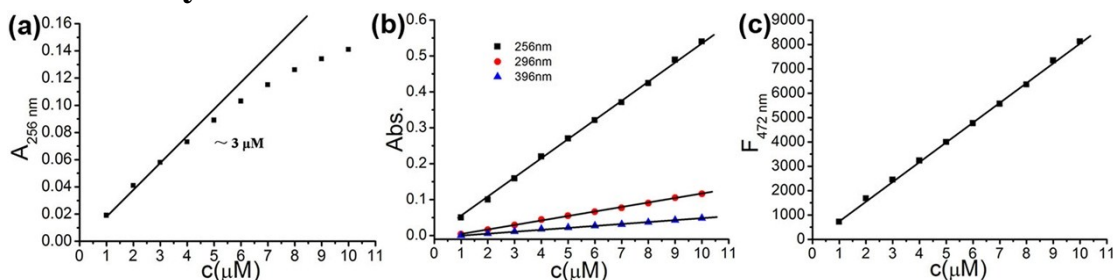
**General Information.** All the chemicals and solvents were purchased from Energy Chemical or Aladdin Industrial Corporation, and used as received with the following exceptions. Dichloromethane (DCM) was distilled from calcium hydride. Pure water (18.25  $\Omega$ ) was used to prepare all aqueous solutions.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were collected on Bruker Avance-400 400 MHz NMR spectrometer and referenced to solvent signals. Electrospray ionization mass spectra (ESI-MS) were obtained using an LC-MS 6120 equipped with Single Quadrupole LC/MS system (Agilent, America) instrument. High resolution mass spectrometric (HRMS) data were obtained on an Agilent 6530 Q-TOF LC/MS spectrometer.



**Scheme S1. Synthesis of compound 2**

Compound **1** (202.3 mg, 1 mmol) <sup>S1</sup> and 2,4-dinitrobenzenesulfonyl chloride (319.9 mg, 1.2 mmol) was dissolved in dry DCM (20 mL). Then trimethylamine (TEA) (0.21 mL, 1.5 mmol) was added to the reaction. The resulting solution was stirred at room temperature for 30 min under argon atmosphere. The reaction mixture was washed with brine, dried over anhydrous sodium sulfate. The solvents were evaporated to give crude solid, which was purified by silica gel column chromatography using DCM/ 0-2% methanol as eluent to afford desired products. Yield: 374.4 mg (86.5%).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , ppm)  $\delta$  8.63 (d,  $J = 2.0$  Hz, 1H), 8.43 (dd,  $J = 2.0$  Hz,  $J = 8.4$  Hz, 1H), 8.20 (d,  $J = 8.8$  Hz, 1H), 7.84 (d,  $J = 9.2$  Hz, 1H), 7.30 (dd,  $J = 2.8$  Hz,  $J = 9.2$  Hz, 1H), 7.21 (s, 1H), 6.87 (d,  $J = 2.8$  Hz, 1H), 2.98 (s, 6H), 2.68 (s, 3H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ , ppm)  $\delta$  154.2, 151.3, 148.8, 143.7, 134.0, 133.7, 129.2, 126.7, 121.5, 120.4, 120.4, 113.7, 97.4, 40.5, 25.0. ESI-MS  $m/z$  for  $\text{C}_{18}\text{H}_{17}\text{N}_4\text{O}_7\text{S}^+$  ( $[\text{M}+\text{H}]^+$ ): calcd: 433.1, found: 433.1.

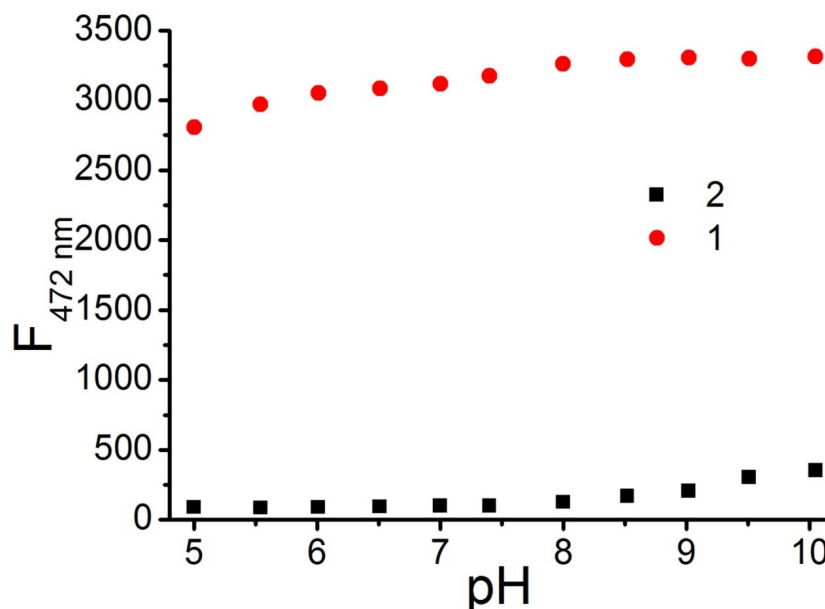
## 2. Solubility



**Fig. S1** (a) Plot of absorbance at 256 nm against **2** concentration in 20 mM HEPES buffer solution (pH 7.4, 25 °C). (b) Plot of absorbance at 256, 296 and 396 nm against **2**

concentration in HEPES buffer solution (20 mM, H<sub>2</sub>O/CH<sub>3</sub>CN, 4:1, v/v, pH 7.4, 25 °C).  
(c) Plot of fluorescence intensity at 472 nm against **1** concentration in HEPES buffer solution (20 mM, H<sub>2</sub>O/CH<sub>3</sub>CN, 4:1, v/v, pH 7.4, 25 °C,  $\lambda_{\text{ex}} = 405 \text{ nm}$ ).

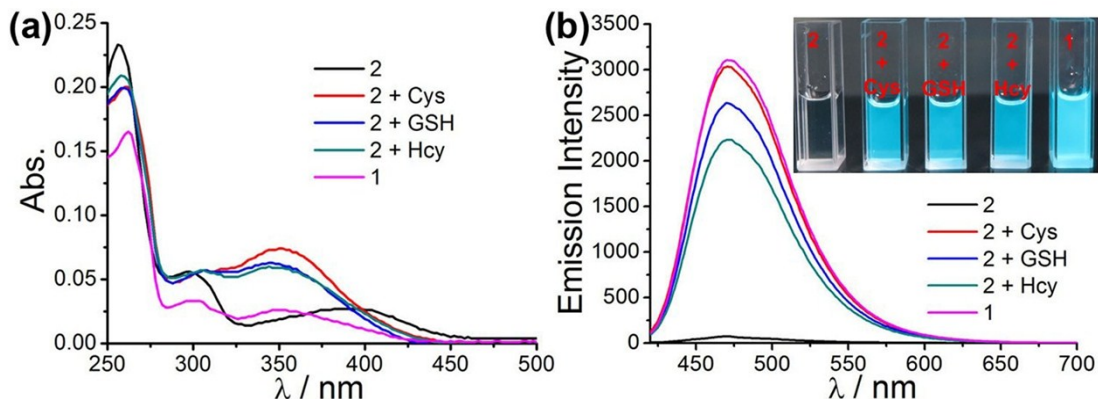
### 3. pH Effect on the Fluorescence Properties



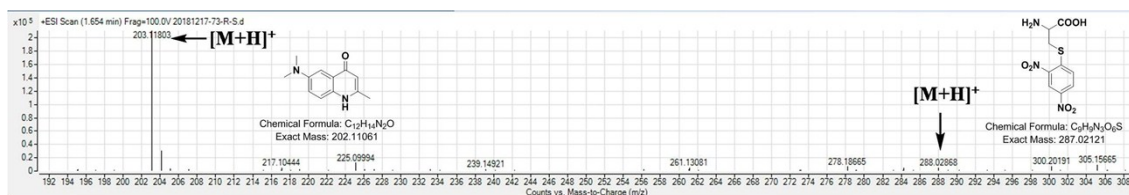
**Fig. S2** Effect of pH on fluorescence intensity at 472 nm for **1** and **2** in HEPES buffer solution (20 mM, H<sub>2</sub>O/CH<sub>3</sub>CN, 4:1, v/v, 25 °C,  $\lambda_{\text{ex}} = 405 \text{ nm}$ ).

### 4. UV-visible Absorbance and Fluorescence Emission

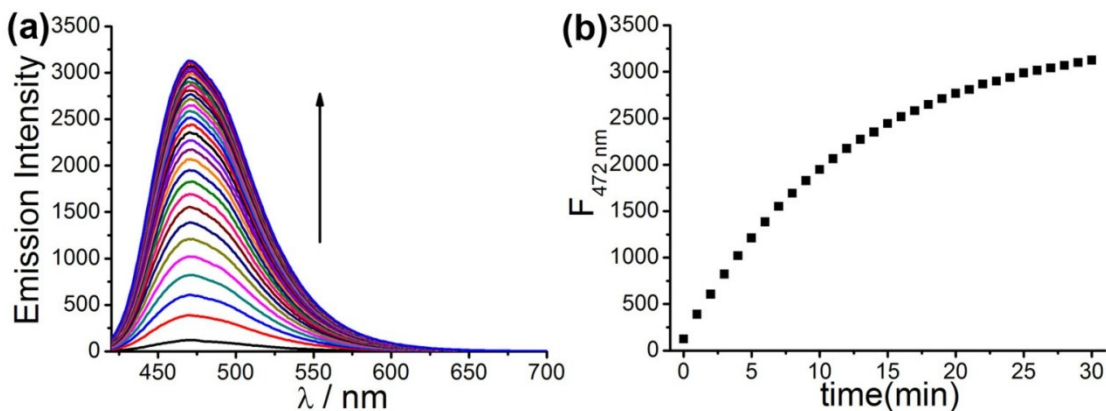
UV-visible spectra were obtained on Thermo Fisher Evolution 300 UV-vis spectrometer. Fluorescence spectra were gathered on a HITACHI F-4600 spectrometer. The PMT voltage was 700 V, excitation slit and emission slit were 5/5 nm, respectively. The path length was 1 cm with cell volume of 3.0 mL. The stock solution of **1** and **2** were prepared in DMSO (2 mM). Aqueous amino acid solutions of 40 mM cysteine (Cys), glutathione (GSH), homocysteine (Hcy), glutamic acid (Glu), proline (Pro), glycine (Gly), asparagine (Asn), serine (Ser), leucine (Leu), phenylalanine (Phe) and methionine (Met) were prepared using deionized water. Stock solutions of 500 mM Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> were prepared from their chloride salts. Stock solutions of 40 mM HS<sup>-</sup>, S<sup>2-</sup>, HSO<sub>3</sub><sup>-</sup>, SO<sub>3</sub><sup>2-</sup>, HSO<sub>4</sub><sup>-</sup> and SO<sub>4</sub><sup>2-</sup> were prepared from their sodium salts. Stock solution of 40 mM CN<sup>-</sup> was prepared from tetrabutylammonium cyanide (Bu<sub>4</sub>NCN). The 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer solution was prepared in deionized water.



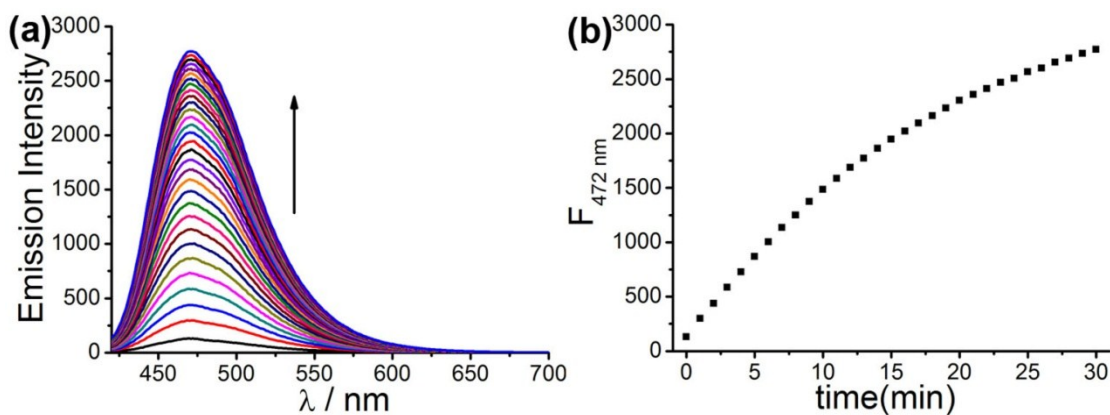
**Fig. S3** (a) Absorption spectra of **1** (5  $\mu\text{M}$ ) (pink line), **2** (5  $\mu\text{M}$ ) (black line) before and after reaction with Cys (30  $\mu\text{M}$ ) (red line), GSH (30  $\mu\text{M}$ ) (blue line) and Hcy (30  $\mu\text{M}$ ) (green line) in HEPES buffer solution (20 mM,  $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ , 4:1, v/v, pH 7.4, 25  $^\circ\text{C}$ ). (b) Fluorescence spectra of **1** (5  $\mu\text{M}$ ) (pink line), **2** (5  $\mu\text{M}$ ) (black line) before and after reaction with Cys (30  $\mu\text{M}$ ) (red line), GSH (30  $\mu\text{M}$ ) (blue line) and Hcy (30  $\mu\text{M}$ ) (green line) in HEPES buffer solution (20 mM,  $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ , 4:1, v/v, pH 7.4, 25  $^\circ\text{C}$ ,  $\lambda_{\text{ex}} = 405$  nm). The fluorescence color changes of **2** before and after the reaction and **1** in HEPES buffer solution under illumination with a 365 nm UV lamp are shown in the inset.



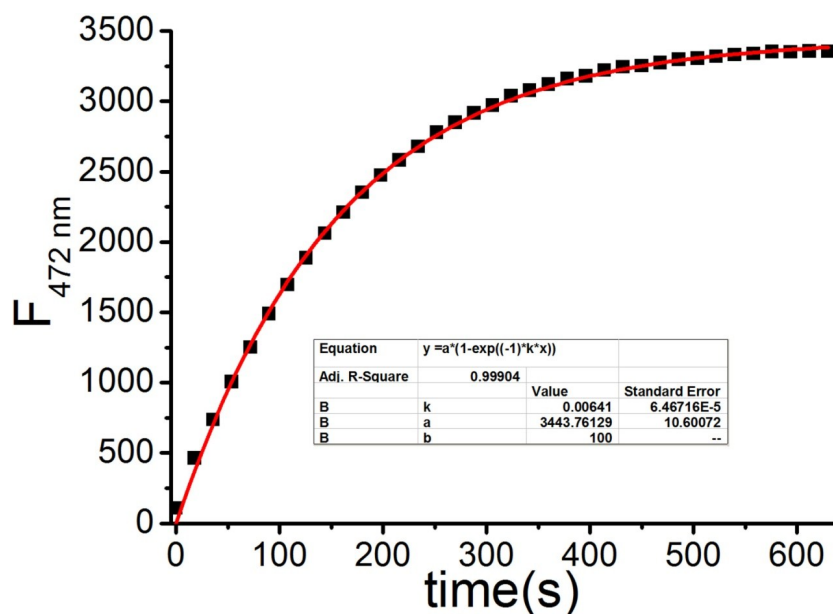
**Fig. S4** HRMS-ESI spectrum of **2** after addition of Cys in HEPES buffer solution (20 mM,  $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ , 4:1, v/v, pH 7.4, 25  $^\circ\text{C}$ ).



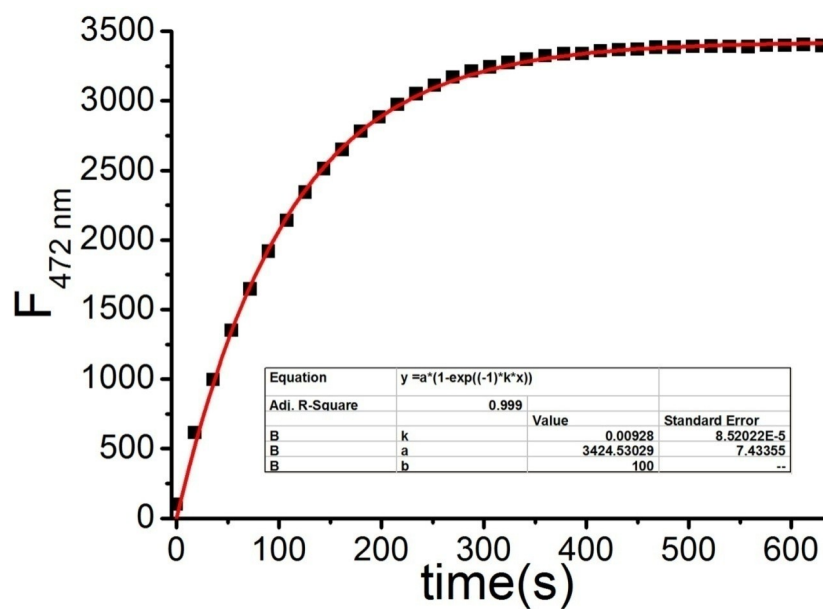
**Fig. S5** (a) Fluorescence spectra of **2** (5  $\mu\text{M}$ ) recorded every 1 min within 30 min after the addition of GSH (30  $\mu\text{M}$ ) in HEPES buffer solution (20 mM,  $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ , 4:1, v/v, pH 7.4, 25  $^\circ\text{C}$ ,  $\lambda_{\text{ex}} = 405$  nm). (b) The fluorescence intensity at 472 nm changes as a function of time.



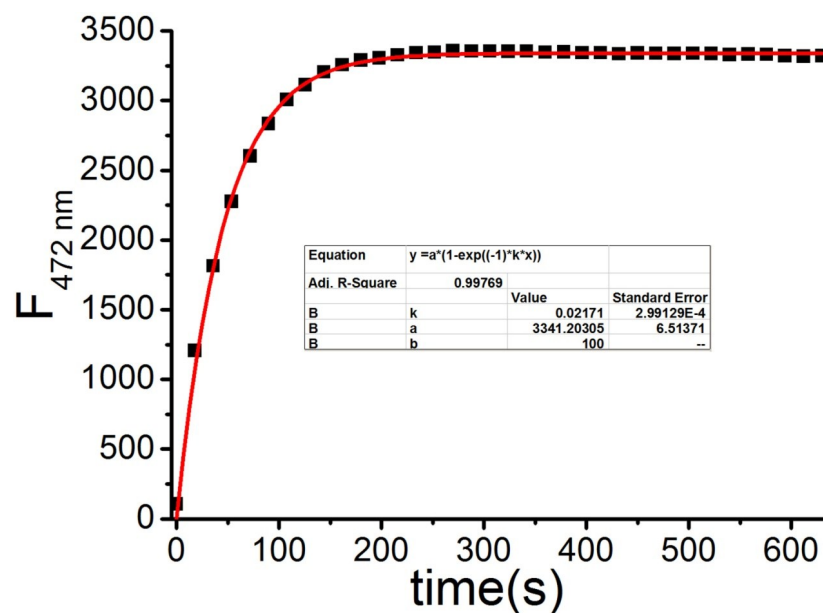
**Fig. S6** (a) Fluorescence spectra of **2** (5  $\mu\text{M}$ ) recorded every 1 min within 30 min after the addition of Hcy (30  $\mu\text{M}$ ) in HEPES buffer solution (20 mM,  $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ , 4:1, v/v, pH 7.4, 25  $^\circ\text{C}$ ,  $\lambda_{\text{ex}} = 405 \text{ nm}$ ). (b) The fluorescence intensity at 472 nm changes as a function of time.



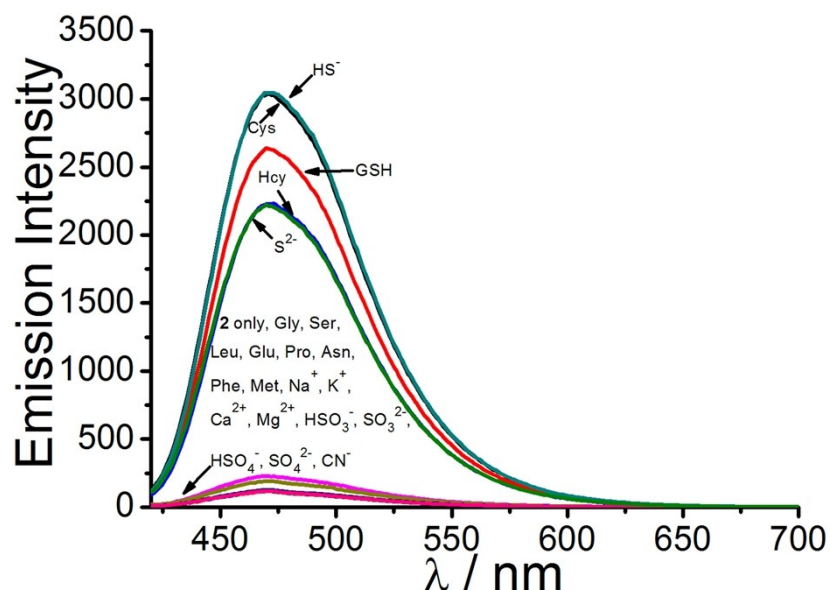
**Fig. S7** Kinetic plot of fluorescence emission intensity at 472 nm of the pseudo-first order reaction of **2** (5  $\mu\text{M}$ ) to Hcy (300  $\mu\text{M}$ ), using excitation wavelength at 405 nm. The slope of the plot corresponds to the observed reaction rate of  $6.41 \times 10^{-3} \text{ s}^{-1}$ .



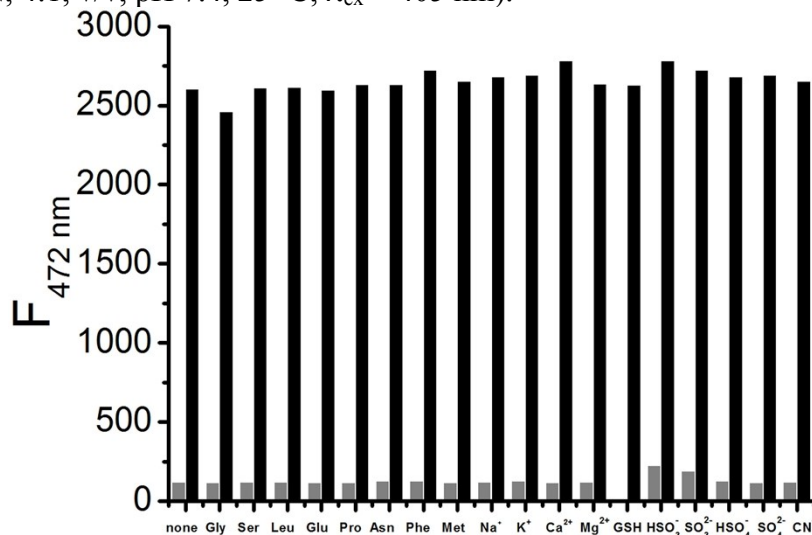
**Fig. S8** Kinetic plot of fluorescence emission intensity at 472 nm of the pseudo-first order reaction of **2** (5  $\mu$ M) to GSH (300  $\mu$ M), using excitation wavelength at 405 nm. The slope of the plot corresponds to the observed reaction rate of  $9.28 \times 10^{-3} \text{ s}^{-1}$ .



**Fig. S9** Kinetic plot of fluorescence emission intensity at 472 nm of the pseudo-first order reaction of **2** (5  $\mu$ M) to Cys (300  $\mu$ M), using excitation wavelength at 405 nm. The slope of the plot corresponds to the observed reaction rate of  $2.17 \times 10^{-2} \text{ s}^{-1}$ .

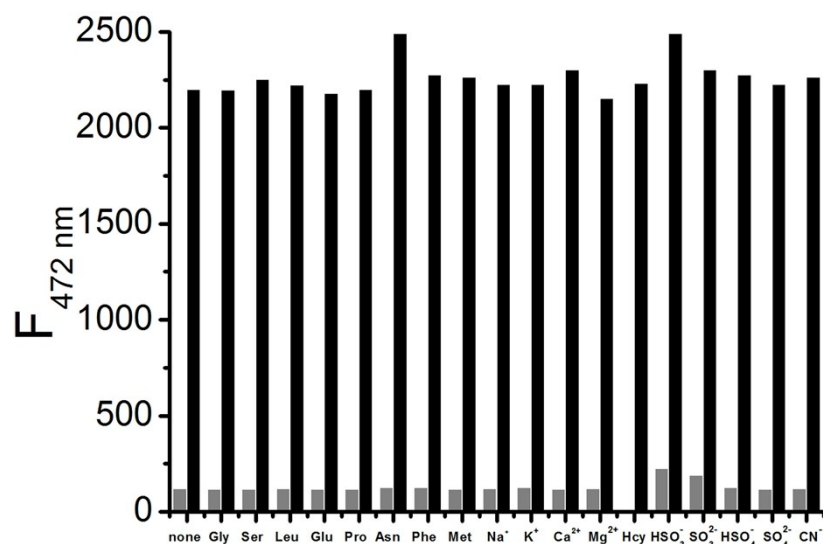


**Fig. S10** Fluorescence spectra of **2** (5  $\mu\text{M}$ ) to biothiols and other analytes: **2** (none), Gly (30  $\mu\text{M}$ ), Ser (30  $\mu\text{M}$ ), Leu (30  $\mu\text{M}$ ), Glu (30  $\mu\text{M}$ ), Pro (30  $\mu\text{M}$ ), Asn (30  $\mu\text{M}$ ), Phe (30  $\mu\text{M}$ ), Met (30  $\mu\text{M}$ ), NaCl (1 mM), KCl (1 mM), CaCl<sub>2</sub> (1 mM), MgCl<sub>2</sub> (1 mM), Cys (30  $\mu\text{M}$ ), GSH (30  $\mu\text{M}$ ), Hcy (30  $\mu\text{M}$ ), NaHS (30  $\mu\text{M}$ ), Na<sub>2</sub>S (30  $\mu\text{M}$ ), NaHSO<sub>3</sub> (30  $\mu\text{M}$ ), Na<sub>2</sub>SO<sub>3</sub> (30  $\mu\text{M}$ ), NaHSO<sub>4</sub> (30  $\mu\text{M}$ ), Na<sub>2</sub>SO<sub>4</sub> (30  $\mu\text{M}$ ), Bu<sub>4</sub>NCN (30  $\mu\text{M}$ ). Data were acquired 15 min after addition of various analytes in HEPES buffer solution (20 mM, H<sub>2</sub>O/CH<sub>3</sub>CN, 4:1, v/v, pH 7.4, 25  $^{\circ}\text{C}$ ,  $\lambda_{\text{ex}}$  = 405 nm).

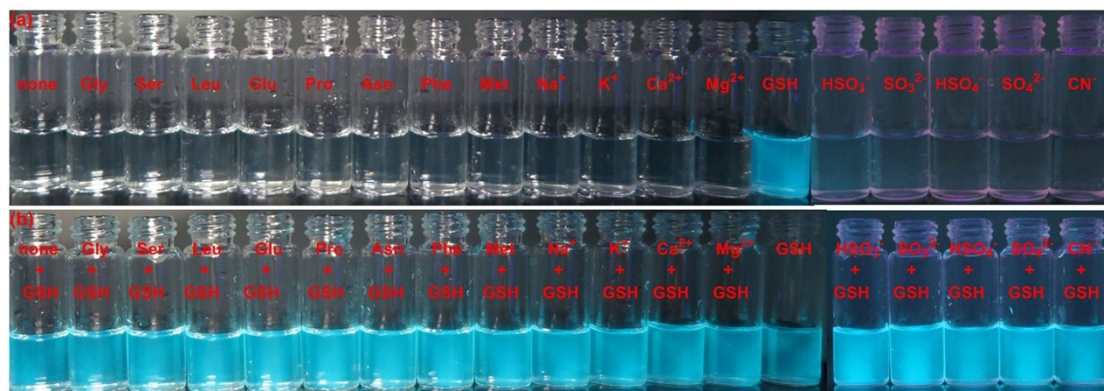


**Fig. S11** Fluorescence responses of **2** (5  $\mu\text{M}$ ) to GSH (30  $\mu\text{M}$ ) and other analytes: **2** (none), Gly (30  $\mu\text{M}$ ), Ser (30  $\mu\text{M}$ ), Leu (30  $\mu\text{M}$ ), Glu (30  $\mu\text{M}$ ), Pro (30  $\mu\text{M}$ ), Asn (30  $\mu\text{M}$ ), Phe (30  $\mu\text{M}$ ), Met (30  $\mu\text{M}$ ), NaCl (1 mM), KCl (1 mM), CaCl<sub>2</sub> (1 mM), MgCl<sub>2</sub> (1 mM), GSH (30  $\mu\text{M}$ ), NaHSO<sub>3</sub> (30  $\mu\text{M}$ ), Na<sub>2</sub>SO<sub>3</sub> (30  $\mu\text{M}$ ), NaHSO<sub>4</sub> (30  $\mu\text{M}$ ), Na<sub>2</sub>SO<sub>4</sub> (30  $\mu\text{M}$ ), Bu<sub>4</sub>NCN (30  $\mu\text{M}$ ). Gray and black bars represent fluorescence intensity at 472 nm in the presence of various analytes before and after addition of GSH, respectively. Data were acquired 15 min after addition of various analytes in HEPES buffer solution (20 mM, H<sub>2</sub>O/CH<sub>3</sub>CN, 4:1, v/v, pH 7.4, 25  $^{\circ}\text{C}$ ,  $\lambda_{\text{ex}}$  = 405 nm).



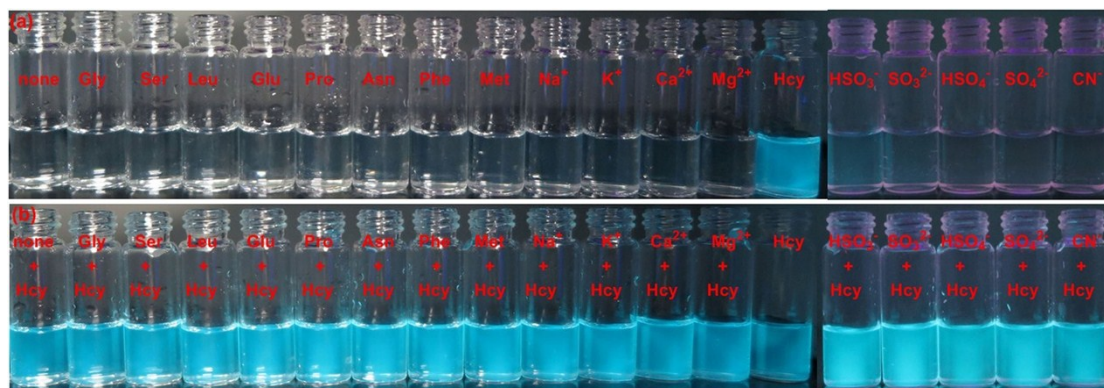


**Fig. S12** Fluorescence responses of **2** (5  $\mu$ M) to Hcy (30  $\mu$ M) and other analytes: **2** (none), Gly (30  $\mu$ M), Ser (30  $\mu$ M), Leu (30  $\mu$ M), Glu (30  $\mu$ M), Pro (30  $\mu$ M), Asn (30  $\mu$ M), Phe (30  $\mu$ M), Met (30  $\mu$ M), NaCl (1 mM), KCl (1 mM), CaCl<sub>2</sub> (1 mM), MgCl<sub>2</sub> (1 mM), Hcy (30  $\mu$ M), NaHSO<sub>3</sub> (30  $\mu$ M), Na<sub>2</sub>SO<sub>3</sub> (30  $\mu$ M), NaHSO<sub>4</sub> (30  $\mu$ M), Na<sub>2</sub>SO<sub>4</sub> (30  $\mu$ M), Bu<sub>4</sub>NCN (30  $\mu$ M). Gray and black bars represent fluorescence intensity at 472 nm in the presence of various analytes before and after addition of Hcy, respectively. Data were acquired 15 min after addition of various analytes in HEPES buffer solution (20 mM, H<sub>2</sub>O/CH<sub>3</sub>CN, 4:1, v/v, pH 7.4, 25 °C,  $\lambda_{\text{ex}}$  = 405 nm).



**Fig. S13** The fluorescence color changes observed before (a) and after (b) the addition of GSH (30  $\mu$ M) to the solution of **2** (5  $\mu$ M) in HEPES buffer solution (20 mM, H<sub>2</sub>O/CH<sub>3</sub>CN, 4:1, v/v, pH 7.4, 25 °C) under illumination with a 365 nm UV lamp in the presence of other analytes: **2** (none), Gly (30  $\mu$ M), Ser (30  $\mu$ M), Leu (30  $\mu$ M), Glu (30  $\mu$ M), Pro (30  $\mu$ M), Asn (30  $\mu$ M), Phe (30  $\mu$ M), Met (30  $\mu$ M), NaCl (1 mM), KCl (1 mM), CaCl<sub>2</sub> (1 mM), MgCl<sub>2</sub> (1 mM), GSH (30  $\mu$ M), NaHSO<sub>3</sub> (30  $\mu$ M), Na<sub>2</sub>SO<sub>3</sub> (30  $\mu$ M), NaHSO<sub>4</sub> (30  $\mu$ M), Na<sub>2</sub>SO<sub>4</sub> (30  $\mu$ M), Bu<sub>4</sub>NCN (30  $\mu$ M).

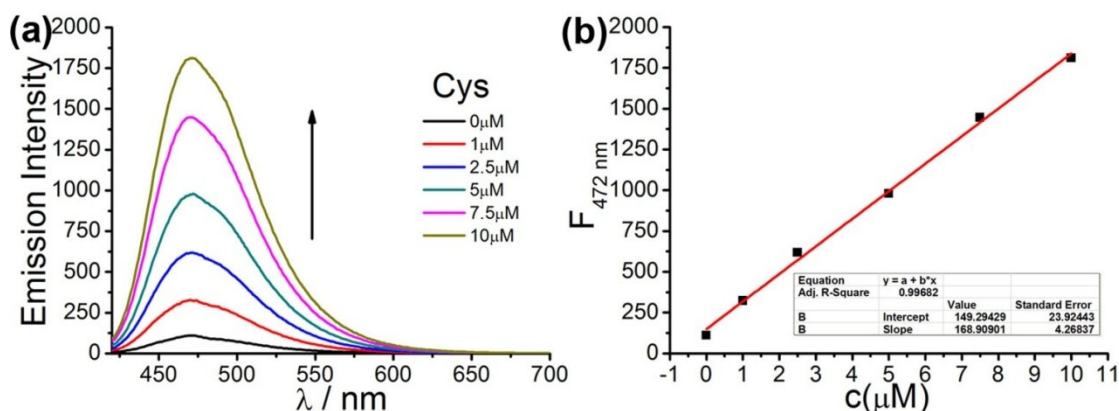




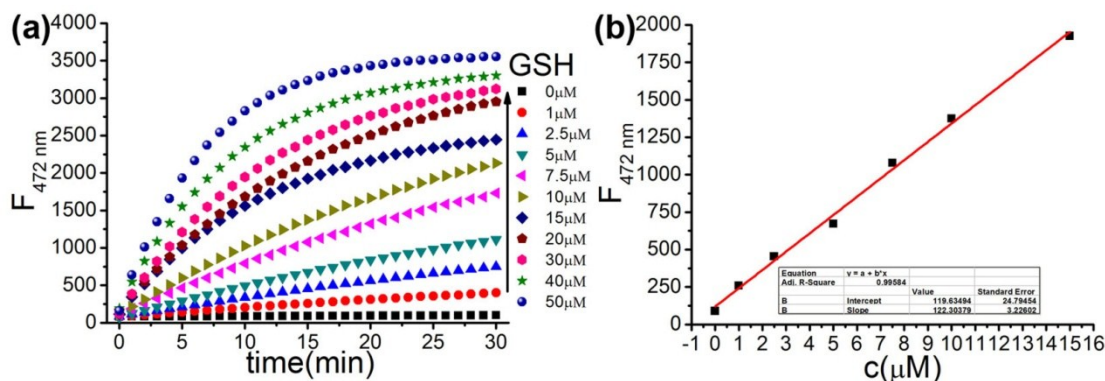
**Fig. S14** The fluorescence color changes observed before (a) and after (b) the addition of Hcy (30  $\mu\text{M}$ ) to the solution of **2** (5  $\mu\text{M}$ ) in HEPES buffer solution (20 mM,  $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ , 4:1, v/v, pH 7.4, 25  $^\circ\text{C}$ ) under illumination with a 365 nm UV lamp in the presence of other analytes: **2** (none), Gly (30  $\mu\text{M}$ ), Ser (30  $\mu\text{M}$ ), Leu (30  $\mu\text{M}$ ), Glu (30  $\mu\text{M}$ ), Pro (30  $\mu\text{M}$ ), Asn (30  $\mu\text{M}$ ), Phe (30  $\mu\text{M}$ ), Met (30  $\mu\text{M}$ ),  $\text{NaCl}$  (1 mM),  $\text{KCl}$  (1 mM),  $\text{CaCl}_2$  (1 mM),  $\text{MgCl}_2$  (1 mM), Hcy (30  $\mu\text{M}$ ),  $\text{NaHSO}_3$  (30  $\mu\text{M}$ ),  $\text{Na}_2\text{SO}_3$  (30  $\mu\text{M}$ ),  $\text{NaHSO}_4$  (30  $\mu\text{M}$ ),  $\text{Na}_2\text{SO}_4$  (30  $\mu\text{M}$ ),  $\text{Bu}_4\text{NCN}$  (30  $\mu\text{M}$ ).

## 5. Determination of the detection limit (LOD)

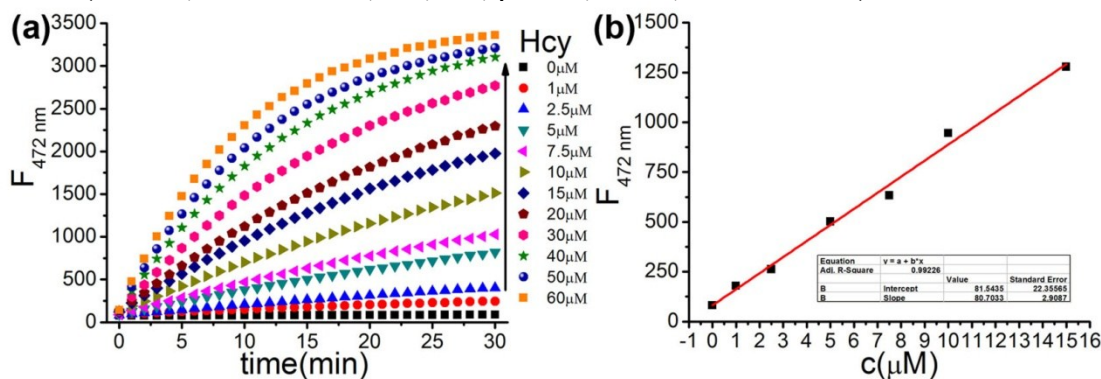
The detection limit was calculated according to the method described in reference.<sup>S2</sup> The emission spectrum of **2** (10  $\mu\text{M}$ ) in HEPES buffer solution (20 mM,  $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ , 4:1, v/v, pH 7.4, 25  $^\circ\text{C}$ ,  $\lambda_{\text{ex}} = 405 \text{ nm}$ ) was collected for 30 times to determine the background noise  $\sigma$ . A linear regression curve was fitted according to the fluorescence intensity at 472 nm as a function of the concentrations of biothiols (Cys, GSH and Hcy), and the slope of the curve was obtained. The detection limit was then determined ( $3\sigma/\text{slope}$ ).



**Fig. S15** (a) Fluorescence spectra of **2** (5  $\mu\text{M}$ ) upon addition of Cys (0 - 10  $\mu\text{M}$ ). (b) Fluorescence intensities of **2** at 472 nm as a function of the concentrations of Cys in the range of 0 - 10  $\mu\text{M}$  upon excitation at 405 nm and the calculation of the detection limit of probe **2** for Cys.



**Fig. S16** (a) Plot of fluorescence intensity at 472 nm versus the reaction time in the presence of varied concentrations of GSH (from bottom to top): 0 (control), 1, 2.5, 5, 7.5, 10, 15, 20, 30, 40 and 50 μM. (b) The fluorescence intensity at 472 nm as a function of the concentrations of GSH in the range of 0 - 15 μM. Data were acquired in HEPES buffer solution (20 mM, H<sub>2</sub>O/CH<sub>3</sub>CN, 4:1, v/v, pH 7.4, 25 °C, λ<sub>ex</sub> = 405 nm).



**Fig. S17** (a) Plot of fluorescence intensity at 472 nm versus the reaction time in the presence of varied concentrations of Hcy (from bottom to top): 0 (control), 1, 2.5, 5, 7.5, 10, 15, 20, 30, 40, 50 and 60 μM. (b) The fluorescence intensity at 472 nm as a function of the concentrations of Hcy in the range of 0 - 15 μM. Data were acquired in HEPES buffer solution (20 mM, H<sub>2</sub>O/CH<sub>3</sub>CN, 4:1, v/v, pH 7.4, 25 °C, λ<sub>ex</sub> = 405 nm).

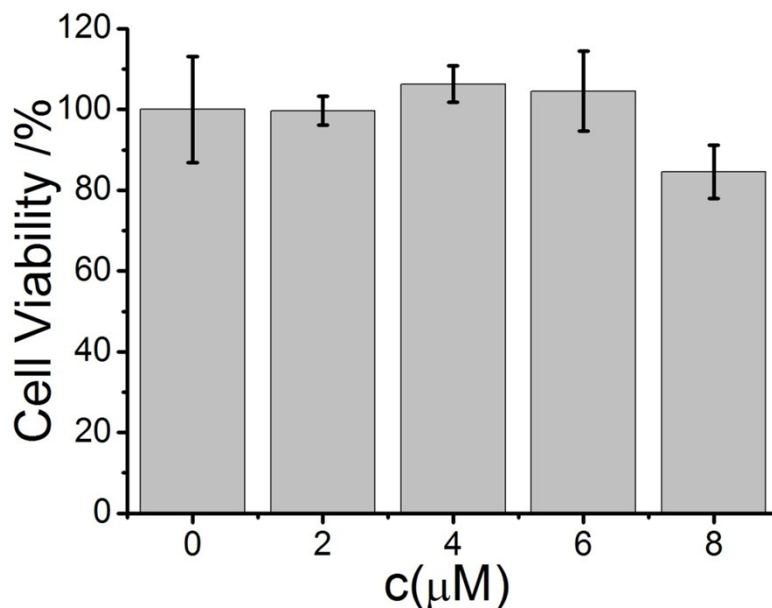
## 6. Methods of cell culture

HeLa cells were purchased from China Center for Type Culture Collection (Wuhan, China). Dulbecco's Modified Eagle Medium (DMEM), penicillin (Hyclone), streptomycin (Hyclone), fetal calf serum (FCS, Gibco) and phosphate-buffered saline (PBS) were purchased from Hangzhou Sijiqing Bioengineering Material Co., Ltd. HeLa cells were cultured in DMEM supplemented with 10% fetal calf serum (FCS, Gibco), 1% streptomycin/penicillin (Hyclone) at 37 °C in a humidified incubator containing 5% CO<sub>2</sub> gas. The cells were plated in a 35 mm glass-bottomed dish and cultured for 2 days before dye loading. Confocal fluorescence imaging experiments were measured on a Leica SP5 laser scanning confocal microscope.

## 7. Cytotoxicity assay

100 μl of Hela cell suspension (10<sup>4</sup> cells/well) were seeded into 96 well plates, and

preincubated for 12 h in a humidified incubator (at 37 °C, 5% CO<sub>2</sub>). Then, various concentrations (0, 2, 4, 6, 8 μM) of **2** were added into the culture media in the plate, and incubated for 24 h. Next, 100 μl of CCK-8 solution was added to each well of the plate, and incubate for 0.5 h. Finally, the absorbance at 450 nm was measured using a microplate reader.

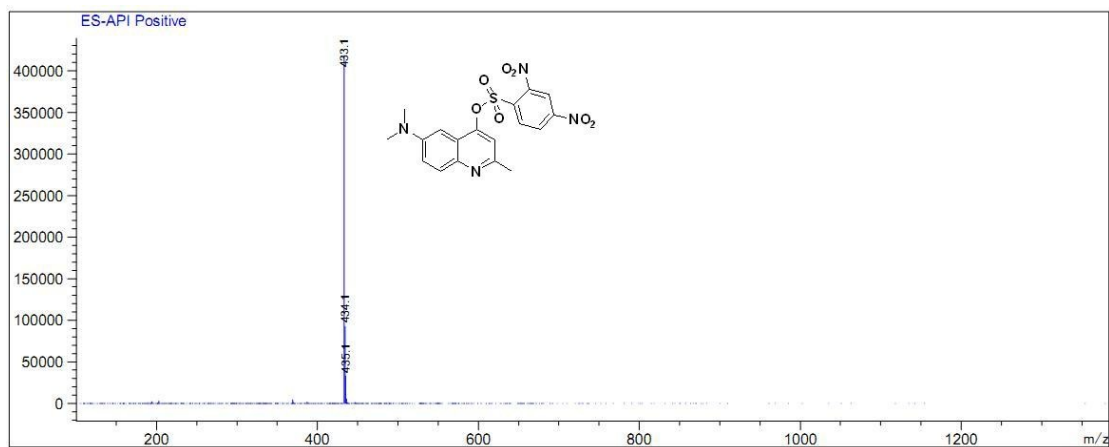


**Fig. S18** CCK-8 assay of Hela cells in the presence of various concentrations of **2** (0, 2, 4, 6, 8 μM) for 24 h at 37 °C.

#### Reference

- (S1) Y.-Y. Qian, L. Xue, D.-X. Hu, G.-P. Li and H. Jiang, *Dyes Pigments*, 2012, **95**, 373.  
 (S2) Y. Chen, C. Zhu, Z. Yang, J. Chen, Y. He, Y. Jiao, W. He, L. Qiu, J. Cen and Z. Guo, *Angew. Chem. Int. Ed.*, 2013, **52**, 1688.

## 8. ESI-MS Spectrum



## 9. NMR Data

