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*Electronic Supplementary Information*

# A turn-on fluorescent sensor for the discrimination of cystein from homocystein and glutathione

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# Contents



## Materials and instruments

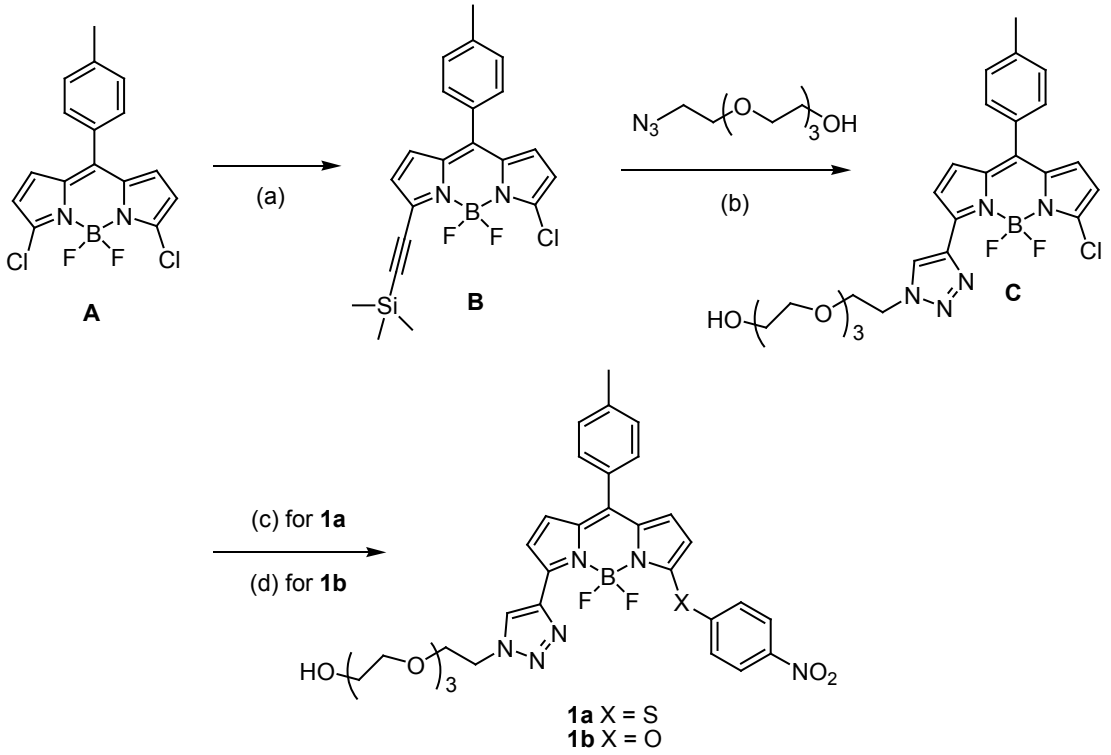
All the reagents and solvents are of commercial quality and without further purification. $^1$H and $^{13}$C NMR spectra were recorded on an Advance Bruker 400M spectrometer and referenced to solvent signals. Mass spectra were obtained on Bruker Apex Ⅳ Fourier Transform Mass Spectrometer. Fluorescence spectra were determined on a Hitachi 4500 spectrophotometer. Absorption spectra were determined on a Shimadzu UV-1601PC UV-Visible spectrophotometer.

## Cell culture and fluorescence imaging

HeLa cells were cultured in culture media (DMEM/F12 supplemented with 10% FBS, 50 unit/mL penicillin, and 50 μg/mL of streptomycin) at 37 °C under a humidified atmosphere containing 5% $CO_2$ for 24 h. The cells were seeded in a 6-well plate at a

density of 104 cells per well in culture media. The cells were treated with 500 μM Cys in culture media for 30 min at 37 °C in a humidified incubator. After washing with PBS, the cells were further incubated with 5 μM of **1a** in culture media for 15 min. For the control experiment, the cells were treated with 500 μM N-ethylmaleimide (NEM) in culture media for 30 min at 37 °C in a humidified incubator. After washing with PBS, the cells were further incubated with 5 μM of **1a** in culture media for 15 min. Confocal fluorescence imaging was performed with Nikon A1R MP multiphoton microscopy with a 60 × oil-immersion objective lens. Fluorescence was excited at 561 nm and emission was collected by a 570-620 nm band pass filter.

**Synthesis**




**Scheme S1.** Synthesis of **1**. (a) trimethylsilylacetylene, $Pd(PPh_3)_2Cl_2$, CuI, toluene, $Et_3N$; (b) TBAF, CuI, $CH_2Cl_2$; (c) 4-nitrothiophenol, $Et_3N$, $CH_3CN$; (d) 4-nitrophenol, $Et_3N$, $CH_3CN$.

Compound **A** was prepared according the reported literature methods.[1]

Compound **A** (350 mg, 1 mmol) was dissolved in toluene (50 mL), then copper(I) iodide (10 mg, 0.05 mmol), bis(triphenylphosphine)palladium(II) chloride (35 mg, 0.05 mmol) were added under nitrogen. 1 mL of triethylamine was added to this mixture through a syringe, followed by trimethylsilylacetylene (142 μL, 1 mmol).

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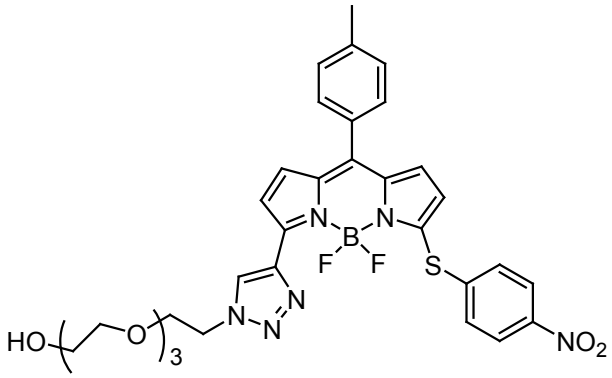
After stirring at 65 °C for 2 h, the solvent was evaporated under reduced pressure. The residue was purified by chromatography on silica gel (dichloromethane / petroleum ether = 1/4 as eluent) to give **B** (185 mg, 45%). $^1$H NMR (400 MHz, $CDCl_3$): 7.39 (d, 2H, $J$ = 8.0 Hz), 7.32 (d, 2H, $J$ = 8.0 Hz), 6.87 (d, 1H, $J$ = 4.4 Hz), 6.81 (d, 1H, $J$ = 4.4 Hz), 6.63 (d, 1H, $J$ = 4.4 Hz), 6.42 (d, 1H, $J$ = 4.4 Hz), 2.46 (s, 3H), 0.32 (s, 9H).

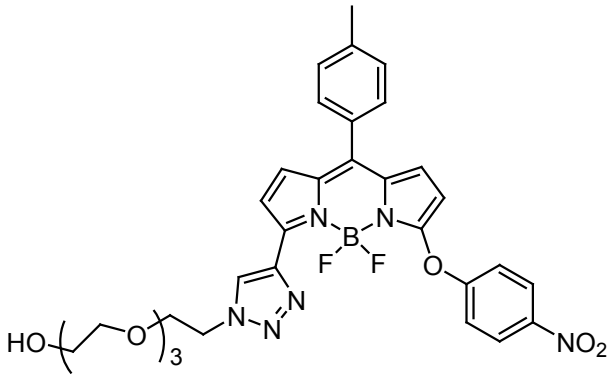
Compound **B** (100 mg, 0.24 mmol) in dichloromethane (15 mL) was added 1-azido-3,6,9-trioxaundecane-11-ol [2] (158 mg, 0.72 mmol) and CuI (4.5 mg, 0.024mmol), the mixture was stirred 10 min under under $N_2$ atmosphere. Then tetrabutylammonium fluoride trihydrate (TBAF, 115 mg, 0.36 mmol) in dichloromethane (5 mL) was added. the reaction mixture was stirred at 0 °C for 5 h, or after the disappearance of **B**, as detected by TLC, the mixture was washed with water (30 mL × 3). The organic layer was dried over $MgSO_4$ and evaporated. The crude residue was purified by column chromatography on silica (dichloromethane/methanol = 1/20 as eluent) to give **C** (71 mg, 53%). $^1$H NMR (400 MHz, $CDCl_3$): δ 8.83 (s, 1H), 7.43 (m, 3H), 7.33 (d, 2H, $J$ = 8.0 Hz), 7.00 (d, 1H, $J$ = 4.4 Hz), 6.78 (d, 1H, $J$ = 4.0 Hz), 6.39 (d, 1H, $J$ = 4.0 Hz), 4.66 (t, 2H, $J$ = 5.2 Hz), 3.96 (t, 2H, $J$ = 5.2 Hz), 3.72-3.65 (m, 10H), 3.59 (t, 2H, $J$ = 4.8 Hz), 2.47 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$): 150.58, 143.63, 141.20, 140.95, 139.11, 137.05, 133.53, 132.83, 130.68, 130.62, 129.32, 127.53, 120.87, 117.49, 72.58, 71.20, 70.87, 70.65, 70.46, 69.56, 61.90, 50.72, 21.58. ESI-HRMS: calculated for $[M+H]^+$ 560.20465, found 560.20376.

## 1a



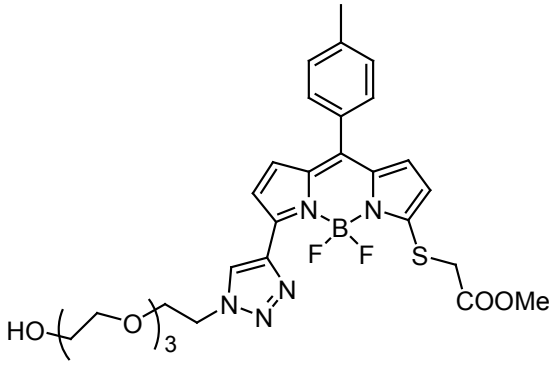
Compound **C** (50 mg, 0.09 mmol) was dissolved in acetonitrile (100 mL) and 4-nitrothiophenol (28 mg, 0.18 mmol) and one drop of $Et_3N$ was added. The mixture was stirred at room temperature for 2 h, and the solvent was evaporated under reduced pressure. The crude residue was purified by column chromatography over silica (dichloromethane/methanol = 1/20 as eluent) to give **1a** (55 mg, 90%). $^1$H NMR (400 MHz, $CDCl_3$): δ 8.79 (s, 1H), 8.19 (d, 2H, $J$ = 9.0 Hz), 7.57 (d, 2H, $J$ = 9.0 Hz), 7.45 (m, 3H), 7.35 (d, 2H, $J$ = 8.0 Hz), 7.04 (d, 1H, $J$ = 4.4 Hz), 6.81 (d, 1H, $J$ = 4.0 Hz), 6.35 (d, 1H, $J$ = 4.0 Hz), 4.64 (t, 2H, $J$ = 5.2 Hz), 3.95 (t, 2H, $J$ = 5.2 Hz), 3.70 (m, 2H), 3.62-3.55 (m, 10H), 2.48 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$): 150.16, 146.97, 145.167, 144.06, 143.10, 141.26, 138.99, 137.68, 136.30, 133.01, 130.87, 130.69, 130.52, 129.38, 128.67, 127.72, 124.45, 122.03, 121.35, 72.57, 71.15, 70.82, 70.63, 70.46, 69.53, 61.88, 50.72, 21.61. ESI-HRMS: calculated for $[M+H]^+$ 679.23217, found 679.23422.

**1b**




Compound **C** (50 mg, 0.09 mmol) was dissolved in acetonitrile (10 mL) and 4-nitrothiophenol (28 mg, 0.18 mmol) and one drop of $Et_3N$ was added. The mixture was refluxed for 5 h, or after the disappearance of **C**, as detected by TLC, the solvent was evaporated under reduced pressure. The crude residue was purified by column chromatography on silica gel (dichloromethane/methanol = 1/20 as eluent) to give **1b** (40 mg, 68%). $^1$H NMR (400 MHz, $CDCl_3$): δ 8.70 (s, 1H), 8.33 (d, 2H, $J$ = 9.2 Hz), 7.44 (m, 4H), 7.38 (d, 1H, $J$ = 4.4 Hz), 7.34 (d, 2H, $J$ = 8.0 Hz), 6.96 (d, 1H, $J$ = 4.4 Hz), 6.86 (d, 1H, $J$ = 4.4 Hz), 5.86 (d, 1H, $J$ = 4.4 Hz), 4.61 (t, 2H, $J$ = 5.2 Hz), 3.94 (t, 2H, $J$ = 5.2 Hz), 3.68 (m, 2H), 3.60-3.53 (m, 10H), 2.48 (s, 3H). ESI-HRMS: calculated for $[M+H]^+$ 663.25501, found 663.25628.

**2**




Compound **1a** (10 mg, 0.015 mmol) and methyl mercaptoacetate (2.7 μL, 0.03 mmol) was dissolved in acetonitrile (10 mL), and one drop of triethylamine was added. The reaction mixture was stirring at room temperature for 2 min, and then evaporated. The crude product was purified through column chromatography over silica (dichloromethane / methanol = 50/1 as eluent) to give **2** as purple solid. $^{1}$H NMR (400 MHz, $CDCl_3$): δ 8.75 (s, 1H), 7.42 (d, 2H, $J$ = 8.0 Hz), 7.35 (d, 1H, $J$ = 4.4 Hz), 7.32 (d, 2H, $J$ = 8.0 Hz), 6.89 (d, 1H, $J$ = 4.4 Hz), 6.83 (d, 1H, $J$ = 4.0 Hz), 6.50 (d, 1H, $J$ = 4.4 Hz), 4.64 (t, 2H, $J$ = 5.2 Hz), 3.96 (t, 2H, $J$ = 5.2 Hz), 3.85 (s, 2H), 3.78 (s, 3H), 3.72-3.63 (m, 10H), 3.59 (t, 2H, $J$ = 4.4 Hz), 2.46 (s, 3H). ESI-HRMS: calculated for $[M+H]^+$ 630.23687, found 630.23672.

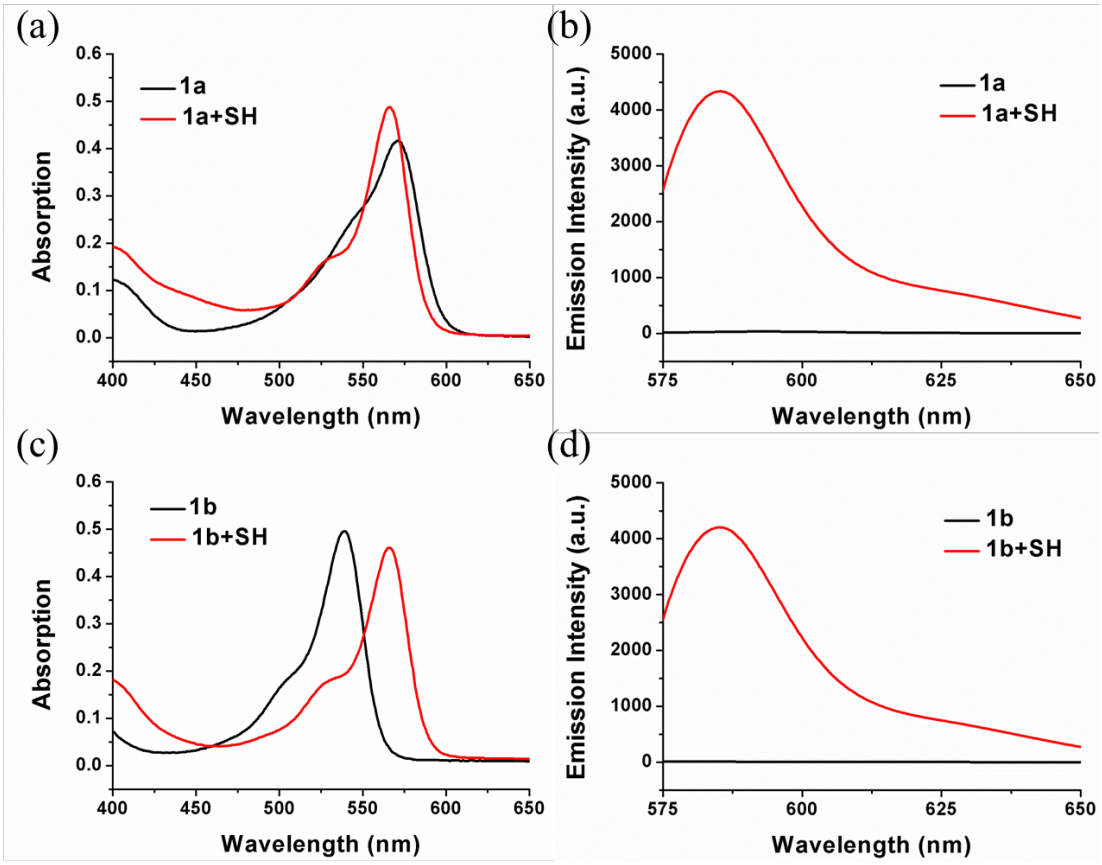



**Figure S1.** Changes of absorption (a, c) and emission (b, d) spectra of **1a** (a, b) and **1b** (c, d) (10 μM) before and after the addition of methyl mercaptoacetate in acetonitrile / HEPES buffer (2:8, v/v, 20 mM, pH 7.4) at 37 °C. $\lambda_{ex}$ = 568 nm.

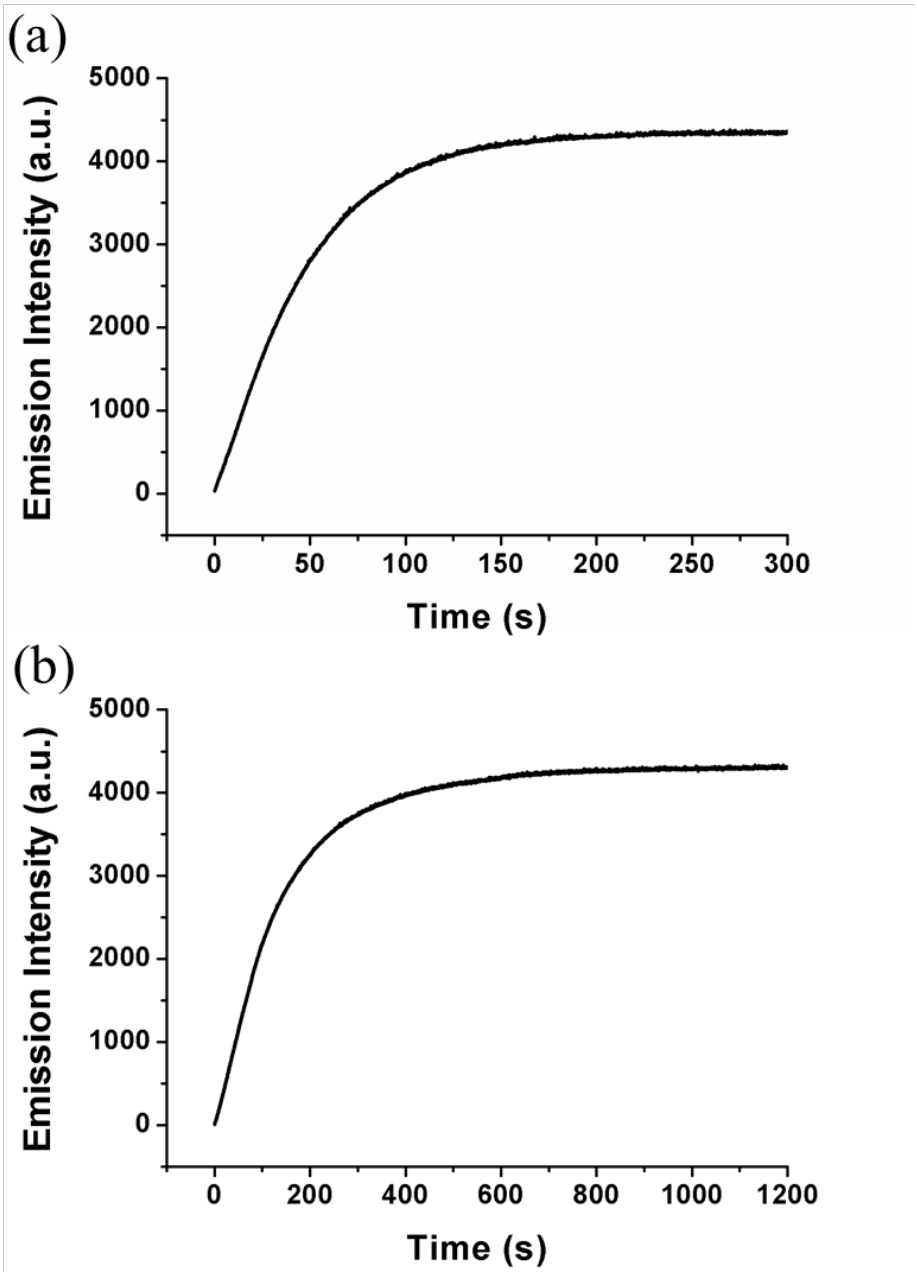



**Figure S2.** Time course of the response at 585 nm of (a) **1a** (10 μM) and (b) **1b** (10 μM) to 100 equiv of methyl mercaptoacetate in acetonitrile / HEPES buffer (2:8, v/v, 20 mM, pH 7.4) at 37 °C.

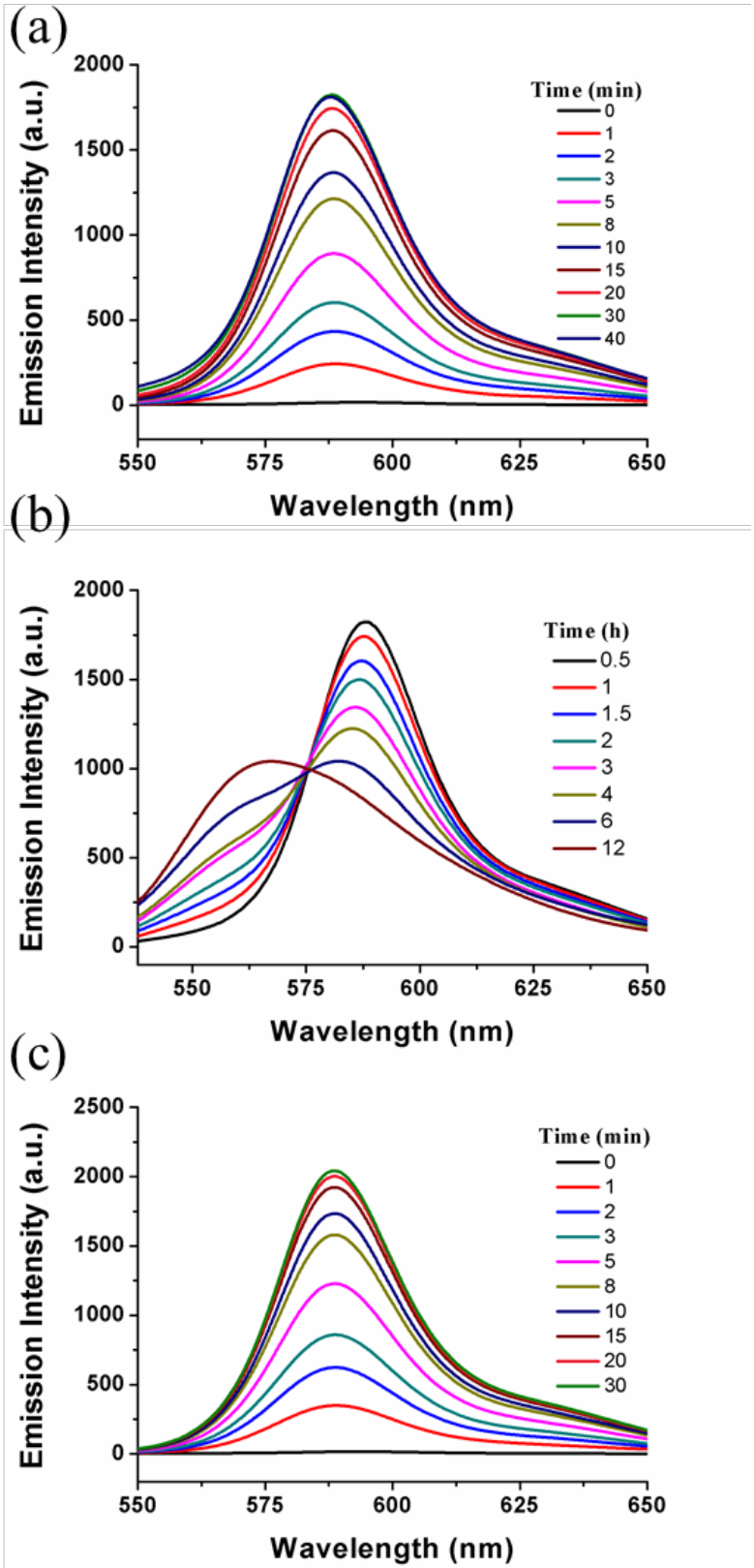



**Figure S3.** Time-dependent fluorescence spectra of **1a** (10 μM) with 100 equiv of (a) (b) Hcy and (c) GSH in acetonitrile / HEPES buffer (2:8, v/v, 20 mM, pH 7.4) at 37 °C. $\lambda_{ex}$ = 528 nm.

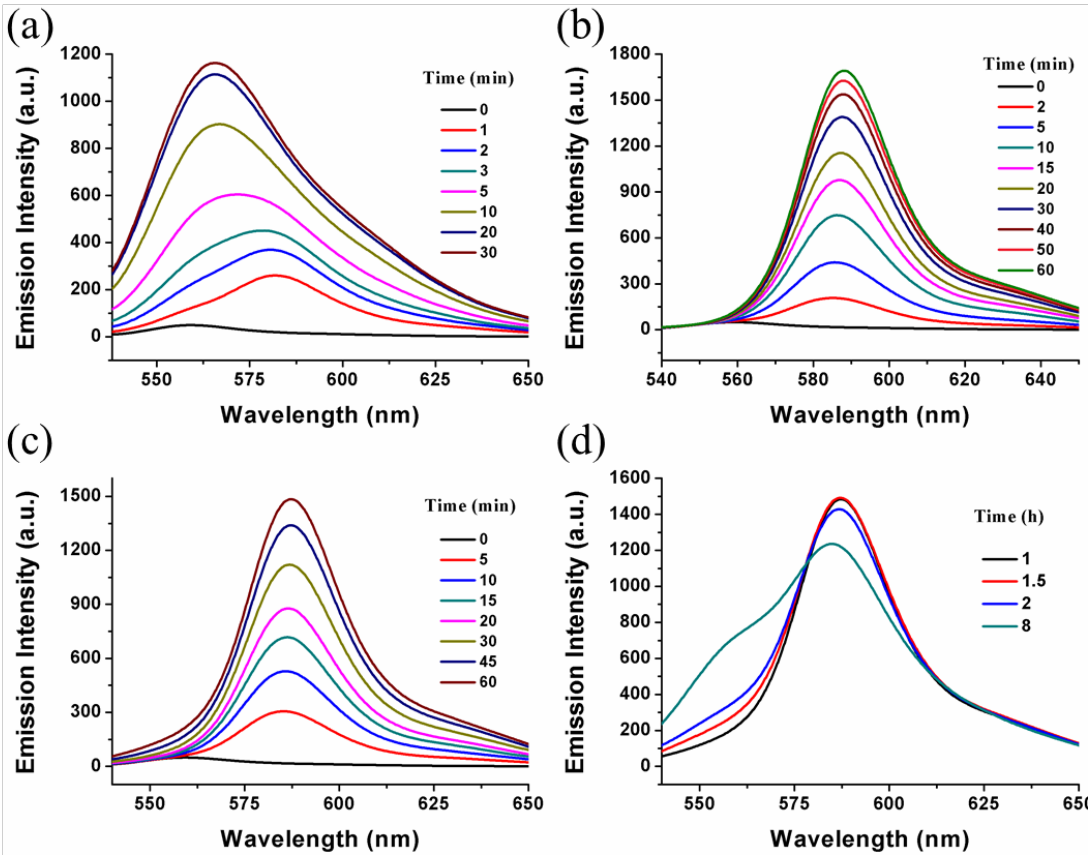



**Figure S4.** Time-dependent fluorescence spectra of **1b** (10 μM) with 100 equiv of (a) Cys, (b) GSH, and (c) (d) Hcy in acetonitrile / HEPES buffer (2:8, v/v, 20 mM, pH 7.4) at 37 °C. $\lambda_{ex}$ = 528 nm

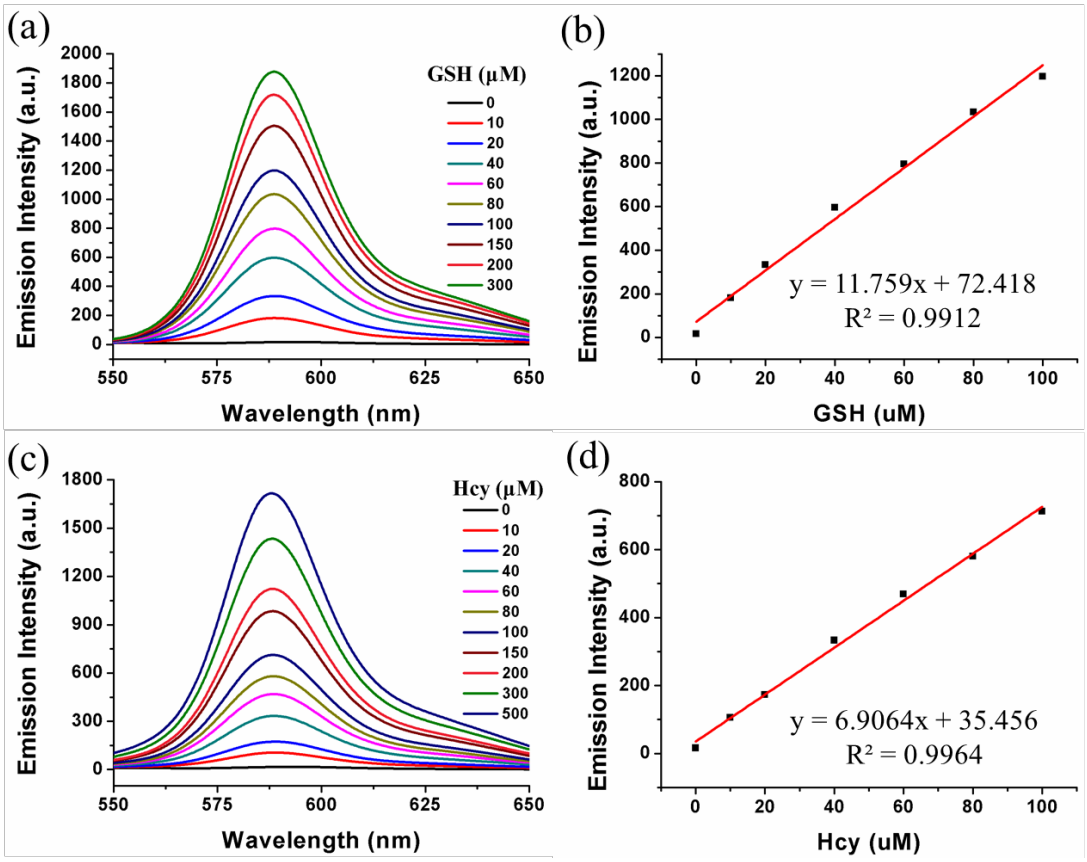
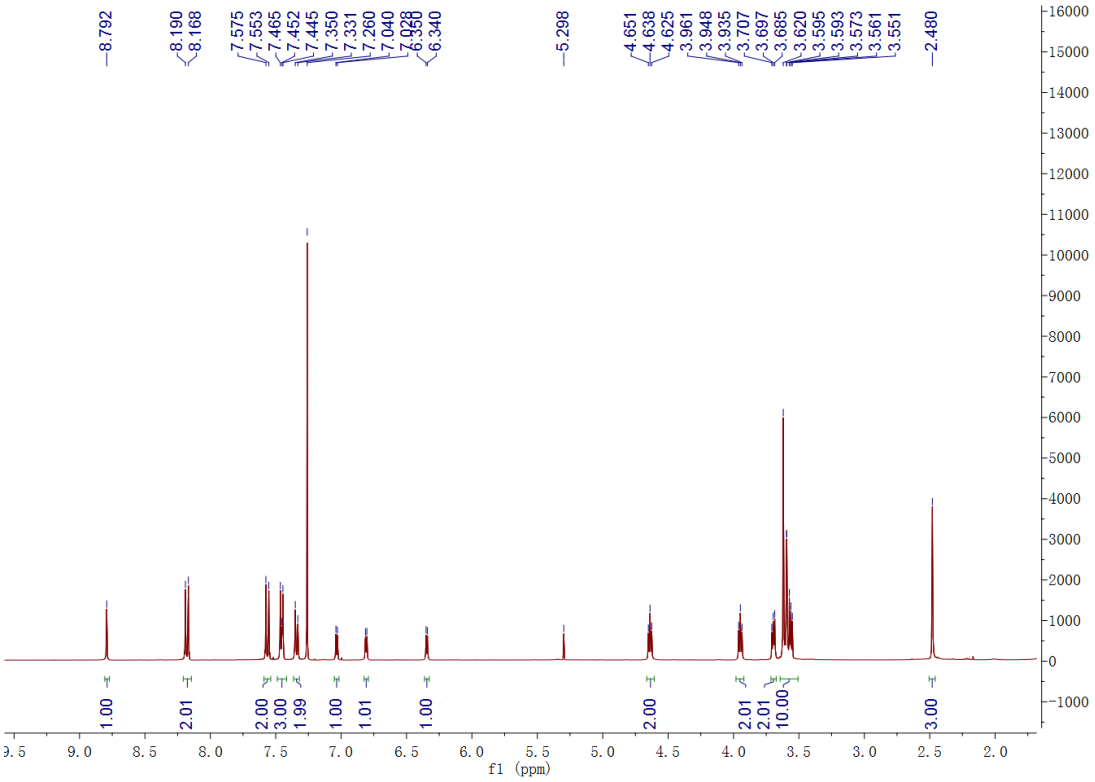
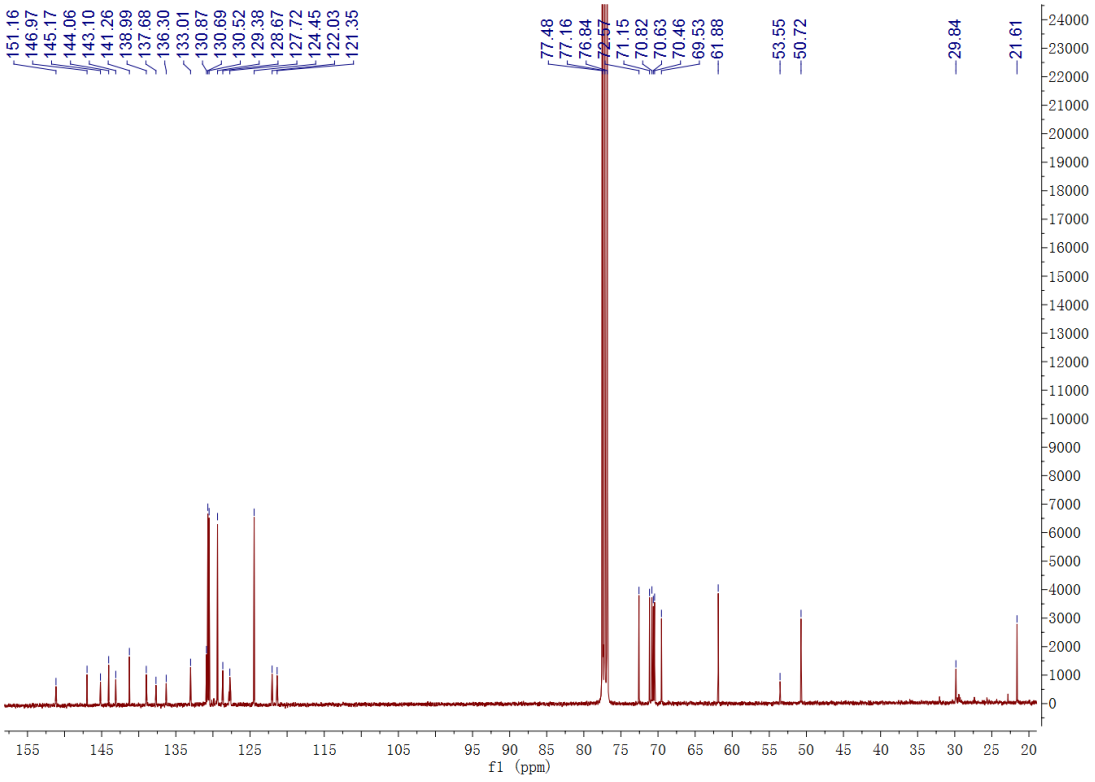



**Figure S5.** Emission spectra of **1a** (10 μM) upon addition of increasing concentrations of (a) GSH or (c) Hcy; (b) Fluorescence intensity at 588 nm as function of the concentrations of (b) GSH and (d) Hcy. Each spectrum was acquired 40 min after GSH or Hcy addition in acetonitrile / HEPES buffer (2:8, v/v, 20 mM, pH 7.4) at 37 °C. $\lambda_{ex}$ = 528 nm.

## $^{1}$H NMR, $^{13}$C NMR and HRMS of 1a



## Peking University Mass Spectrometry Sample Analysis Report

**Analysis Info**

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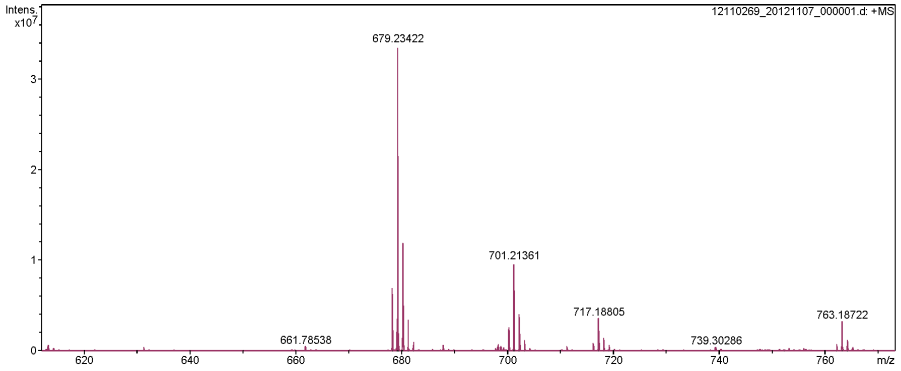
| Comment | ESI Positive | Operator | Peking University |

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|---|---|---|---|---|---|---|---|---|---|---|
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Bruker Compass DataAnalysis 4.0 printed: 11/7/2012 11:02:28 AM Page 1 of 1

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## References

(1) (a) M. Baruah, W Qin.; N. Basarić, W. M. De Borggraeve and N. Boens, *J. Org. Chem.* 2005, **70**, 4152; (b) D. W. Domaille, L. Zeng and C. J. Chang, *J. Am. Chem. Soc.* 2010, **132**, 1194.

(2) M. Gonçalves, K. Estieu-Gionnet, T. Berthelot, G. Laïn, M. Bayle, X. Canron, N. Betz, A. Bikfalvi and G. Déléris, *Pharm. Res.* 2005, **22**, 1411.