

Supplementary Information

A colorimetric and ratiometric fluorescent probe for thiols and its bioimaging applications

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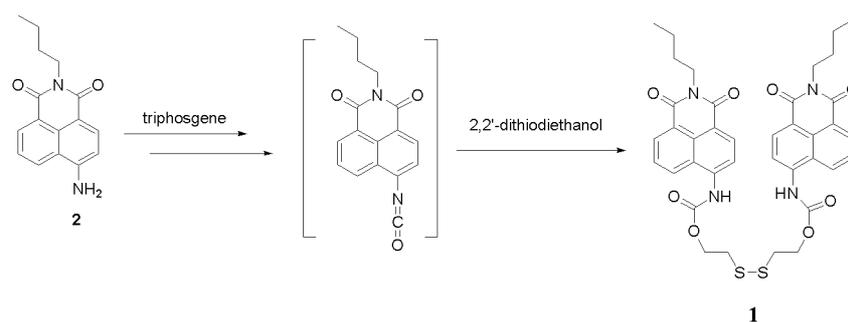
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1. Materials and general methods

All chemicals used in this paper were commercial products of analytical grade. $^1\text{H-NMR}$ was recorded on a Bruker AV-400 spectrometer with chemical shifts reported as ppm (in $\text{DMSO-}d_6$, TMS as internal standard). Mass spectral analyses were carried out on a MALDI-TOF spectrometer. High-resolution mass data were measured with fourier transform ion cyclotron resonance mass spectrometer (APEX IV). Absorption spectra were recorded on TU-1901 UV-vis spectrophotometer. Fluorescence emission and excitation spectra were measured on Hitachi F-7000. All pH measurements were made with a Sartorius basic pH-meter PB-10.

2. Synthesis of probe 1



Scheme S1. Synthesis of probe 1

To a mixture of **2** (536.6 mg, 2 mmol) and DIPEA (878.8 mg, 6.8 mmol) in 30 mL toluene was added a solution of triphosgene (611.8 mg, 2 mmol) in 8 mL toluene dropwise. The resulting solution was heated to reflux for 3 h. After cooling to room temperature, the reaction mixture was diluted with 30 mL THF and filtered. After removal of the solvents, to the residues was added the 2,2'-dithiodiethanol (154.3 mg, 1 mmol) in 10 mL THF. The solution was stirred at room temperature for an additional 18 h. After removal of THF, the residues were purified by silica gel column chromatography using chloroform as eluent to afford **1**. $^1\text{H-NMR}$ (400 MHz,

DMSO- d_6) δ ($\times 10^{-6}$): 0.92(t, $J = 7.4$ Hz, 6H), 1.31-1.37(m, 4H), 1.57-1.60(m, 4H), 3.17(t, $J = 6.4$ Hz, 4H), 3.98(t, $J = 7.4$ Hz, 4H), 4.48(t, $J = 6.4$ Hz, 4H), 7.71(t, $J = 7.8$ Hz, 2H), 8.04(d, $J = 8.4$ Hz, 2H), 8.27(d, $J = 8.4$ Hz, 2H), 8.37(d, $J = 6.8$ Hz, 2H), 8.56(d, $J = 8.4$ Hz, 2H), 10.19(s, 2H). MALDI-TOF calcd for $C_{38}H_{38}N_4NaO_8S_2$ $[M+Na]^+$ 765.2, found 765.2; $C_{38}H_{38}KN_4O_8S_2$ $[M+K]^+$ 781.2, found 781.2. HRMS (ESI positive) calcd for $C_{38}H_{39}N_4O_8S_2$ $[M+H]^+$ 743.22038, found 743.21947; $C_{38}H_{38}N_4NaO_8S_2$ $[M+Na]^+$ 765.20233, found 765.20083.

3. Fluorescence responses of **1** to different thiols

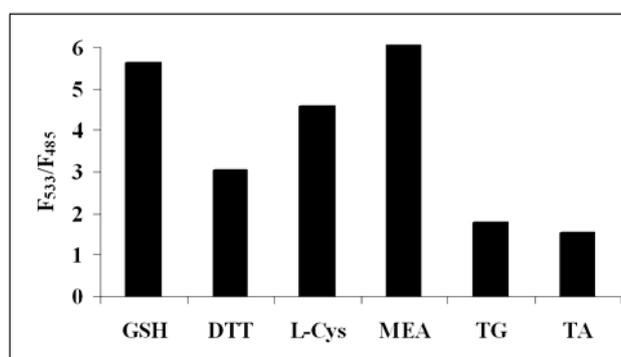


Fig. S1 Fluorescence responses of **1** ($5 \mu\text{M}$) to different thiols (10 mM) including GSH, DTT, L-Cys, MEA, TG, and TA. Excitation wavelength was 400 nm. Each spectrum was acquired 1 h after various thiols addition at 30 \square .

4. Effects of the pH on the fluorescence spectrum of 1 and the reaction between 1 and GSH

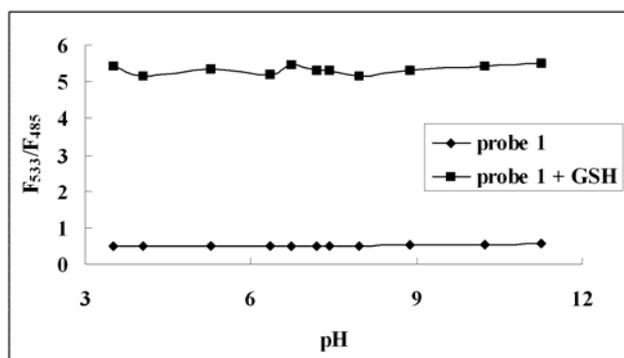


Fig. S2 Effects of the pH on the fluorescence spectrum of 1 (5 μ M) and the reaction between 1 and GSH (10 mM) in the mixture of ethanol and water (1:9, v/v). Excitation wavelength was 400 nm. Each spectrum was acquired 1 h after various analytes addition at 30 $^{\circ}$ C.

5. Fluorescence responses of 1 toward NEM, GSH and a mixture of NEM and GSH

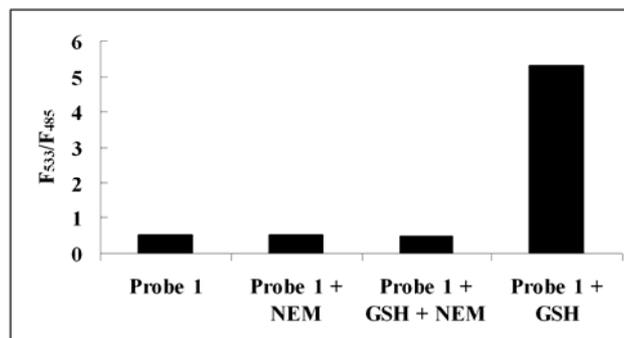


Fig. S3 Fluorescence responses of 1 (5 μ M) toward NEM, GSH and a mixture of NEM and GSH in PBS (20 mM) solution (ethanol/water = 1:9, v/v, pH 7.4). Excitation wavelength was 400 nm. Each spectrum was acquired 1 h after various analytes addition at 30 $^{\circ}$ C.

6. Photos of color and fluorescence changes of **1** in the absence and presence of GSH

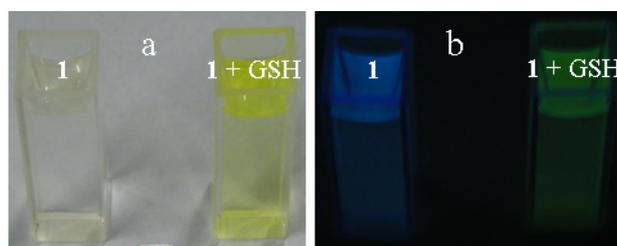


Fig. S4 Color (a) and Fluorescence (excited by UV lamp (ex. 365 nm)) (b) changes of **1** (5 μ M) in PBS (20 mM) solution (ethanol/water = 1:9, v/v, pH 7.4) in the absence (left) and presence (right) of GSH (10 mM).

7. Determination of the detection limit

The detection limit was calculated based on the fluorescence titration. The fluorescence emission spectrum of probe **1** was measured by five times and the standard deviation of blank measurement was achieved. To gain the slop, the ratio of the fluorescence intensity at 533 nm to the fluorescence intensity at 485 nm (F_{533}/F_{485}) was plotted as a concentration of GSH. So the detection limit was calculated with the following equation:

$$\text{Detection limit} = 3\sigma/k$$

Where σ is the standard deviation of blank measurement, k is the slop between the fluorescence intensity ratio versus GSH concentration.

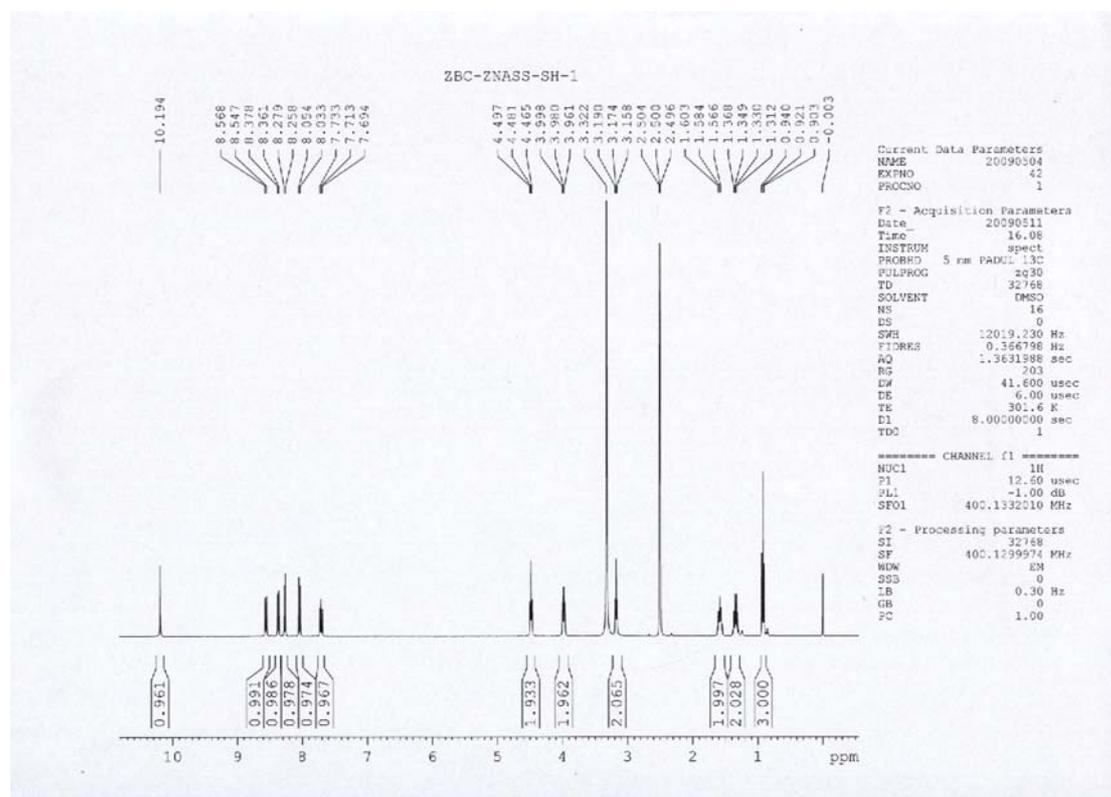
8. Cell culture

HeLa cells (gifted from the center of cells, Peking Union Medical College) were cultured in culture media (DMEM/F12 supplemented with 10% FBS, 50 unit/mL penicillin, and 50 μ g/mL of streptomycin) at 37 $^{\circ}$ C under a humidified atmosphere containing 5% CO₂. HeLa cells were seeded in a 6-well plate at a density of 10⁴ cells per well in culture media. After 24 h, the cells were incubated with 10 μ M **1** in culture media for 20 min at 37 $^{\circ}$ C. HeLa cells were pretreated with 2.5 mM NEM for 1 h to

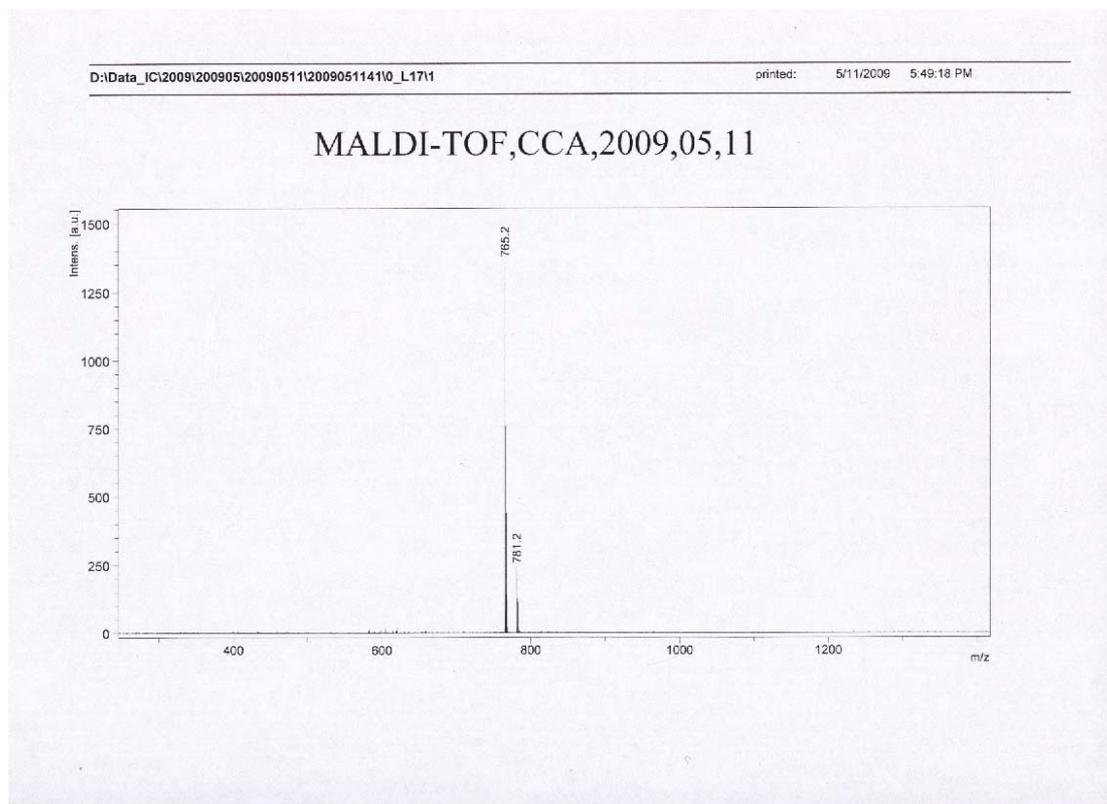
reduce the concentration level of biothiols, and then they were incubated with the probe **1** (10 μ M) for another 20 min. After the medium was removed and the cells were carefully washed with PBS for twice, fluorescence imaging of living HeLa cells was observed under Nikon CI Si confocal and multi-photo system (confocal excitation: a diode laser at 408 nm).

9. The characterization data of **1**

^1H NMR of **1**



MS of 1



HRMS of 1

