Supporting Information

Fluorescent detection of biothiols based on a novel cascade reaction

Jing Liu, Yuan-Qiang Sun, Xin Lv, and Wei Guo*

School of Chemistry and Chemical Engineering, Shanxi University, Taiyuan 030006, China.
E-mail: guow@sxu.edu.cn

1. Experimental Section

General information and methods. All reagents and solvents were purchased from commercial sources and were of the highest grade. Solvents were dried according to standard procedures. All reactions were magnetically stirred and monitored by thin-layer chromatography (TLC). Flash chromatography (FC) was performed using silica gel 60 (200–300 mesh). Absorption spectra were taken on an Agilent 8453 spectrophotometer. Fluorescence spectra were taken on SHIMADZU RF-5301PC fluorescence spectrometer. The $^1$H NMR and $^{13}$C NMR spectra were recorded at 300 and 75 MHz, respectively. The following abbreviations were used to explain the multiplicities: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; br = broad. High resolution mass spectra were obtained on a Varian QFT-ESI mass spectrometer.

Procedures for thiol sensing

Deionized water was used throughout all experiments. A stock solution of 1 (1 mM) was prepared in CH$_3$CN. The stock solution of 1 was then diluted to 2 μM with the
solution of HEPES (10 mM, pH 7.4 containing 5% CH$_3$CN). Spectra data were recorded in an indicated time after the addition of amino acids. Normally, excitation was at 465 nm. The excitation and emission slit width was 5 nm and 10 nm respectively.

**Cell culture and fluorescence imaging:** The HeLa cell line was provided by Key Laboratory of Chemical Biology and Molecular Engineering of Ministry of Education (China). Cells were grown in RPMI 1640 medium supplemented with 10 % FBS (Fetal Bovine Serum) and 1% antibiotics at 37 ºC in humidified environment of 5% CO$_2$. Cells were plated on 6-well plate at 5×106 cells per well and allowed to adhere for 12 hours. We investigated the living cell bioimaging of Cys by using DMSO–PBS (1:500, v/v, pH 7.4) as a staining medium. HeLa cells were treated with 20 µM of probe 1 in the media for 30 min at 30 ºC, and washed 3 times with PBS. For the control experiment, HeLa cells were pretreated with 500 µM NEM in the media for 30 min at 30 ºC. After washing with phosphate buffered saline (PBS) to remove the remaining NEM, the cells were further incubated with 20 µM of 1 in the media for 30 min at 30 ºC. Fluorescence imaging was performed with by a Olympus FV1000 Laser Scanning Confocal Microscope (Japan).

**Quantum yield determination of probe 1 and methylfluorescein 2**

Fluorescence quantum yields of 1 and methylfluorescein 2 were determined in HEPES buffer (pH 7.4, 10 mM, containing 5% CH$_3$CN) with fluorescein (Φ = 0.95, in 0.1 M NaOH) as a reference.$^{53}$ Methylfluorescein 2 was obtained in the experiment by addition of 50 equiv of Cys to the solution of probe 1. The quantum yields were calculated using Eq.1:

$$\Phi_u = \frac{[(A_sFA_u\eta^2)/ (A_uFA_s\eta_0^2)]\Phi_s}{\Phi_u}. \quad \text{Eq.1}$$

Where $A_s$ and $A_u$ are the absorbance of the reference and sample solution at the reference excitation wavelength, $FA_s$ and $FA_u$ are the corresponding integrated fluorescence intensity, and $\eta$ and $\eta_0$ are the solvent refractive indexes of sample and reference, respectively. Absorbance of sample and reference at their respective
excitation wavelengths was controlled to be lower than 0.05.

Quantum yield of 1: $\Phi = 0.073$

Quantum yield of methylfluorescein 2: $\Phi = 0.265$

**Synthesis**

\[
\text{C} + \text{O} \xrightarrow{\text{AlCl}_3} \text{HO} 
\]

**Compound 2:** 2 was synthesized according to the literature report without modification.\(^{S1}\) To a stirred solution of maleic anhydride (2.45 g, 25 mmol) in 15 ml benzene was added AlCl\(_3\) (7.2 g, 54 mmol) portionwise at room temperature. The mixture was stirred at 80 °C for 30 min. Then the content of the flask was poured onto 30 ml of ice-water and 7.5 ml of concentrated hydrochloric acid was added. The solution was extracted with 2×35 ml of EtOAc, dried with Na\(_2\)SO\(_4\) and evaporated under reduced pressure to give 4.0 g of the product as a yellow solid. \(^1\)H NMR (CDCl\(_3\), 300 MHz): $\delta$ 10.68 (br, 1H), 7.98–8.03 (m, 3H), 7.62–7.67 (m, 1H), 7.47–7.55 (m, 2H), 6.90 (d, $J = 15.6$ Hz, 1H).

\[
\text{HO} + \text{HO} \xrightarrow{\text{EDC, DMAP}} \text{HO} 
\]

**Compound 1:** 3 was synthesized according to the literature report.\(^{S2}\) To a mixture of compounds 2 (0.264 g, 1.5 mmol), 3 (0.173 g, 0.5 mmol), EDC (0.288 g, 1.5 mmol), and DMAP (18.3 mg, 0.15 mmol) was added CH\(_2\)Cl\(_2\) (20 ml) at room temperature. The mixture was stirred for 12 hours. Then solvent was evaporated under reduced pressure and resulted residue was subjected to silica gel chromatography with CH\(_2\)Cl\(_2\)/EtOAc (30:1), giving 0.14 g of probe 1 (55.6 %) as a light yellow solid. \(^1\)H NMR (CDCl\(_3\), 300 MHz): $\delta$ 8.02–8.30 (m, 4H), 7.70–7.77 (m, 3H), 7.59 (t, $J = 7.5$ Hz, 2H), 7.34–7.40 (m, 2H), 6.89–7.07 (m, 4H), 6.69–6.77 (m, 2H), 3.83 (s, 3H); \(^{13}\)C NMR (DMSO, 75 MHz): 188.6, 168.0, 162.8, 160.7, 151.8, 151.0, 150.5, 138.2,
135.3, 133.8, 129.9, 128.5, 125.1, 124.4, 123.5, 117.6, 116.3, 111.9, 109.8, 100.3, 81.1, 55.2; HRMS: calcd for 505.1282 (M+H)$^+$, found 505.1278; calcd for 527.1107 (M+Na)$^+$, found 527.1096.

References
2. Supplementary Spectra

Fig. S1. Time-dependent fluorescence spectra of the probe 1 (2 μM) to several sulfydryl-containing compounds (100 μM) in HEPES buffer (pH 7.4, 10 mM, containing 5% CH₃CN) at 20 °C. (A) N-acetyl-protected Cys (NAC); (B) thiophenol; (C) mercaptoacetic acid. λₓₑₓ = 465 nm, λₑₜₐₐₙ = 512 nm. Slit: 5 nm/5 nm.

Fig. S2. Time-dependent fluorescence spectra of the probe 1 (2 μM) to Cys (10 μM) in HEPES buffer (pH 7.4, 10 mM, containing 5% CH₃CN) at 20 °C.
**Fig. S3** The fluorescent intensities at 512 nm for probe 1 (2 μM) in the absence or presence of Cys (10 μM) at varied pH values.

**Fig. S4** (A) Fluorescence spectral changes of 1 (2 μM) upon addition of Cys (0, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2, 3.6, 4.0 μM) (λ_ex = 465 nm, λ_em = 512 nm; slit, 5 nm/5 nm). (B) The fluorescence intensity of 1 at 512 nm as a function of the concentration of Cys. Each spectrum was recorded after 15 min upon addition Cys in HEPES buffer (pH 7.4, 10 mM, containing 5% CH3CN) at 20 °C.
Fig. S5 The kinetic study of the response of the probe 1 (2 μM) to 100 μM of Cys, Hcy and GSH, respectively, in HEPES buffer (pH 7.4, 10 mM, containing 5% CH₃CN) at 20 °C.

Fig. S6 HRMS charts of probe 1 upon addition of Cys obtained after 0.5 h (A) and within 1 min (B).
Fig. S7 $^1$H NMR chart of 1 (DMSO-$d_6$) (300 MHz).

Fig. S8 $^{13}$C NMR chart of 1 (DMSO-$d_6$) (75 MHz).
Fig. S9 HRMS chart of 1.