Supporting Information

Two Emission Channels for Sensitive Discrimination of GSH over Cys/Hcy and detection of GSH in Plasma and Cells

Yuanlin Zhang,[a] Xiangmin Shao,[a] Yue Wang,[a] Fuchao Pan,[a] Ruixue Kang,[a] Fangfang Peng,[a] Zhentao Huang,[a] Weijuan Zhang*[a] and Weili Zhao*[ab]

[a] Key Laboratory for Special Functional Material of the Ministry of Education, Henan University, Kaifeng, 475004, P. R. China

[b] School of Pharmacy, Fudan University, 826 Zhangheng Rd., Shanghai, 201203, P. R. China

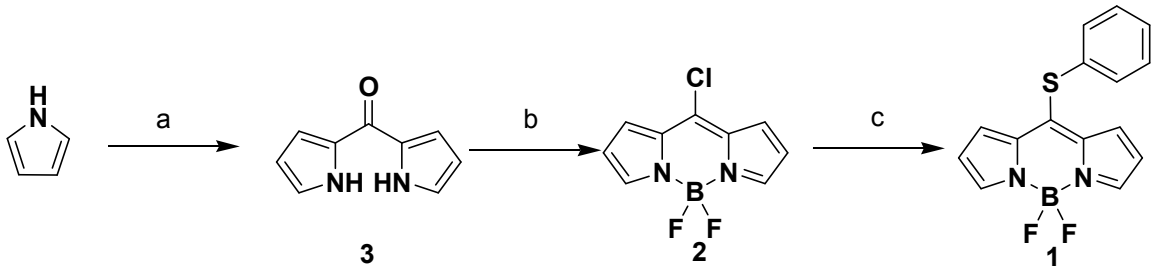
Content

General Information

All reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. Acetonitrile in chromatographic purity and deionized water were used in detection. ^{1}H NMR spectra were recorded on a VARIAN Mercury 400 MHz spectrometer. ^{1}H NMR chemical shifts (δ) are given in ppm (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet) downfield from Me_4Si, determined by chloroform (δ = 7.26 ppm) and dimethyl sulfoxide (δ = 2.5 ppm). ^{13}C NMR spectra were recorded on a VARIAN Mercury 100 MHz spectrometer. ^{13}C NMR chemical shifts (δ) are reported in ppm with the internal $CDCl_3$ at δ 77.0 as standard, respectively. Mass spectrometric measurements were performed by the mass spectrometry service of Institute of Organic Chemistry, Chinese Academy of Sciences on a Bruker Reflex MALDI as matrix (20 kV). Fluorescence spectra were recorded on FluoroSENS spectrophotometer. UV/Vis spectra were recorded on Perkin-Elmer Lambda 35 UV/Vis spectrophotometer at room temperature. Confocal laser scanning microscopic studies were recorded on OLYMPUS FV1000.

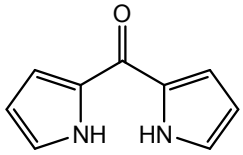
Synthesis

Probe **1** was synthesized by S_N2 replacement of 8-chloro-BODIPY (**2**) which was prepared from dipyrryl ketone (**3**) obtained from pyrrole and triphosgene (see Scheme 1).



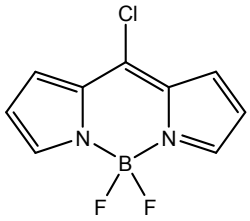
Scheme S1. Synthesis of probe **1**. a) (i) triphosgene (0.33 equiv.), 1,2-dichloroethane, 0 °C, 2 h; (ii) pyrrole (1 equiv.), 80 °C, 30 min; (b) (i) $POCl_3$ (2.3 equiv.), 1,2-dichloroethane, 80 °C, 2 h. (ii) Et_3N (10.0 equiv.), BF_3•Et_2O (11.0 equiv.); (c) Thiophenol (1.0 equiv.), Et_3N (1.0 equiv.), dichloromethane, 25 °C, 30 min.

Dipyrrolyl-2-yl methanone (**3**)



A solution of triphosgene (2.97 g, 10 mmol) in 1,2-dichloroethane (30 ml) was added to a stirred solution of freshly distilled pyrrole (2.08 mL, 30 mmol) and ethyldiisopropylamine (4.96 mL, 30 mmol) in 1,2-dichloroethane (10 ml) over 20 min at 0°C under nitrogen. After stirred for 2 h at 0 °C, a second portion of pyrrole (2.08 mL, 39 mmol) was added. The mixture was heated under reflux for 30 min. The resulting solution was poured into diethyl ether (300 ml). The organic layer was separated and was washed with water. After drying over anhydrous Na_2SO_4, filtration, and evaporation, the crude product was purified by column chromatography on silica gel (Eluent: 20% EtOAc in CH_2Cl_2). Pure product (1.6 g, 33%) was obtained as a pale-yellow needles. ^{1}H-NMR (400 MHz; d-DMSO) 3.40 (2H, m), 6.27 (2H, m), 7.11 (2H, m), 11.85 (2H, s). ^{13}C-NMR (400 MHz; d-DMSO) 110.3, 115.9, 124.8, 173.0.

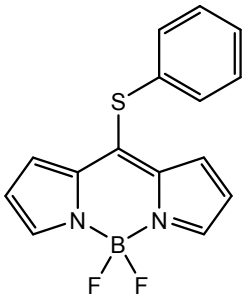
8-Chloro-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (**2**)



Dipyrrolyl-2-yl methanone (**3**) (160 mg, 1 mmol) was dissolved in 1,2-dichloroethane (10 ml). Phosphorusoxychloride (0.18 ml, 2 mmol) was added, and the reaction mixture was heated to reflux for 3 h, then cooled in an ice bath. Triethylamine (1.4 ml, 10 mmol) was added, and the reaction was stirred at 0 °C for 5 min. Boron trifluoride etherate (1.4 ml, 11 mmol) was added dropwise while maintaining the temperature at 0 °C. The reaction mixture was allowed to warm up to room temperature and stirred for additional 2 h. The resulting solution was poured into diethyl ether (300 ml). The organic layer was separated and was washed with water. After drying over Na_2SO_4, filtration, and evaporation, the crude product was purified by flash chromatography on silica gel

(CH_2Cl_2/petroleum ether 1 : 1, v/v) to give **2** (120 mg, 53%) as red crystalline solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ7.88 (s, 2H), 7.41 (d, 2H, J = 4.00 Hz), 6.58 (d, 2H，J = 4.00 Hz) ppm; ^{13}C-NMR (400 MHz, $CDCl_3$): δ145.1, 141.2, 134.1, 129.3, 119.1 ppm.

8-(Phenylmercapto)-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (**1**)



8-Chloro BODIPY **2** (22.6 mg, 0.1 mmol) was dissolved in dichloromethane (10 ml), thiophenol (10.2 μL, 0.1 mmol) and triethylamine (13.9 μL, 0.1 mmol) were added. The reaction mixture was purged with nitrogen and stirred at room temperature for 10 min. The crude mixture was poured into diethyl ether, washed with aqueous Na_2CO_3, and the organic layer was evaporated to dryness. Compound **1** was obtained as a red crystalline solid in 87% yield (26.0 mg) after purification by column chromatography on silica gel (Eluents: CH_2Cl_2/petroleum ether 1 : 1, v/v).

^{1}H-NMR(300MHz, $CDCl_3$): δ7.77, (s, 2H); 7.60 (d, 2H); 7.44 (m, 3H); 6.96 (s, 2H); 6.38 (s, 2H) ppm; ^{13}CNMR (75 MHz, $CDCl_3$): δ150.4, 142.2, 134.3, 133.1, 131.9, 130.3,130.1, 128.7, 118.1 ppm; HRMS: Calculated for $C_{15}H_{11}BF_2N_2S$: 300.0704, measured: 300.0700.

Procedure for GSH detection in plasma

The detection of GSH was performed in 10% deproteinized human plasma. Plasma proteins were precipitated using acetonitrile (two thirds of the reconstitution volume) and removed by centrifugation at 8,000 rpm for 30 min. The supernatant liquid was diluted in HEPES buffer (pH 7.4, 10 mM). The GSH content in the plasma sample was determined from the regression equation of a standard calibration curve. The GSH content of the plasma sample was determined to be 4.5 μM, which is well within the reported GSH concentration range for human plasma samples from healthy individuals.

Procedures for GSH imaging in cells

Probe **1** (20 μM, 2 mL) in acetonitrile/HEPES buffer (2 : 8, v/v, 10 mM, pH 7.4) was added to Human Hepatoma SMMC-7721 cells in 35 mm cell plates that contained 1.0 mL DMEM culture medium supplemented with 10% FBS (Fetal Bovine Serum) and 1% antibiotics at 37 °C in humidified environment of 5% CO_2 and was incubated at 37 °C for 60 min. Cells were plated on cell plates at 5×10^6 cells per cell plate and allowed to adhere for 24 hr. After removing the culture medium and washing with PBS twice, the conforcal laser scanning fluorescence images of cells were recorded using the green channel (λ_{ex} = 488 nm). For the trapping experiment, the cells in the cell culture plate that contained 2.0 mL culture medium were treated with 5 mM Nethylmaleimide (NEM) in culture media for 60 min at 37 °C in a humidified incubator. After washing with PBS for twice, the cells were further incubated with 10 μM of the probe for 60 min. After washing with PBS for twice, the confocal laser scanning fluorescence images of cells were taken.

MTT assay experiment

MTT experiment was performed in 96-well plate to assess the cytotoxicity of the probe. The MTT assy in Human Hepatoma SMMC-7721 cells with probe concentrations from 2.5 to 80 μM in comparison with the blank and negative control. Cells were plated on cell plates at 4×10^3 cells per well and allowed to incubate for 24 hr. The probe **1** in various concentrations was added to the well and incubated for 24 hr followed by classical MTT treatment and data acquiring.

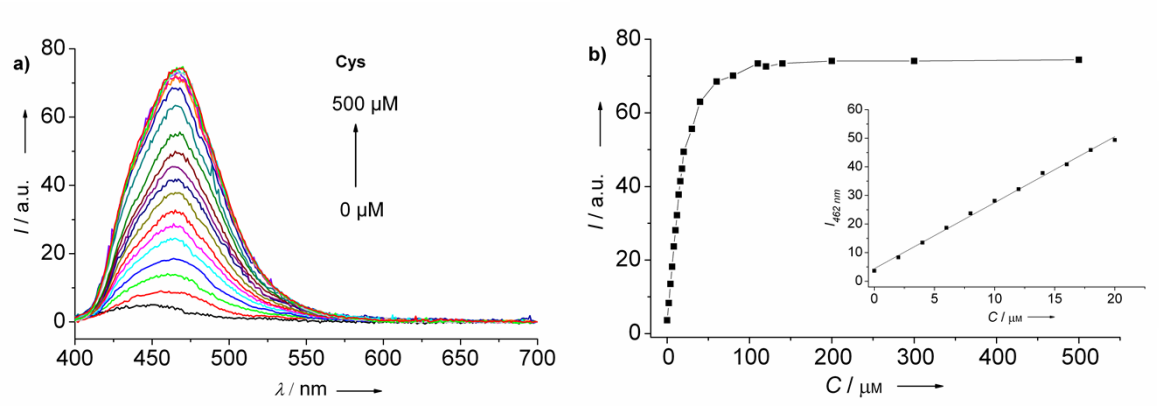


Figure S1. (a) Fluorescence spectral changes of probe **1** (10 μM) upon addition of Cys (0-500 μM) (λ_{ex} = 390 nm, λ_{em} = 467 nm) in acetonitrile/HEPES buffer (2 : 8, v/v, 10 mM, pH 7.4) at 37 °C. Each spectrum was recorded after 10 min; (b) Fluorescence response of 10 μM probe **1** at 467 nm upon reacting with Cys in 0–500 μM concentrations after 10 min of incubation in acetonitrile/HEPES buffer (2 : 8, v/v, 10 mM, pH 7.4) at 37 °C. The excitation wavelength was 390 nm. The inner panel displays the linear fluorescence enhancement of probe **1** toward Cys in 1-20 μM.

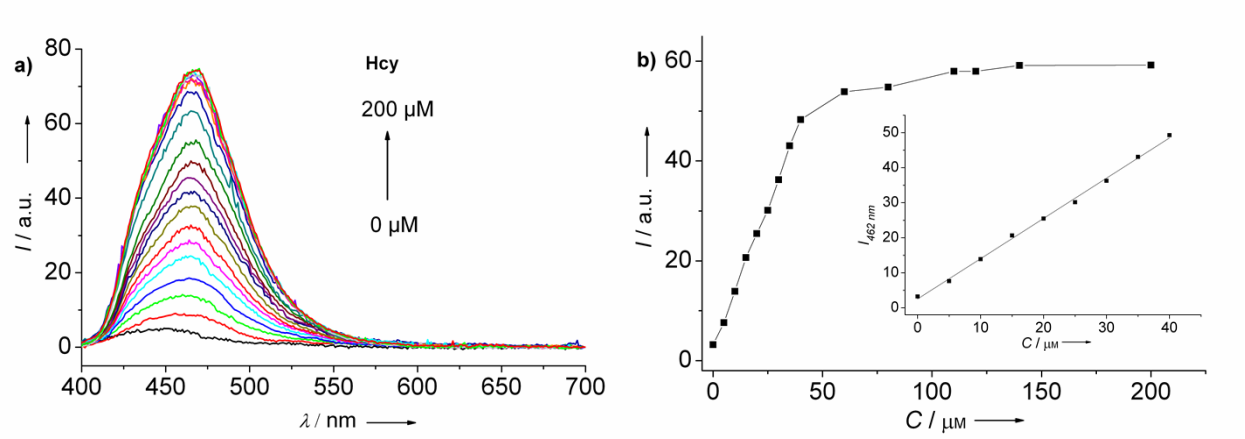


Figure S2. (a) Fluorescence spectral changes of probe **1** (10 μM) upon addition of Hcy (0-200 μM) (λ_{ex} = 390 nm, λ_{em} = 462 nm) in acetonitrile/HEPES buffer (2 : 8, v/v, 10 mM, pH 7.4) at 37 °C. Each spectrum was recorded after 10 min; (b) Fluorescence response of 10 μM probe **1** at 462 nm upon reacting with Cys in 1-200 μM concentrations after 10 min of incubation in acetonitrile/HEPES buffer (2 : 8, v/v, 10 mM, pH 7.4) at 37 °C. The excitation wavelength was 390 nm. The inner panel displays the fluorescence enhancement of probe **1** toward Hcy in 1-40 μM.

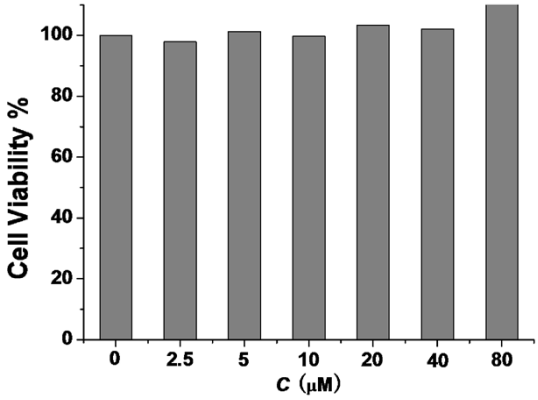


Figure S3. Cell viability assay of probe **1** in MTT assay.

Copies of NMR Spectra