Electronic supplementary information

Low Background D-A-D Type Fluorescent Probe for Imaging of Biothiols in Living Cells

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Contents

Materials and instruments

All the solvents and reagents were obtained commercially, and the solvents including THF and DMF were used after appropriate distillation and purification. UV–vis absorption spectra were recorded on a Schimadzu 160A spectrophotometer. Fluorescence spectra were recorded on a Hitachi F-4500 spectrometer. The pH measurements were made with a Sartorius basic pH-meter PB-10. HPLC analyses were performed on Shimadzu LC-20A high performance liquid chromatography. Fluorescence imagings were carried out by a confocal laser scanning microscope (CLSM, Zeiss LSM 880, Jena, Germany). ^{1}H NMR spectra were recorded on Bruker Ascend 400 MHz spectrometers, and ^{13}C NMR spectra were recorded on 100 MHz spectrometers. Mass spectra (MS) were recorded on an Ion Spec 4.7 T FTMS instrument.

Synthesis of M-1

Curcumin (0.37 g, 1 mmol) was dissolved in 20 mL of dichloromethane, followed by the addition of boron trifluoride diethyl ether (0.17 g, 1.2 mmol). The mixture was stirred overnight at room temperature. The solvent was evaporated and the resulting solid was suspended into diethyl ether. The precipitate was filtered off and the black powder was washed with diethyl ether (20 mL) yielding the pure borondifluoride curcumin (M-1) as red solid (0.39g, 93%). ^{1}H NMR (400 MHz, DMSO) δ [ppm]: 10.14 (s, 2H), 7.92 (d, *J* = 16 Hz, 2H), 7.48 (s, 2H), 7.35 (d, *J* = 8Hz, 2H), 7.03 (d, *J* = 16Hz, 2H), 6.88 (d, *J* = 8 Hz, 2H), 6.45 (s, 1H), 3.85 (s, 6H).

Preparation of solutions

Stock solution of CBFB and CBFM (1 mM) were prepared in analytical grade DMSO. Other analytes including Cys, Hcy, GSH, H_2O_2, Vc (ascorbic acid), NaClO, t-BuOOH, Gly (glycine), Glu (glutamic acid), His (histidine), Pro (proline), and Lys (lysine) were dissolved in deionized water to afford 10 mM aqueous solution.

Measurements of absorption and fluorescence spectra

The test solution were prepared by adding 30 μL of stock solution of probes and an appropriate volume of each analytical solution in a 3 mL volumetric flask. The hybrid solutions were diluted to the corresponding concentration with the mixed solution of HEPES buffer (20 mM, pH 7.4)/ethanol (v/v = 1/4). The final concentration of probe was 10 μM. All the measurements were conducted after incubation with analytes for 120 min.

Cytotoxicity assays

Cells were seeded in 96-well microplates at a density of 5×10^3 cells/well in 100 μL of complete DMEM media and incubated at 37 ºC in an atmosphere containing 5% CO_2 and 95% air. After the cells reached about 80% confluence, the cells were incubated with 0, 10, 20, 30, 40 and 50 μM of **CBFB** in the transfection liquid solution contained 0.9% Lipofectamine 2000 Reagent for 12 h. After that, 20 μL of MTT solution (5 mg mL^{-1}) in PBS was added to each well and further cultured for another 4 h at 37 ºC. Then the DMEM solution was removed and 150 μL of DMSO was added to dissolve the formed purple crystals derived from MTT. The plates were then analyzed with a microplate reader (Tecan M200 PRO, Austria) at the absorbance wavelength of 570 nm.

Cell culture and cell imaging

HeLa cells were obtained from Xiangya Central Experiment Laboratory (Hunan Province, China). HeLa cells were maintained under the standard culture conditions (atmosphere of 5% CO_2 and 95% air at

37°C) in RPMI 1640 medium, supplemented with 10% FBS (fetal calf serum) and 100 IU/mL penicillin−streptomycin.

Before confocal microscopy imaging of cells with probes, HeLa cells in the exponential phase were plated on 35 mm glass-bottom culture dishes for 2 days to reach around 80% confluency, respectively. HeLa cells were starved with 1 mL of serum-free medium Opti-MEM for 2 h before they were transfected with the appropriate concentrations of probe by liposome transfection. Then the cells were incubated with the prepared transfection liquid solution in an atmosphere of 5% CO_2 and 95% air for desired time at 37 °C. Cells were washed three times with 1 mL of PBS at room temperature, and then, they were added to 1 mL of PBS culture medium and observed under confocal microscopy (Zeiss LSM 880) with a 63× oil-immersion objective.

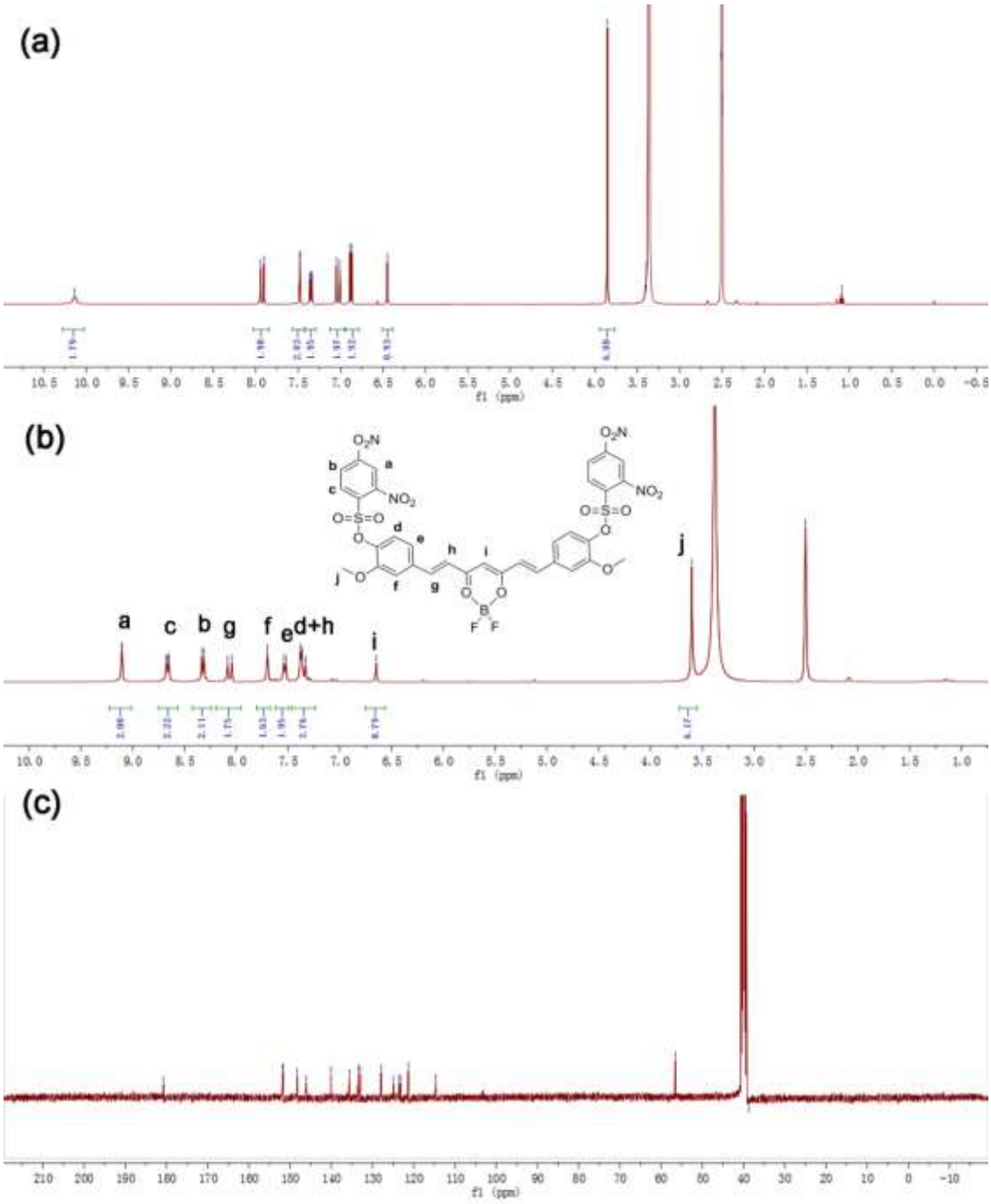

Figure S1. ^{1}H NMR spectra of (a) **M-1** and (b) **CBFB**, and (c) ^{13}C NMR spectra of **CBFB** in DMSO.

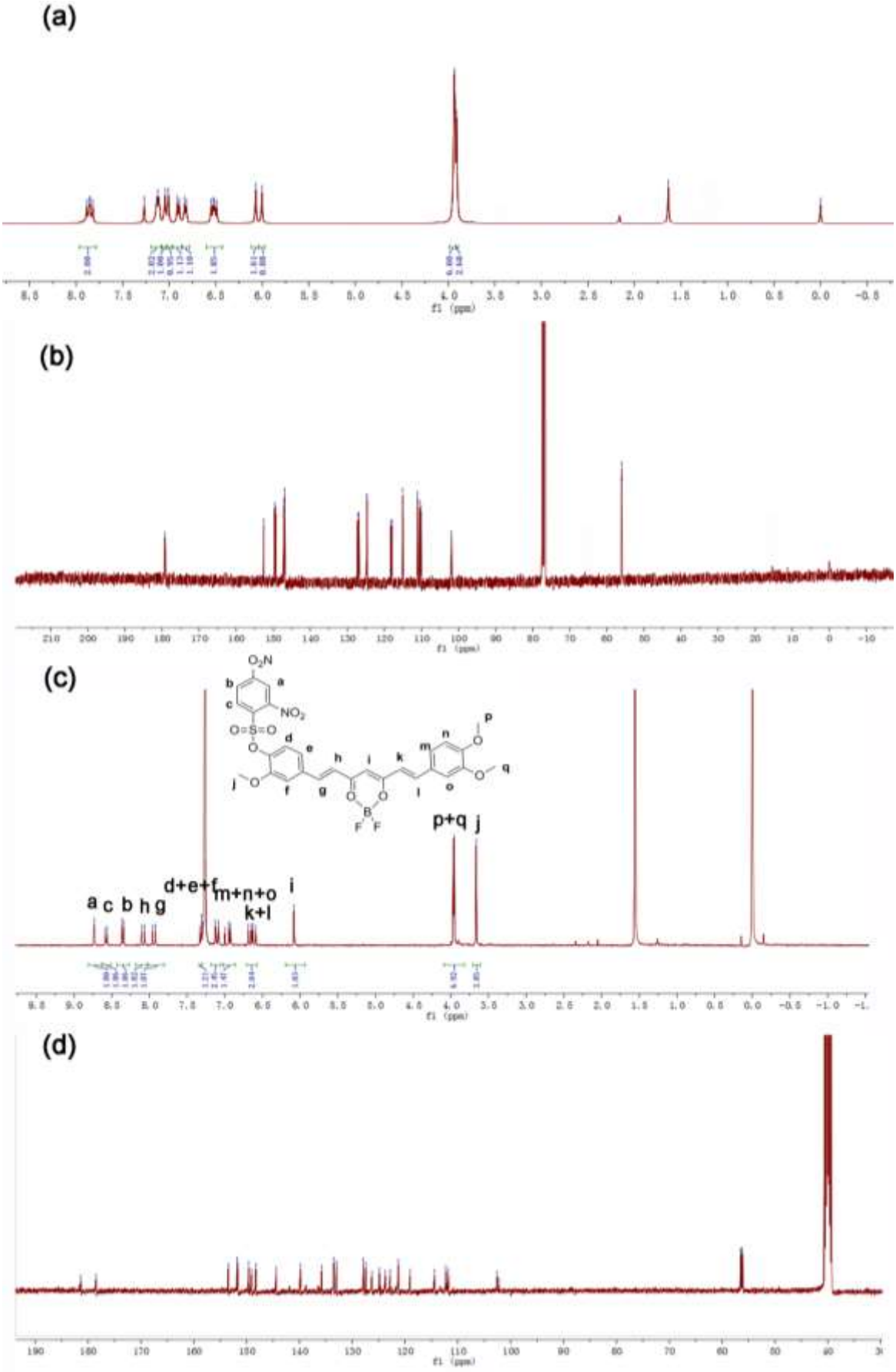

Figure S2. ^{1}H NMR spectra of (a) **M-2** and (c) **CBFM** in $CDCl_3$, and ^{13}C NMR spectra of (b) **M-2** in $CDCl_3$ and (d) **CBFM** in DMSO.

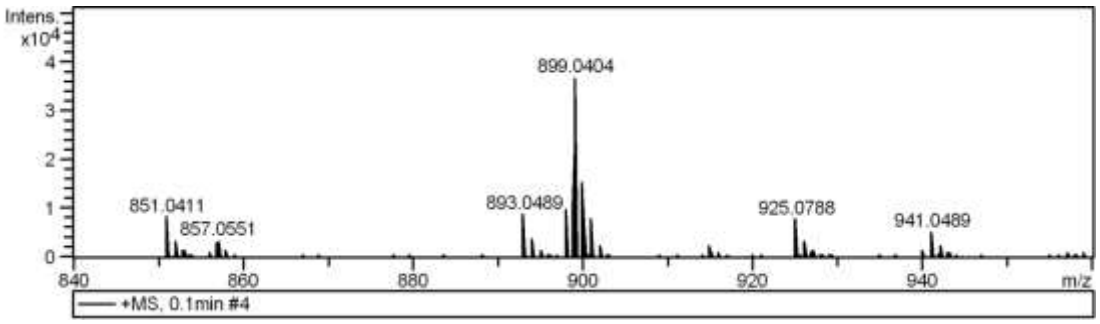

Figure S3. Mass spectrum (positive ion mode) of **CBFB**. $[M+Na]^+$: Calculated: 899.0408; Found: 899.0404.

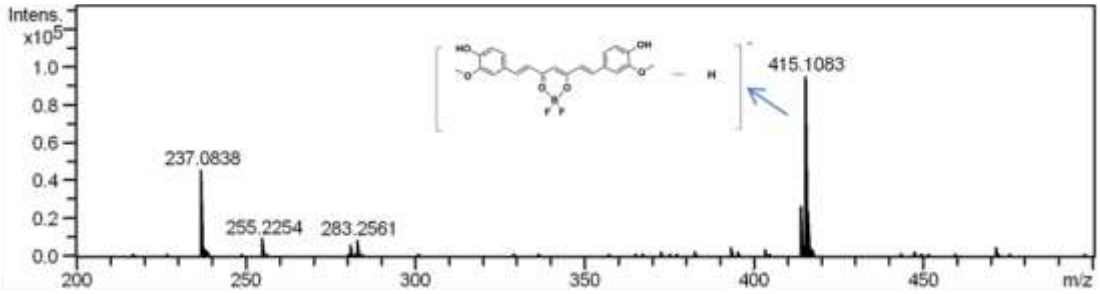

Figure S4. Mass spectrum (negative ion mode) of **CBFB** (10 μM) after treatment with Cys (200 μM) in pH 7.4 HEPES buffer/methanol (v/v, 1/4) for 120 min. Calculated: 415.1170; Found: 415.1083.

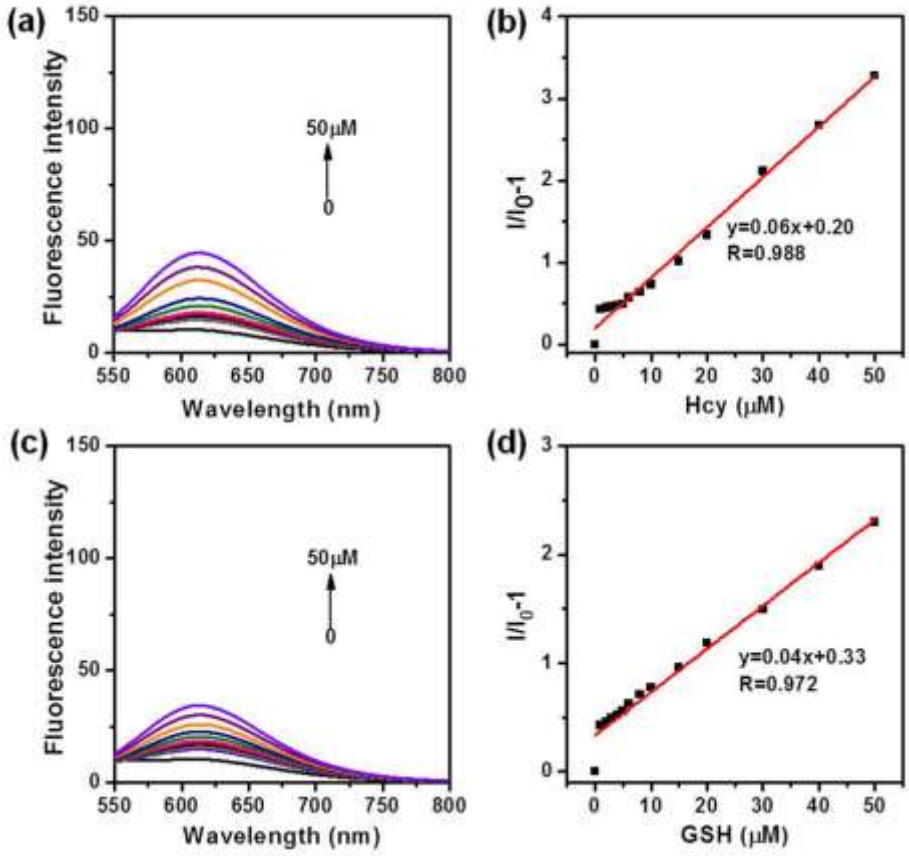

Figure S5. The fluorescence spectra of **CBFB** with addition of (a) Hcy and (c) GSH, and the changing curve of fluorescence intensity during titration of **CBFB** at 610 nm with (b) Hcy and (d) GSH in ethanol/HEPES buffer (pH 7.4, v/v = 4/1). λ_{ex} = 510 nm.

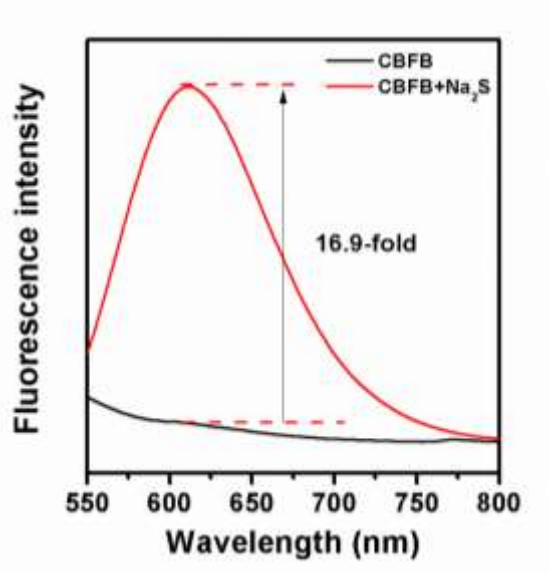

Figure S6. The response of **CBFB** (10 μM) to Na_2S (100 μM) in EtOH/HEPES buffer (pH 7.4, v/v = 4/1).

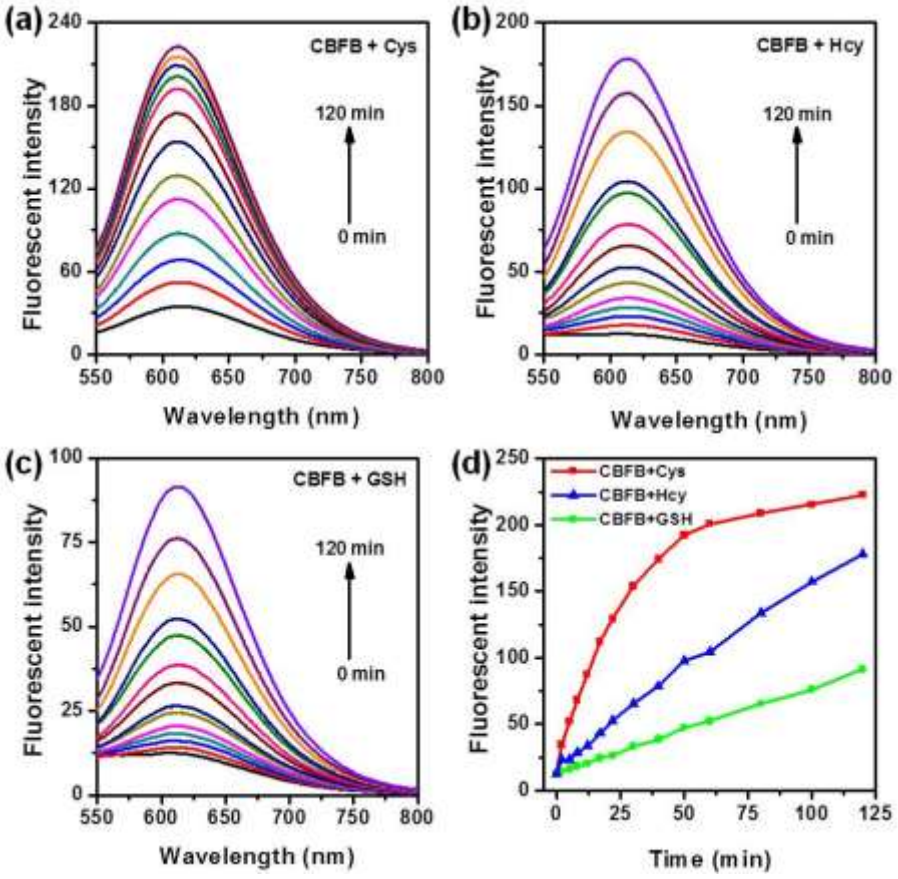

Figure S7. Fluorescence intensity change over time of probe **CBFB** towards (a) Cys, (b) Hcy and (c) GSH in ethanol/HEPES buffer (pH 7.4, v/v, 4/1), and (d) the corresponding time-dependent fluorescent intensity curve. Relative fluorescence intensity of 10 μM **CBFB** in the presence of 100 μM analytes (final concentration). λ_{ex} = 510 nm. λ_{em} = 610 nm.

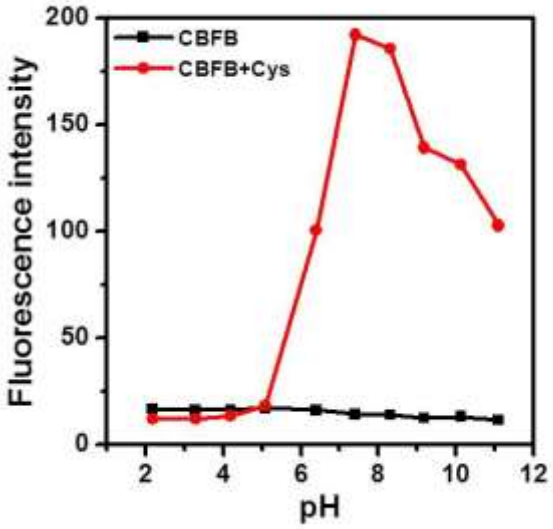

Figure S8. Fluorescence intensity of probe **CBFB** (10 μM) with addition of Cys (100 μM) in the pH range of 2 to 11. λ_{ex} = 510 nm. λ_{em} = 610 nm.

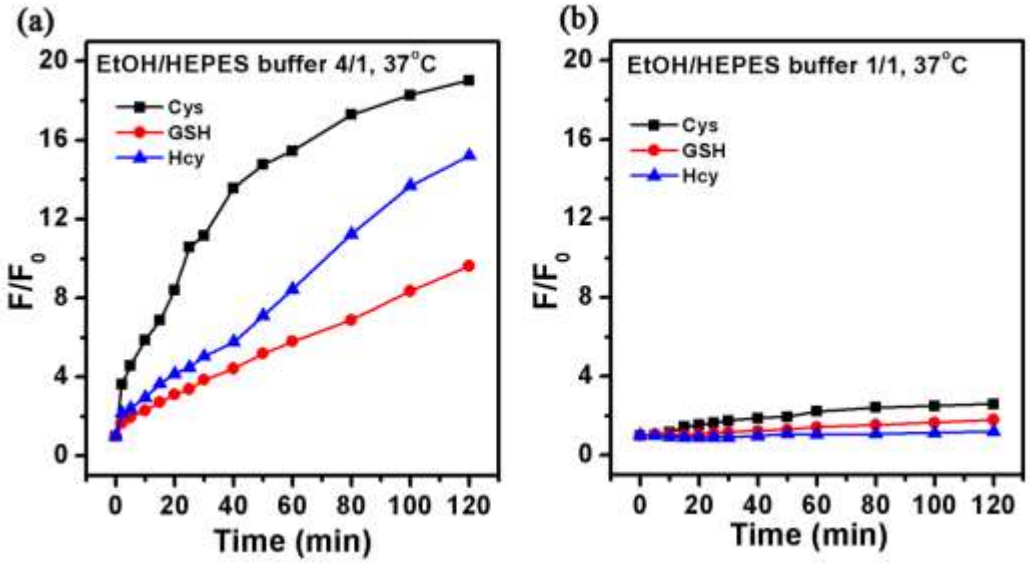

Figure S9. The time-dependent fluorescence response of **CBFB** to biothiols at 37 °C in ethanol/HEPES buffer (pH 7.4) with the volume ratio (v/v) of (a) 4/1 and (b) 1/1.

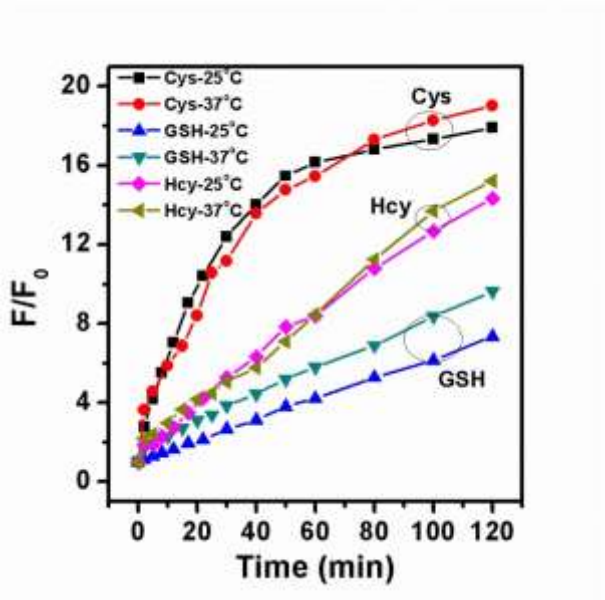

Figure S10. The comparison of fluorescence responses of **CBFB** to Cys, Hcy and GSH at 25 and 37 ^{o}C, respectively.

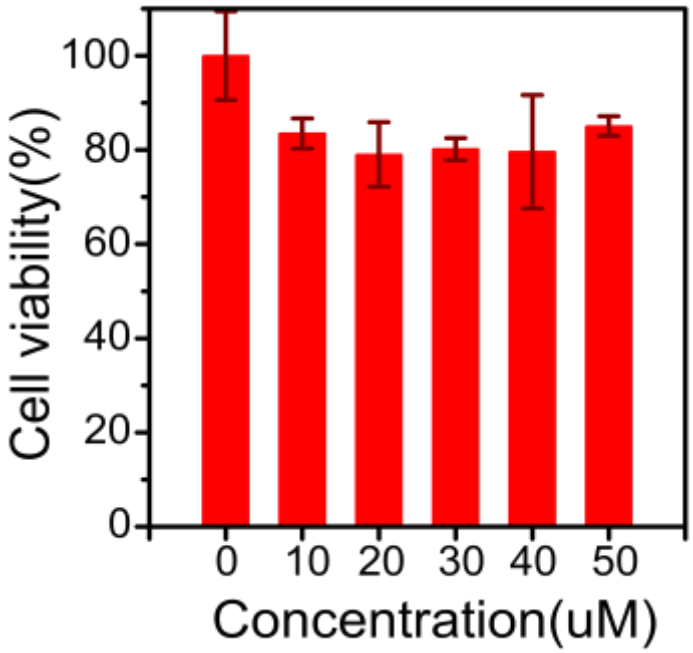

Figure S11. Cell viability (%) estimated by MTT assay versus incubation concentrations of **CBFB**. HeLa cells were incubated with 0 - 50 μM **CBFB** at 37 °C for 12 h.

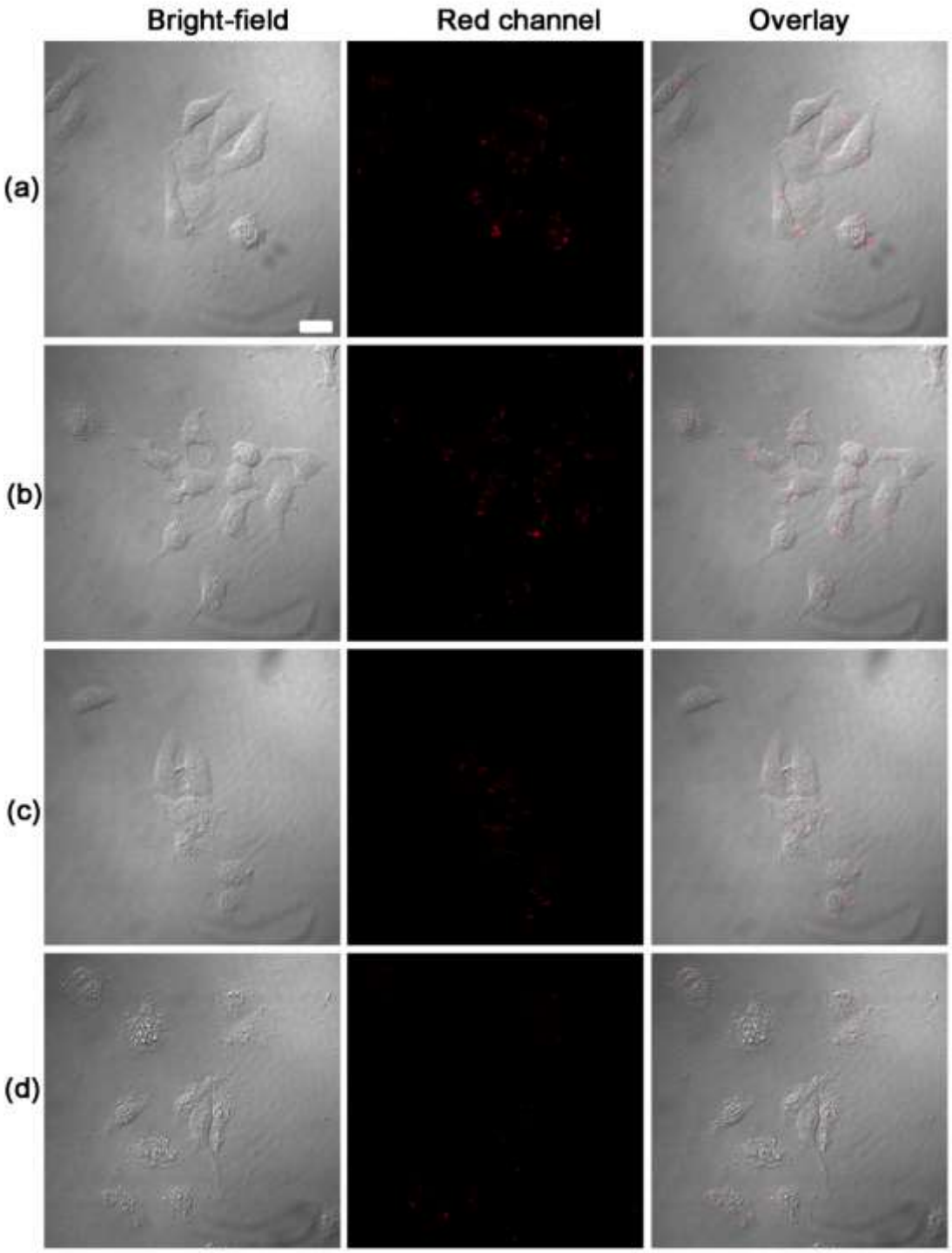

Figure S12. Bright-field, red channel and overlay images of the living HeLa cells incubated with **CBFB** probe (20 μM) for 60 min in the transfection liquid solution contained (a) 0.6%, (b) 0.9%, (c) 1.2%, and (d) 1.5% Lipofectamine 2000 Reagent. Excitation at 488 nm. Emission collected at 550-650 nm. Scale bar, 20 μm.

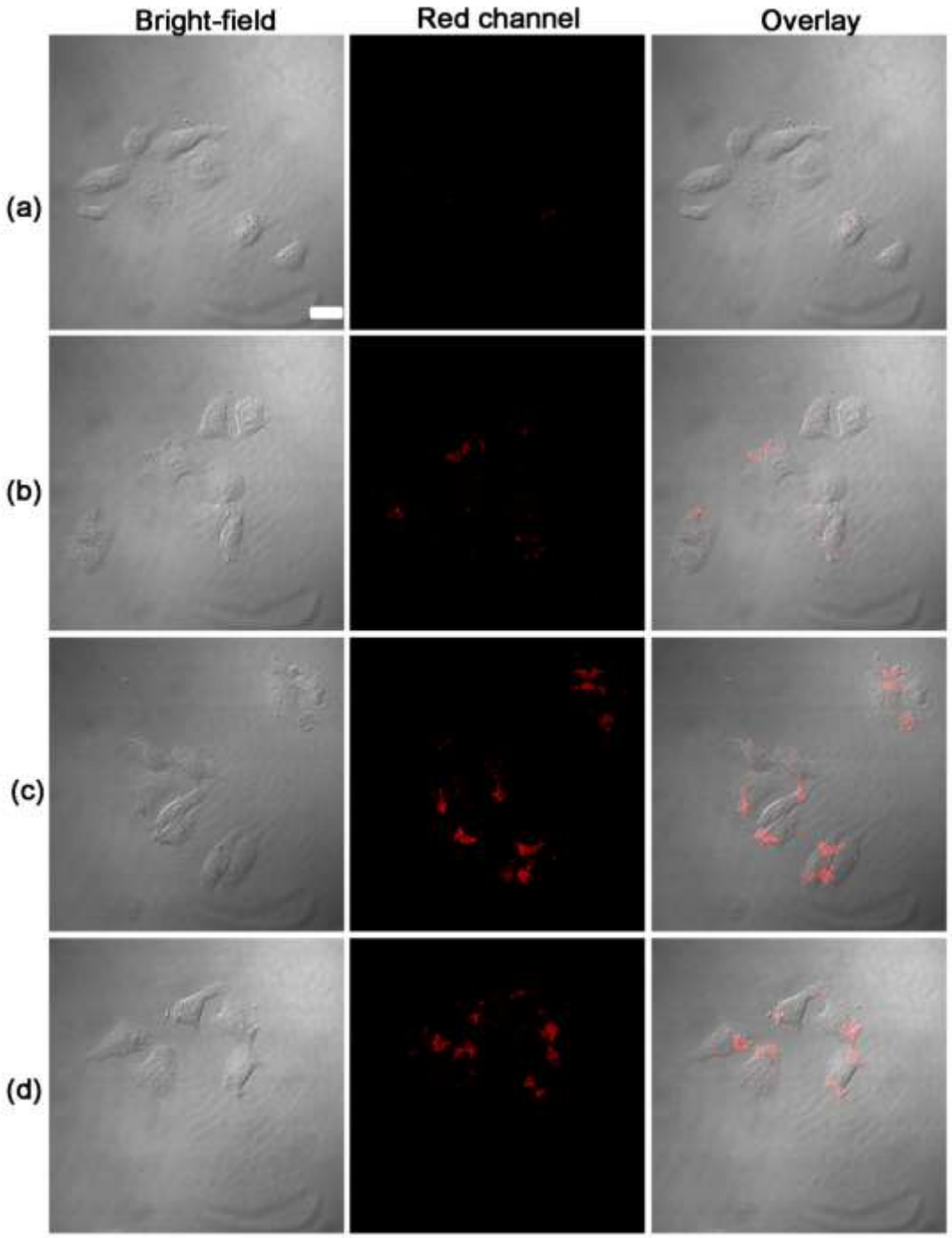

Figure S13. Bright-field, red channel, and overlay images of the living HeLa cells incubated with **CBFB** probe with concentration of (a) 5 μM, (b) 10 μM, (c) 20 μM, and (d) 50 μM for 60 min in the transfection liquid solution contained 0.9% Lipofectamine 2000 Reagent. Excitation at 488 nm. Emission collected at 550-650 nm. Scale bar, 20 μm.

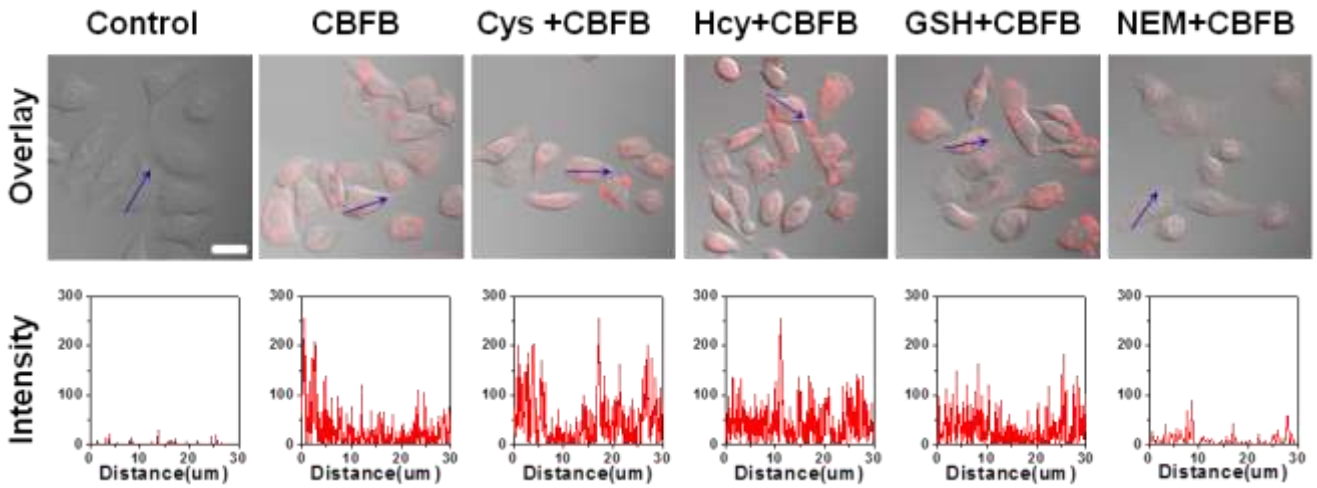

Figure S14. The fluorescence intensity changes across a certain cell in different experimental groups. Excitation at 488 nm. Emission collected at 550-650 nm. Scale bar, 20 μm.

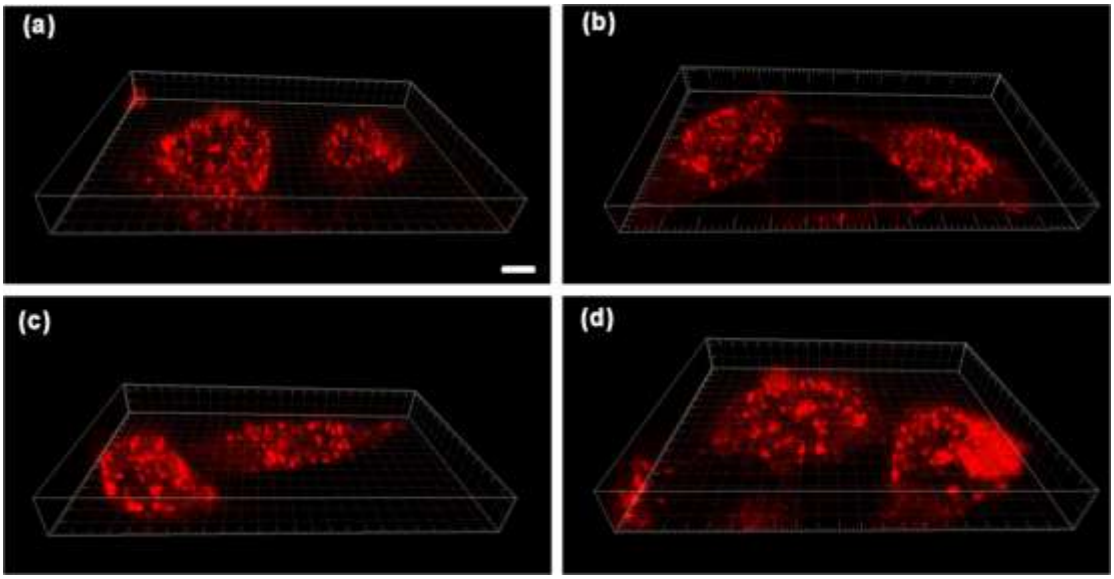

Figure S15. 3D image of living HeLa cells (a) incubated with **CBFB** probe (20 μM) for 60 min, (b) pre-treated with Cys (100 μM) for 60 min, then incubated with **CBFB** (20 μM) for 60 min, (c) pretreated with Hcy (100 μM) for 60 min, then incubated with **CBFB** (20 μM) for 60 min, (d) pretreated with GSH (100 μM) for 60 min, then incubated with **CBFB** (20 μM) for 60 min. Excitation at 488 nm. Emission collected at 550-650 nm. Scale bar 5 μm.