Electronic Supplementary Material (ESI) for Chemical Communications.

Mitochondria-targeted fluorescent probe for monitoring endogenous cysteine in living cells and zebrafish

Yingshuang Chen[a §], Xiuli Zhong[a §], Xinrui Yang[a], Shanmei Zhu[a], Yuliang Jiang[a]*, Can Jin[b]*

a Nanjing Normal Univ, Jiangsu Collaborat Innovat Ctr Biomed Funct Mat, Jiangsu Key Lab Biofunct Mat, Sch Chem & Mat Sci, Nanjing 210023, Jiangsu, Peoples R China

b Institute of chemical Industry of Forest Products, Key Lab Biomass Energy & Mater Jiangsu Prov, National Engineering Lab Biomass Chem Utilizat, CAF, Nanjing 210042, Jiangsu, Peoples R China

Corresponding Authors

Email: 07205@njnu.edu.cn (Y. L. Jiang)

Email: envis@163.com (C. Jin)

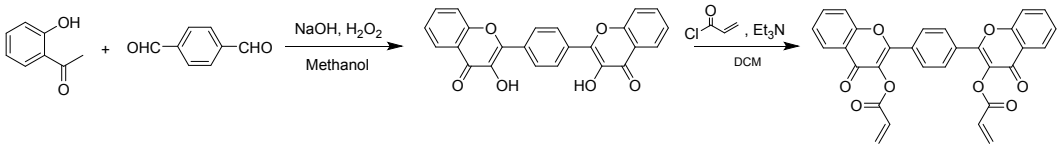
List of contents

1. Experimental section

Materials and Methods

All chemicals and instrumentation were obtained from commercial suppliers and all starting materials were used without further purification, unless otherwise noted. ^{1}H NMR and ^{13}C NMR spectra were obtained by using an AVANCE Ⅲ HD AN-400 MHz spectrophotometer (Bruker, Germany). UV-vis spectra were acquired with a Lambda 650s spectrophotometer (PerkinElmer, USA), and fluorescence studies were performed with an F-7100 fluorescence spectrophotometer (Hitachi, Japan). Field-emission scanning electron micrographs (SEM) were recorded on a JSM-7600F ultrahigh resolution scanning electron microscope (Hitachi, Japan). High-resolution mass spectroscopy data were acquired with a high-resolution Orbitrap Fusion Lumos (Thermo Fisher Scientific, USA) instrument, and fluorescence imaging of the cells and zebrafish was performed with an A1 confocal laser scanning microscope (Nikon, Japan).

Synthesis



Intermediate 1 BFD

Scheme.S1 The synthetic route of 4-(2-benzothiazol)benzaldehyde

Synthesis of intermediate 1

A mixture containing terephthalaldehyde (0.67 g, 5 mmol), 2-hydroxyacetophenone (0.68 g, 5 mmol), and NaOH (1.5 g) was dissolved in 40 mL methanol and refluxed for 6 h; the reaction was carried out at room temperature overnight. Thereafter, NaOH (0.5 mol L^{-1}, 5 mL) and H_2O_2 (30%, 5 mL) were added to the mixture and stirring for 6 h; the mixture was subsequently poured into ice-water (200 mL). Thereafter, the mixture was left to stand overnight. The precipitate was collected by filtration and washed with cold methanol. The red solid was dried to afford compound 1 (yield: 73.7%). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.71 (s, 4H), 8.05 (d, J = 8.0 Hz, 2H), 7.58 (dd, J = 20.2, 7.9 Hz, 4H), 7.24 (t, J = 7.6 Hz, 2H).

Synthesis of probe BFD

Triethylamine (300 μL, 2 mmol) was added to a suspension of compound 1 (0.19 g, 0.5 mmol) in 30 mL anhydrous di-chloromethane, and the mixture was stirred for 40 min in an ice bath. Thereafter, acryloyl chloride (180 μL, 2 mmol) was added dropwise to the cooled reaction system and stirred for 2 h at 0°C. The mixture was further stirred at room temperature for 10 h and the reaction progress was monitored by thin layer chromatography (TLC). The resulting solution was washed with H_2O (10 mL × 3), the organic phase was collected and dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with ethyl acetate/petroleum ether = (v/v, 1:5) as the eluent to give probe BFD as a white solid (yield: 41.1%). ^{1}H NMR (400 MHz, chloroform-d) δ 8.31 (dd, J = 8.0, 1.7 Hz, 2H), 8.06 (s, 4H), 7.78 (ddd, J = 8.7, 7.1, 1.7 Hz, 2H), 7.62 (dd, J = 8.6, 1.0 Hz, 2H), 7.49 (ddd, J = 8.1, 7.1, 1.1 Hz, 2H), 6.69 (dd, J = 17.4, 1.2 Hz, 2H), 6.46–6.38 (m, 2H), 6.12 (dt, J = 10.4, 1.3 Hz, 2H). ^{13}C NMR (101 MHz, chloroform-d) δ 172.00, 163.05, 155.65, 154.86, 134.34, 134.23, 132.53, 128.59, 126.71, 126.31, 125.54, 123.65, 118.15. MS: m/z calcd. for $C_{30}H_{19}O_8^+$ [M^+], 507.1074; found: 507.1075.

Preparation of probe and analytes

The stock solution of the probe BFD was prepared in DMSO at concentration of 1 mM and other analytes (Zn^{2+}, Ag^+, Cu^{2+}, Fe^{3+}, Fe^{2+}, Pb^{2+}, Mg^{2+}, NO_2^-, NO_3^-, S^{2-}, H_2PO_4-, HPO_4^{2-}, HSO_4^-, $S_2O_3^{2-}$, Hcy, GSH, His, Thr, Trp, Tyr, Lys, Met,

Phg, Phe, Leu) were prepared in deionized water (10 mM). Fresh stock solutions were used, and were diluted with PBS buffer to the desired concentrations when needed.

Cell culture

HeLa cells were received from the cell bank of the Chinese Academy of Sciences, and the ability of the probe for sensing Cys in living cells was tested. HeLa cells were cultured in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% FBS (fetal bovine serum) at 37°C under 5% CO_2 humidified atmosphere. The cells were treated in the following ways: (1) four groups of cells were prepared for exogenous Cys detection and they were all initially incubated with BFD (10 μM) for 30 min. One group was directly treated with the probe and the other three groups were incubated separately with Cys (50, 200, 300 μM) for another 30 min, and then washed with PBS buffer and imaged under a laser scanning confocal microscope. (2) As a control group, the endogenic Cys and other thiols such as Hcy and GSH were further investigated. The cells were treated with the free probe (10 μM) for comparison. For the experimental groups, all cells were first treated with N-ethylmaleimide (NEM; 0.2 mM) for 30 min and then separated into four groups. One group was only treated with probe BFD (10 μM) for 30 min; the remaining groups were treated with exogenous Cys (300 μM), Hcy (300 μM), and GSH (500 μM), respectively, and finally incubated with the probe (10 μM). All experimental images were acquired after excitation at 405 nm and the data were collected in the red channel (570–620 nm).

Co-localization fluorescence imaging of probe BFD

HeLa cells were cultured and grown on Petri dishes for 24 h before treatment. All cells were cultured with BFD (10 μM) for 30 min and cleaned with PBS solution. The cells were incubated with Mito-Tracker Green (100 nM), on the plate for another 30 min. Afterward, the cells were washed three times with PBS buffer before acquiring the fluorescence images of the cells (laser wavelength: BFD: 405 nm; Mito-Green: 488 nm; emission filter-BFD: 570–620 nm; Mito-Green: 500-550 nm).

Fluorescence imaging in zebrafish

Zebrafish studies were approved by the Ethics Committee and IACUC of Qilu Health Science Center, Nanjing Normal University, and were conducted in compliance with European guidelines for the care and use of laboratory animals. To test the practical applicability of the probe, 3 to 5-day-old zebrafish as a vertebrate model were used for laser scanning confocal microscopy imaging. First, the zebrafish were treated with 10 μM of probe BFD and co-incubated with different concentrations of exogenous Cys (50, 200, 300 μM) for another 30 min. The fish were then anaesthetized and imaged by confocal microscopy. The thiol scavenger NEM (0.2 mM) was further added, and the medium was seeded with Cys (300 μM), Hcy (300 μM), and GSH (500 μM) and the probe as control groups.

2. ^{1}H NMR spectroscopy of BFD

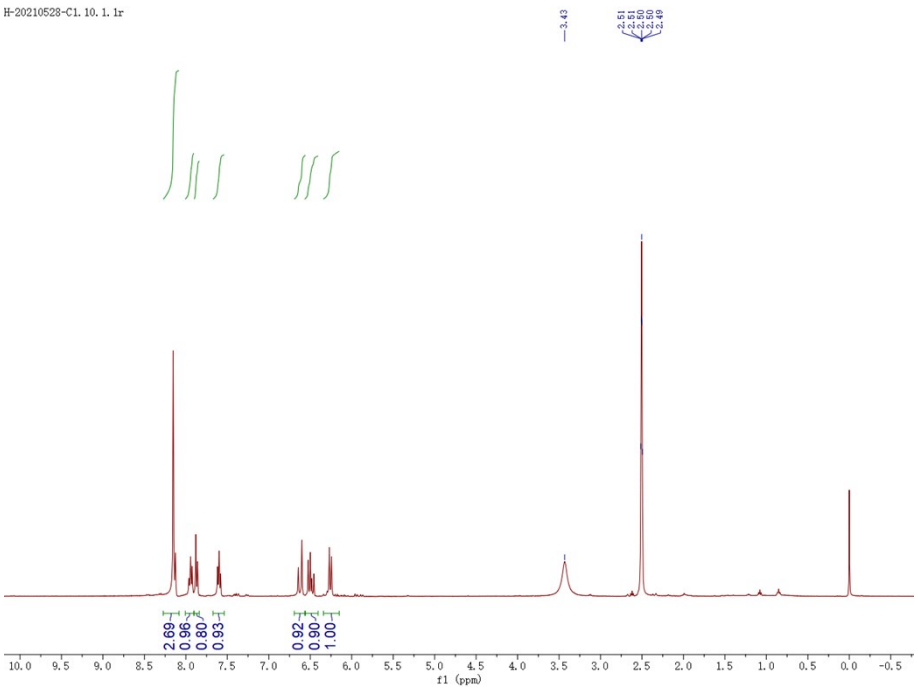


Fig. S1 ^{1}H NMR spectroscopy of BFD

3. ^{13}C NMR spectroscopy of BFD

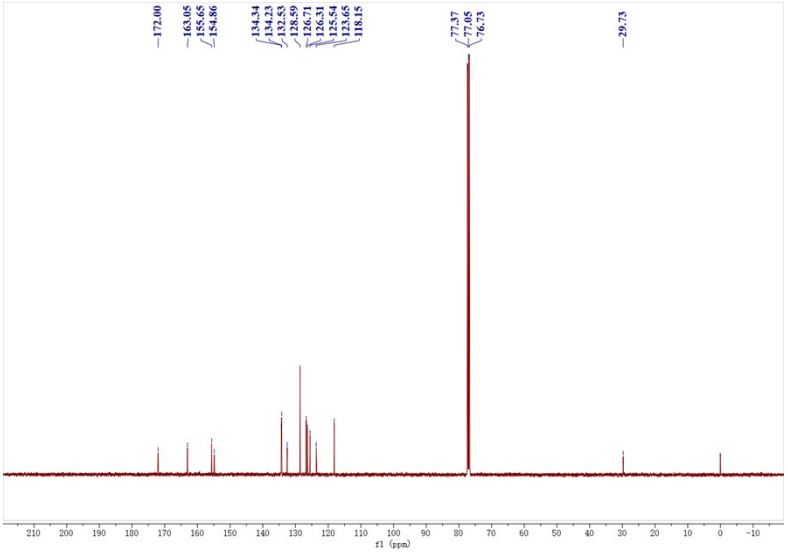


Fig.S2 ^{13}C NMR spectroscopy of BFD

4. HRMS spectroscopy of BFD

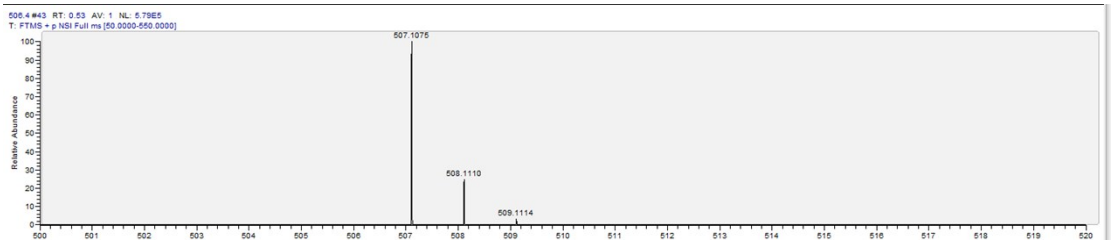


Fig.S3 HRMS spectroscopy of BFD

5. Fluorescence spectra of probe BFD with Cys, Hcy and GSH

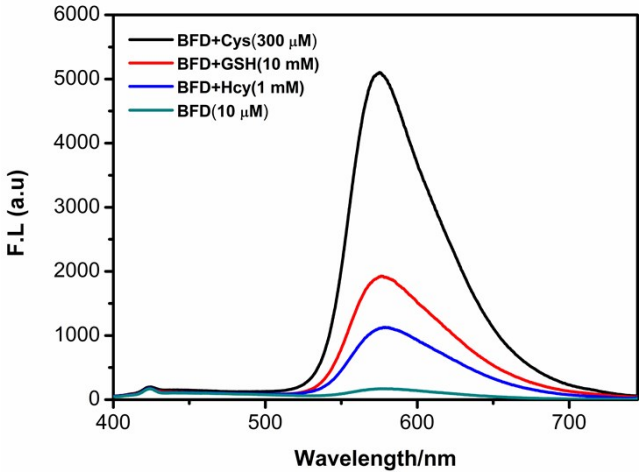


Fig. S4 Fluorescence spectrum of probe BFD (10 μM) with 300 μM Cys, 1 mM Hcy and 10 mM GSH within 2 min separately.

6.Selective experiment

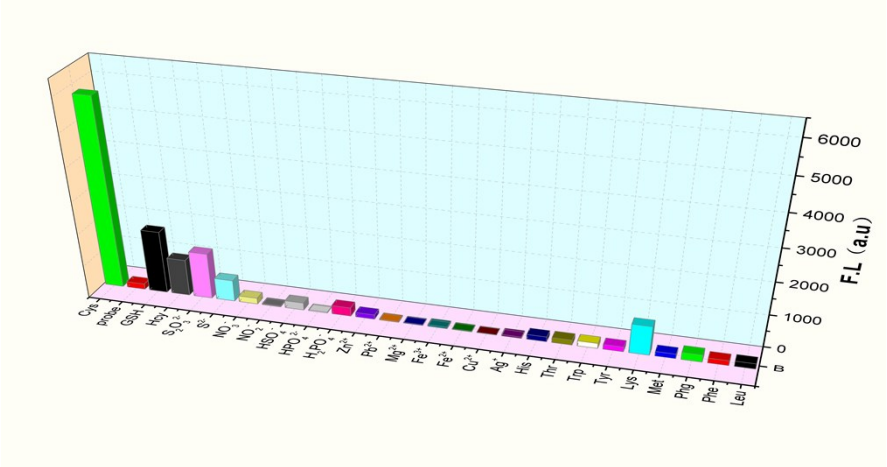


Fig.S5 The selectivity of BFD for Cys (300 μM) was examined in the presence of other relevant species including Zn^{2+}, Ag^{+}, Cu^{2+}, Mg^{2+}, Fe^{3+}, Pb^{2+}, Fe^{2+}, NO_2^-, NO_3^-, $H_2PO_4^-$, HSO_4^-, HPO_4^{2-}, S^{2-}, $S_2O_3^{2-}$, His, Thr, Trp, Tyr, Lys, Met, Phg, Phe, Leu(500 μM), respectively, and Hcy (1 mM), GSH (10 mM).

7. Time-dependent response

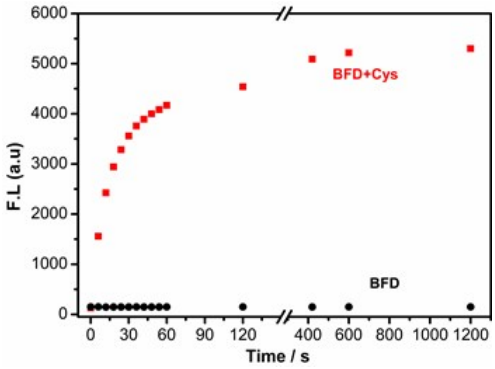


Fig.S6 Time-dependent response of BFD and BFD-Cys.

8. The NMR titration experiment

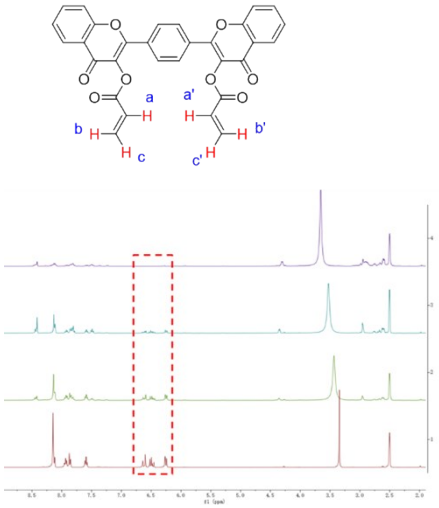


Fig. S7 NMR titration

9.The ^{1}H NMR of BFD-Cys

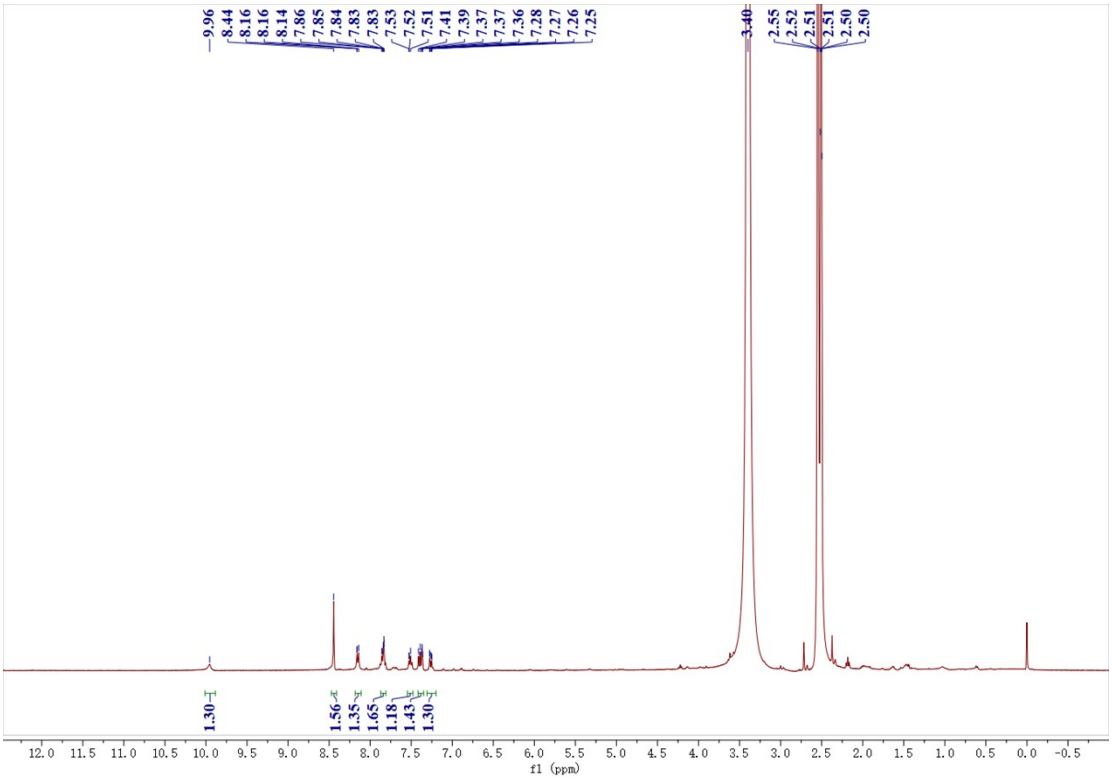


Fig. S8 The ^{1}H NMR of BFD-Cys

10. The HRMS spectroscopy of BFD /Cys.

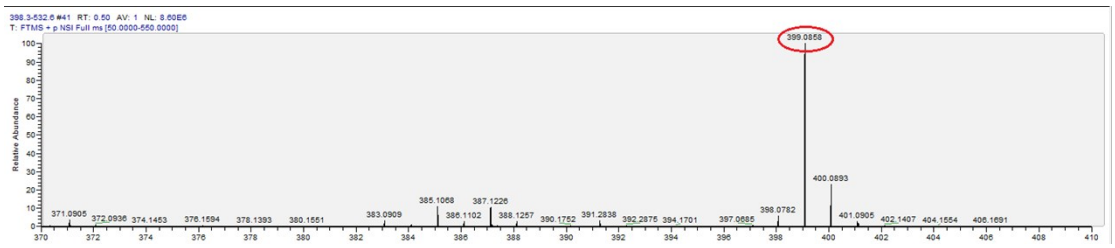


Fig. S9 The HRMS spectroscopy of BFD /Cys.

11. Cytotoxicity of BFD

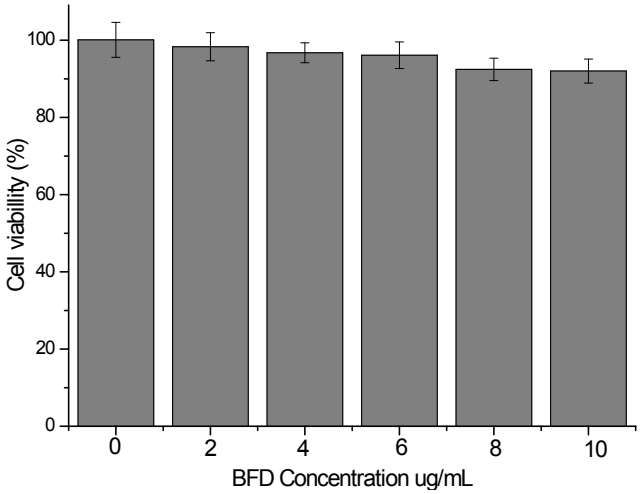


Fig. S10 Cytotoxicity of BFD by a MTT assay (n = 3).

12. Different concentrations of Cys detection in cell

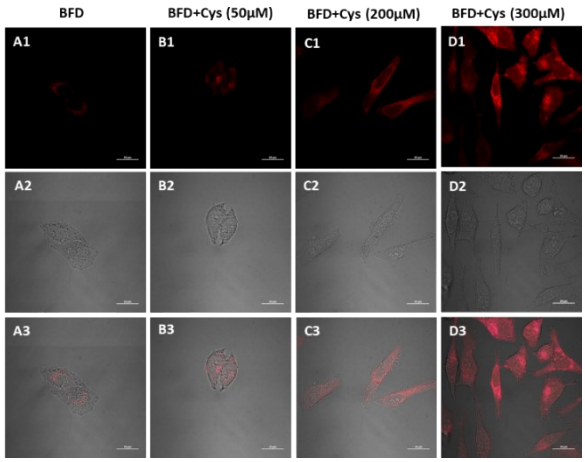


Fig.S11 Fluorescence images of Cys in living HeLa cells with 10 μM probe BFD. From left to right: free probe BFD; incubate of 50, 200, 300 μM Cys. The fluorescence images were captured from the red channel of 570-620 nm with excitation at 405 nm. Scar bar: 20 μm.

13. Endogenous cysteine detection

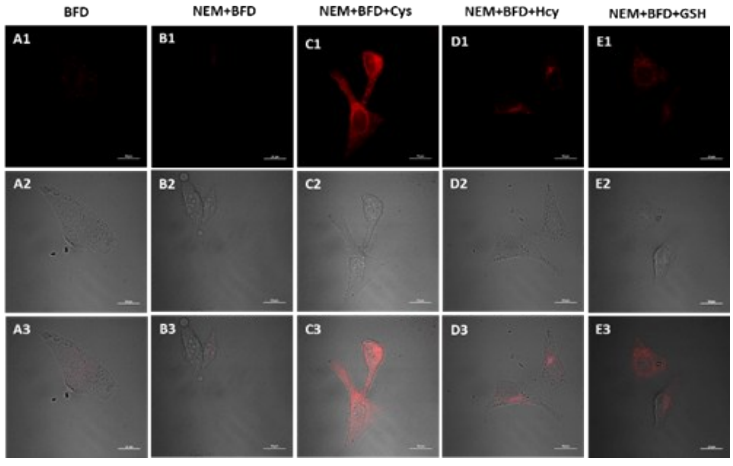


Fig.S12 Cell imaging of BFD responding to endogenous Cys. From top to bottom: red channel, bright field and merge (A1-A3) HeLa cells were incubated with BFD (10 μM). (B1-B3) NEM (0.2 mM) and BFD (10 μM) incubated with HeLa cells. (C1-C3) NEM (0.2 mM), BFD (10 μM) and Cys (300 μM) incu-bated with HeLa cells. (D1-D3) NEM (0.2 mM), BFD (10 μM) and Hcy (300 μM) incubated with HeLa cells. (E1-E3) NEM (0.2 mM), BFD (10 μM) and GSH (500 μM) incubated with HeLa cells.

14. SEM of BFD

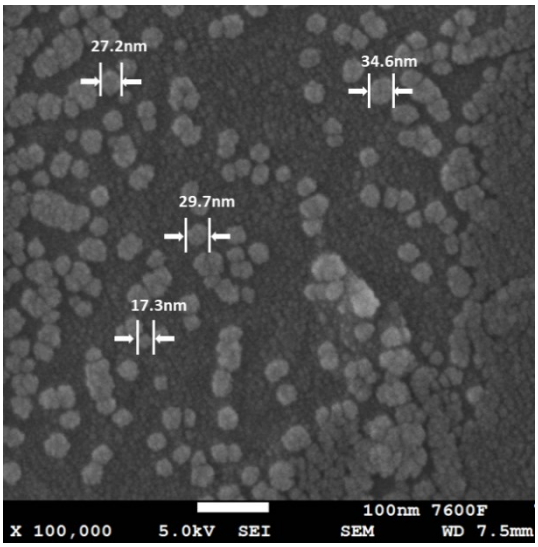


Fig. S13 The SEM of BFD in THF solution.

15.Different concentrations of Cys detection in zebrafish

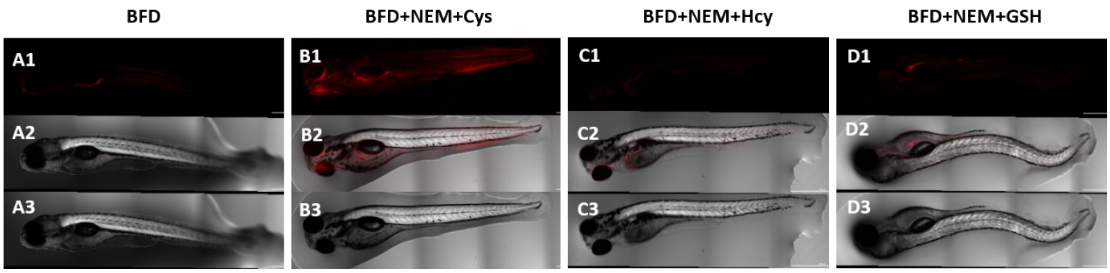


Fig. S14. Cell imaging of BFD responding to Cys. Yellow channel, bright field and merge of (A1-A3) HeLa cells were incubated with BFD (10 μM). (B1-B3) NEM (0.5 mM), BFD (10 μM) and Cys (200 μM) incubated with HeLa cells. (C1-C3) NEM (0.5 mM), BFD (10 μM) and Hcy (200 μM) incubated with HeLa cells. (D1-D3) NEM (0.5 mM), BFD (10 μM) and GSH (200 μM) incubated with HeLa cells.