Supporting Information

A novel highly sensitive fluorescent probe for bioimaging biothiols and its applications in distinguishing cancer cells from normal cells

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1. Materials and instruments

1.1 Reagents and instruments

Except for special labels, chemical reagents were obtained from commercial vendor and employed without further purification. Chemical reagents were purchased from Tianjin Fuyu Fine Chemical Co., Ltd. *N*-ethylmaleimide (NEM) was bought from Shanghai Civic Chemical Technology Co., Ltd. High resolution mass spectra (HRMS) were obtained by LC-MS2010A instrument produced in Shimadzu. ^{1}H and ^{13}C NMR data were obtained by Bruker AV-400 NMR spectrometer produced in America. Absorption spectra were obtained by UV-3101PC spectrophotometer produced in Shimadzu. Fluorescence spectra were obtained by Horiba FluoroMax-4 spectrophotometer produced in Japan. Fluorescence imaging of biothiols in live cells and zebrafish were carried out on a Fluoview FV1000 confocal laser scanning biological microscope (Olympus Corporation, Tokyo, Japan).

1.2 Live subject statement

All experimental procedures were carried out in strict accordance with the National Institute of Health (NIH) guidelines for the Care and Use of Laboratory Animals and the regulations of Qilu University of Technology on the ethical use of animals. All experimental procedures were approved by the faculty Ethical Committee of the Biology Institute of the Shandong Academy of Sciences. All efforts were made to minimize the number of animals used and their suffering. The sources of biological samples (HeLa, HUVEC, RAW 264.7, MGC803, A549, HepG2, and MCF-7 cells) in our experiments were all from Shanghai Institute of Biochemistry and Cell Biology, China Academy of Sciences (Shanghai, China).

1.3 Spectral experiments

4.3 mg of probe **HN-NBD** was dissolved in acetonitrile to make a 1 mM stock solution, and the probe concentration in the testing solution was 5 μM. Cys and Hcy were dissolved in ultrapure water, GSH was dissolved in PBS buffer (pH = 7.4), and their stock concentrations were 10 mM. All optical measurements in this paper were carried out in PBS buffer (10 mM, pH = 7.4, ultrapure water contains 0.5% acetonitrile).

2. Determination of the detection limit

The detection limit was calculated based on the fluorescence titration. The fluorescence spectra of free probe **HN-NBD** were measured by five times and its standard deviation was obtained. To gain the slope, the fluorescence intensities at 550 nm were plotted as the increasing concentrations of the corresponding biothiols. So, the detection limit was calculated with the following equation (1):

$$\text{Detection limit} = 3\sigma/k \quad (1)$$

Where σ is the standard deviation of blank measurement, k is the slope between the fluorescence intensities versus the concentrations of Cys, Hcy and GSH.

3. Excitation and emission spectra of 4-hydroxynaphthalimide, NBD-N-Cys/Hcy, and NBD-S-GSH

Cys (50 μM), Hcy (50 μM), and GSH (70 μM) were added into NBD-Cl (20 μM) solution respectively. In addition, 4-hydroxy-1,8-naphthalimide (10 μM) solution was prepared. Spectrum determination was performed 10 minutes later (Figure S1).

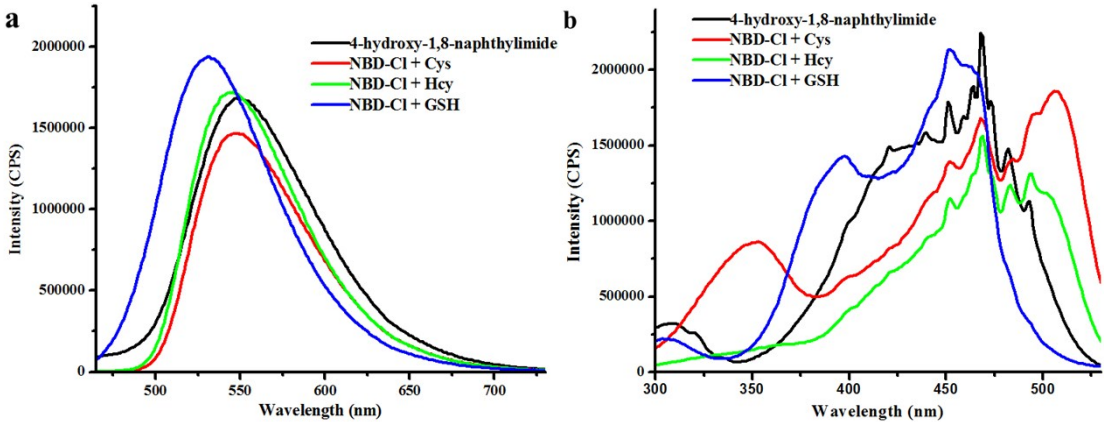
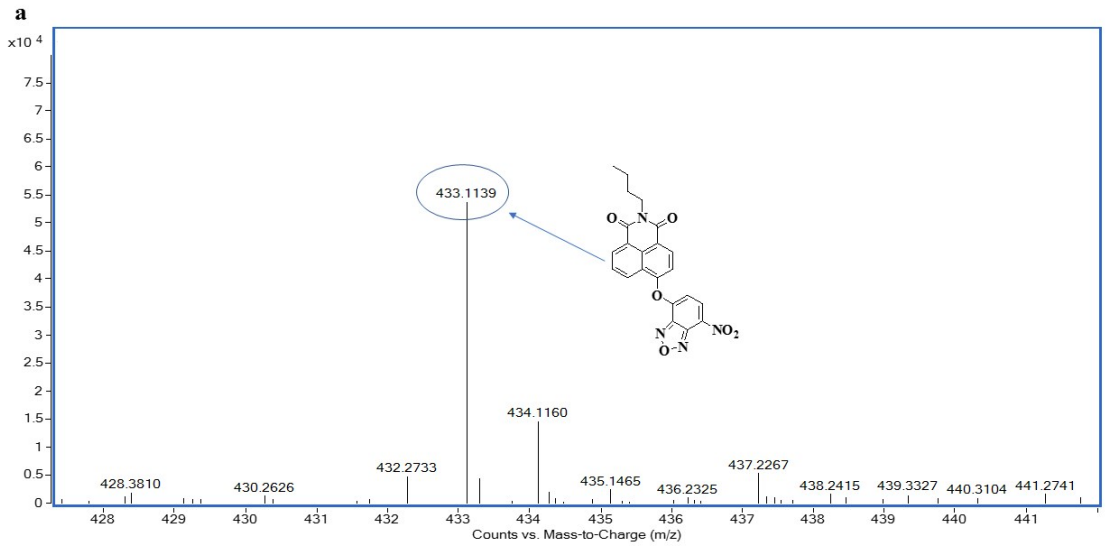


Figure S1. (a) Emission spectra of 4-hydroxy-1,8-naphthalimide fluorescence dye, NBD-N-Cys/Hcy, and NBD-S-GSH. λ_{ex}: 450 nm; dye: $W_{ex} = W_{em} = 2$ nm; NBD-N-Cys: $W_{ex} = W_{em} = 4$ nm; NBD-N-Hcy: $W_{ex} = W_{em} = 4$ nm; NBD-S-GSH: $W_{ex} = W_{em} = 5$ nm. (b) Excitation spectra of 4-hydroxy-1,8-naphthalimide fluorescence dye, NBD-N-Cys/Hcy, and NBD-S-GSH. dye: $W_{ex} = W_{em} = 2$ nm; NBD-N-Cys: $W_{ex} = W_{em} = 4$ nm; NBD-N-Hcy: $W_{ex} = W_{em} = 4$ nm; NBD-S-GSH: $W_{ex} = W_{em} = 5$ nm.

4. HRMS data for probe HN-NBD and its reaction products with Cys



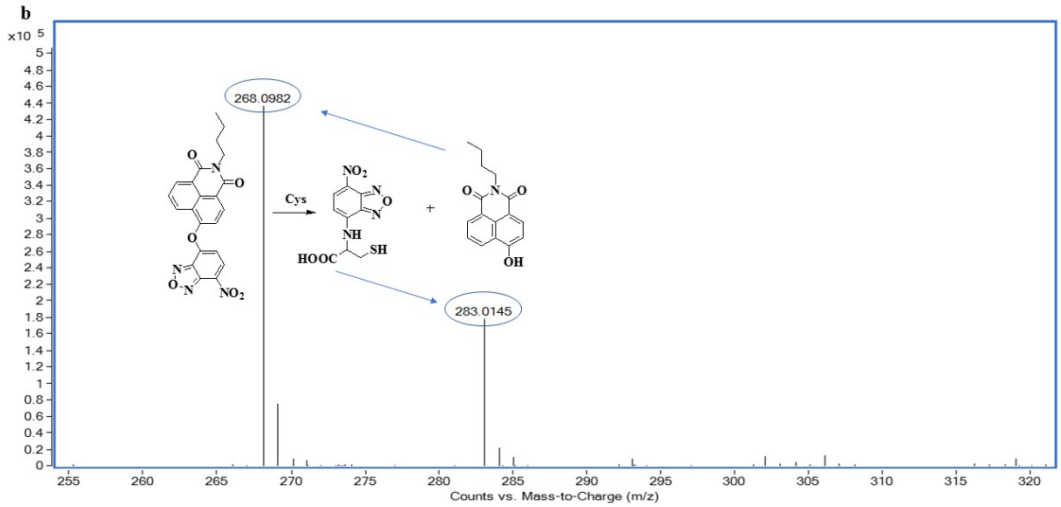


Figure S2. (a) HRMS data for probe **HN-NBD**. (b) HRMS data for the reaction products of probe **HN-NBD** with Cys.

5. Cytotoxicity assays

The cell viability of Hela cells, treated with probe **HN-NBD**, was assessed by a cell counting kit-8 (CCK-8; Dojindo Molecular Technologies, Tokyo, Japan). Briefly, Hela cells, seeded at a density of 1×10^6 cells·mL^{-1} on a 96-well plate, were maintained at 37 °C in a 5% CO_2 / 95% air incubator for 12 h. Then the live Hela cells were incubated with various concentrations (0, 5, 10, and 20 μM) of probe **HN-NBD** suspended in culture medium for 24 h. Subsequently, CCK-8 solution was added into each well for 2 h, and absorbance at 450 nm was measured.

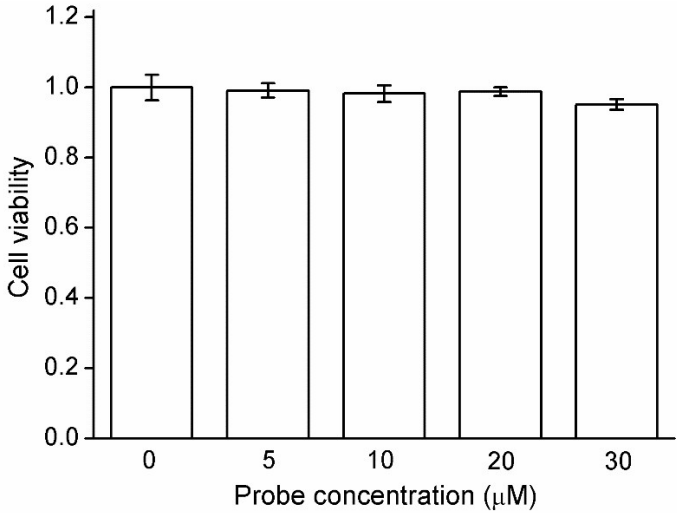


Figure S3. Cytotoxicity assays of probe **HN-NBD** at different concentrations for Hela cells.

6. Stability test of probe HN-NBD

The stability of probe **HN-NBD** under physiological conditions is crucial for bioimaging. As shown in Figure S4, the emission spectra of probe **HN-NBD** were collected at 0, 1, 12, and 24 h in PBS buffer (10 mM, pH = 7.4, ultrapure water contains 0.5% acetonitrile). The results showed that probe **HN-NBD** was stable under simulated physiological conditions and had good potential for bioimaging applications.

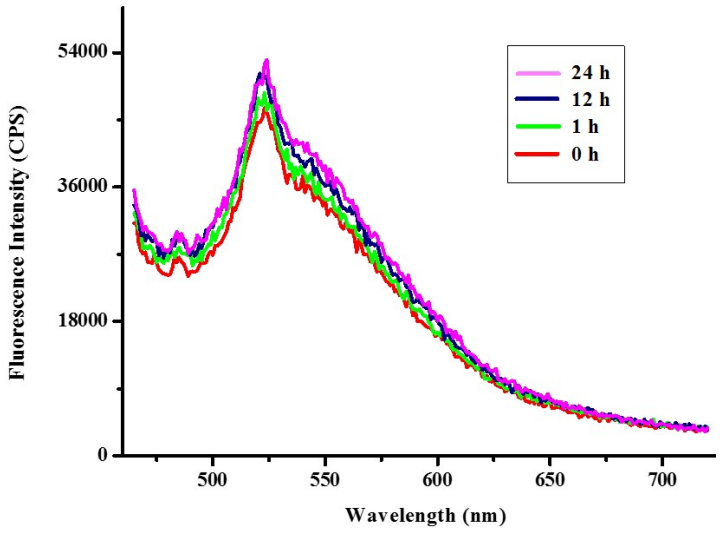


Figure S4. Stability test. Emission spectra of probe **HN-NBD** (5 μM) were tested in PBS buffer (10 mM, pH = 7.4, ultrapure water contains 0.5% acetonitrile) after 0, 1, 12, and 24 h. λ_{ex}: 450 nm. Slit widths: $W_{ex} = W_{em} = 4$ nm.

7. Imaging studies of live cells

The Hela cells were cultured in Dulbecco's modified Eagle's medium (DMEM) and incubated under an atmosphere containing 5% CO_2 at 37 °C humidified air for 24 h. DMEM contains 10% fetal bovine serum and 1% penicillin-streptomycin. Before

imaging by confocal fluorescence microscope, probe **HN-NBD** (10 μM) was used as a bioimaging reagent to incubate cells for 30 min, the culture medium was then removed and rinsed with phosphate buffered saline for three times before fluorescence imaging was performed. On the other hand, the cells treated with NEM for 30 minutes were incubated with probe **HN-NBD** (10 μM) for 30 minutes, and then the culture medium was washed with phosphate buffer saline for three times before imaging. The fluorescence imaging of cells was carried out by confocal fluorescence microscope.

Similarly, other bioimaging applications of probe **HN-NBD** in different cells were performed.

8. Imaging studies of zebrafish

Healthy male and female zebrafish (AB stain) were maintained in different tanks with a 14 h light / 10 h dark cycle at 28 °C. Then, sexually mature zebrafish were selected to induce spawning in tanks and the zebrafish eggs were obtained by giving light stimulation in the morning. After sterilizing and cleaning, the fertilized eggs were added to zebrafish embryo culture water (5 mM NaCl, 0.17 mM KCl, 0.4 mM $CaCl_2$, 0.16 mM $MgSO_4$) and cultured in illumination incubator at 28 °C. The 3-day-old zebrafish were incubated with probe **HN-NBD (**10 μM) for 30 min, the culture medium was used to clean and remove the residual probe, and then confocal fluorescence microscopy was used to observe. In addition, three groups of zebrafish were treated with NEM (200 μM, 500 μM, 1 mM) for 30 minutes, everyone group was washed with culture medium and then incubated with probe **HN-NBD** for 30

minutes before imaging with confocal microscope. Finally, the fluorescence imaging of zebrafish was carried out by confocal fluorescence microscope.