Supporting Information

*for*

# Nitrobenzoxadiazole Ether-Based Near-Infrared Fluorescent Probe with Unexpected High Selectivity for $H_2S$ Imaging in Living Cells and Mice


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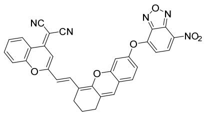
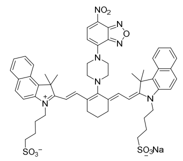
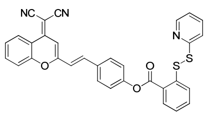
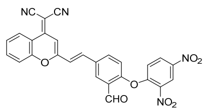
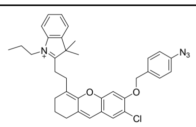
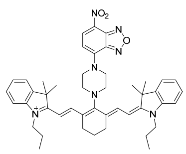
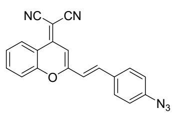
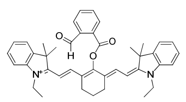
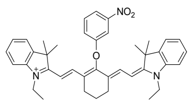
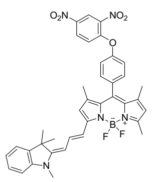
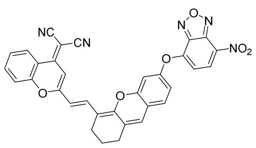
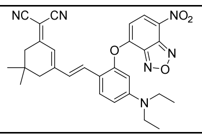
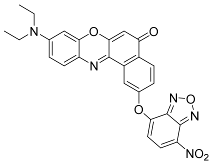
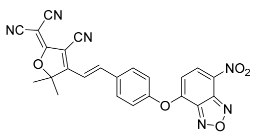
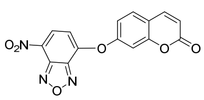
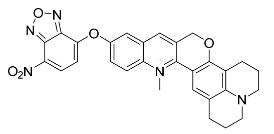
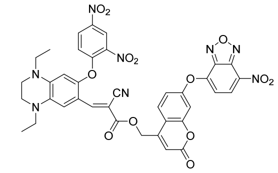
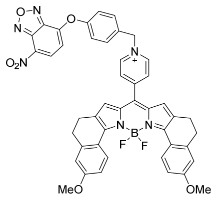
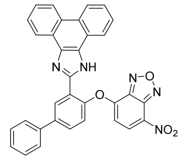
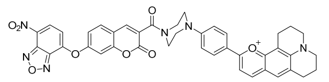
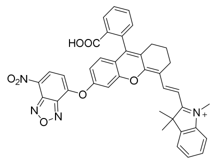
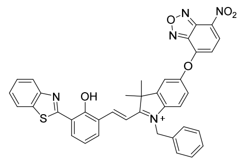
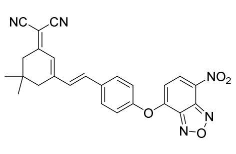
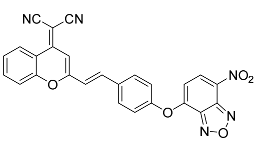
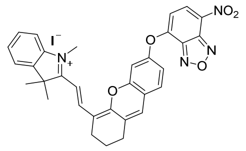
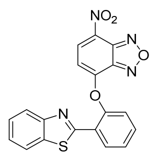
## 1. **Table S1.** Comparison of NIR fluorescent probes for $H_2S$.

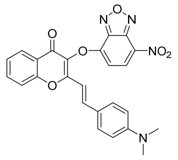
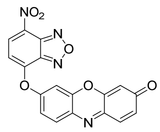
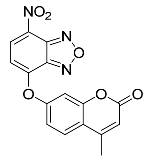
| Probe | $\lambda_{abs}/\lambda_{em}$ nm | Stokes shift | Response time | Detection conditions | Application in living cells | Application in vivo |
|---|---|---|---|---|---|---|
|  This work | 578/744 | 166 nm | 3 min | Tris-HCl (10 mM, pH 7.4, 20% THF, v/v) | Yes | Yes |
|  Talanta 2018, 184, 109–114. | 730/830 | 100 nm | 60 min | aqueous solution (pH 7.4, 10 mM) | Yes | Yes |
|  Dyes Pigm. 2018, 153, 206–212. | 560/680 | 130 nm | 30 min | PBS buffer (pH 7.4) with 50% DMSO | Yes | No |
|  Sens. Actuators B 2018, 255, 2347–2355. | 518/655 | 137 nm | 8 min | PBS buffer (10 mM, pH 7.4, with 50% DMSO) | Yes | No |
|  Biosens. Bioelectron. 2017, 89, 919–926. | 680/720 | 40 nm | 30 min | PBS buffer (pH 7.4) with 30% $CH_3CN$ | Yes | Yes |
|  Chem. Sci. 2017, 8, 2776–2781. | 740/796 | 56 nm | 30 min | PBS buffer (pH 7.4) | Yes | Yes |
|  Chem. Commun. 2013, 49, 3890-3892. | 520/670 | 150 nm | 60 min | PBS buffer (pH 7.4) with 50% DMSO | Yes | No |

| | | | | | | |
|---|---|---|---|---|---|---|
|  Chem. Sci. 2013, 4, 2551-2556. | 765/780 NIR fluorescence off | 15 nm | 35 min | HEPES buffer (pH 7.4, 0.5% $CH_3CN$). | Yes | No |
|  Chem. Commun. 2012, 48, 11757–11759. | 755/809 | 54 nm | 60 min | HEPES buffer, (pH 7.4). | Yes | No |
|  Chem. Commun. 2012, 48, 10529–10531. | 650/708 | 58 nm | 8 min | PBS buffer (pH 7.0) with 3 mM CTAB and 10% ethanol | Yes | No |

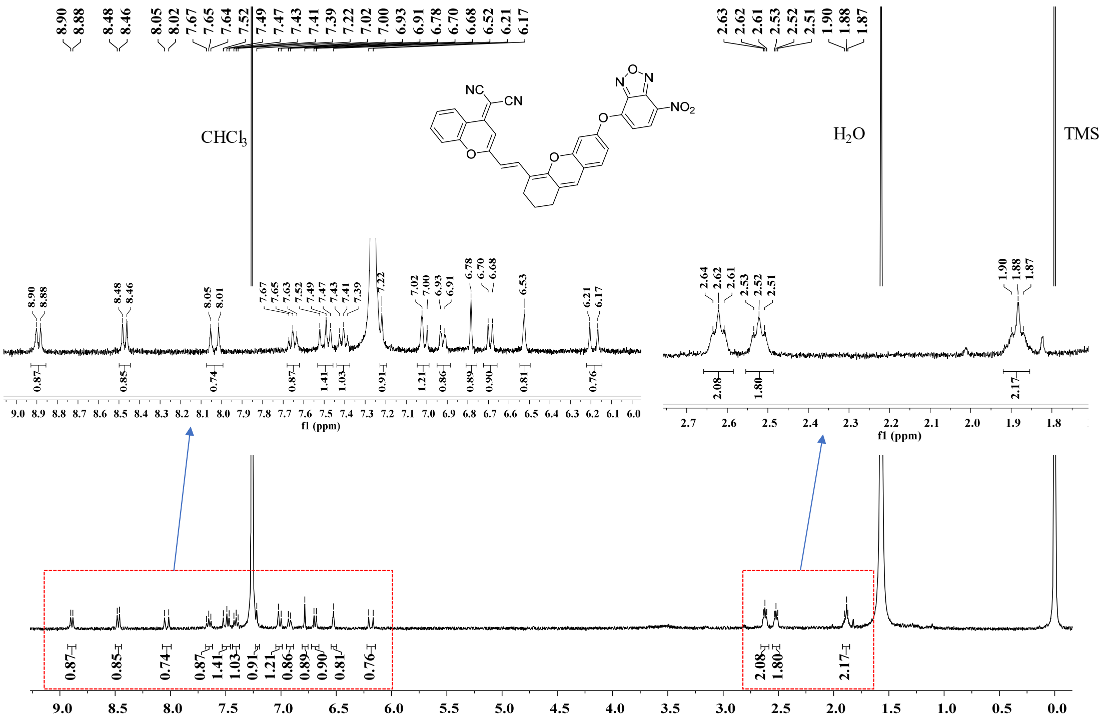
## 2. **Table S2.** Comparison of NBD-ether based fluorescent probes.

| Probe | Target detected | Detection condition | reference |
|---|---|---|---|
|  | $H_2S$ | Tris-HCl (10 mM, pH 7.4, 20% THF, v/v) at 37 °C | This work |
|  | Cys, Hcy, GSH | PBS (10 mM, pH pH 7.4 50% DMSO, v/v) at 25 °C | Anal. Chim. Acta 2019, 1074, 123−130. |
|  | Cys, $H_2S$, GSH | PBS (10 mM, pH 7.4, 40% $CH_3CN$, v/v). | Talanta 2019, 196, 145–152. |
|  | Cys, Hcy, GSH | PBS (10 mM, pH 7.4, 10% DMF) at 37 °C | Dyes Pigm. 2019, 168, 189–196. |
|  | Cys, Hcy, GSH | PBS (10 mM, pH 7.4, 20% $CH_3CN$, v/v) | Dyes Pigm. 2019, 165, 164–171. |

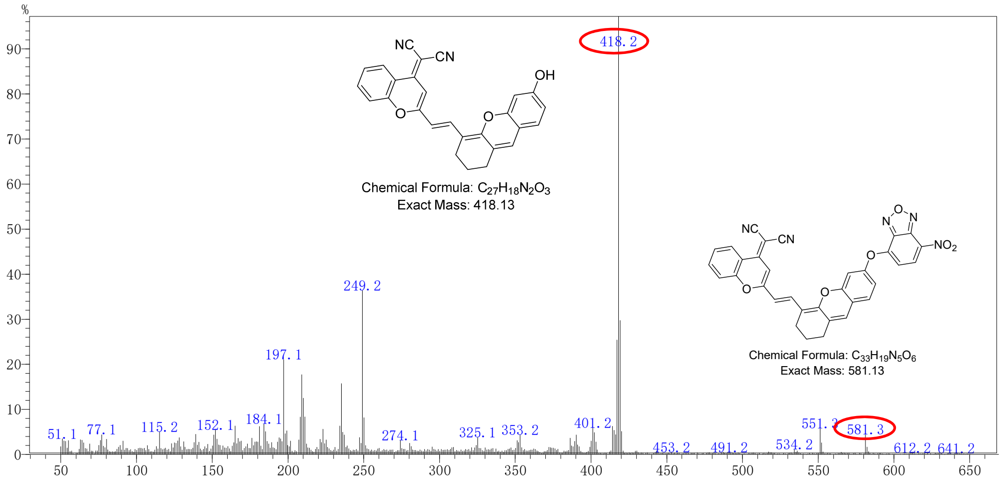
| | | | |
|---|---|---|---|
|  | Cys, Hcy, GSH | PBS (10 mM, pH 7.4) at 25 °C | Sens. Actuators B 2018, 273, 1170–1178. |
|  | Cys, Hcy, GSH, | PBS (10 mM, pH 7.4, 30% $CH_3CN$) | ACS Sens. 2018, 3, 1863−1869. |
|  | Cys, Hcy, GSH, $H_2S$ | PBS (10 mM, pH 7.4, 50% DMSO, v/v). | Sens. Actuators B 2018, 257, 1076–1082. |
|  | Cys, Hcy, GSH | PBS (20 mM, pH 7.4, 40% DMF, v/v). | J. Mater. Chem. B 2018, 6, 8221—8227. |
|  | Cys, Hcy, GSH, $H_2S$ | PBS (25 mM, pH 7.4, 20% DMSO, v/v) | Chem. Commun. 2017, 53, 13168–13171. |
|  | GSH, $H_2S$ | PBS (25 mM, pH 7.4, 1% EtOH) | Anal. Chim. Acta 2017, 981, 86–93. |
|  | Cys, Hcy, GSH, $H_2S$ | PBS (25 mM, pH 7.4, 20% $CH_3CN$, v/v). | Chem. Sci. 2017, 8, 6257–6265. |
|  | Cys, Hcy, GSH, | HEPES (10 mM, pH 7.4, 60% EtOH, v/v). | Dyes Pigm. 2017, 140, 212–221. |
|  | Cys, Hcy, GSH | PBS (50% DMSO v/v). | Sens. Actuators B 2017, 245, 297–304. |
|  | Cys, Hcy, GSH | PBS (10mM, pH 7.4, 5% DMSO) at 37 °C | Biosens. Bioelectron. 2016, 81, 341–348. |
|  | Cys, Hcy, GSH, $H_2S$ | PBS (10 mM, pH 7.4, 20% DMSO) at 25 °C | Sens. Actuators B 2016, 235, 691–697. |

| | | | |
|---|---|---|---|
|  | Cys, Hcy, GSH | PBS (10 mM, pH 7.4, 30% $CH_3CN$, v/v) | Anal. Chem. 2016, 88, 3638−3646. |
|  | Cys, Hcy, GSH | PBS (10 mM, pH 7.4) at 25 °C. | Chem. Commun. 2015, 51, 9388–9390. |
|  | Cys, Hcy, $H_2S$ | PIPES (50 mM, 100 mM KCl, pH 7.4). | Anal. Chem. 2014, 86, 7135−7140. |

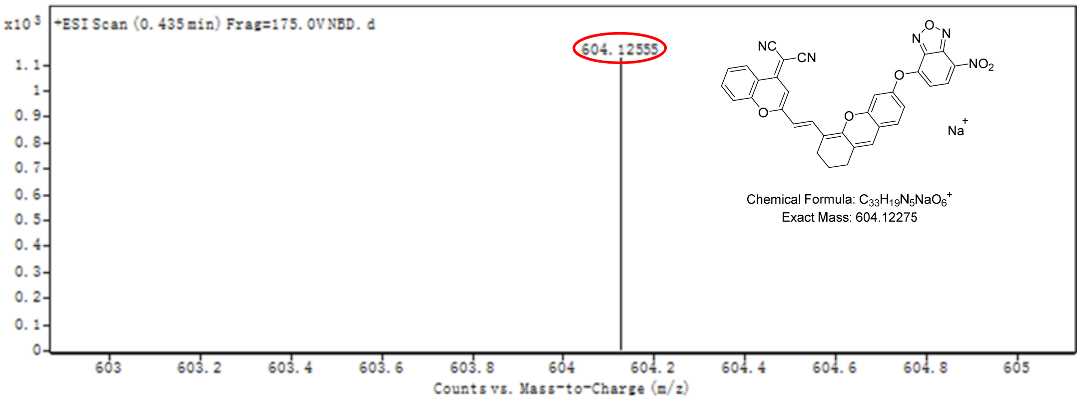
## 3. Structure characterizations for probe DC-NBD.




$^1$H NMR spectrum of DC-NBD in $CDCl_3$

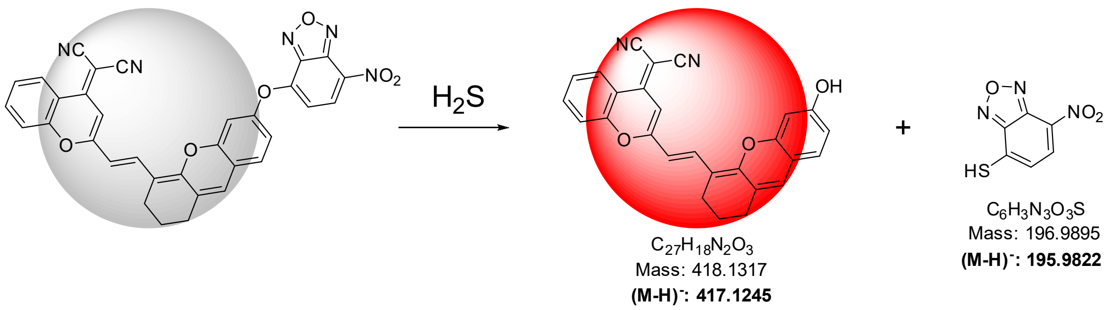



EI-MS spectrum of DC-NBD




HR-MS spectrum of DC-NBD

## 3. Additional data and spectra.




**Scheme S1** The proposed sensing mechanism of **DC-NBD** for the detection of $H_2S$.

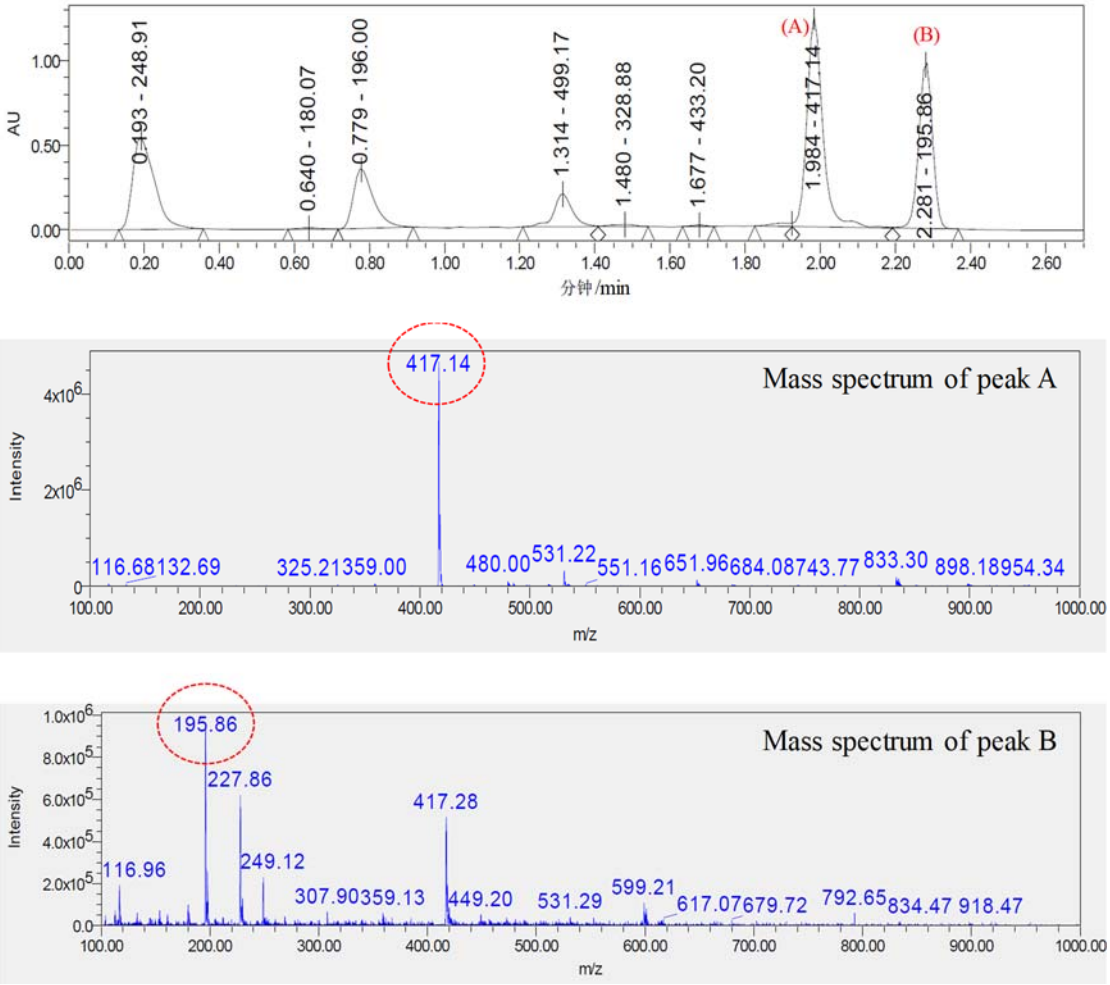



**Figure S1.** The LC-MS analysis of the mixture of probe **DC-NBD** (5 μM) and NaHS (50 μM) in Tris-HCl (10 mM, pH 7.4, with 20% THF).

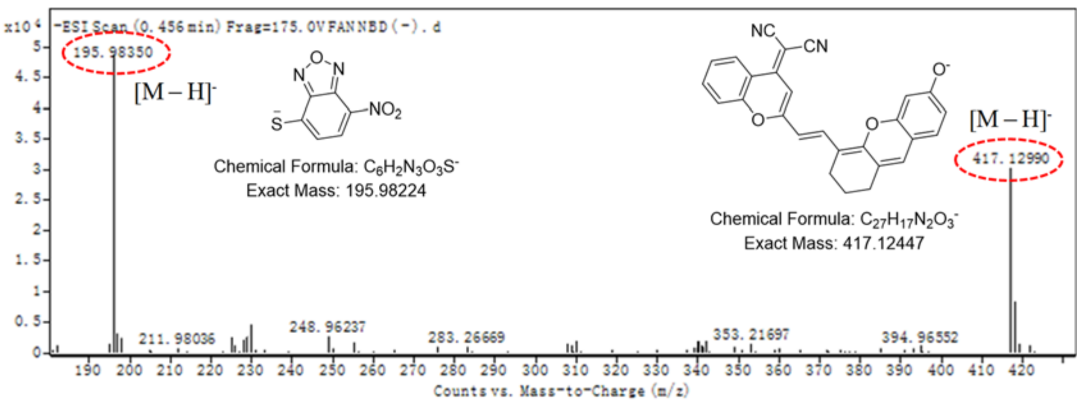



**Figure S2.** High resolution mass spectrometry (HRMS) analysis of the mixture of probe **DC-NBD** (5 μM) and NaHS (50 μM) in Tris-HCl (10 mM, pH 7.4, with 20% THF). The peak at m/z = 417.12990 can be assigned to the produced **DC-OH** (Calcd. for $[M - H]^-$: 417.12447). The peak at m/z = 195.98224 can be assigned to the produced NBD-SH (Calcd. for $[M - H]^-$: 195.98224).

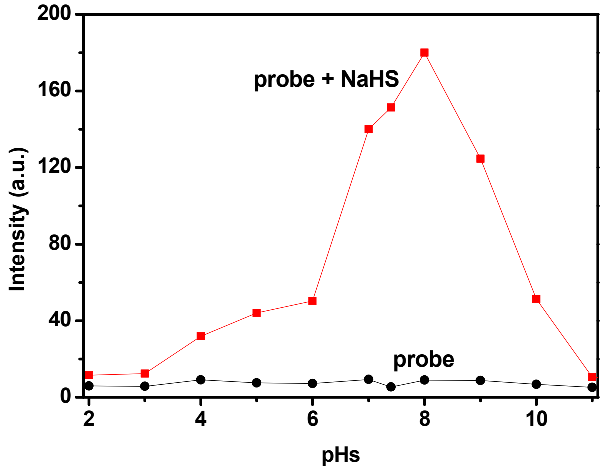



**Figure S3.** Fluorescence response of probe DC-NBD (2 μM) in the absence and presence of NaHS (100 μM) under different pH values. All data were collected at 744 nm in Tris-HCl (10 mM, with 20% THF, v/v) at 37 °C. Each data was obtained 3 min after mixing. $\lambda_{ex}$ = 613 nm, slit width: $d_{ex}$ = $d_{em}$ = 10 nm.

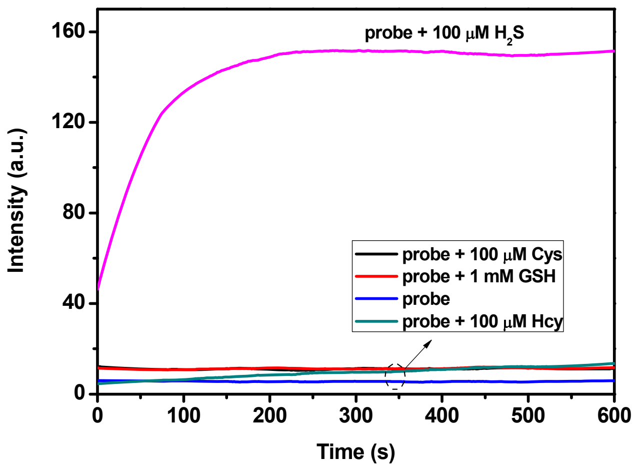



**Figure S4.** Fluorescence kinetics of probe DC-NBD (2 μM) upon addition of NaHS and biothiols. All data were collected at 744 nm in Tris-HCl buffer (10 mM, with 20% THF, v/v) at 37 °C. $\lambda_{ex}$ = 613 nm, slit width: $d_{ex}$ = $d_{em}$ = 10 nm.

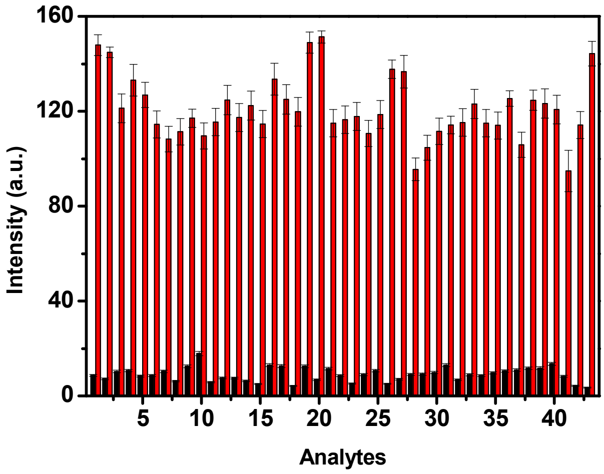



**Figure S5.** Fluorescent intensity responses of probe DC-NBD (2 μM) at 744 nm to $H_2S$ (100 μM) in the presence of various analytes (100 μM unless otherwise stated) including:(1) $K^+$, (2) $Na^+$, (3) $F^-$, (4) $Cl^-$, (5) $Br^-$, (6) $I^-$, (7) $N_3^-$, (8) $NO_2^-$, (9) $HSO_3^-$, (10) $SO_3^{2-}$, (11) $HSO_4^-$, (12) $SCN^-$, (13) $S_2O_7^{2-}$, (14) $S_2O_3^{2-}$, (15) $OCN^-$, (16) $AcO^-$, (17) $HCO_3^-$, (18) $C_2O_4^{2-}$, (19) $NO_3^-$, (20) $ClO^-$, (21) Gln, (22) Ile, (23) Pyr, (24) Thr, (25) Trp, (26) Ala, (27) Asp, (28) Ser, (29) Phe, (30) Lys, (31) His, (32) Val, (33) Met, (34) Glu, (35) Leu, (36) NAC, (37) Tyr, (38) Gly, (39) Arg, (40) Hcy, (41) Cys, (42) 1 mM GSH, (43) $H_2S$. Black bars represent the addition of a single analyte. Red bars represent the subsequent addition of NaHS (100 μM) to the mixture.

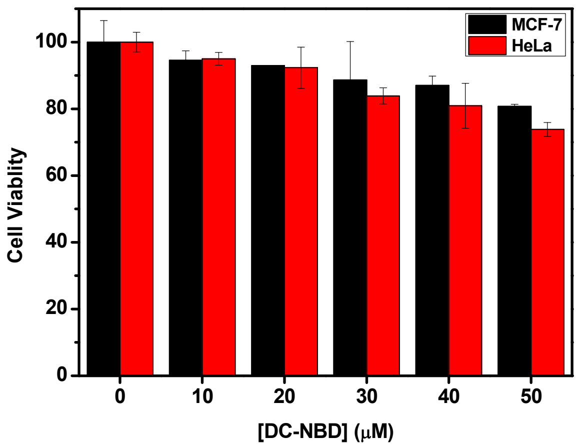



**Figure S6.** The percentage of viable MCF-7 and HeLa cells after treatment with different concentrations of DC-NBD after 12 hours. The cell viability was obtained via MTT assay.

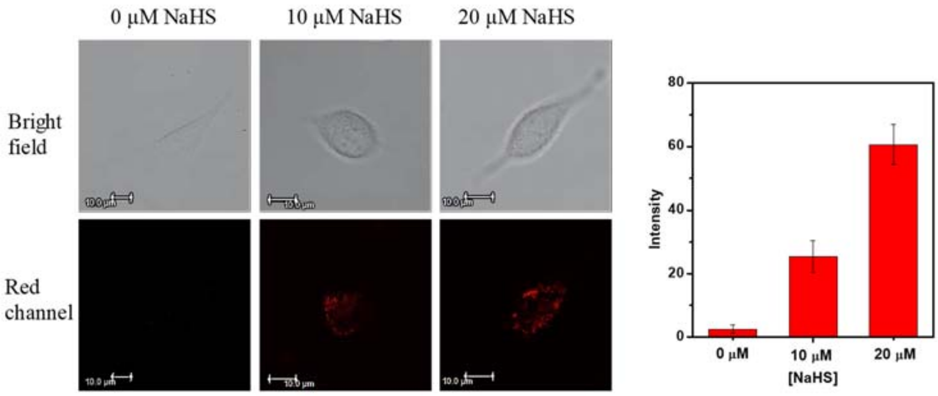



**Figure S7.** Confocal imaging of exogenous $H_2S$ in HeLa cells with probe DC-NBD (5 μM). Cells were incubated respectively with 0, 10, and 20 μM of NaHS for 30 min, and then incubated with DC-NBD for 15 min. For fluorescent images, $\lambda_{ex}$ = 633 nm, $\lambda_{em}$ = 700-780 nm.

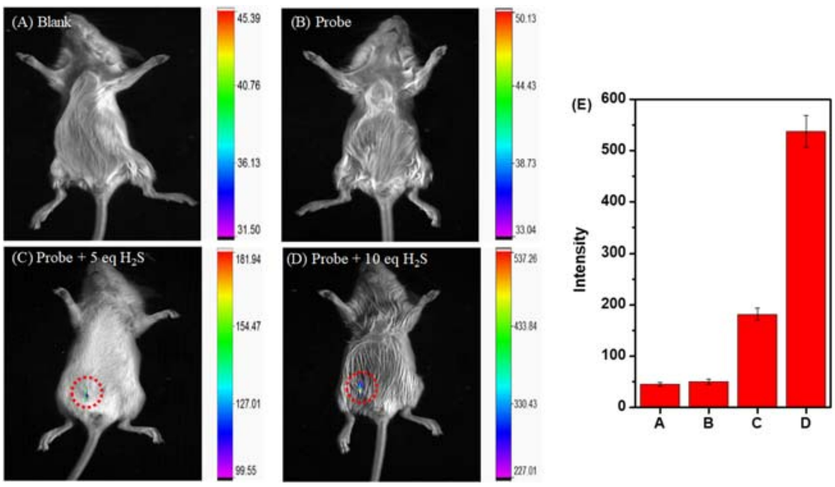



**Figure S8.** Probe DC-NBD for imaging of $H_2S$ in living mice with injection of less amount of DMSO. (A) Mouse blank. (B) The mouse was given an intraperitoneal injection of only DC-NBD (100 μL, 100 μM in PBS buffer with 15% DMSO, v/v) and imaged after 15 min. (C) The mouse was given an intraperitoneal injection of $H_2S$ (100 μL, 500 μM in PBS buffer) and followed by injection with DC-NBD (100 μL, 100 μM in PBS buffer with 15% DMSO, v/v) and then imaged after 3 min. (D) The mouse was given an intraperitoneal injection of $H_2S$ (100 μL, 1 mM in PBS buffer) and followed by injection with DC-NBD (100 μL, 100 μM in PBS buffer with 15% DMSO, v/v) and then imaged after 3 min. (E) Relative fluorescence intensity the mice A-D. Excitation was set at 610 nm and emission was collected around 750 nm.

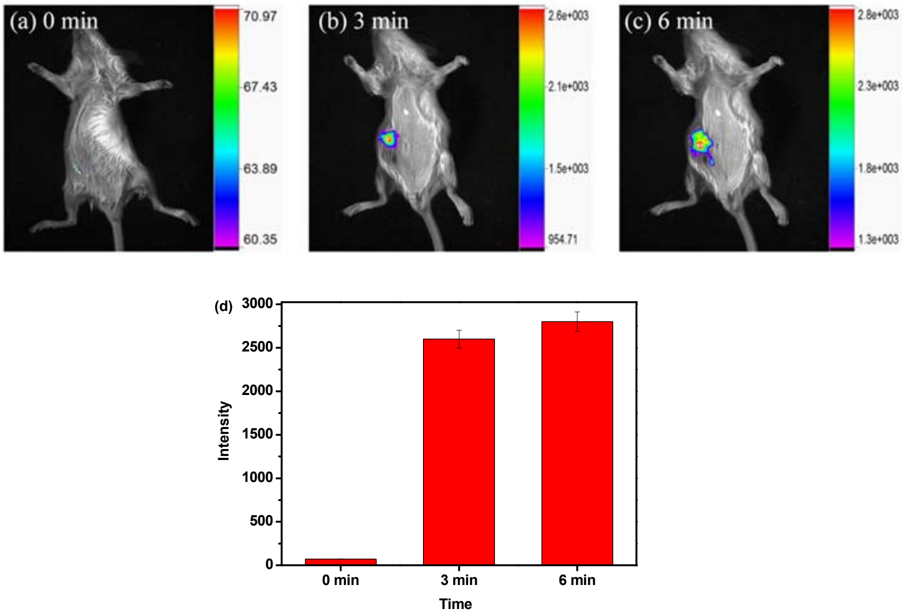



**Figure S9.** Imaging of $H_2S$ in living mouse with probe DC-NBD over time. The mouse was given an intraperitoneal injection of NaHS (100 µL, 1 mM in PBS buffer) followed by injection with DC-NBD (100 µL, 100 µM in DMSO). The mouse was imaged at (a) 0 min, (b) 3 min, (c) 6 min, respectively. (d) Relative fluorescence intensity from the abdominal area of the mice at different times. Excitation was set at 610 nm and emission was collected around 750 nm.