

Supporting Information

Fluorogenic sensing of H₂S in blood and living cells based on reduction of aromatic dialkylamino N-oxide

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General experimental procedure

All solvents and chemicals were purchased from Sigma-Aldrich, J&K, or Alfa Aesar, et.al. All the buffers used are degassed with N₂. Column chromatography was performed on silica gel (200~300 mesh). NMR spectra (¹H at 400 MHz and ¹³C at 100 MHz) were recorded on a Bruker instrument using tetramethylsilane as the internal reference. Mass spectra were measured with a Waters SQD or Bruker APEX IV FT-MS (7.0 T). UV-Visible spectra were obtained on a DU800 ultraviolet spectrometer. The fluorescence spectra were performed on a Cary Eclipse fluorometer or Molecular Devices Flex Station III microplate. pH values of the buffers were obtained on METTLER TOLEDO FiveEasy pH FE20. MCF-7 cells were analyzed using a confocal fluorescence microscope PerkinElmer Ultraview.

Synthesis of N-oxide of 7-diethylamino-4-methylcoumarin (COU):



Scheme S1 Synthetic method of COU

3-Chloroperbenzoic acid (800 mg) and 7-diethylamino-4-methylcoumarin (1 g) were dissolved in CH₂Cl₂. The solution was stirred at room temperature for 2 h and then evaporated in vacuum. The residue was purified by silica gel column chromatography to give 501 mg of product (yield: 45%). ¹H-NMR (400 MHz, CD₃OD): 7.97 (d, 1H, J = 2.04 Hz), 7.95 (s, 1H), 7.82 (dd, 1H, J₁ = 8.8 Hz, J₂ = 2.28 Hz), 3.97 (m, 2H), 3.74 (m, 2H), 2.54 (d, 3H, J = 1.00 Hz), 1.14 (t, 6H, J = 7.06 Hz). ¹³C-NMR (100 MHz, CD₃OD): 160.51, 153.46, 152.77, 151.13, 125.94, 120.39, 117.44, 115.33, 111.18, 66.61, 17.22, 7.17. LC-MS (C₁₄H₁₇NO₃): Calculated (M+H)⁺: 248.13; found: 248.44.

Quantum yields:

Fluorescence quantum yields were determined in pH 7.4 PBS buffer (20 mM) using quinine sulfate (0.1M H₂SO₄, Q = 54%) as a standard according to a published method using the following equation:

$$\Phi = \Phi_R \times \frac{I}{I_R} \times \frac{A_R}{A} \times \frac{\eta^2}{\eta_R^2}$$

Where A_R and A are the absorbance of reference and sample solutions at the same excitation wavelength. I and I_R are the corresponding integrated fluorescence intensity. And η and η_0 are the solvent refractive indexes of sample and reference, respectively. Quantum yield of **COU**: $\Phi = 0.1\%$; Quantum yield of 7-diethylamino-4-methylcouamrin: $\Phi = 3.56\%$.

Synthesis and separation of reduction product of COU with NaHS

0.4 g of **COU** was dissolved in DMF followed by addition of NaHS (1 g). The resulting solution was stirred at room temperature overnight. After the reaction was stopped, it was poured into deionized water and extracted with CH_2Cl_2 . The combined organic layer was dried over anhydrous Na_2SO_4 . Then the solvent was removed in vacuum. The crude product was purified by silica gel chromatography and characterized by NMR and HRMS spectrum.

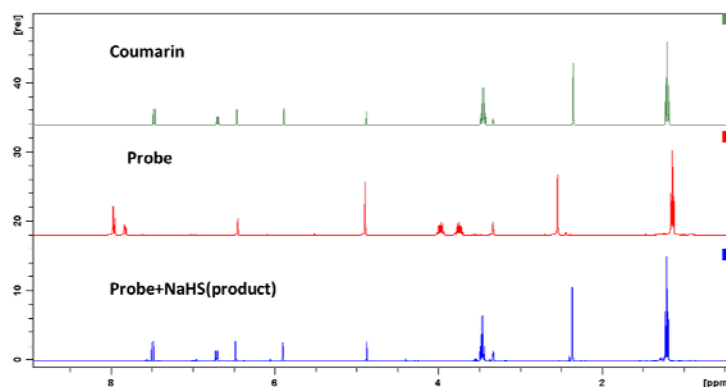


Fig S1. ^1H -NMR spectra of 7-diethyl-4-methylcoumarin, **COU** and isolated product of **COU** with NaHS in CD_3OD

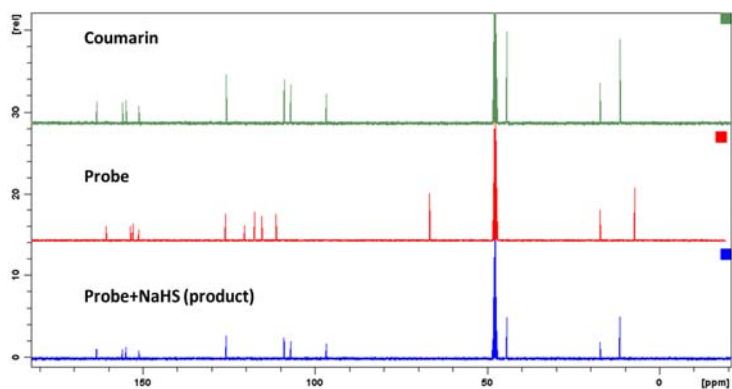


Fig. S2 ¹³C-NMR spectra of 7-diethyl-4-methylcoumarin, **COU** and isolated product of **COU** with NaHS in CD₃OD

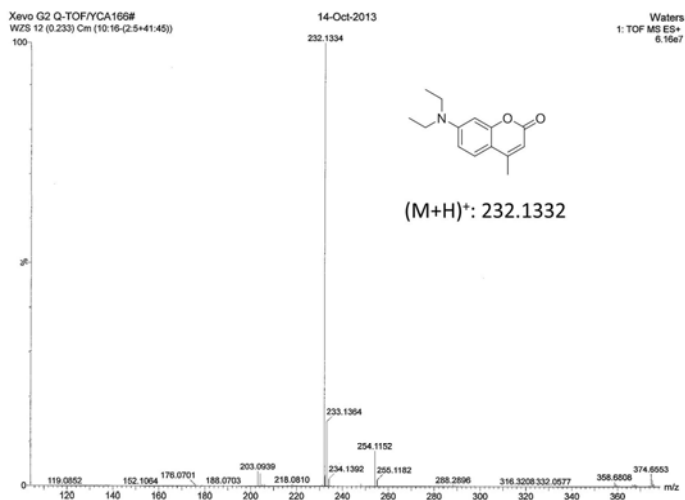


Fig. S3 HRMS of isolated product of probe's reaction with NaHS

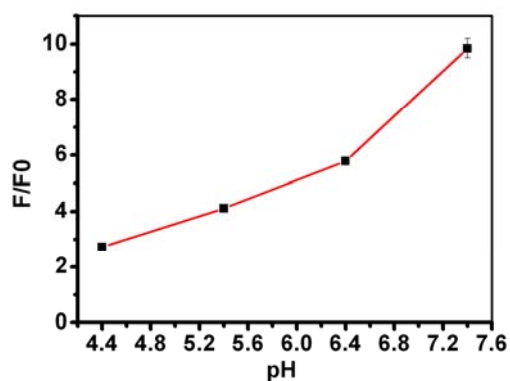


Fig. S4 Fluorescence enhancement (F/F_0) of probe ($5 \mu\text{M}$) toward NaHS ($40 \mu\text{M}$) at different pH (4.4, 5.4, 6.4, 7.4). $\lambda_{\text{ex}} = 360 \text{ nm}$, $\lambda_{\text{em}} = 455 \text{ nm}$, Cut off: 420 nm, PMT: medium.

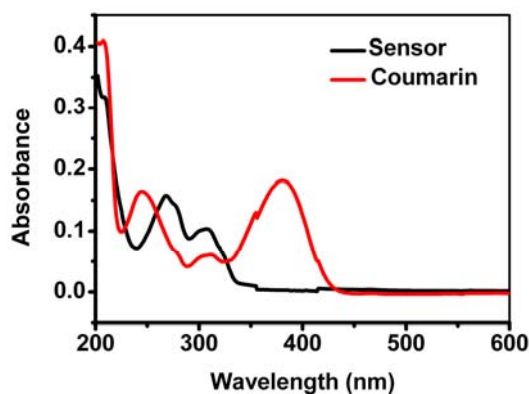


Fig. S5 Absorbance spectra of sensor (COU, $10 \mu\text{M}$) and 7-diethylamino-4-methylcoumarin ($10 \mu\text{M}$) in pH 7.4 buffer.

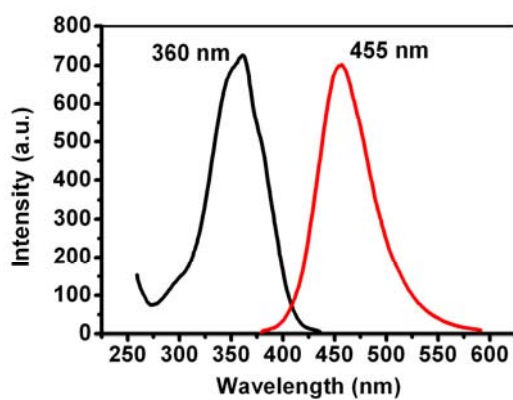


Fig. S6 Excitation and emission spectra of COU solution (pH = 7.4) in the presence of NaHS.

Fluorescence response of resazurin towards H₂S:

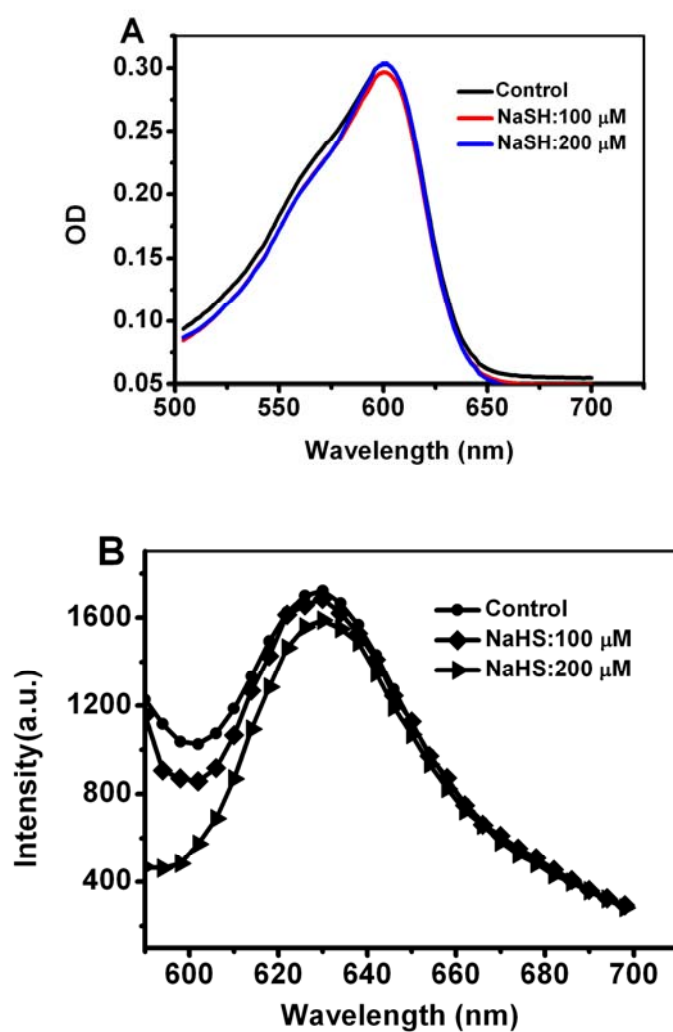
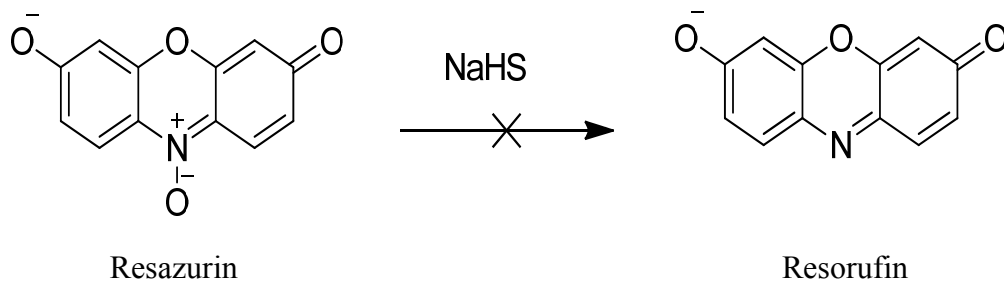


Fig. S7 Absorbance spectra change (A) and fluorescence spectra change of resazurin (10 μ M) upon addition of NaHS in pH 7.4 buffer. Data was obtained after 120 min incubation time. $\lambda_{\text{ex}} = 560$ nm.

Sensitivity of fluorescence assay of COU for H₂S detection

Various amounts of NaHS stock solution were added into a serial of pH 7.4 solutions containing fixed COU probe (100 μ M) to a final concentration of 0, 5, 10, 30, 50, 60 or 100 μ M. The reaction solutions were incubated at room temperature for 60 minutes and then directly analyzed for fluorescence intensity.

Selectivity of fluorescence assay of COU for H₂S detection

Relvant Species	Fluorescence enhancement(F/F ₀)	Stand error
Control	1	0.10341
Hcy	1.06762	0.04846
Cys	1.37181	0.08673
GSH	1.25296	0.03906
SO ₄ ²⁻	0.96852	0.07669
HCO ₃ ⁻	0.92685	0.15292
N ₃ ⁻	0.74639	0.04736
H ₂ PO ₄ ⁻	1.10149	0.62004
NO ₂ ⁻	0.8759	0.06194
CO ₃ ²⁻	0.88167	0.01549
Br ⁻	1.13391	0.33878
Cl ⁻	0.77859	0.02084
citrate	1.0736	0.11471
AcO ⁻	0.90034	0.1707
HS ⁻	10.05511	0.18181

Table. S1 Selectivity of COU with relvant species. Data was acquired after incubation 60 min. Condition: 5 μ M probe, 1 mM anions, 5 mM Cys, Hcy and GSH, pH 7.4 PBS buffer. $\lambda_{\text{ex}} = 360$ nm, $\lambda_{\text{em}} = 455$ nm, Cut off: 420 nm, PMT: medium.

H₂S detection in blood:

COU was dissolved in anhydrous EtOH (100 μ M) then coating in 96-well plate (costar 3610, white color). EtOH was evaporated through air-dry and COU was coated on the bottom surface of 96-well plate for fluorescence detection of further

control and blood samples. For standard curve of fluorescence intensity to sulfide concentrations, sulphide saturated solution (5 mM) was diluted to various concentrations (100 μM , 50 μM , 25 μM , 12.5 μM , 6.25 μM , 3.125 μM , 0 μM) then was added to 96-well plate. The mixed solution was incubated for 60 min then was analysed by Ffuostar OPTIMA (Excitation: 355 nm; Emission: 460 nm; gain: 650).

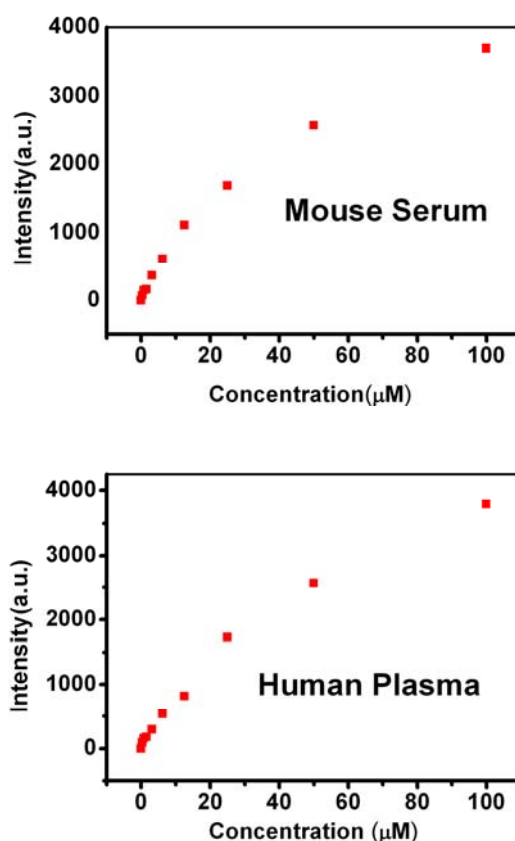


Fig. S8 Assay sensitivity of probe (100 μM) in mouse blood serum (up) and human blood plasma (down).

Cell culture and imaging:

MCF-7 cells were cultured in DMEM with 10% FBS. NaHS solution was prepared in degassed PBS buffer (by bubbling N_2 for 30 min). Cells were passaged and allowed to grown on glass coverslips containg 2 mL DMEM, and incubated in 37 $^\circ\text{C}$ with 5% CO_2 for 24 h. To start the experiment, MCF-7 cells were preloaded with 100 μM probe for 30 minutes, and then washed twice with PBS buffer to remove the extracellular probe. Cells were then treated with or without 250 μM NaHS in PBS

solutions for 30 minutes. After treatment, cells were rinsed with PBS three times and fresh DMEM was added for imaging. Differential interference contrast (DIC) and fluorescence images were obtained using a confocal fluorescence microscope PerkinElmer Ultraview.

Cytotoxicity analysis:

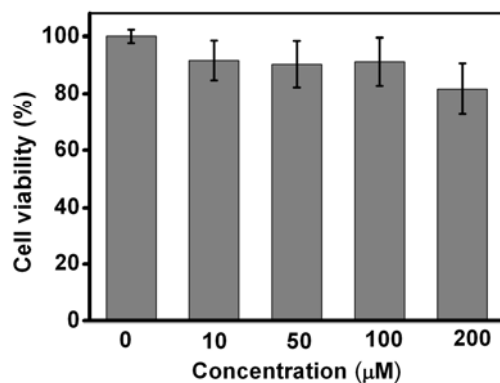


Fig. S9 Cell viability of COU for MCF-7 cells through SRB assay in 24 h at 37°C

NMR spectra and mass spectra of COU

