Supporting information

A Fluorescent Probe for H_2S in vivo with Fast Response and High Sensitivity

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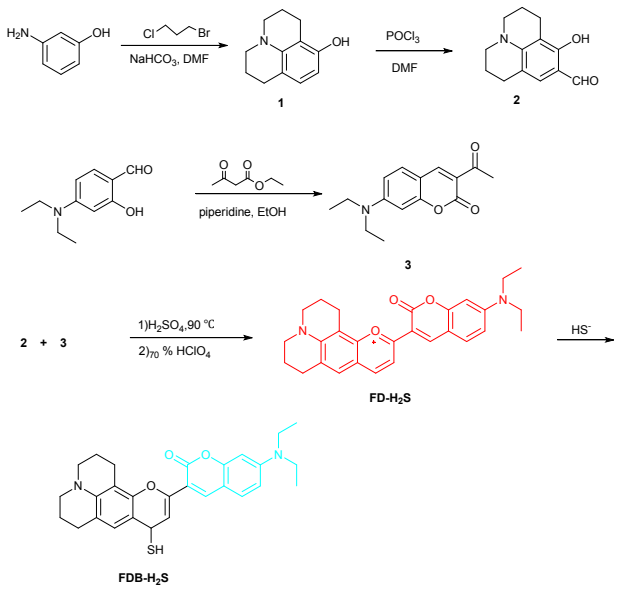
Content

Materials and methods

All solvents and reagents used were reagent grade and were used without further purification unless otherwise stated. The solution of **FD-H_2S** was dissolved in CH_3CN at a concentration of 2mM as the stock solution and stored in a refrigerator for use. ^1H NMR and ^{13}C NMR spectra were recorded on a VARIAN INOVA-400(or a Bruker Avance II 400 MHz or 500 MHz) spectrometer. Chemical shifts (δ) were reported as ppm (in $CDCl_3$ or DMSO, with TMS as the internal standard). Fluorescence spectra were performed on a VAEIAN CARY Eclipse fluorescence spectrophotometer (Serial No. FL0812-M018). Excitation and emission slit widths were modified to adjust the fluorescence intensity to a suitable range. Absorption spectra were measured on a Perkin Elmer Lambda 35 UV/VIS spectrophotometer (Perkin Elmer). Mass spectrometric data were achieved with HP1100LC/MSD MS and an LC/Q-TOF-MS instruments. All pH measurements were performed using a Model PHS-3C meter. Slight pH variations in the solutions were achieved by adding the minimum volumes of HCl or NaOH (1 M). The fluorescence quantum yields for compounds with Absolute PL Quantum Yield Spectrometer. Stock solutions or generation of ROS were prepared according to our previous reported[1]. Flash column chromatography was performed using silica gel (100 - 200 mesh) and aluminum oxide (neutral. 100-200 mesh) obtained from Qingdao Ocean Chemicals. Mito Tracker Green FM, Lyso Tracker Green and ER Tracker Green were purchased from Life Technologies Co. (USA). Doubly purified water used in all experiments was from Milli-Q systems. Each experiment was carried out in three replicates (n = 3).

Synthesis of Compounds



Scheme S1. the synthesis and the proposed H_2S sensing mechanism of FD-H_2S.

The synthesis of 1

1, 2 was synthesized from 3-(dimethylamino) phenol by the procedure published in literature[2].

The synthesis of 3

3 was synthesized from 4-(diethylamino)-2-hydroxybenzaldehyde by the procedure published in literature[3].

The synthesis of FD-H_2S

2 (108 mg, 0.5 mM) and **3** (129 mg, 0.5 mM) were dissolved in conc. H_2SO_4 (5 ml). The mixture was stirred at 90 ºC for 4.5 h. After cooling to room temperature, the solution was added ice (5 g) the 70% $HClO_4$ (0.25 ml), filtered, and washed with water to afford crude product. The crude product was purified by silica gel column chromatography with CH_2Cl_2/CH_3OH (30:1 to 20:1) and get the desired black solid product (216 mg, 80%). ^{1}H NMR (400 MHz, DMSO), δ: 8.69 (s, 1H), 8.37 (d, 1H, J = 8.3), 8.12 (d, 1H, J = 8.1), 7.85 (d, 1H, J = 9.1), 7.51 (s, 1H), 6.94 (d, 1H, J = 9.2), 6.67 (s, 1H), 3.57 (dd, 8H, J = 14.0, 6.9), 3.06 (d, 2H, J = 6.0), 2.85 (s , 2H), 2.09 – 2.00 (m, 2H), 1.95 (s, 2H), 1.19 (t, 6H, J = 7.0). ^{13}C NMR (125 MHz, DMSO) δ:12.47, 18.84, 18.95, 19.73, 26.90, 44.79, 50.28, 50.70, 96.18, 104.75, 106.24, 108.98, 109.77, 111.17, 118.27, 127.16, 127.82, 132.91, 144.33, 145.37, 152.13, 152.88, 153.94, 157.53, 157.91, 160.47 ppm; TOF HRMS: m/z calcd for $C_{28}H_{29}N_2O_3^+$ $[M]^+$: 441.2173, found: 441.2171.

Determination of the detection limit

The detection limit was calculation based on the fluorescence titration (Figure 1b) of **FD-H_2S** in the presence of NaHS (0-30 equiv). The fluorescence intensity of **FD-H_2S** was measured by three times and standard deviation of the blank measurement was achieved. The detection limit was calculated by using detection limit was calculated with the following equation:

Detection limit = $3\sigma/k$

Where σ is the standard deviation of the blank measurement, k is the slope between the fluorescence ratios (F_{487}/F_{707}) versus NaHS concentration.

Absolute PL Quantum Yield Spectrometer

Quantaurus-QY was developed as a compact, easy-to-system with a small footprint based on Hamamatau's established C9920-02/03 systems for measuring absolute photoluminescence quantum yields. Operating this system is simple. Load a sample and press the start button to measure the photoluminescence quantum yields, excitation wavelength dependence, PL excitation spectrum and other properties in a short time.

The PL Quantum Yield (Φ) is expressed as the ratio of the number of photons emitted from molecules (PN_{em}) to that absorbed by molecules (PN_{abs}).

$\Phi = PN_{em}/ PN_{abs}$

Live cell imaging experiments

Human breast cancer cells (MCF-7) were purchased from Institute of Basic Medical Sciences

(IBMS) of the Chinese Academy of Medical Sciences. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) supplemented with 10 % fetal bovine serum (Invitrogen). The cells were seeded in 24-well flat-bottomed plates and then incubated for 24 h at 37 ºC under 5% CO_2. Before imaging, the live cells were incubated with **FD-H_2S** (10 μM) for another 30 min and then washed with phosphate-buffered saline (PBS) three times. Fluorescence imaging was performed using an OLYMPUSFV-1000 inverted fluorescence microscope with a 60 × objective lens. Under the confocal fluorescence microscope, **FD-H_2S** was excited at 405 nm and emission was collected at 460-510 nm (green channel) and 655-755 nm (red channel). For the detection of exogenous H_2S, MCF-7 cells were incubated with **FD-H_2S** (10 μM) 37 ºC for 30 min and then NaHS (20 equiv) was added at 37 ºC for 20 min. For the detection of endogenous produced H_2S, MCF-7 cells were pre-incubated with L- cysteine (30 equiv) at 37 ºC for 30 min. For the detection of basal H_2S, MCF-7 cells were treated with NMM (50 equiv) at 37 ºC for 60 min and then further incubated with **FD-H_2S** (10 μM) for 30 min.

MTT assay

Measurement of cell viability was evaluated by reducing of MTT (3-(4, 5)-dimethylthiahiazo (-2-yl)-3, 5-diphenytetrazoliumromide) to formazan crystals using mitochondrial dehydrogenases (Mosmann, 1983). MCF-7 cells were seeded in 96-well microplates (Nunc, Denmark) at a density of 1×10^5 cells/mL in 100 μL medium containing 10 % FBS. After 24 h of cell attachment, the plates were then washed with 100 μL / well PBS. The cells were then cultured in medium with 1, 5, 10, 20 μM of **FD-H_2S** for 12 h. Cells in culture medium without **FD-H_2S** were used as the control. Six replicate wells were used for each control and test concentration. 10 μL of MTT (5 mg/mL) prepared in PBS was added to each well and the plates were incubated at 37ºC for another 4 h in a 5% CO_2 humidified incubator. The medium was then carefully removed, and the purple crystals were lysed in 200 μL DMSO. Optical density was determined on a microplate reader (Thermo Fisher Scientific) at 570 nm with subtraction of the absorbance of the cell-free blank volume at 630 nm. Cell viability was expressed as a percent of the control culture value, and it was calculated using the following equation:

Cells viability (%) = $(OD_{dye} - OD_{K\ dye}) / (OD_{control} \cdot OD_{K\ control}) \times 100$

Fluorescence imaging in mice

All procedures were carried out in compliance with the Guide for the Care and Use of Laboratory Animal Resources and the National Research Council, and were approved by the Institutional Animal Care and Use Committee of the NIH. The bioimaging experiments on living mice utilized the NightOWL II LB983 small animal in *vivo* imaging system equipped with a sensitive Charge Coupled Device (CCD) camera, with the excitation at 630 nm and the 700 nm emission filter. Healthy mice (seven weeks old, 20-25 g) were used, and animals had free access to food and water. The mice were anesthetized, and the abdominal fur was removed.

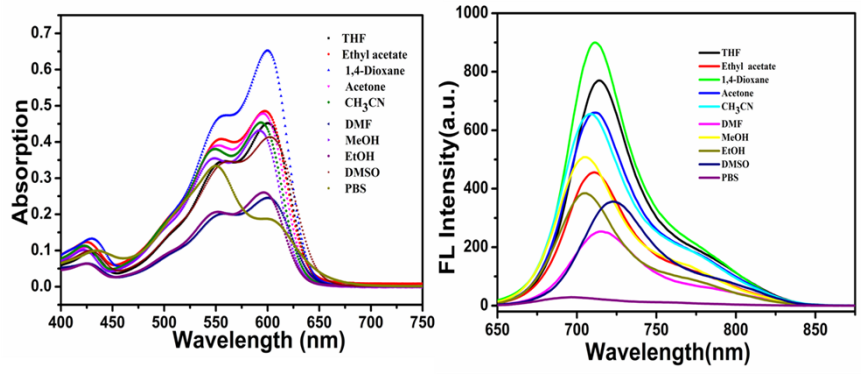


Fig. S1 The absorbance and emission spectra of **FD-H_2S** (10 μM) in different solutions.

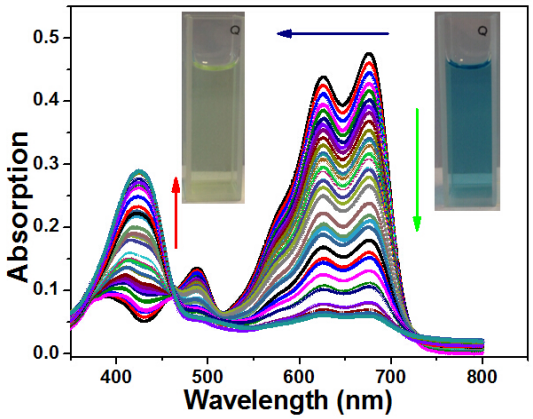


Fig. S2 Absorption spectra of **FD-H_2S** (10 μM) toward different concentrations of NaHS (titration concentration: 0-138 equiv of NaHS in in PBS (0.01 M) solution (acetonitrile /water = 3:7, pH 7.4). Inset: the color of **FD-H_2S** (10 μM) in absence and presence of NaHS.

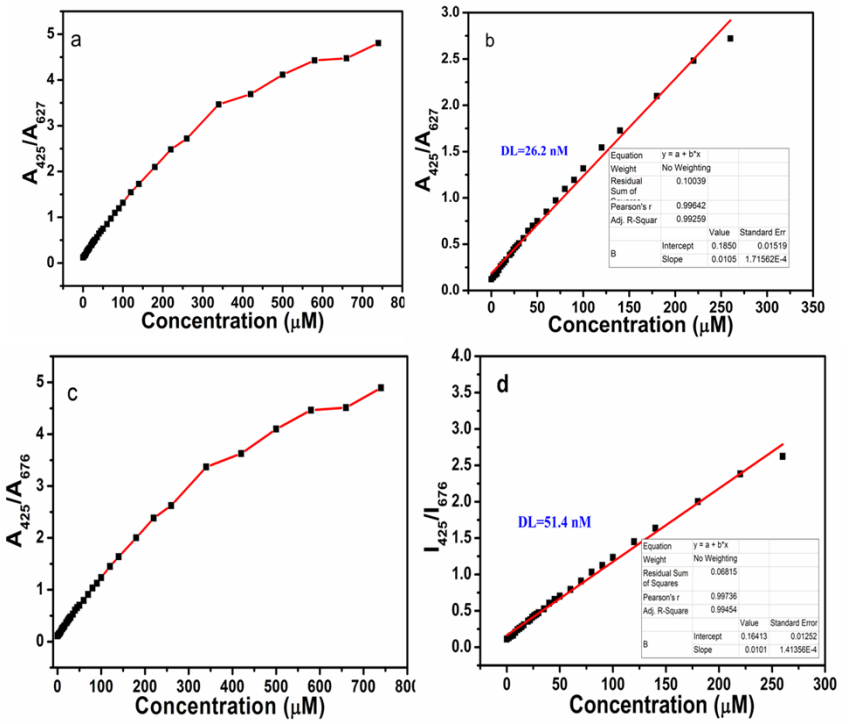


Fig. S3 (a) Absorbance intensity ratio (A_{425}/A_{627}) of **FD-H_2S** (10 μM) toward different concentrations of NaHS (titration concentration: 0-138 equiv of NaHS in in PBS (0.01 M) solution (acetonitrile /water = 3:7, pH 7.4). (b) The linear relationship of absorbance intensity ratio (A_{425}/A_{627}) changes of **FD-H_2S** (10 μM) with 0-30 equiv of NaHS. (c) Absorbance intensity ratio (A_{425}/A_{676}) of **FD-H_2S** (10 μM) toward different concentrations of NaHS (titration concentration: 0-138 equiv of NaHS in in PBS (0.01 M) solution (acetonitrile /water = 3:7, pH 7.4). (d) The linear relationship of absorbance intensity ratio (A_{425}/A_{676}) changes of **FD-H_2S** (10 μM) with 0-30 equiv of NaHS.

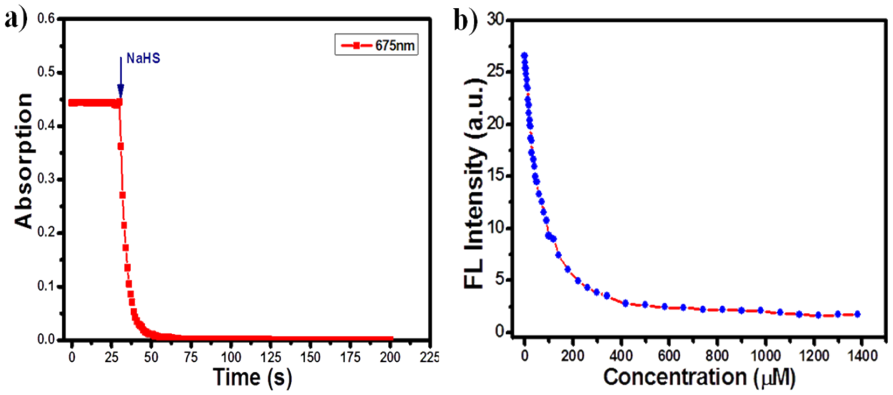


Fig. S4 (a) Time dependence of absorption (675nm) profiles of **FD-H_2S** (10 μM) with 100 equiv NaHS. (b) The fluorescence intensity of F_{707} of **FD-H_2S** (10 μM) were plotted as a function of NaHS concentration, inset: the liner relationship of fluorescence intensity F_{707} of **FD-H_2S** (10 μM) vs. increasing concentrations of NaHS.

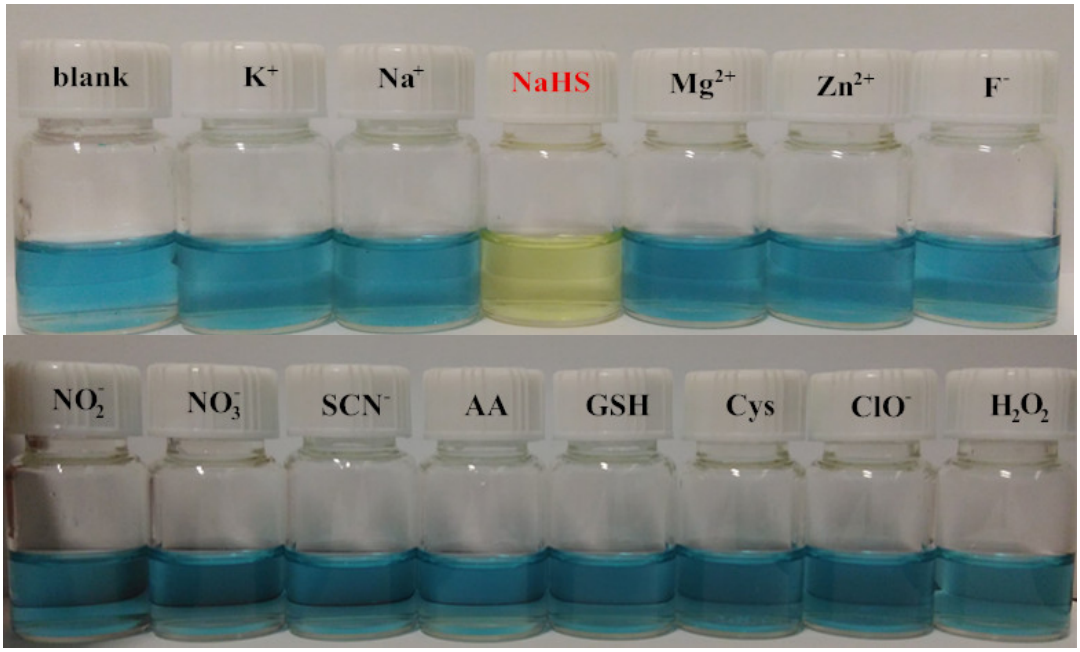


Fig. S5 The color change of **FD-H_2S** (10 μM) with different analytes in PBS (0.01 M) solution (acetonitrile /water = 3:7, pH 7.4). The pictures were recorded at 3h after of the analytes (1, probe alone; 2, K^+; 3, Na^+; 4,Mg^{2+}; 5, Zn^{2+}, 6, F^-; 7,NO_2^-; 8, NO_3^-; 9, SCN^-; 10, ascorbic acid; 11,GSH; 12,Cys; 13, NaClO; 14, H_2O_2; 15, NaHS (0.4 mM)).

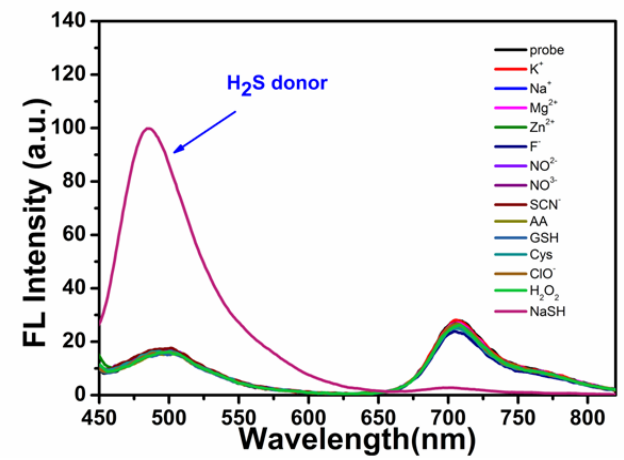


Fig. S6 Fluorescence spectra of **FD-H_2S** (10 μM) toward various biospecies (1 mM except for notation) in PBS (0.01 M) solution (acetonitrile /water = 3:7, pH 7.4). 1, probe alone; 2, K^+; 3, Na^+; 4,Mg^{2+}; 5, Zn^{2+}; 6, F^-; 7,NO_2^-; 8, NO_3^-; 9, SCN^-; 10, ascorbic acid; 11, GSH; 12, Cys; 13, NaClO; 14, H_2O_2; 15, NaHS (0.40 mM) (λ_{ex} = 430 nm, slit: 5/10 nm).

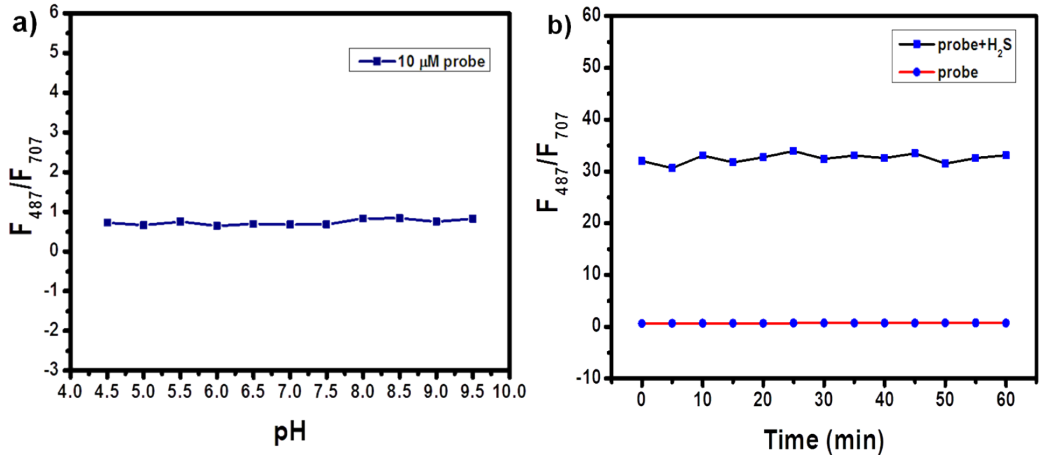


Fig. S7 (a) Influence of pH on fluorescent intensity ratio (F_{487}/F_{707}) of **FD-H_2S** (10 μM) in PBS (0.01 M) solution (acetonitrile /water = 3:7, pH 7.4). (b) The stability of time dependence of **FD-H_2S** (10 μM) with 40 equiv (black line) or not (red line) in PBS (0.01 M) solution (acetonitrile /water = 3:7, pH 7.4) were measured with a spectrophotometer every 6 min from 0 to 60 min. λex = 430, slit: 5/10 nm.

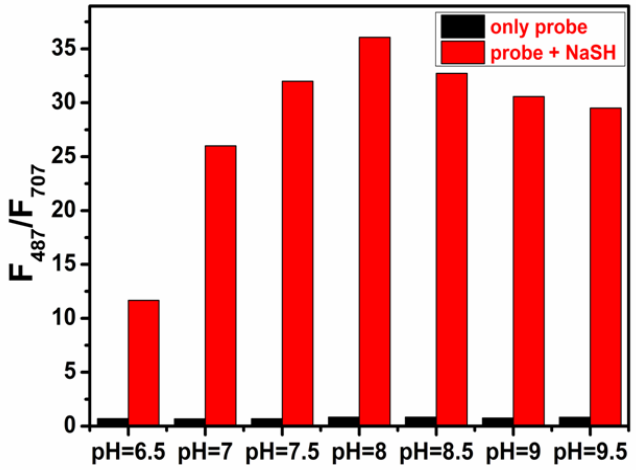


Fig. S8 Fluorescence intensity retio (F_{487}/F_{707}) of **FD-H_2S** (10 μM) in the absence and presence of 40 equiv NaHS in PBS (0.01 M) solution (acetonitrile /water = 3:7) (λ_{ex} = 430 nm, slit: 5/10 nm).

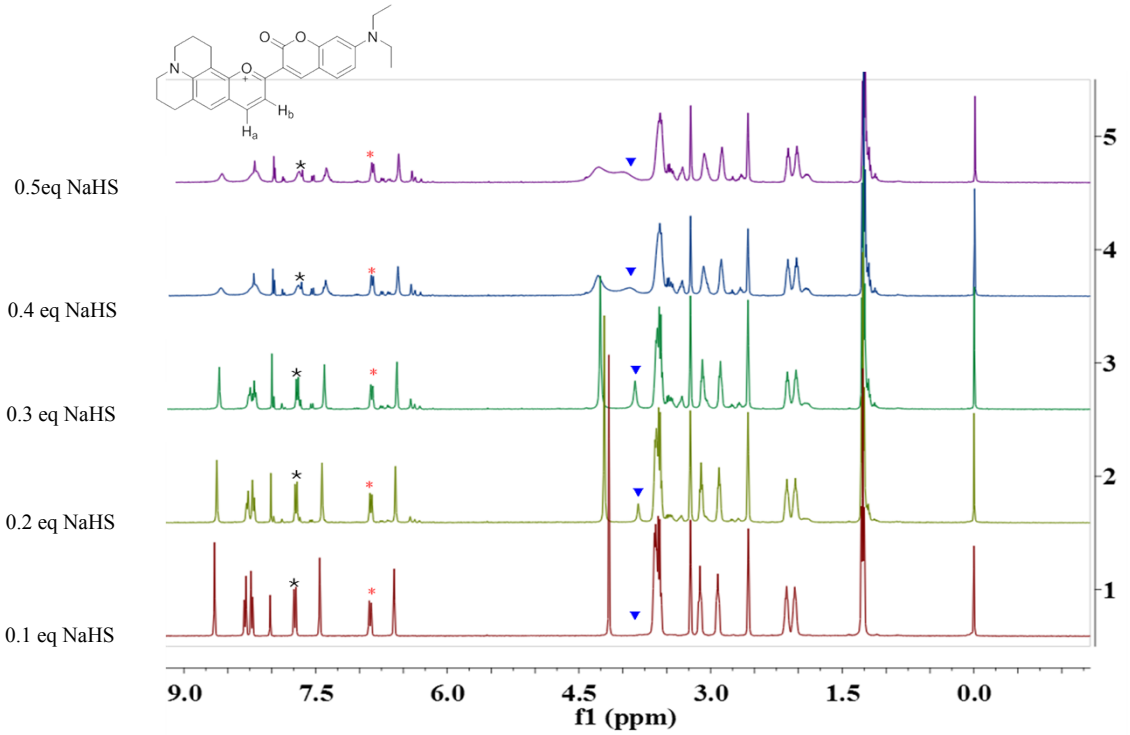


Fig. S9 ^{1}H NMR spectra of **FD-H_2S** (12 mM) in presence of different equivalents of NaHS in DMSO/$CDCl_3$/CD_3OD (1/1/1,v/v/v). Peaks marked with (*) (*) (▾) represent different chemical environment of hydrogen.

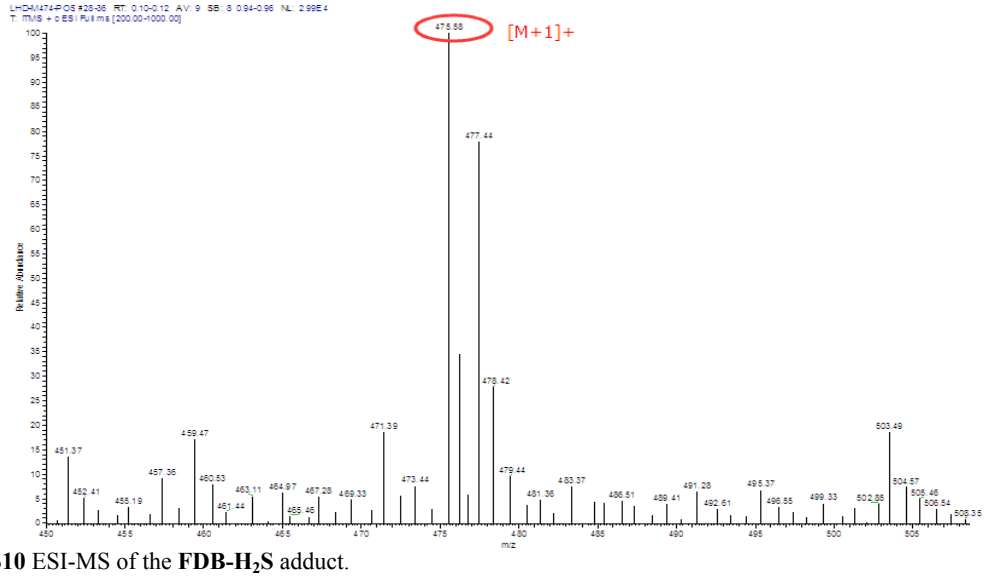


Fig. S10 ESI-MS of the **FDB-H_2S** adduct.

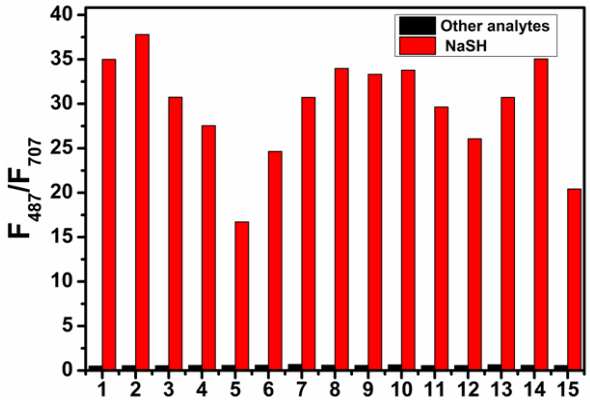


Fig. S11 Relative fluorescent intensity ratio (F_{487}/F_{707}) of the system. (black bar: various analyses, red bar: probe +

various analytes + NaHS). Each spectrum was obtained 2 min after NaHS addition. λ_{ex} = 430 nm, slit: 5/10 nm.

^{1}H-NMR, ^{13}C-NMR, and TOF-MS spectra

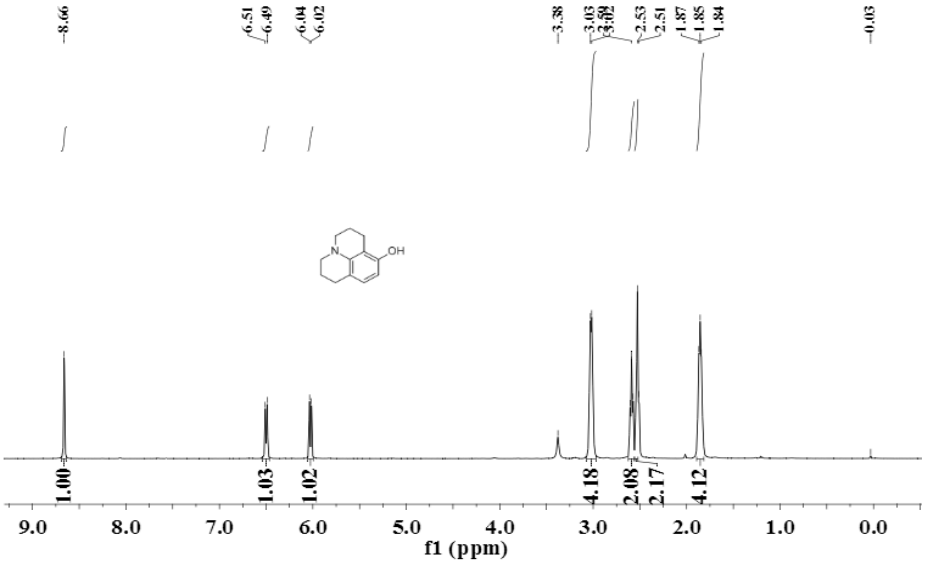


Fig. S12 ^{1}H NMR of **1** recorded in DMSO-d_6

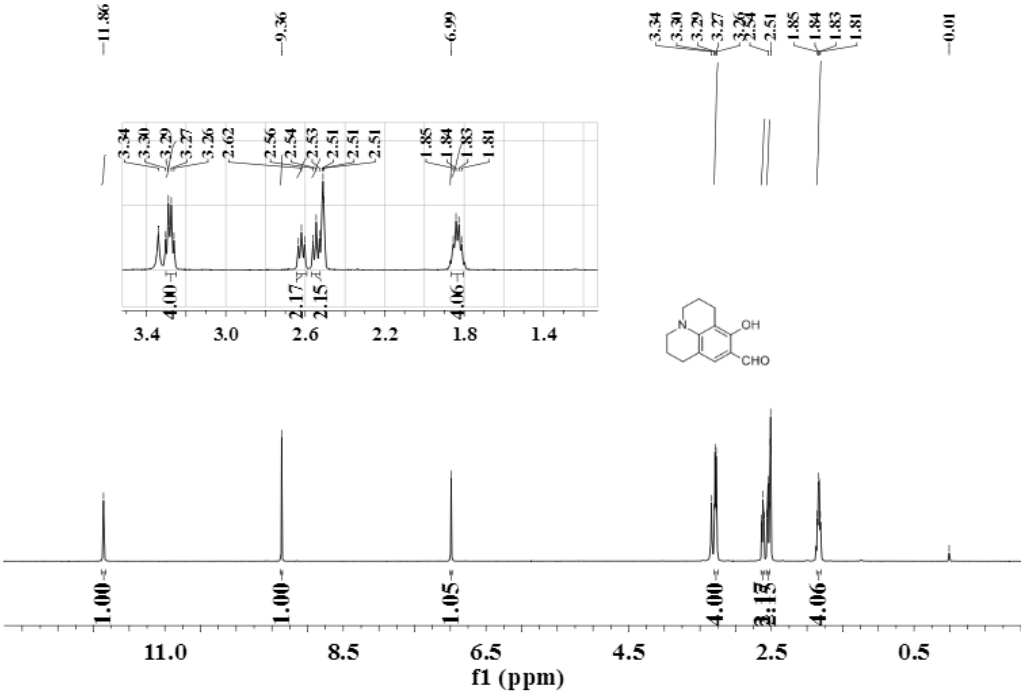


Fig. S13 ^{1}H NMR spectrum of **2** recorded in DMSO-d_6

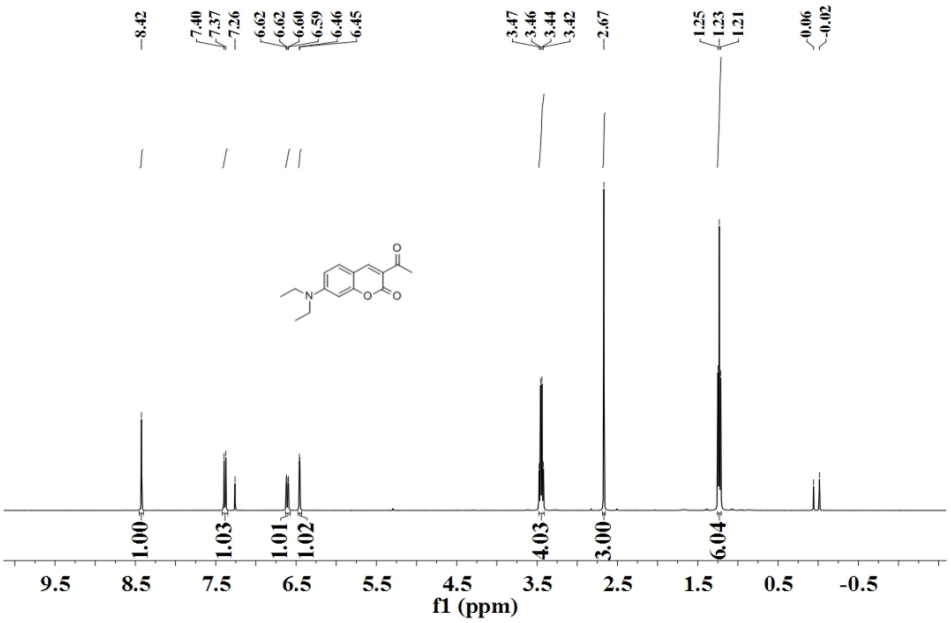


Fig. S14 ^{1}H NMR spectrum of **3** recorded in $CDCl_3$

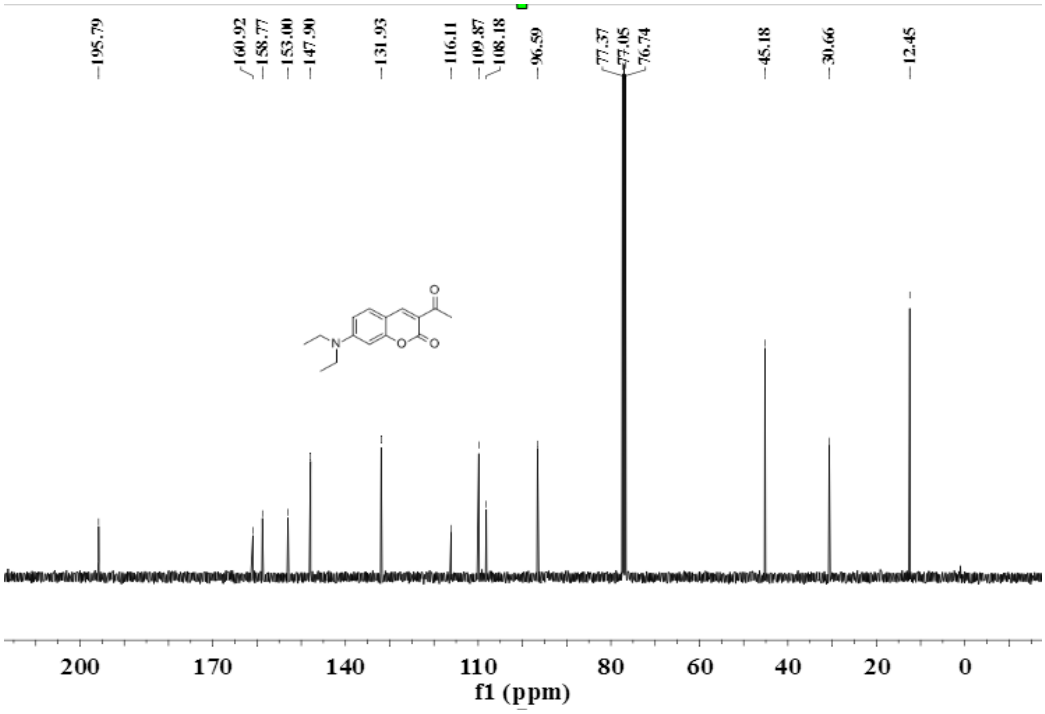


Fig. S15 ^{13}C NMR spectrum of **3** recorded in $CDCl_3$

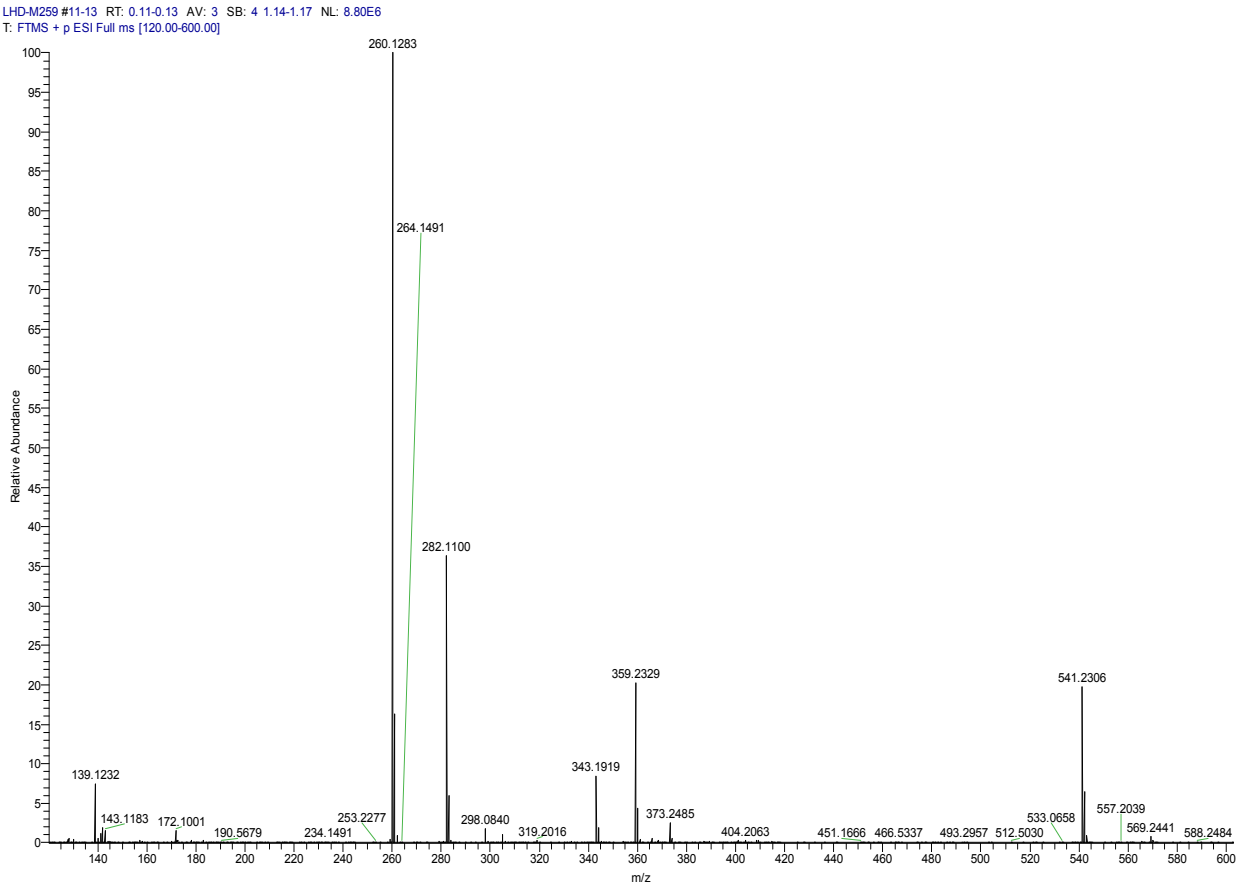


Fig. S16 TOF mass of compound **3**

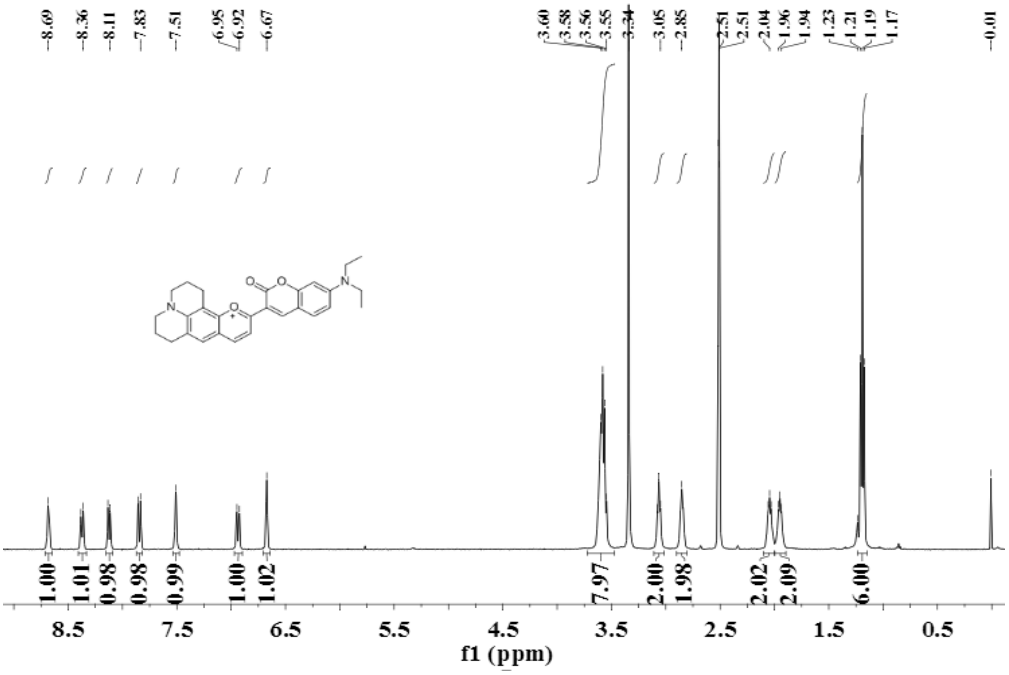


Fig. S17 ^{1}H NMR spectrum of **FD-H_2S** recorded in DMSO-d_6

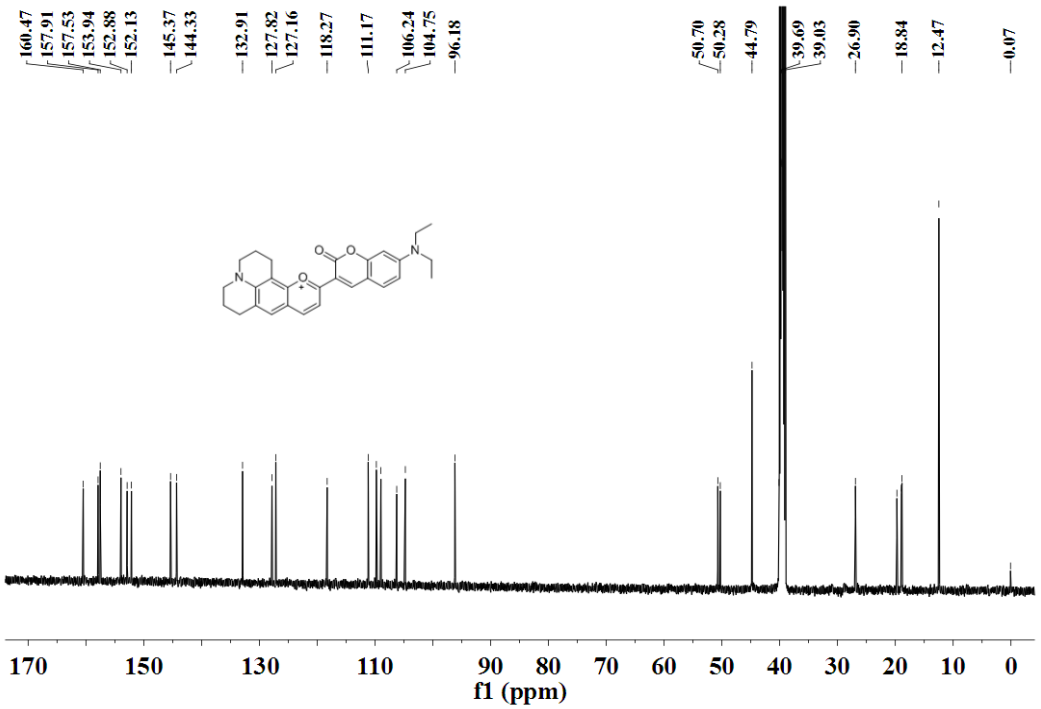


Fig. S18 ^{13}C NMR spectrum of **FD-H_2S** recorded in DMSO-d_6

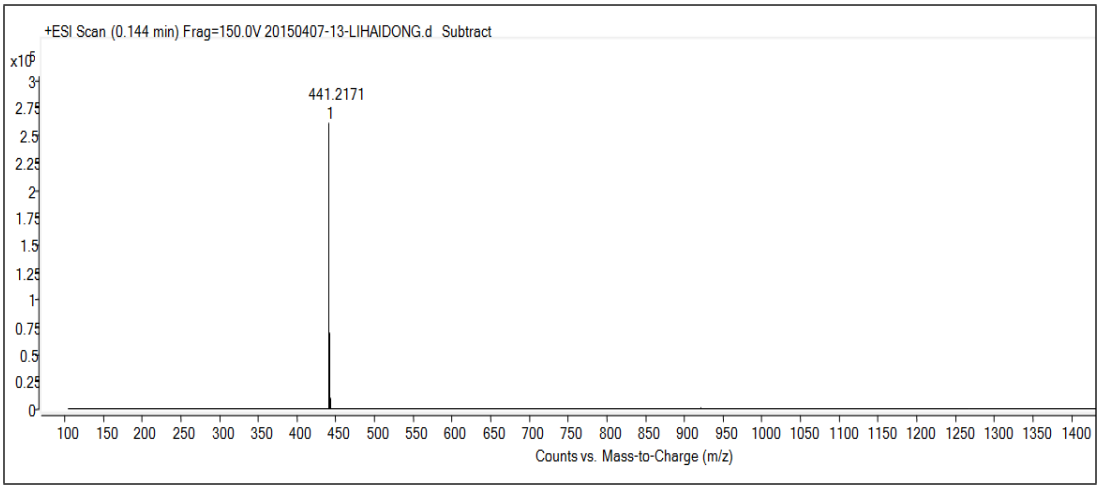
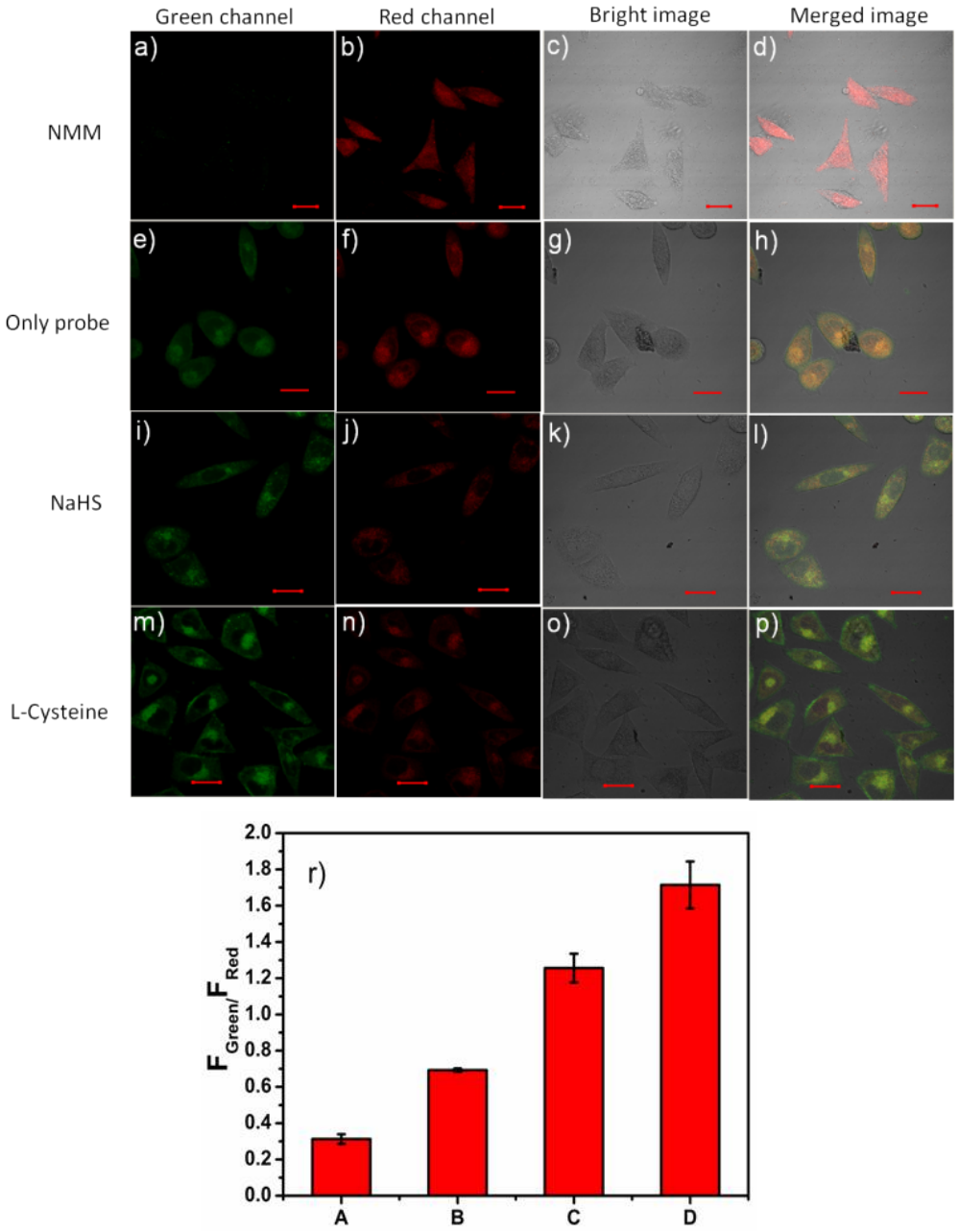


Fig. S19 TOF mass of compound **FD-H_2S**



The data indicated that the MCF-7 cells incubated only **FD-H_2S** (10 μM) have approximately 2.23-fold higher fluorescence intensity ratio (F_{Green}/F_{Red}) than with NMM, and the MCF-7 cells loaded with **FD-H_2S** (10 μM) and NaHS have about 1.83-fold higher fluorescence intensity ratio (F_{Green}/F_{Red}) than only **FD-H_2S** (10 μM), and the corresponding fluorescence intensity ratio (F_{Green}/F_{Red}) of the MCF-7 cells incubated L-cysteine and **FD-H_2S** (10 μM) is 2.48-fold than the MCF-7 cells loaded with only **FD-H_2S** (10 μM).

Fig. S20 Fluorescence image of H_2S in MCF-7 cells. the excitation wavelength was 405 nm and the emission was collected at 460-510 nm (green channel) and 655-755 nm (red channel), merged image generated from green to red channel: a-d) Cells were pre-incubated with 500 μM NMM at 37 °C for 60 min, and further incubated with **FD-H_2S** (10 μM) for 30 min. e-h) Cells were incubated with **FD-H_2S** (10 μM) at 37 °C for 30 min. i-l) Cells were pre-incubated with **FD-H_2S** (10 μM) for 30 min, and then treated with 200 μM NaHS at 37 °C for 20 min. m-p) Cells were pre-incubated with 300 μM L-Cysteine at 37 °C for 30 min, and then treated with **FD-H_2S** (10 μM) at 37 °C for 30 min. r) Quantitative image analysis of the average total fluorescence of cells, determined from analysis of 10 regions of interest (ROIs) across MCF-7 cells. Scale bar = 20μm.

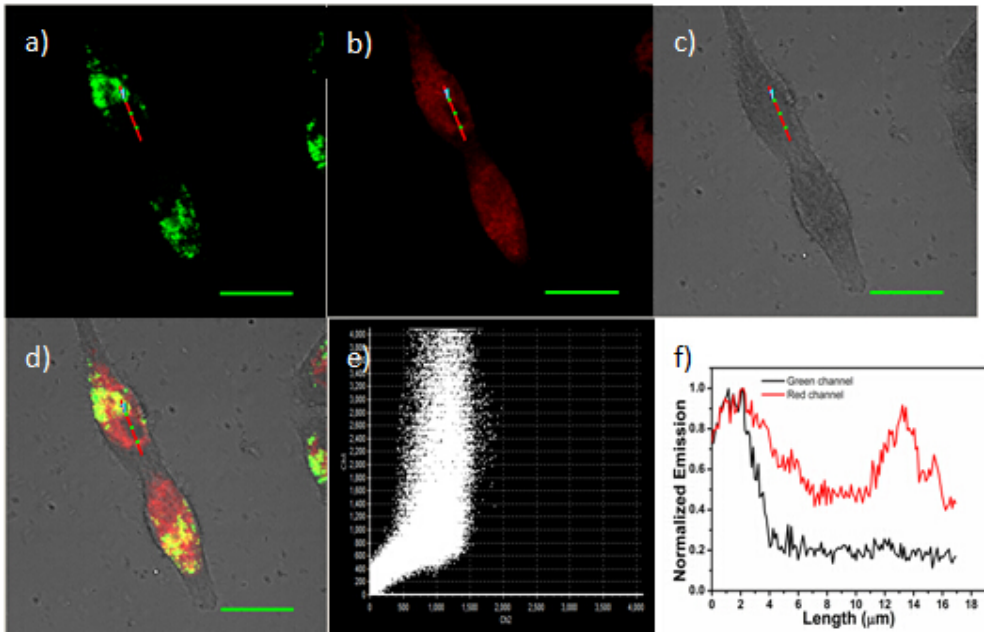


Fig. S21 FD-H_2S co-localizes to mitochondria in MCF-7 cells. a, b) Confocal fluorescence images of MCF-7 cells incubated with Lyso Tracker Green DND-26 (1.0 μM) at 37 °C for 20 min (Green channel: λex = 488 nm)(a) and FD-H_2S (10μM) at 37 °C for 20 min (Red channel: λex=635 nm) (b). c) Bright-field image. d) Merged image of (a) and (b). e) Correlation plot of the intensities of Lyso Tracker Green DND-26 and FD-H_2S (Rr = 0.86). f) Normalized intensity profile of regions of interest (ROIs) across MCF-7 cells. Scale bar = 20μm.

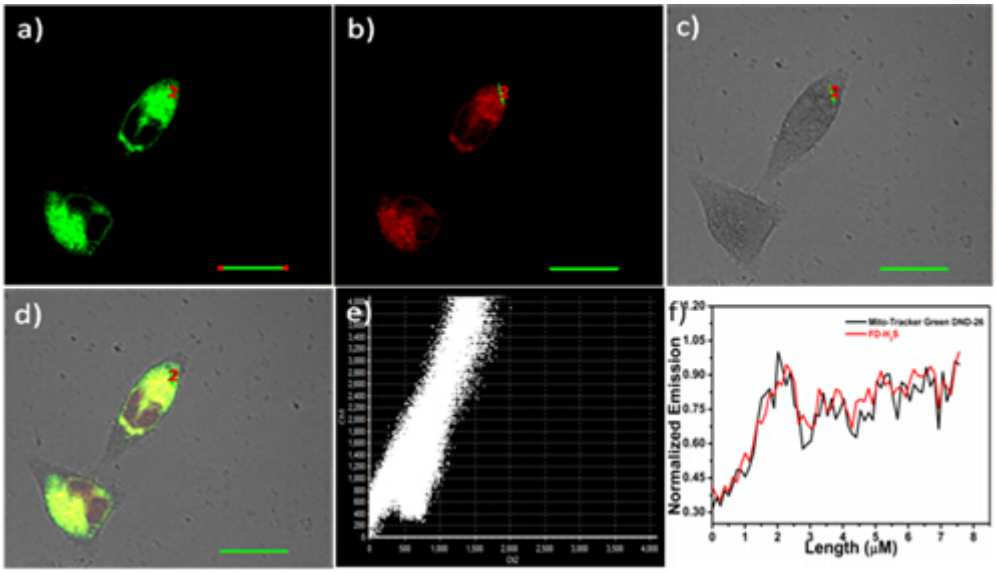


Fig. S22 FD-H_2S co-localizes to mitochondria in MCF-7 cells. a, b) Confocal fluorescence images of MCF-7 cells incubated with Mito Tracker Green FM (1.0 μM) at 37 °C for 20 min (Green channel: λex = 488 nm)(a) and **FD-H_2S** (10μM) at 37 °C for 20 min (Red channel: λex = 635 nm) (b). c) Bright-field image. d) Merged image of (a) and (b). e) Correlation plot of the intensities of Mito Tracker Green FM and **FD-H_2S** (R_r = 0.97). f) Normalized intensity profile of regions of interest (ROIs) across MCF-7 cells. Scale bar = 20μm.

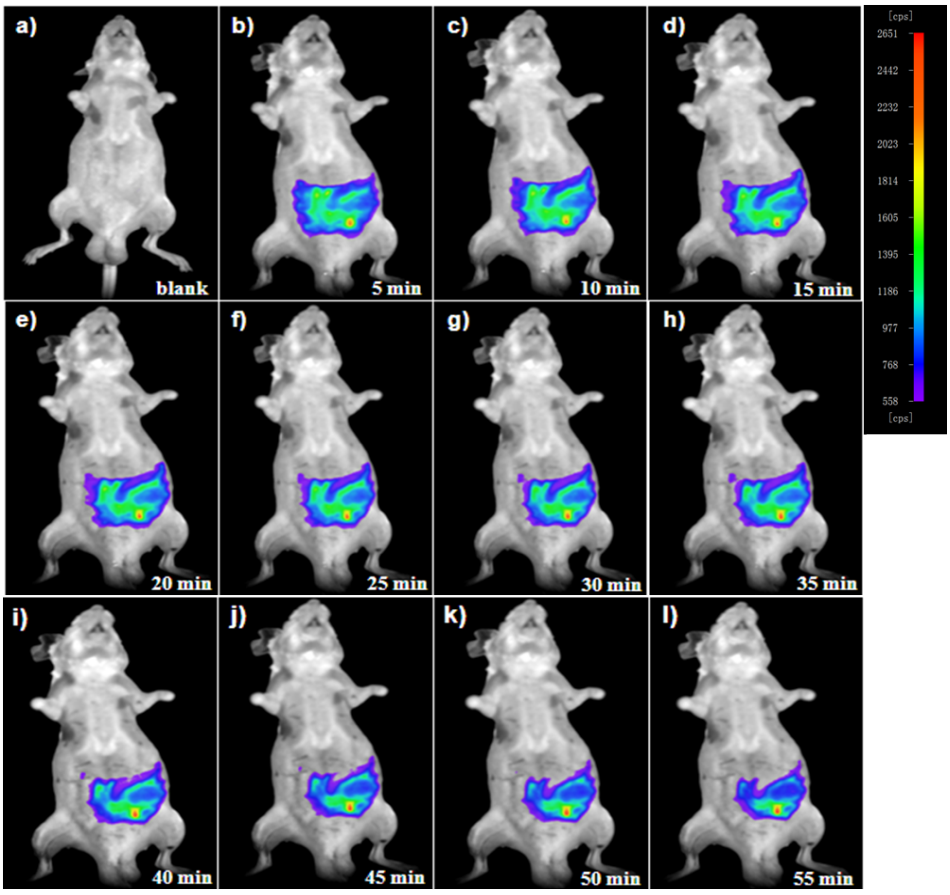


Fig. S23 Representative fluorescent images of H_2S in mice. (a) Without any treatment of mouse was as control; (b-l) only the probe **FD-H_2S** was injected in the peritoneal cavity of the mouse; Images were taken after incubation of different times (5 min (b), 10 min (c), 15 min (d), 20 min (e), 25 min (f), 30 min (g), 35 min (h), 40 min (i), 45 min (j), 50 min (k), 55 min (l)) with the excitation filter at 630 nm and the emission filter set at 700 ± 20 nm.

References

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3 J.-T. Hou, J. Yang, K. Li, Y.-X. Liao, K.-K. Yu, Y.-M. Xie and X.-Q. Yu, *Chem. Commun*., 2014, **50**, 9947-9950.