Supporting Information

A novel FRET-based ratiometric fluorescent probe for highly sensitive detection of hydrogen sulfide

Kunzhu Huang, Lun Yu, Pengfei Xu, Xintong Zhang, and Wenbin Zeng*.

School of Pharmaceutical Sciences, Central South University, 172 Tongzipo Road, Changsha, 410013, P. R. China.

*Author of correspondence: Prof. Dr. Wenbin Zeng, Tel/Fax: (86)731-8265-0459; Email: wbzeng@hotmail.com.

Table of Contents

1. Materials and instruments

Unless otherwise stated, all reagents and solvents were purchased from commercial suppliers and used without further purification. Twice-distilled water was used throughout all experiments. ^{1}H NMR spectra were recorded on a 600 MHz Varian Unity Inova NMR spectrophotometer, using TMS as an internal standard. ^{13}C NMR spectra were recorded on the same instrument (150 MHz) with total proton decoupling. Mass spectra were recorded with Thermo Scientific Orbitrap Velos Pro MS spectrometer or Agilent 6410 Triple Quad LC/MS mass spectrometer. UV-Vis absorption spectra were measured with a Shimadzu UV-2450 UV-visible spectrophotometer. Fluorescence spectra were recorded on Shimadzu RF-5301 PC spectrofluorometer with the excitation and emission slit widths at 3.0 and 5.0 nm respectively. Cell imaging was performed with a Nikon Eclipse TE300 inverted microscope. TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200-300), both of which were obtained from the Qingdao Ocean Chemicals.

2. Preparation of the test solution

A stock solution of probe **H_2S-CR** (200 μM) was prepared in CH_3CN. The test solution of the probe (10 μM) in 30 mM potassium phosphate buffer (pH 7.4, containing 10% CH_3CN as a co-solvent) was prepared by placing 0.15 mL of the probe stock solution, 0.15 mL of CH_3CN, and 2.7 mL of 33.4 mM sodium phosphate buffer (pH=7.4). The solutions of various testing species were prepared from Na_2CO_3, $MgCl_2$, $CaCl_2$, $ZnCl_2$, $CuCl_2$, CuCl, KI, $FeCl_3.6H_2O$, $FeCl_2.4H_2O$, NaF, CH_3COONa, NaN_3, tetrabutylammonium cyanide, $NaNO_2$, $NaNO_3$, Na_2SO_4, $NaHCO_3$, $Na_2S_2O_3.5H_2O$, ascorbic acid, $NaHSO_3$, Na_2SO_3, H_2O_2, NaClO, GSH, Cys, Hcy, Lys, Ser, Glu, Pro, Phe, Arg, Tyr, Asn, respectively. Nitric oxide (NO) was generated in-situ before use by dissolving sodium nitroferricyanide (III) dihydrate in deionizer water at room temperature. ROO· was generated from ammonium peroxydisulfate. $\cdot O^{2-}$ was generated by dissolving of KO_2 in DMSO. Hydroxyl radicals (·OH) were

generated by reaction of Fe^{2+} with H_2O_2. Singlet oxygen ($^1O_2$) was generated from ClO^- and H_2O_2. The $ONOO^-$ source was the donor 3-morpholinosydnonimine hydrochloride (SIN-1). The resulting solution was shaken well and incubated for 20 min at room temperature before recording the spectra.

3. Spectral overlap between the energy donor and acceptor

The selection of **coumarin** and **rhodamine/fluorescein** as the fluorophores was based on the consideration that the emission of **coumarin** has a strong overlap with the absorption of **rhodamine/fluorescein**. Thus, they are suitable as a **FRET dyad** in which **coumarin** and **rhodamine/fluorescein** acts as the energy donor and acceptor, respectively.

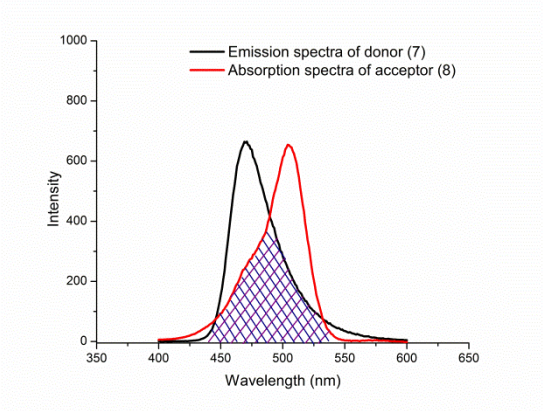
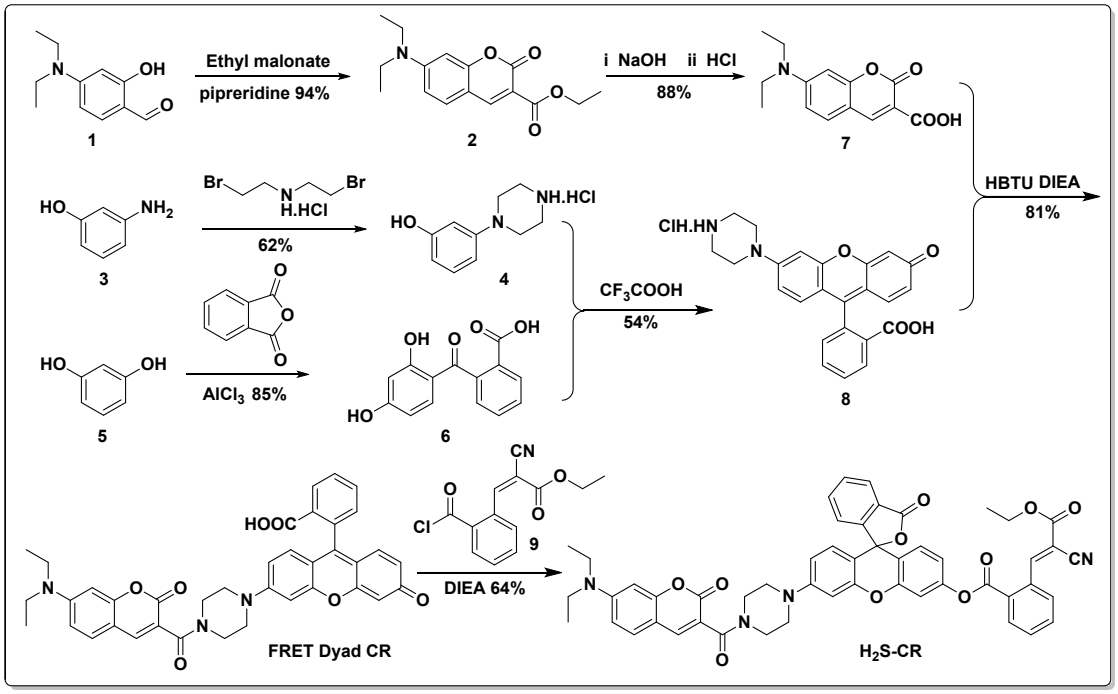


Fig. S1 Spectral overlaps between the energy donor **coumarin** (10 μM) emission (black line) and acceptor ring-opened **rhodamine/fluorescein** (10 μM) absorption (red line).

4. Synthesis and characterization of compounds



Scheme S1 The synthesis of the probe **H_2S-CR**.

4.1 Ethyl 7-(diethylamino)-2-oxo-2*H*-chromene-3-carboxylate (2)

To a solution of 4-diethylaminosalicyl aldehyde (955.0 mg, 5.0 mmol) in 3.3 mL of ethanol was added diethyl malonate (1 mL, 6.5 mmol) and piperidine (0.4 mL) at room temperature. The mixture was heated to reflux, and stirred for 2 h. The solvent was evaporated in vacuo. The crude product was purified by silica gel chromatography to provide the final product as yellow liquid (1358.0 mg, 94.0%). ^{1}H NMR (500 MHz, $CDCl_3$, TMS): δ 8.46 (s, 1H), 7.36 (d, 1H, J = 9.0 Hz), 6.60 (d, 1H, J = 8.5 Hz), 6.46 (s, 1H), 3.91 (s, 3H), 3.44 (q, 4H, J = 7.0 Hz), 1.23 (t, 6H, J = 7.0 Hz); ^{13}C NMR (300 MHz, $CDCl_3$, TMS): δ 165.02, 158.54, 158.33, 152.96, 149.66, 131.11, 109.58, 108.54, 107.72, 96.70, 52.33, 45.12, 12.41.

4.2 7-(Diethylamino)-2-oxo-2*H*-chromene-3-carboxylic acid (7)

To a solution of **2** (289.0 mg, 1.0 mmol) in 10 mL of ethanol was added 10 mL of 0.5 M aqueous NaOH solution. The mixture was stirred at room temperature for 12 h. The solvent was evaporated in vacuo. H_2O (5 mL) was added to dissolve the residue, and then pH was adjusted to acidic by 1.0 M aqueous HCl solution. The

precipitate was filtered off to afford the final product as orange red solid (230.0 mg, 88.0%). ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 8.65 (s, 1H), 7.45 (d, 1H, J = 9.0 Hz), 6.70 (dd, 1H, J_1 = 8.7 Hz, J_2 = 1.8 Hz), 6.52 (d, 1H, J = 1.5 Hz), 3.49 (q, 4H, J = 7.1 Hz), 1.26 (t, 6H, J = 7.1 Hz); ^{13}C NMR (300 MHz, $CDCl_3$, TMS): δ 165.39, 164.30, 157.88, 153.65, 150.05, 131.80, 110.82, 108.37, 105.28, 96.65, 45.22, 12.25.

4.3 3-(Piperazin-1-yl)phenol (4)

In an atmosphere of dry N_2, a mixture of 3-aminophenol (327.0 mg, 3.0 mmol), *bis*(2-chloroethyl)amine hydrochloride (795.0 mg, 3.0 mmol), and diethylene glycol monomethyl ether (0.8 mL) was heated at 150 °C for 12 h. After being cooled to room temperature, the mixture was dissolved in MeOH (4.0 mL) followed by addition of Et_2O (150.0 mL). The precipitate was filtered off and washed with Et_2O to provide the final product as a white solid (1017.0 mg, 62.0%). ^{1}H NMR (300 MHz, MeOD, TMS): δ 7.03 (t, 1H, J = 8.1 Hz), 6.45 (dd, 1H, J_1 = 8.4 Hz, J_2 = 1.8 Hz), 6.39 (t, 1H, J = 2.1 Hz), 6.30 (dd, 1H, J_1 = 8.4 Hz, J_2 = 2.1 Hz), 3.07 (t, 4H, J = 2.7 Hz), 2.95 (t, 4H, J = 2.7 Hz); ^{13}C NMR (300 MHz, MeOD, TMS): δ 159.22, 154.63, 130.75, 109.21, 108.40, 104.69, 51.30, 46.48.

4.4 2-(2,4-dihydroxybenzoyl)benzoic acid (6)

$AlCl_3$ (10000.0 mg, 75.0 mmol) was added to a solution of phthalic anhydride (5000.0 mg, 33.8 mmol) and resorcinol (3600.0 mg, 32.5 mmol) in 56.0 mL of nitrobenzene and purged with N_2. After stirring overnight, the solution was poured into a vigorously stirred biphasic solution of 187.5 mL hexanes and 250.0 mL of 0.5 M aqueous HCl. The solution was allowed to stir for 2 h and the light tan precipitate that formed was filtered and washed with 50.0 mL of aqueous 0.1 M HCl and 75.0 mL of hexanes. The crude product was crystallized from EtOAc/PE (7100.0 mg, 85.0%). ^{1}H NMR (600 MHz, CD_3OD, TMS): δ 8.12 (d, 1H, J = 6.7 Hz), 7.65-7.80 (m, 2H), 7.45 (d, 1H, J = 7.5 Hz), 7.0 (d, 1H, J = 9.0 Hz), 6.39 (d, 1H, J = 2.3 Hz) 6.33 (dd, 1H, J_1 = 9.0 Hz, J_2 = 2.3 Hz); ^{13}C NMR (600 MHz,

CD_3OD, TMS): δ 200. 5, 166. 7, 165. 0, 164. 4, 134. 7, 134. 0, 132. 3, 129. 9, 129. 7, 129. 4, 127. 4, 113. 2, 108. 3, 102. 5.

4.5 2-(3-oxo-6-(piperazin-1-yl)-3*H*-xanthen-9-yl)benzoic acid hydrochloride (8)

To compound **6** (1740.0 mg, 6.7 mmol) in 10 mL of TFA was added compound **4** (1000.0 mg, 5.6 mmol), then heated to reflux and stirred for 36 h. The solvent was removed by rotavapor. The crude product was further purified by column chromatograph, and obtained as a red solid (1320.0 mg, 54.0%). ^{1}H NMR (600 MHz, CD_3OD, TMS): δ 8.23 (d, 1H, J = 12.6 Hz), 7.88 (m, 2H), 7.35 (d, 1H, J = 10.8 Hz), 7.19-6.99 (m, 5H), 6.85 (dd, 1H, J_1 = 13.2 Hz, J_2 = 3 Hz) 3.85 (t, 4H, J = 7.8 Hz), 3.43 (t, 4H, J = 7.8 Hz); ^{13}C NMR (600 MHz, CD_3OD, TMS): δ 169.65, 161.96, 161.51, 153.65, 153.31, 153.19, 152.75, 135.95, 130.63, 129.87, 1 29.58, 127.69, 125.32, 124.81, 119.77, 115.88, 113.36, 113.09, 111.26, 111.13, 103.35, 103.21, 84.34, 63.92, 54.86, 45.97, 43.85; HRMS-EI ($C_{24}H_{21}ClN_2O_4$) m/z: calculated for $[M]^+$: 436.1190, found $[M\text{-}Cl]^+$: 401.1477.

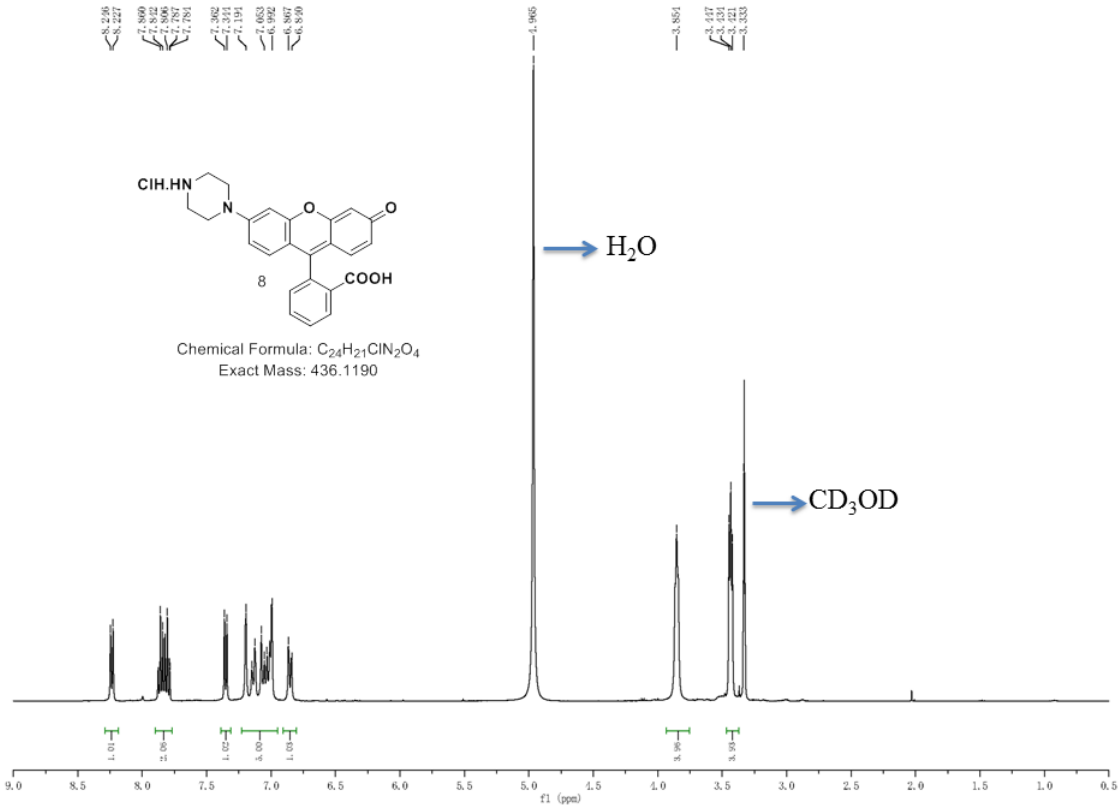


Fig. S2-1 ^{1}H NMR spectrum of **8**.

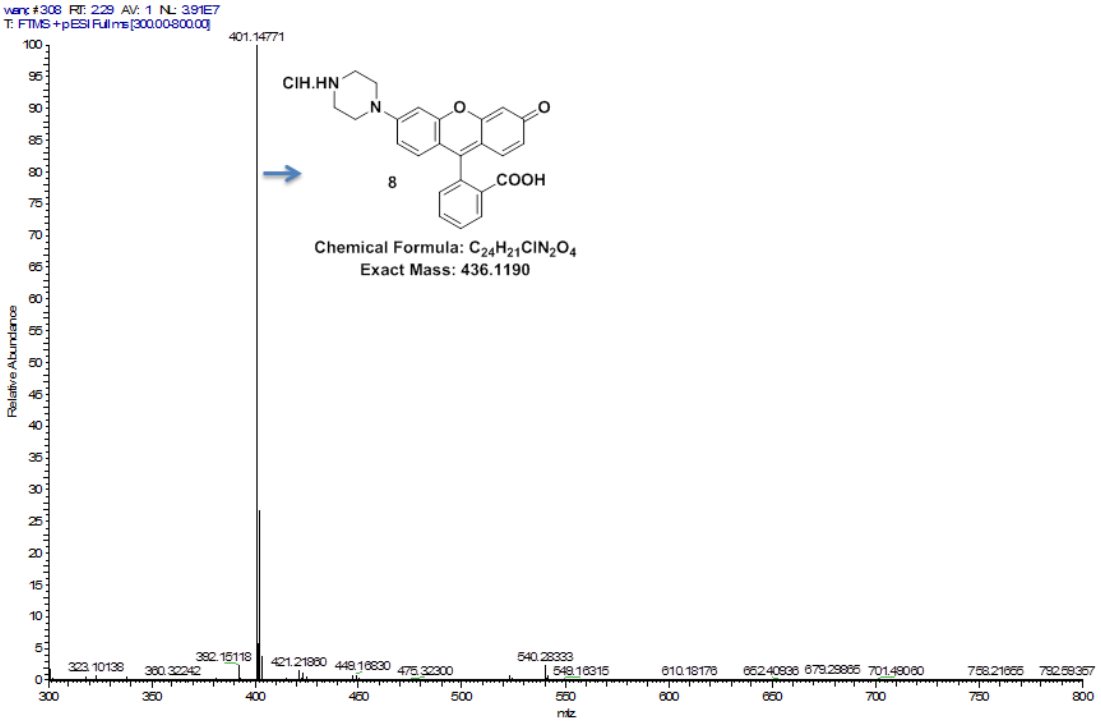


Fig. S2-2 HRMS spectrum of **8**.

4.6 2-(6-(4-(7-(diethylamino)-2-oxo-2*H*-chromene-3-carbonyl)piperazin-1-yl)-3-oxo-3*H*-xanthen-9-yl)benzoic acid (FRET Dyad CR)

To a solution of compound **7** (261.0 mg, 1.0 mmol) in 30 mL of DCM/DMF (v/v, 5:1) was added compound **8** (436.0 mg, 1.0 mmol), DMAP (244.0 mg, 2.0 mmol) and DCC (247.0 mg, 1.2 mmol), then stirred at room temperature for 24 h. The solvent was removed by rotavapor, and then the product was purified through column chromatograph and obtains as a red solid (521.0 mg, 81.0%). ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 7.97 (dd, 1H, J_1 = 6.6 Hz, J_2 =1.2 Hz), 7.88(s, 1H), 7.65-7.54 (m, 2H), 7.27 (d, 1H, J = 8.7 Hz), 7.11 (d, 1H, J = 6.9 Hz), 6.74 (d, 1H, J = 1.8 Hz), 6.65-6.52 (m, 6H), 6.45 (d, 1H, 2.1Hz), 3.87 (s, 2H), 3.55 (s, 2H), 3.41 (q, 4H, J = 7.2 Hz), 3.29 (s, 4H), 1.2 (t, 6H, J = 7.2 Hz); ^{13}C NMR (300 MHz, $CDCl_3$, TMS): δ 169.95, 165.44, 159.40, 157.36, 152.80, 152.70, 152.54, 151.95, 145.81, 134.80, 130.10, 129.59, 129.24, 128.91, 127.30, 125.10, 124.26, 115.21, 112.87, 112.26, 110.75, 109.97, 109.61, 107.75, 103.16, 102.39, 96.88, 48.47, 48.00, 46.94, 44.99, 42.14,

12.42; MS-ESI ($C_{38}H_{33}N_3O_7$) m/z: calculated for $[M]^+$: 643.2391, found $[M+H]^+$: 644.4.

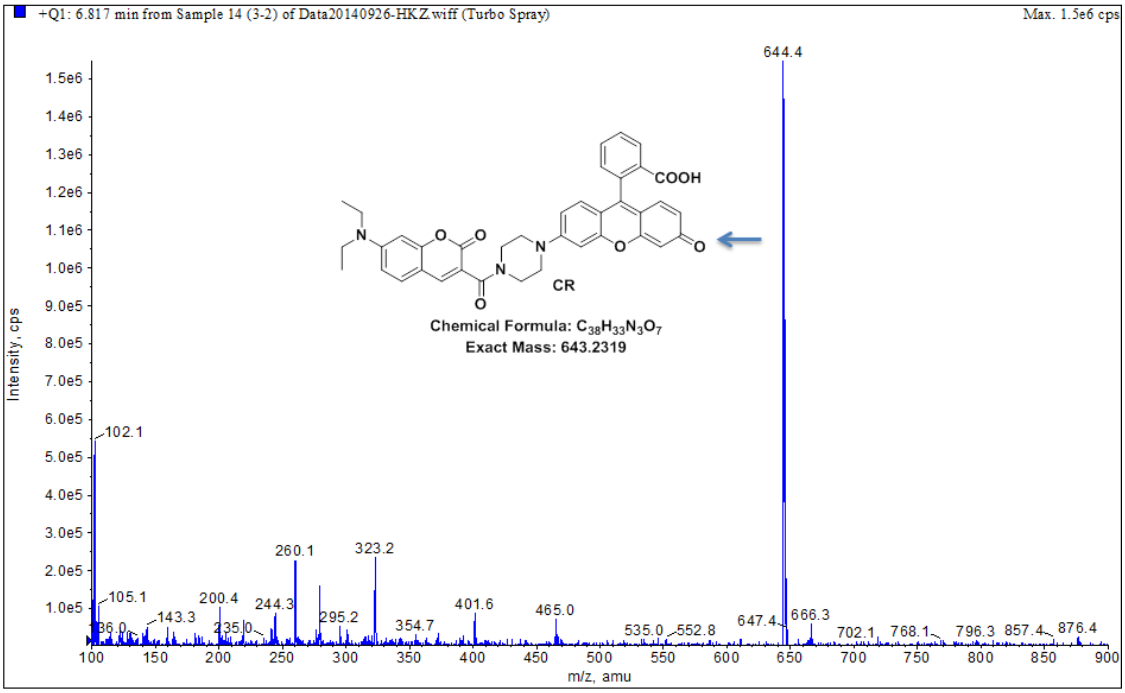


Fig. S3 MS spectrum of **FRET Dyad CR**.

4.7 2-(2-cyano-3-ethoxy-3-oxoprop-1-en-1-yl)benzoic acid

2-Formylbenzoic acid (1500.0 mg, 10.0 mmol) and ethyl cyanoacetate (1130.0 mg, 10.0 mmol) were dissolved in methanol (2.0 mL) and cooled to 0 °C. Sodium hydroxide (400.0 mg, 10.0 mmol) in methanol (5.0 mL) was added and after a few minutes the precipitated sodium salt was filtered off and washed with diethyl ether. After acidification of an aqueous solution of this salt, the product was obtained as a red solid (2210.0 mg, 90.0%). ^{1}H NMR (600 MHz, $CDCl_3$, TMS): δ 7.57-8.01 (m, 4H), 6.02-6.03 (d, 1H, J = 3.6 Hz), 4.29-4.42 (q, 2H, J = 11 Hz), 4.16-4.17 (d, 1H J = 3.6 Hz), 1.29-1.36 (t, 3H, J = 11 Hz); ^{13}C NMR (600 MHz, $CDCl_3$, TMS): δ 187.0, 164.4, 161.1, 156.9, 133.1, 132.2, 131.2, 130.5, 128.7, 114.0, 105.3, 53.4, 14.1.

4.8 3'-(4-(7-(diethylamino)-2-oxo-2*H*-chromene-3-carbonyl)piperazin-1-yl)-3-oxo-3*H*-spiro[isobenzofuran-1,9'-xanthen]-6'-yl (H_2S-CR)

To a suspension of 2-(2-cyano-3-ethoxy-3-oxoprop-1-en-1-yl)benzoic acid

(1225.0 mg, 5.0 mmol) in toluene (50.0 mL) was added $SOCl_2$ (2975.0 mg, 25.0 mmol). The reaction mixture was heated to reflux and stirred for 4 h. The volatiles were evaporated under reduced pressure and subsequently dried with vacuum pumping. The residue was dissolved in dry dichloromethane (10.0 mL). The solution was slowly added into the dichloromethane solution (50.0 mL) containing **FRET Dyad CR** (643.0 mg, 1.0 mmol) and triethylamine (1010.0 mg 10.0 mmol) and kept stirring at 0 ^{o}C for 30 min. Then the mixture was warmed to room temperature and stirred overnight. The solvent was removed in vacuo to obtain a crude mixture. Finally, **H_2S-CR** was isolated by silica chromatography as a pale yellow solid (547.8 mg, 64.0%). ^{1}H NMR (600 MHz, $CDCl_3$, TMS): δ 8.93 (s, 1H), 8.29 (dd, 1H, J_1 = 7.8 Hz, J_2 = 1.2 Hz), 7.97 (d, 1H, J = 7.8 Hz), 7.85 (s, 1H), 7.81 (d, 1H, J = 7.8 Hz), 7.71 (t, 1H, J = 7.2 Hz), 7.62 (t, 2H, J = 7.8 Hz), 7.57 (t, 1H, J = 7.8 Hz), 7.26 (d, 1H, J = 9.0 Hz), 7.13 (d, 1H, J = 2.4 Hz), 7.12 (d, 1H, J = 7.8 Hz), 6.84-6.79 (m, 3H), 6.65 (d, 2H, J = 9.0 Hz), 6.57 (d, 1H, J = 8.4 Hz), 6.44 (s, 1H), 4.30 (q, 2H, J = 7.2 Hz), 3.91 (s, 2H), 3.57 (s, 2H), 3.37 (q, 4H, J = 7.2 Hz), 3.32 (s, 4H), 1.30 (t, 3H, J = 7.2 Hz), 1.16 (t, 6H, J = 7.2 Hz); ^{13}C NMR (150 MHz, $CDCl_3$, TMS): δ 169.39, 165.14, 163.89, 161.55, 159.22, 157.37, 157.03, 156.59, 152.96, 152.16, 152.11, 151.78, 151.63, 145.62, 135.18, 134.73, 134.04, 131.81, 131.46, 129.99, 129.93, 129.90, 129.31, 128.89, 128.23, 126.53, 125.13, 124.06, 117.42, 117.23, 115.80, 114.54, 112.68, 110.47, 109.54, 107.85, 107.32, 102.76, 97.02, 82.58, 62.88, 49.31, 45.05, 33.76, 25.55, 24.89, 14.14, 12.41; HRMS-EI ($C_{51}H_{42}N_4O_{10}$) m/z: calculated for $[M]^+$: 870.9000, found $[M+H]^+$: 871.3015.

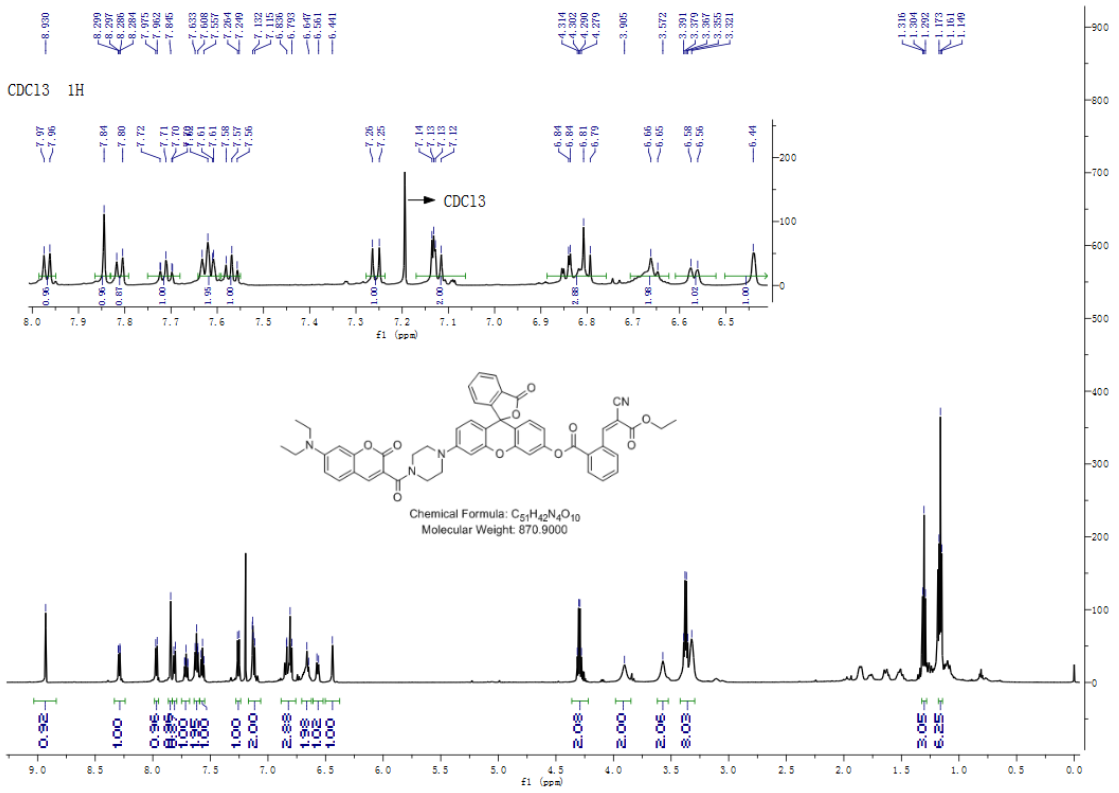


Fig. S4-1 ^{1}H NMR spectrum of **H_2S-CR**.

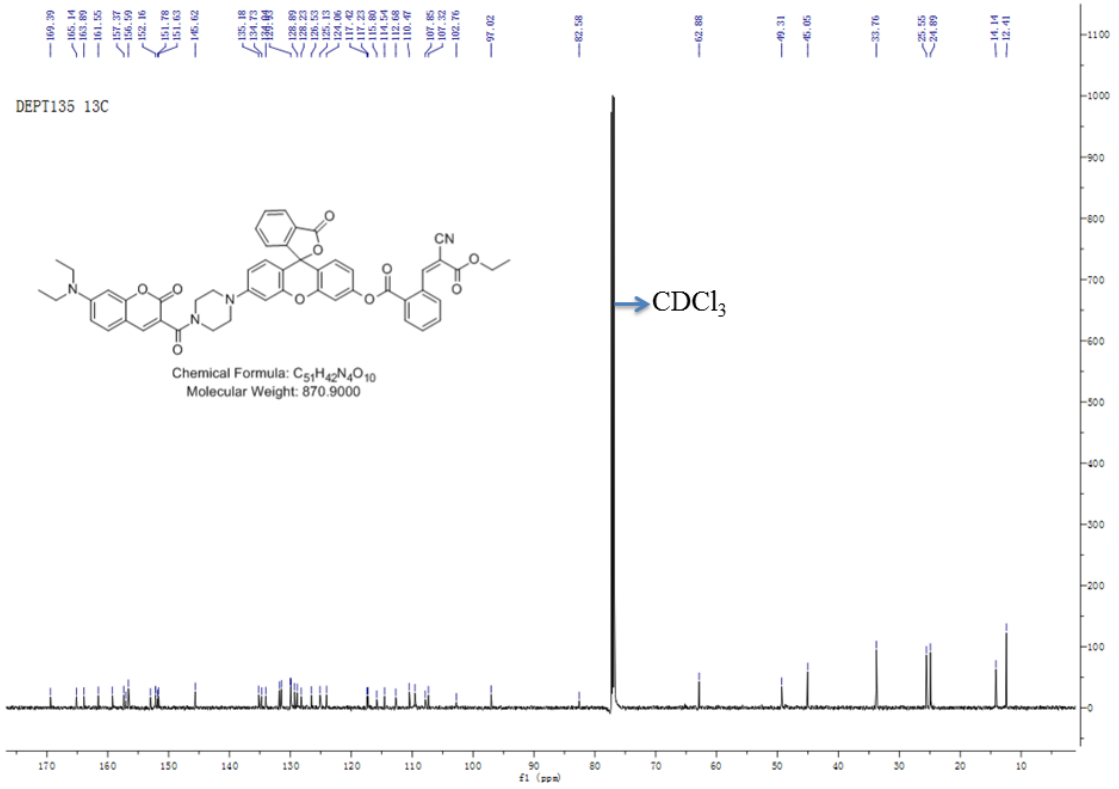


Fig. S4-2 ^{13}C NMR spectrum of **H_2S-CR**.

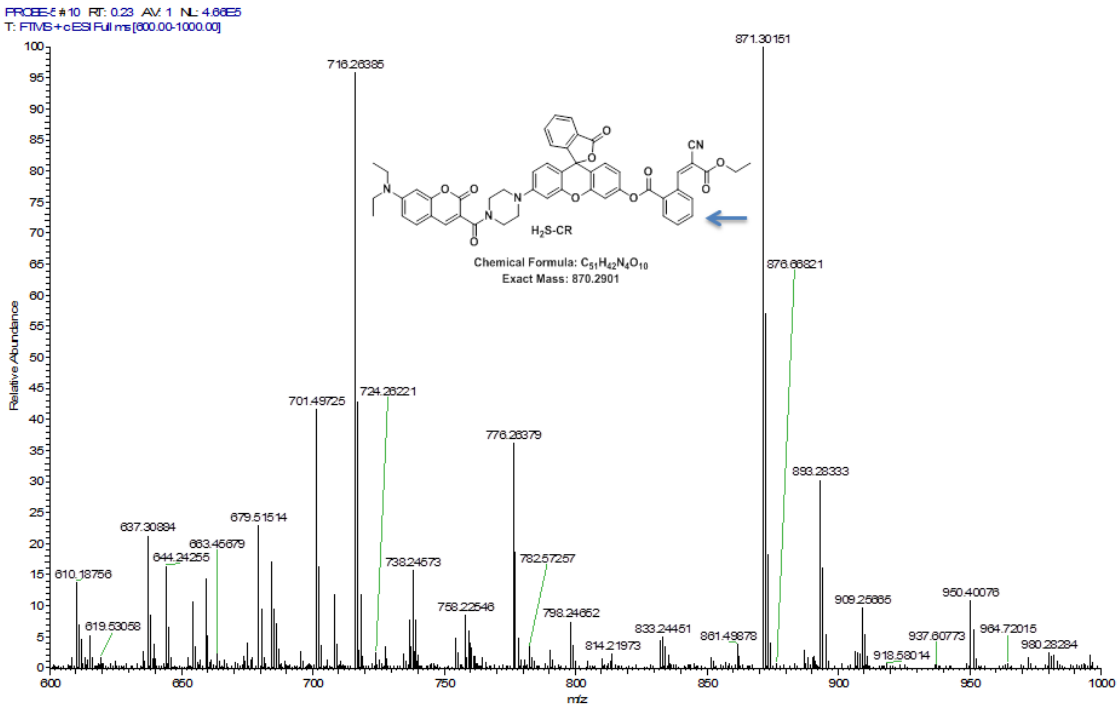


Fig. 4-3 HRMS spectrum of **H_2S-CR**.

4 Effect of buffer solution

Because the type of buffer solution plays an important role in the detection, we investigated the effect of two types of buffer solutions: HEPES and PBS. The results are obtained and shown in Fig. S5. The best condition for the fluorescence response was in the case of 30 mM PBS.

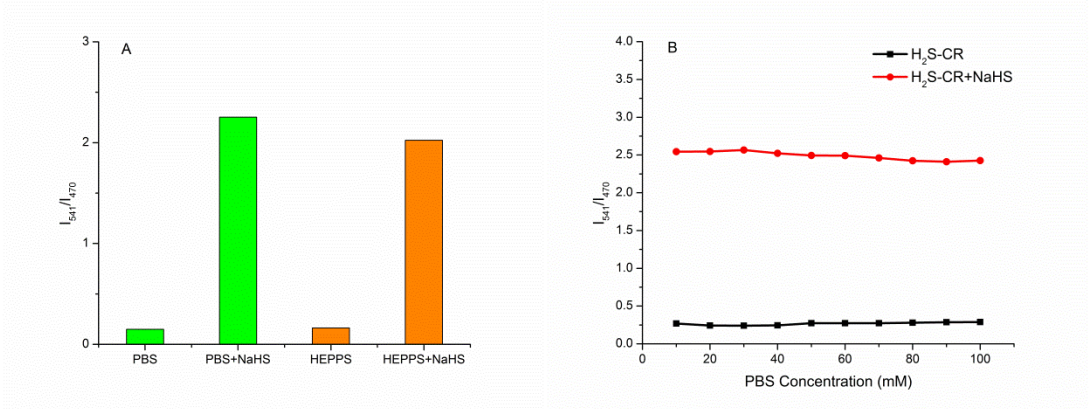


Fig. S5 Effect of buffer solution on the reaction system of **H_2S-CR** (10 μM) with the addition of NaHS (80 μM). (A) Effect of HEPES (30 mM) and PBS (30 mM). (B) Effect of PBS concentration. Conditions: excitation wavelength is 414 nm,

acetonitrile-PBS buffer solution (pH 7.4, 1:9 v/v) at 25 °C for 30 min.

6. The intramolecular energy transfer efficiency

The intramolecular energy transfer efficiency (*E*) was determined according to a published method using the following equation.[1-3] Conditions: H_2S-CR (10 μM) in the presence of 80 μM NaHS, excitation wavelength is 414 nm, acetonitrile–PBS buffer solution (30 mM, pH 7.4, 1:9 v/v) at 25 °C.

$$E = 1 - F_{DA} / F_D$$

Where, F_{DA} and F_D denote the donor fluorescence intensity with and without an acceptor, respectively. The intramolecular energy transfer efficiency of **H_2S-CR**: *E*=70.7%.

7. Detection limit

Fluorescence titrations were employed to determine detection limit of probe **H_2S-CR**. According to reported method by measuring of emission intensity ratio (I_{541}/I_{470}) of probe **H_2S-CR** in absence of NaHS by seven times, standard deviation of blank measurements were recorded.[4-5] A linear relationship between the fluorescence intensity ratio (I_{541}/I_{470}) and NaHS concentration was acquired. The detection limit of probe **H_2S-CR** was determined according to a published method using the following equation.

$$\text{Detection limit} = 3s/n$$

Where *s* is the standard deviation of seven blank measures, *n* is the slope of emission intensity ratio (I_{541}/I_{470}) versus NaHS concentration graph. This calculation gives the detection limit of probe **H_2S-CR** as 19 nM.

8. Quantum Yields

The quantum yield of the probe **H_2S-CR** (10 μM) before and after interaction with NaSH (80 μM) were calculated in acetonitrile-PBS buffer solution (30 mM, pH 7.4, 1:9 v/v) with quinine sulfate (Φ = 0.58, in 0.1 M H_2SO_4) as a reference. The quantum yields were calculated using

$$\Phi u = [(A_s F A_u \eta^2)/ (A_u F A_s \eta_0^2)]\Phi s.^{6\text{-}8}$$

Where A_s and A_u are the absorbance of the reference and sample solution at the reference excitation wavelength, FA_s and FA_u are the corresponding integrated fluorescence intensity, and η and η_0 are the solvent refractive indexes of sample and reference, respectively. Quantum yield of **H_2S-CR**: Φ = 0.132; Quantum yield of **H_2S-CR** interaction with NaSH: Φ = 0.100.

9. Monitoring the response of H_2S-CR towards NaHS

We testified the reaction mechanism shown in Fig. S6 by HPLC-MS. The probe **H_2S-CR** was dissolved in acetonitrile and then diluted to 10 μM with 30 mM PBS buffer of pH 7.4. Then 80 μM NaHS was injected into the probe solution. 20 min later, the reaction product was analyzed by HPLC-MS. The mass spectra of **H_2S-CR** exhibited characteristic peaks at m/z 871.5 corresponding to [**H_2S-CR**+H] $^+$, and the mass spectra of the reaction solution exhibited characteristic peaks at m/z 644.4 corresponding to [**CR**+H]$^+$, and at m/z 279.1 corresponding to [Cyclization product+H]$^+$, respectively. The characteristic peak of **qH_2S-CR** was not found because the nucleophilic addition and nucleophilic substitution of H_2S towards **H_2S-CR** was so fast that no **qH_2S-CR** was remained. The results agreed well with the conclusions drawn from scheme 1.

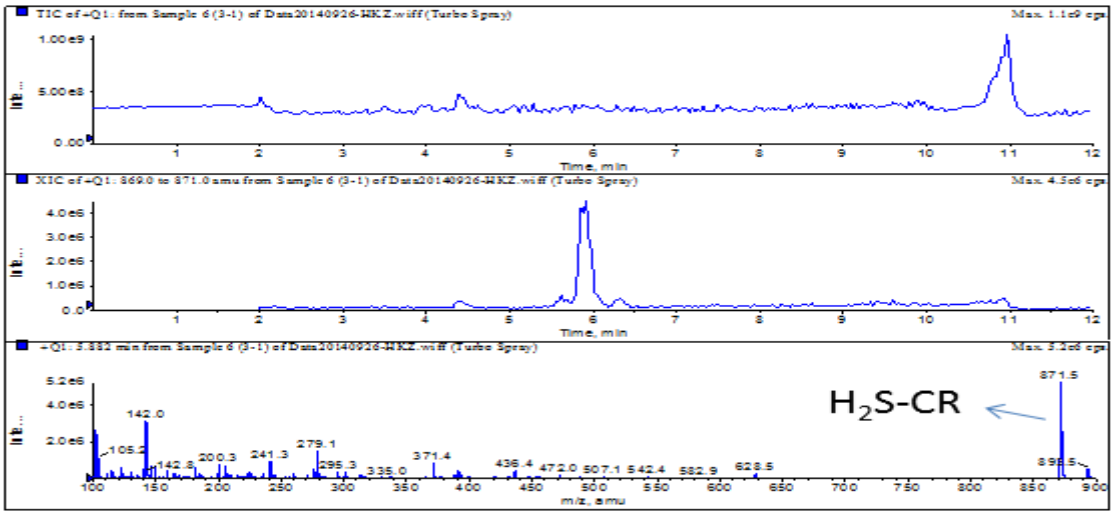


Fig. S6-1 HPLC-MS spectrum of **H_2S-CR**.

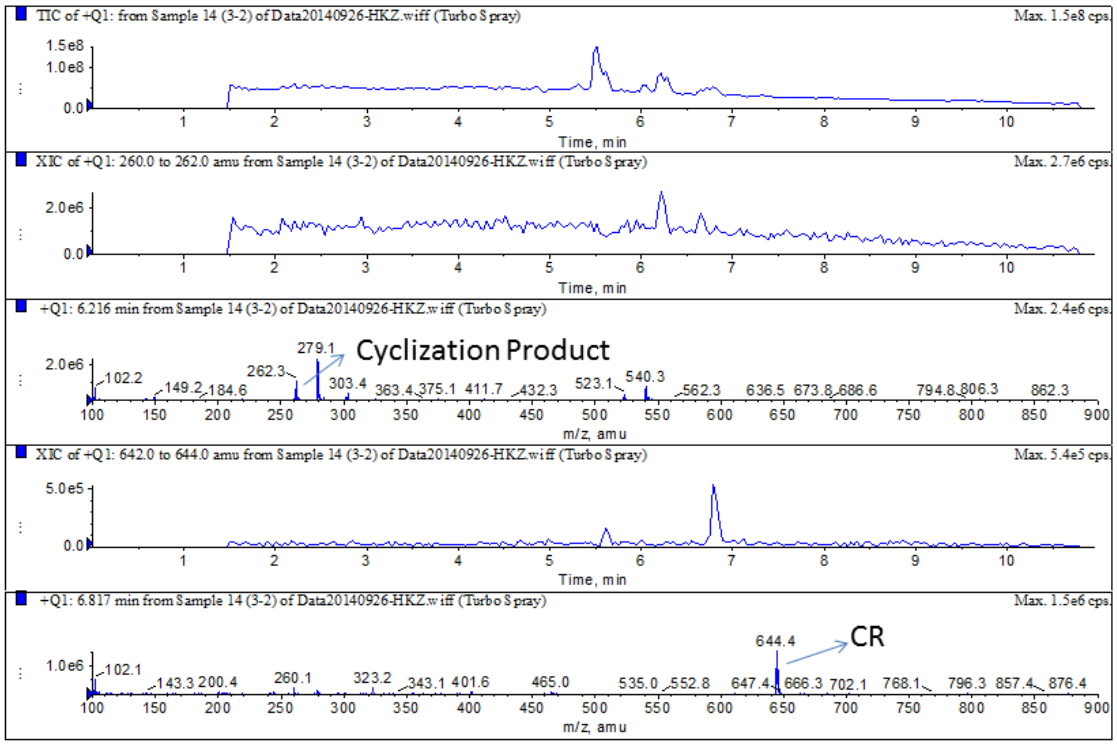


Fig. S6-2 Monitoring the response of **H_2S-CR** towards NaHS via HPLC-MS.

10. Selectivity experiments

High selectivity toward H_2S in the presence of other competitive species is a very important feature to evaluate the performance of the fluorescent probe **H_2S-CR**. Therefore, the competition experiments were also conducted when CN^-/biothiols/(reactive sulfur species) and NaHS co-existed in the system. To our delighted, when NaHS and these species co-existed, almost the same I_{541}/I_{470} enhancement as that only treated by NaHS was observed.

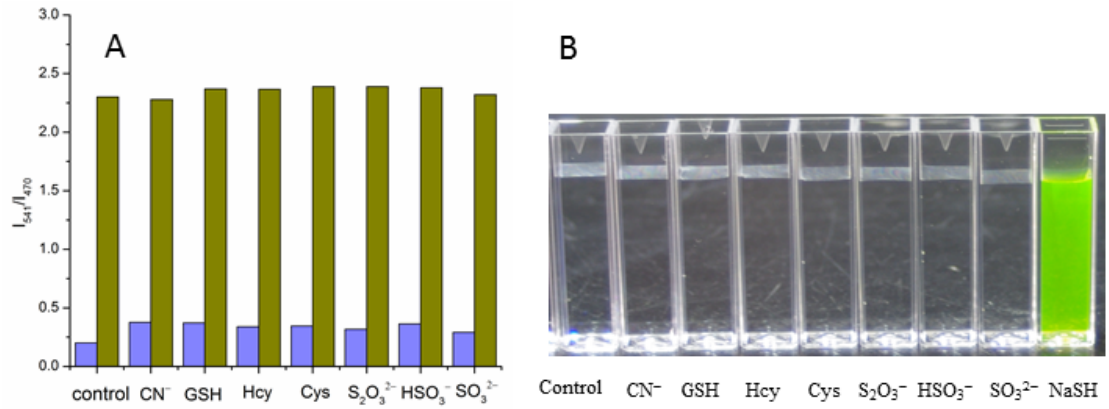


Fig. S7 (A) Fluorescence ratio (I_{541}/I_{470}) of **H_2S-CR** (10 μM) to NaHS (80 μM), CN^-

(0.8 mM), Cys (0.8 mM), Hcy (0.8 mM), GSH (0.8 mM), SO_3^{2-} (0.8 mM), HSO_3^- (0.8 mM) , and $S_2O_3^{2-}$ (0.8 mM) in acetonitrile-PBS buffer solution (30 mM, pH 7.4, 1:9 v/v) after 30 min at 25 °C. λex = 414 nm. Black bar: **H_2S-CR** + CN^-/biothiols/ROS; red bar: **H_2S-CR** + CN^-/biothiols/ROS + NaHS. (B) Colorimetric detection of NaHS. **H_2S-CR** (10 μM) to physiological saline, CN^- (0.8 mM), Cys (0.8 mM), Hcy (0.8 mM), GSH (0.8 mM), SO_3^{2-} (0.8 mM), HSO_3^- (0.8 mM) , and $S_2O_3^{2-}$ (0.8 mM), and NaHS (80 μM).

11. Effect of reaction pH

To verify whether the probe is suitable for the physiological detection, we evaluated the effect of pH on the fluorescence of the probe **H_2S-CR**. As shown in Fig. S8, in the absence of NaHS, almost no change in fluorescence ratio (I_{541}/I_{470}) was observed in the free probe **H_2S-CR** over a wide pH range of 2-11 indicating excellent pH stability. Furthermore, upon treatment with NaHS, the maximal fluorescence ratio (I_{541}/I_{470}) displayed constant in the pH range of 6-11. Thus, the observation that **H_2S-CR** had the maximal sensing response at physiological pH, suggested that **H_2S-CR** is promising for biological applications.

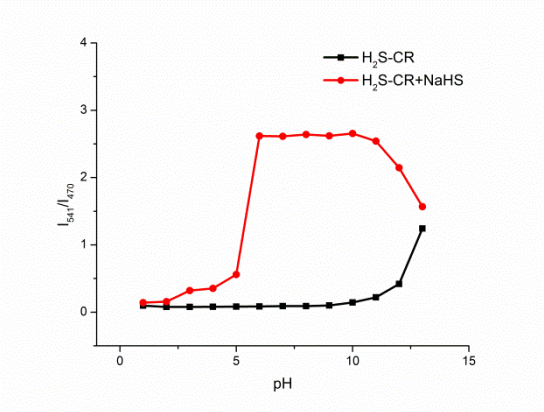


Fig. S8 Fluorescence ratio (I_{541}/I_{470}) of **H_2S-CR** (10 μM) alone (black curve) and **H_2S-CR** (10 μM) treated with NaHS (80 μM) (red curve) in different pH. Conditions: excitation wavelength is 414 nm, acetonitrile-PBS buffer solution (30 mM, 1:9 v/v) at 25 °C for 30min.

12. Cell culture and fluorescence imaging

H9C2 cells were seeded in a 24-well plate in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum in an atmosphere of 5% CO_2 and 95% air at 37 ^oC for 36 h. Immediately before the experiments, the cells were washed with physiological saline. H9C2 cells were then incubated with the probe **H_2S-CR** (10 μM) for 20 min at 37 °C. After washing with physiological saline three times to remove the remaining probes, the cells were then incubated with NaHS (0, 10, 20, 30, 40 and 80 μM) for another 30 min. The fluorescence images were acquired with a Nikon Eclipse TE300 equipped with a CCD camera.

13. References

1. D. Seth, A. Chakraborty, P. Setua, D. Chakrabarty, and N. Sarkar, *J. Phys. Chem. B*., 2005, **109**, 12080-12088.
2. S. L. Gilat, A. Adronov, and J. M. J. Fréchet, *Angew. Chem., Int. Ed*., 1999, **38**, 1422-1427.
3. M. H. Lee, D. T. Quang, H. S. Jung, J. Yoon, C. H. Lee, and J. S. Kim, *J. Org. Chem*., 2007, **72**, 4242-4245.
4. T. Saha, D. Kand, and P. Talukdar, *Org. Biomol. Chem***.**, 2013, **11**, 8166-8170.
5. R. Wang, F. Yu, L. Chen, H. Chen, L. Wang, and W. Zhang, *Chem. Commun.*, 2012, **48**, 11757-11759.
6. T. Ozdemir, F. Sozmen, S. Mamur, T. Tekinay, and E. U. Akkaya, *Chem. Commun*., 2014, **50**, 5455-5457.
7. B. Wang, P. Li, F. Yu, J. Chen, Z. Qu, and K. Han, *Chem. Commun*., 2013, **49**, 5790-5792.
8. J. Zhang, and W. Guo, *Chem. Commun*., 2014, **50**, 4214-4217.