Supporting Information

A FRET-Based NCA Fluorescent Probe for Highly Sensitive and Selective Detection of Hydrogen Sulfide

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Experimental section

Instruments

^{1}H NMR spectra were obtained from Bruker Avance NEO 400 MHz spectrometer and tetramethylsilane (TMS) used as the standard substance. DMSO-d_6 was used as deuterium reagent. The UV–Vis absorption spectra were determined using a Shimadzu UV-2700 Spectrophotometer. Fluorescence data was measured on a Hitachi F-2700 Fluorescence spectrophotometer. The fluorescence absolute quantum yields were determined using an integrating sphere on an Edinburgh Instruments FLS-1000 steady-state/transient fluorescence spectrometer. Cell imaging were performed using a Nikon Ts2-FL inverted fluorescence microscope.

Materials

All chemical and biological reagents were purchased from commercial suppliers and used without further purification. Sodium sulfide (Na_2S) could serve as the source of H_2S. The NCA solution was dissolved in THF to prepare a 1 mM stock solution, and stored in a refrigerator at 2 °C. The Britton Robinson (BR) buffer was composed of 0.04 M H_3PO_4, H_3BO_3, and HOAc, with the pH adjusted using NaOH (0.2 M). The test solution was diluted to 10 μM with BR buffer solution (pH 8). The stock solutions of various cations (Na^+, Ca^{2+}, K^+, Mg^{2+}, Cu^{2+}, Al^{3+}, Zn^{2+}, NH_4^+, Fe^{3+}, Fe^{2+}, Ni^{2+}, Li^+, Ba^{2+}), anions, (SO_3^{2-}, SO_4^{2-}, Br^-, HCO_3^-, Cl^-, PO_4^{3-}, CO_3^{2-}), amino acids (Cys, Hcy, GSH), and reactive oxygen species ($S_2O_3^{2-}$) were prepared in deionized water. Unless otherwise specified, the excitation and emission slit widths are 2.5 nm and 2.5 nm, respectively, and the PMT voltage is 700 V.

Synthesis of compound NB

A solution of mono-t-boc-piperazine (558 mg, 3.0 mmol) and 4-chloro-7-nitrobenzofurazan (NBD-Cl, 498 mg, 2.5 mmol) in CH_2Cl_2 (50 mL) was treated with N,N-diisopropylethylamine (DIPEA, 625 μL). The reaction mixture was stirred at room temperature under argon atmosphere for 4 h. After completion, the solvent was evaporated under reduced pressure to afford a crude residue. Purification by silica gel column chromatography (gradient elution: 1.5% MeOH in CH_2Cl_2) yielded an intermediate as a red solid. Subsequent Boc-deprotection with TFA/CH_2Cl_2 (1:1, v/v, 20 mL) for 4h, followed by solvent removal under vacuum, afforded compound NB as a red solid (913 mg, 83.5%).The product was characterized by ^{1}H NMR (DMSO-d_6) (Fig. S1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.41 (s, 2H), 8.56 (d, J = 9.0 Hz, 1H), 6.77 (d, J = 9.1 Hz, 1H), 4.32 (t, J = 5.2 Hz, 4H).

Synthesis of compound CA

A mixture of 2,4-dihydroxysalicylaldehyde (276 mg, 2.0 mmol) and Meldrum's acid (288 mg, 2.0 mmol) was dissolved in distilled water (10 mL), followed by the addition of K_2CO_3(20 mol%, 55.3 mg). The reaction system was stirred at room temperature for 20 h. After completion of the reaction, the mixture was acidified with cooled dilute HCl and the resulting precipitate was filtered, washed with deionized water, and subsequently recrystallized from ethyl acetate to afford pure CA as a light-yellow solid (371 mg 90%). The product was characterized by ^{1}H NMR (DMSO-d_6) (Fig. S2). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.96 (d, J = 401.3 Hz, 2H), 8.69 (s, 1H), 7.75 (d, J = 8.6 Hz, 1H), 6.85 (dd, J = 8.6, 2.3 Hz, 1H), 6.74 (d, J = 2.2 Hz, 1H).

Synthesis of compound NCA

Under an inert nitrogen atmosphere, compound CA (206 mg, 1.0 mmol) was added to anhydrous thionyl chloride ($SOCl_2$, 10 mL) and the suspension was stirred at room temperature for 4 h. Following acid chloride formation, the excess $SOCl_2$ was removed through vacuum distillation using a high-vacuum oil pump at 40 °C temperature, yielding dark-yellow solid.

The crude product was immediately dissolved in anhydrous CH_2Cl_2 (15 mL) under nitrogen atmosphere. To this solution NB (274 mg, 1.1 mmol) and NEt_3 (200 μL) were sequentially added. The reaction mixture was stirred at 40 °C for 20 h, then purified by silica gel chromatography using a CH_2Cl_2/MeOH gradient to yield the compound NCA (320 mg, 73%) as a red solid. The product identity was confirmed by ^1H NMR analysis (Fig. S3).^1H NMR (400 MHz, DMSO-d_6) δ 8.55 (d, J = 9.0 Hz, 1H), 8.20 (s,1H), 7.65 (d, J = 8.6 Hz, 1H), 6.87 (dd, J = 8.6, 2.2 Hz, 1H), 6.80 (d, J = 2.2 Hz, 1H), 6.67 (d, J = 9.2 Hz, 1H), 4.23 (d, J = 50.3 Hz, 4H), 3.82 (d, J = 58.4 Hz, 4H).

Determination of Absolute Fluorescence Quantum Yield

The absolute fluorescence quantum yields (Φ) of NCA before and after reaction with H_2S were determined using an integrating sphere coupled to an Edinburgh Instruments FLS-1000 steady-state/transient fluorescence spectrometer. Samples were prepared at 10 μM in Britton–Robinson buffer (pH 8.0). Prior to measurement, the integrating sphere was calibrated according to the manufacturer's standard protocol.

For each sample, both the excitation scattering and fluorescence emission signals collected within the sphere were recorded under identical excitation conditions. The quantum yields were calculated using the integral method implemented in the FLS-1000 software suite. All measurements were performed at room temperature.

Determination of the detection limit

The detection limit was calculated based on the fluorescence titration. The fluorescence emission spectra of NCA were measured by ten times and the standard deviation of blank measurement was obtained. To gain the slope, the fluorescence intensity was plotted as the increasing concentrations of Na_2S. The detection limit was calculated with the following equation:

Limit of Detection (LOD) = $3\delta/K$

where δ is the standard deviation of the control sample and K is the slope of the linearity of the equation.

Figures and tables

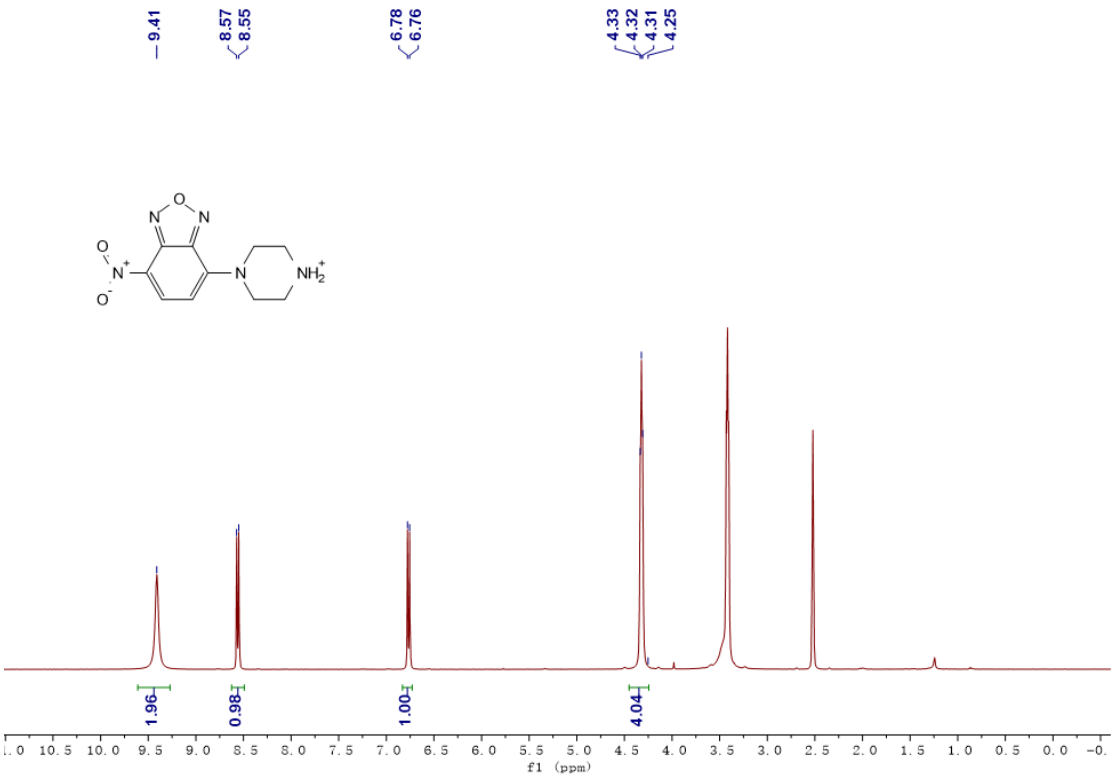

Fig. S1 ^{1}H NMR spectrum of NB (400 MHz, DMSO-d_6).

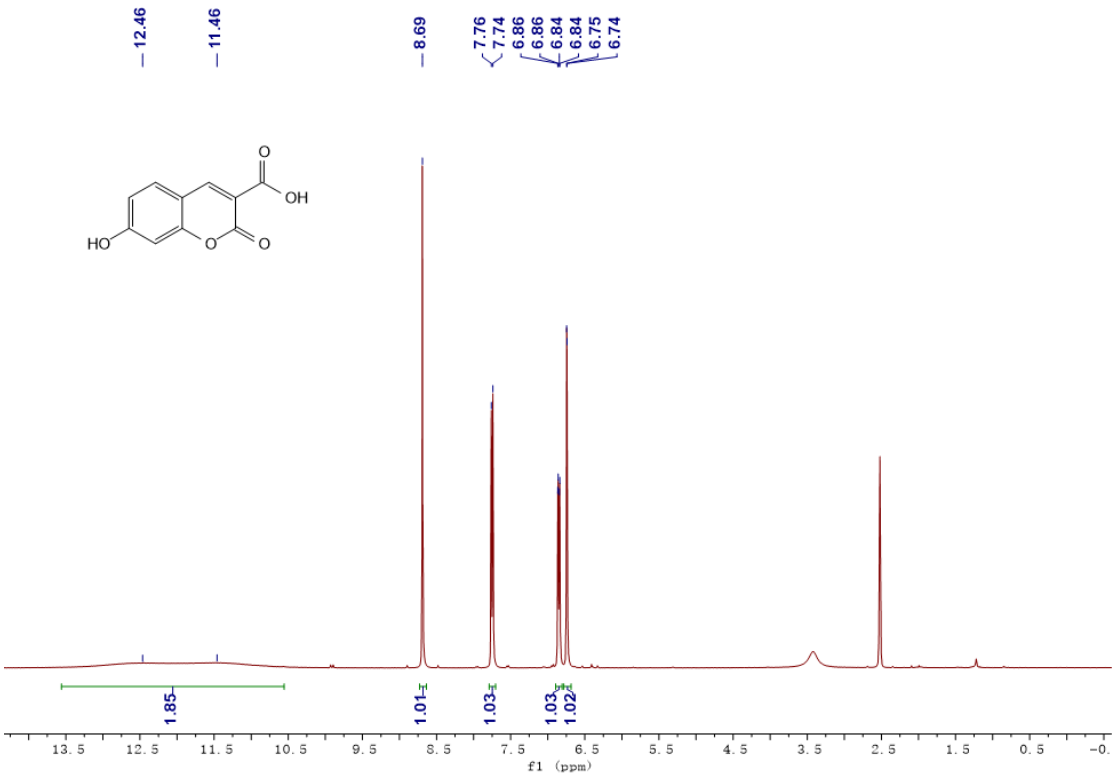

Fig. S2 ^{1}H NMR spectrum of CA (400 MHz, DMSO-d_6).

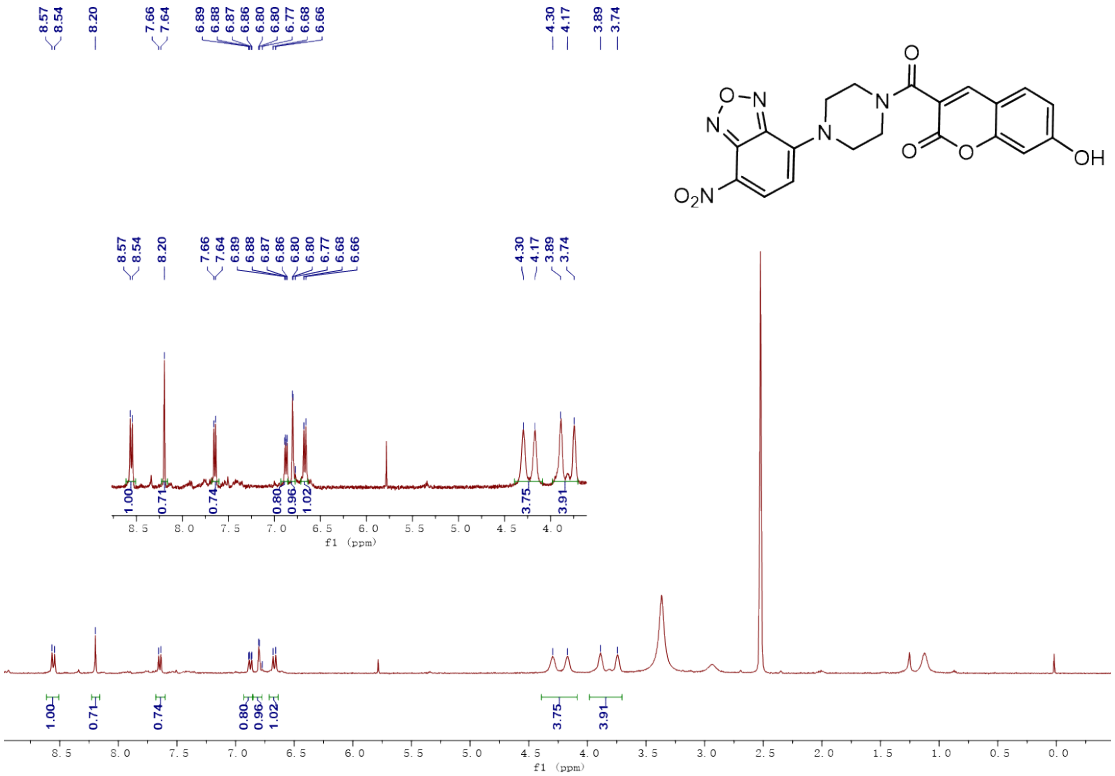

Fig. S3 ^{1}H NMR spectrum of NCA (400 MHz, DMSO-d_6).

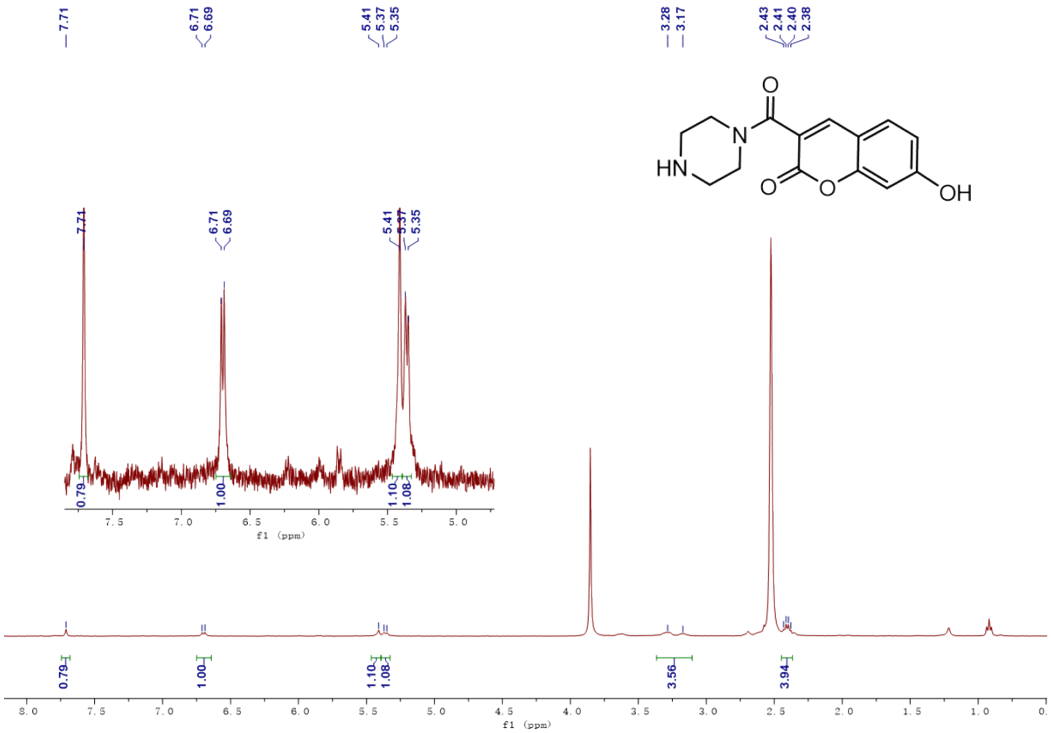

Fig. S4 ^{1}H NMR spectrum of NCA after reaction with Na_2S (400 MHz, DMSO-d_6).

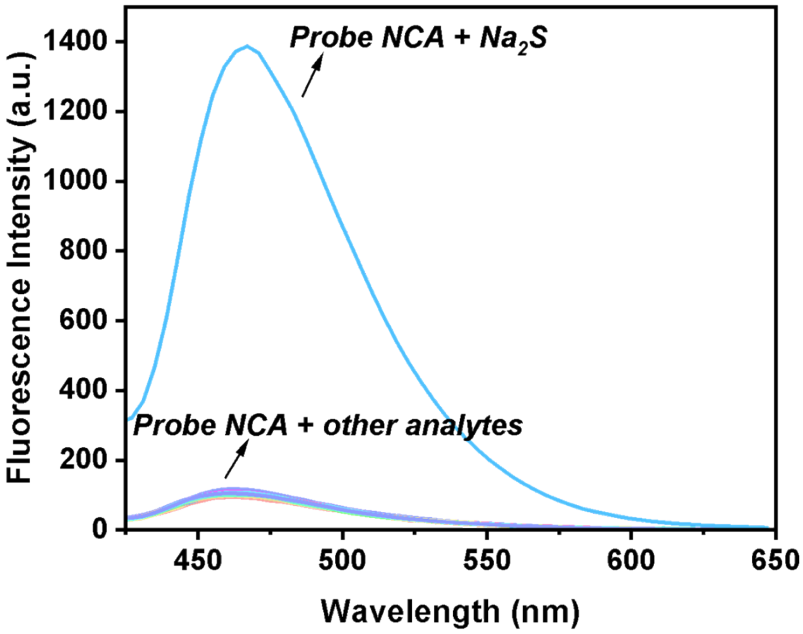

Fig. S5. Fluorescence spectral changes of NCA (10.0 μM) upon the addition of different analytes (250 μM).

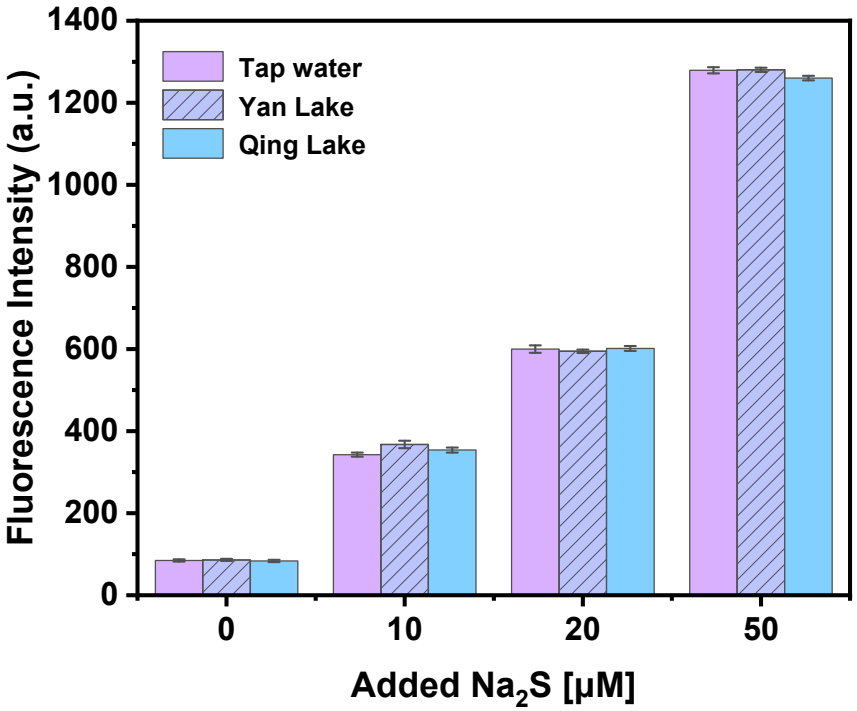

Fig. S6 The fluorescence intensity of NCA (10 μM) in Tap water, Yan Lake, and Qing Lake with the addition of Na_2S (10, 20, 50 μM), λex = 403 nm.

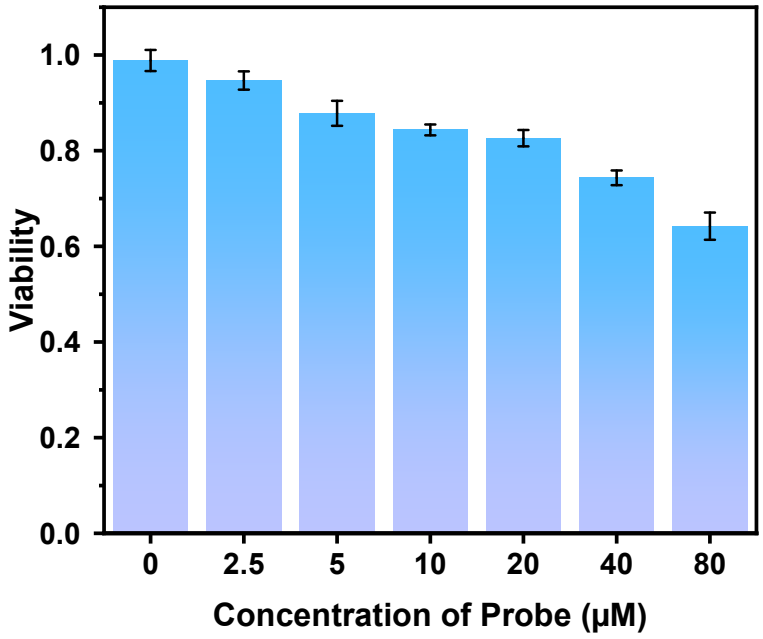

Fig. S7 Cell viability of A549 cells after incubation with different concentrations of NCA (0-80 μM).

Table S1 The comparison between NCA and previous reports.

Probe	LOD (μM)	Response Time	Linear range (μM)	Application	Reference
TPABF-HS	0.42	90 min	0-1000	cells; caenorhabditis elegans	SI[1]
BTDA-DNB	0.99	-	0-40	cells; tumor tissue; Arabidopsis	SI[2]
DCM-H_2S	0.13	12 min	0-35	cells	SI[3]
Rho-HS	0.5	15 min	0-30	cells; caenorhabditis elegans	SI[4]
NBT	0.082	60 min	5-70	water; beer	SI[5]
QM-RSH	44.6	10 min	1-40	cells; zebrafish	SI[6]
FL-N_3	3.99	30 min	0-800	cells	SI[7]
BPO-N_3	0.03	30 min	0.1-20	cells	SI[8]
CIM-SDB	0.12	40 min	0-50	cells; test strips	SI[9]
Cy-H_2S	0.18	3 min	0-50	cells; zebrafish	SI[10]
WFP-PC	0.47	-	3.3-20	cells; mitochondrial; mice	SI[11]
NCA	0.11	15 min	0.5-100	cells; test strips; water samples; gaseous H_2S	This work

References

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