Supporting information

Selective Fluorescent Detection of Hydrogen Sulfide in the Brain microdialysate Using an ESIPT-Activated Probe

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Contents

Experimental section

Synthesis of Compound 2. Compounds 2 were synthesized according to reported methods.[1] 4-Chlororesorcinol (1, 14.6 g, 0.10 mol) was dissolved in a solution of KOH (7.4 g, 0.132 mol) in 20 mL of water and 80 mL of ethanol. Isoamyl nitrite (12.2 g, 16.0 mL, 0.104 mol) was added dropwise to the dark brown solution with stirring at 0-5 °C over 30 min. The reaction mixture was then allowed to warm to room temperature and stirred for an additional 1 h. The solution was acidified to pH = 2 by addition of concentrated aqueous HCl, resulting in the formation of a yellow precipitate, which was filtered, washed with 50 mL of cold water, and air-dried under reduced pressure overnight to afford compound 2 as a yellow powder (1, 14.52 g, 84%). Mp 125-128 °C; IR (KBr): 3281, 3072, 1612, 1566, 1440, 1327, 1215, 1056, 998, 852 cm^{-1}; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 13.86 (br s, 1H, Ar-OH), 11.41 (br s, 1H, Ar-OH), 7.67 (s, 1H, Ar-H), 5.81 (s, 1H, Ar-H); ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ 178.8, 162.5, 144.3, 135.7, 119.5, 105.6; HRMS (ESI^-) m/z: $[M-H]^-$ calcd for $C_6H_3O_3NCl$, 172.0; found 171.8.

Synthesis of Compound 3. Compounds 3 were synthesized according to reported methods.[1] 4-Chlororesorcinol (1.45 g, 10.0 mmol) was dissolved in 10 mL of concentrated H_2SO_4 and heated to 85 °C with stirring. After 10 min, a homogeneous dark red solution formed, to which compound 2 (1.74 g, 10.0 mmol) was added portionwise over 10 min. The mixture was then heated to 110 °C and stirred for 20 h. After cooling to room temperature, it was poured into 300 mL of ice-water with vigorous stirring. The resulting precipitate was filtered, washed with water (2 × 200 mL), air-dried for 3 h, and then dried in vacuo at 80 °C to afford dichlororesorufin 3 as a dark brown powder (2.83 g, >99%). The product was insoluble in most organic solvents and was used without further purification. Mp >300 °C; ^{1}H NMR (400 MHz, CD_3COOD): δ 7.90 (s, 2H, Ar-H), 6.87 (s, 2H, Ar-H); HRMS (ESI^-) m/z: $[M-H]^-$ calcd for $C_{12}H_4O_3NCl_2$, 280.0; found 279.8.

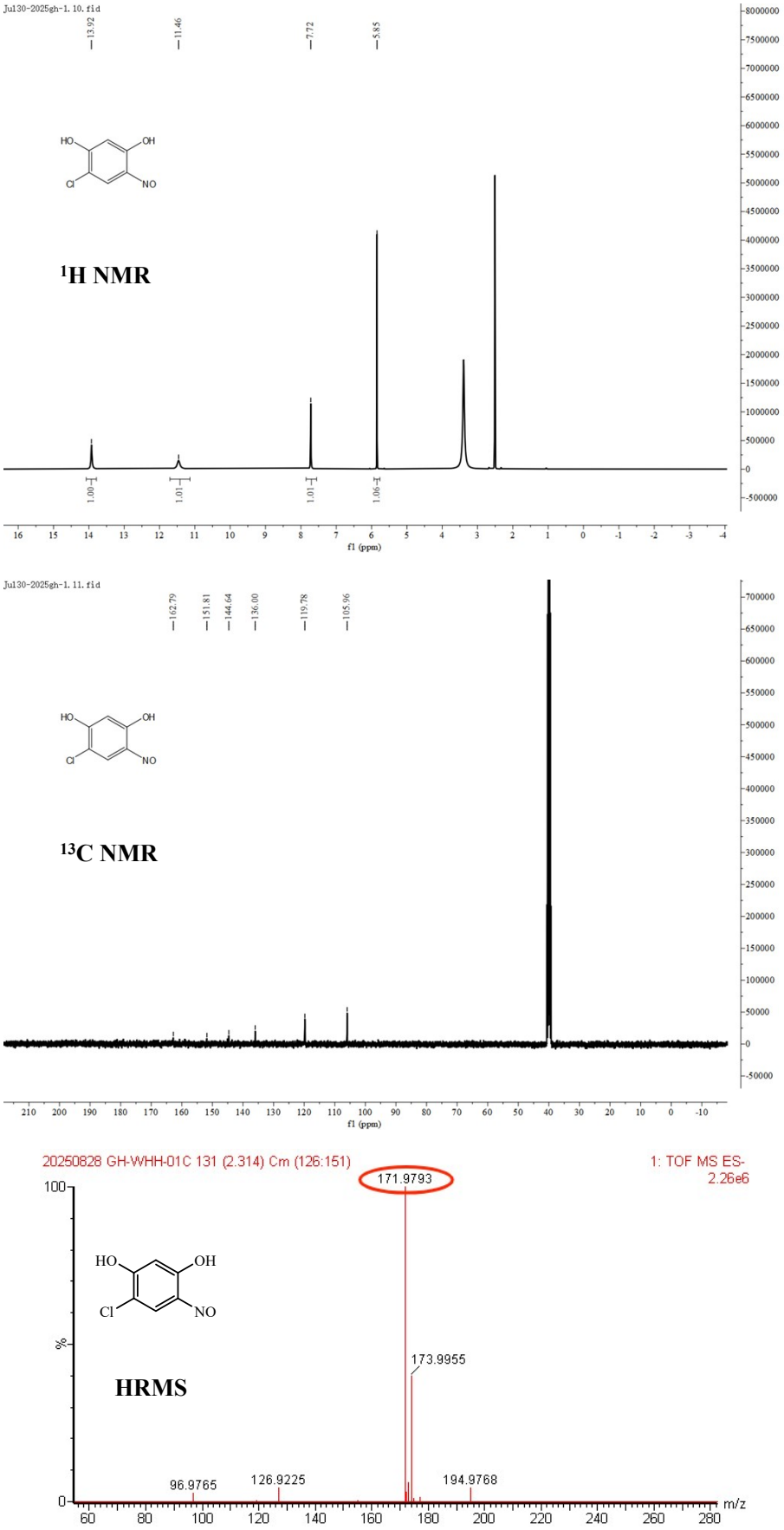


Figure S1. ^{1}H NMR, ^{13}C NMR, and HRMS spectra of Compound 2.

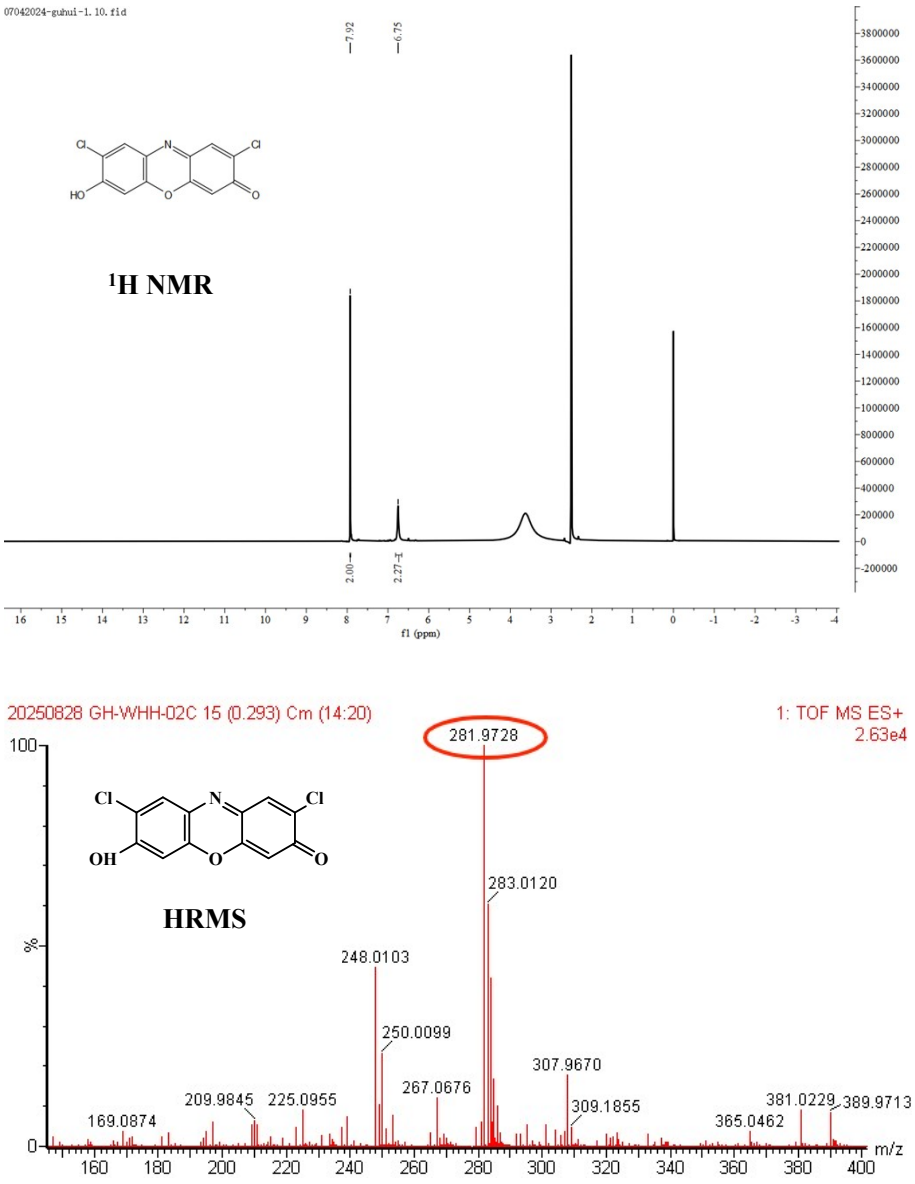


Figure S2. [1]H NMR and HRMS spectra of Compound 3.

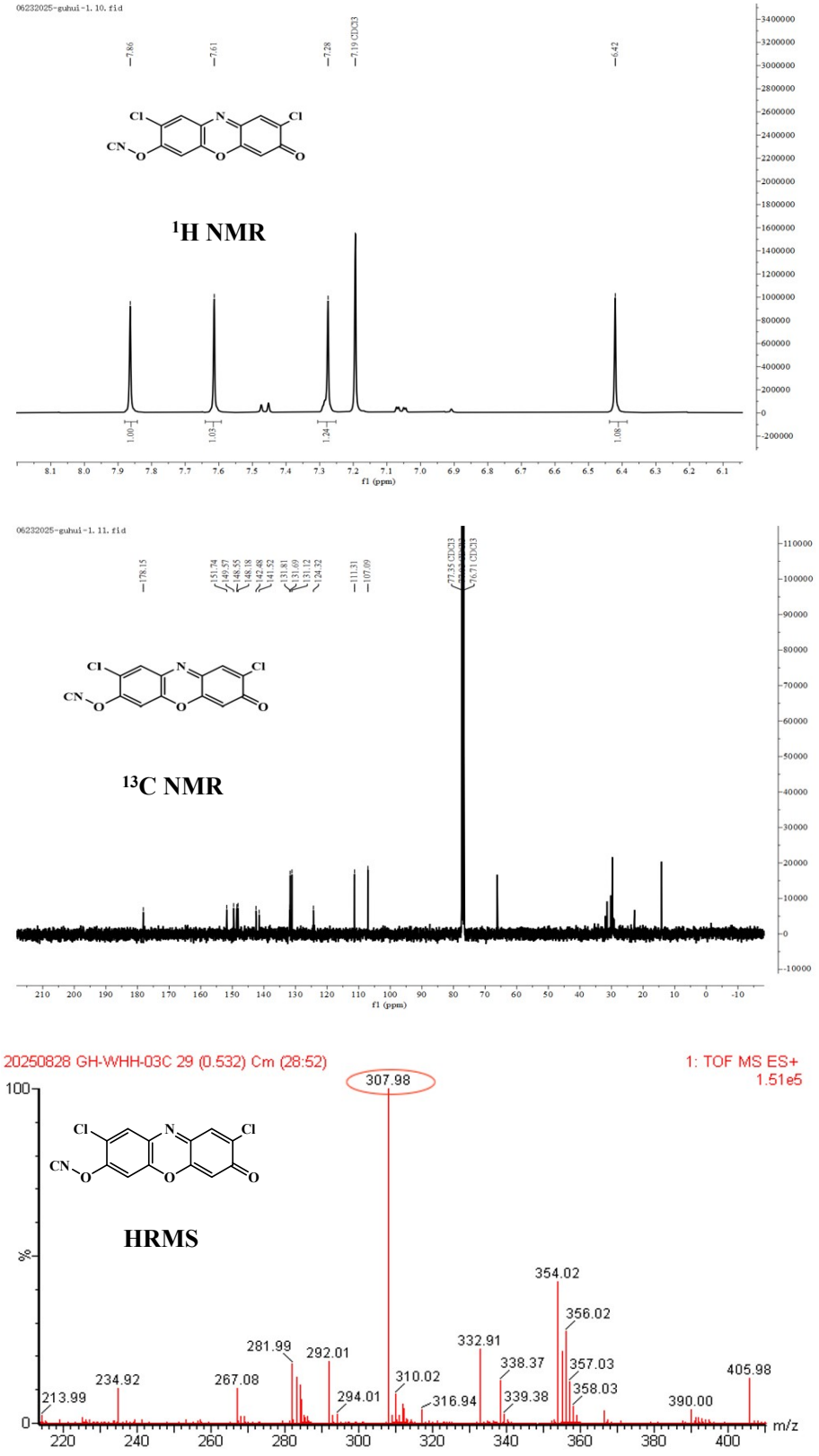


Figure S3. Structural characterization of Probe. ^{1}H NMR, ^{13}C NMR, and HRMS spectra of Probe.

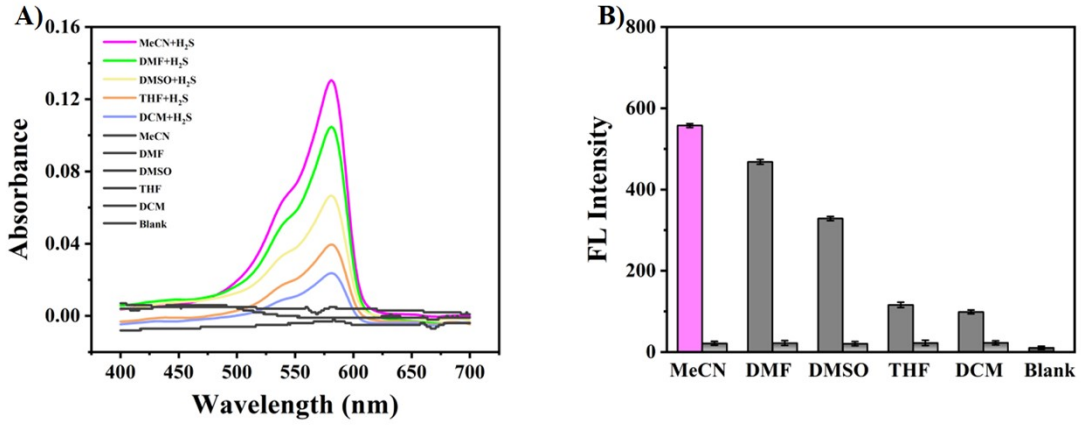


Figure S4. Solvent effect of Probe toward H_2S in five organic solvents at ≤ 0.1‰ (v/v).

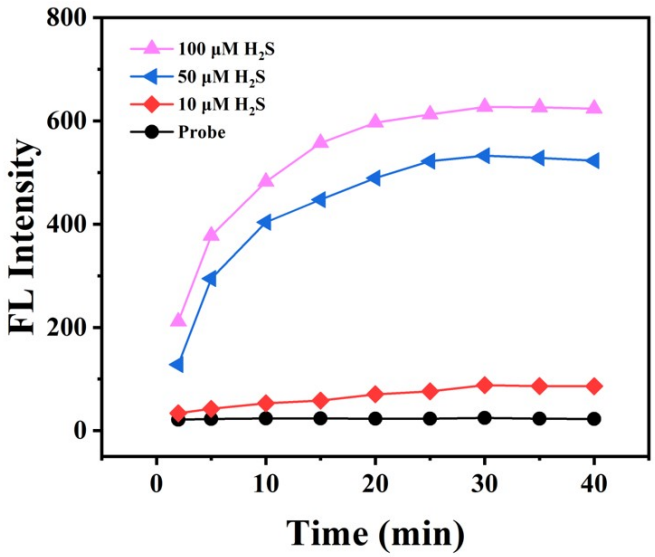


Figure S5. (A) Time dependent fluorescence response to H_2S with different concentration.

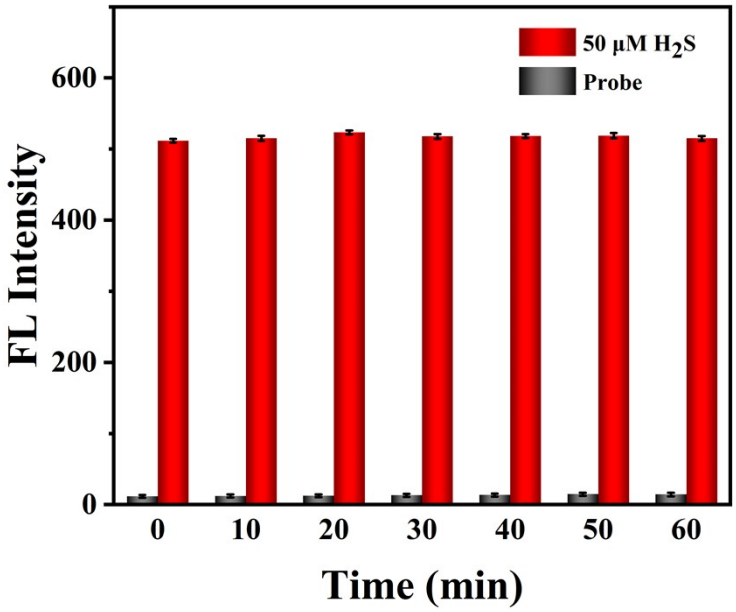


Figure S6. Anti-photobleaching performance of the probe before and after reaction with H_2S.

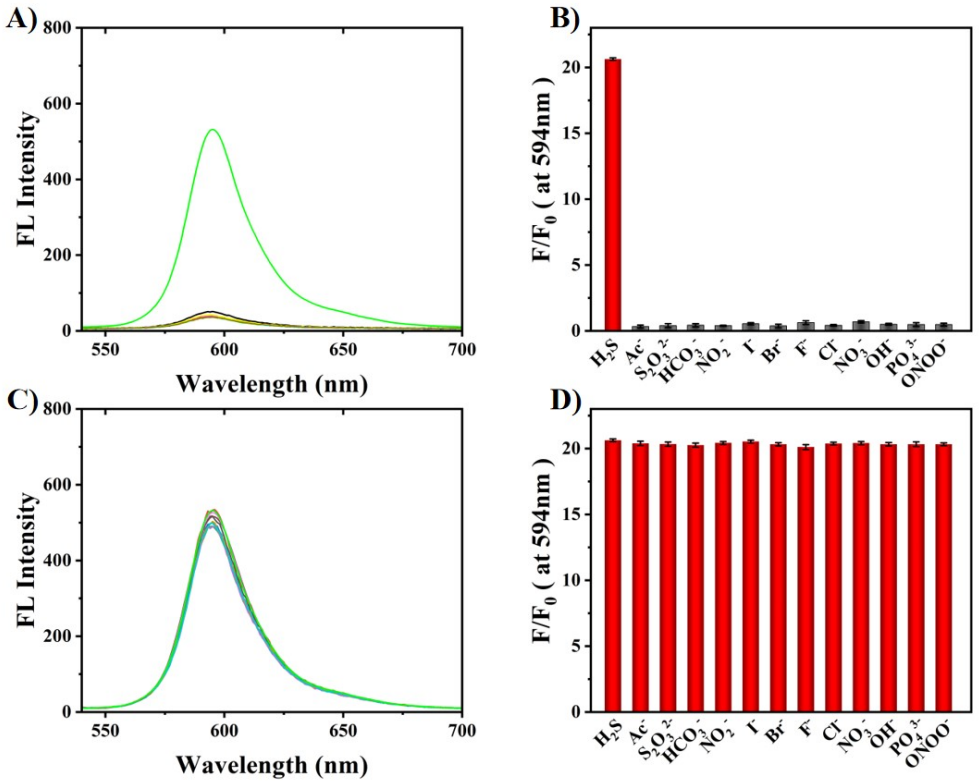


Figure S7. Selectivity and competition assays of Probe (10 μM) toward H_2S (50 μM) in aCSF solution (pH 7.4) with anions (A-D): Ac^-, $S_2O_3^{2-}$, HCO_3^-, NO_2^-, I^-, Br^-, F^-, NO_3^-, OH^-, PO_4^{3-}, $ONOO^-$ (100 μM each, except NO_3^- at 50 μM and HCO_3^- at 1 mM).

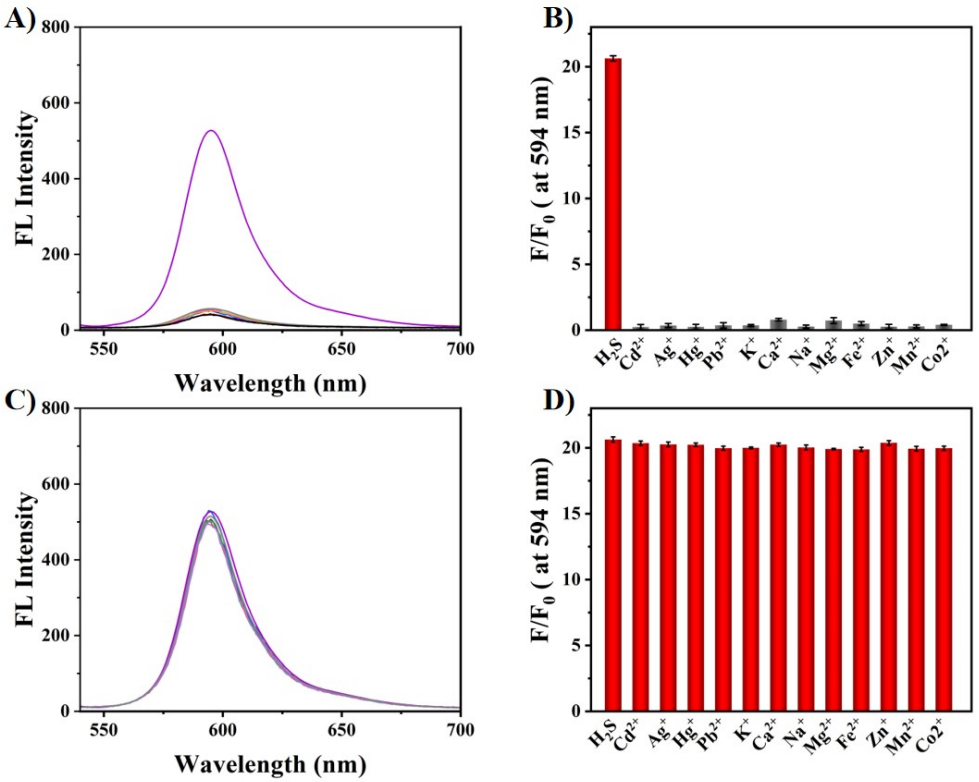


Figure S8. Selectivity and competition assays of Probe (10 μM) toward H_2S (50 μM) in aCSF solution (pH 7.4) with Cations (A-D): Cd^{2+}, Ag^{+}, Hg^{+}, Pb^{2+}, K^{+}, Ca^{2+}, Na^{+}, Mg^{2+}, Fe^{2+}, Mn^{2+}, Co^{2+} (100 μM each, except Na^{+} at 5 mM, K^{+} at 3 mM, Ca^{2+} and Mg^{2+} at 1 mM, and Mn^{2+} at 50 μM).

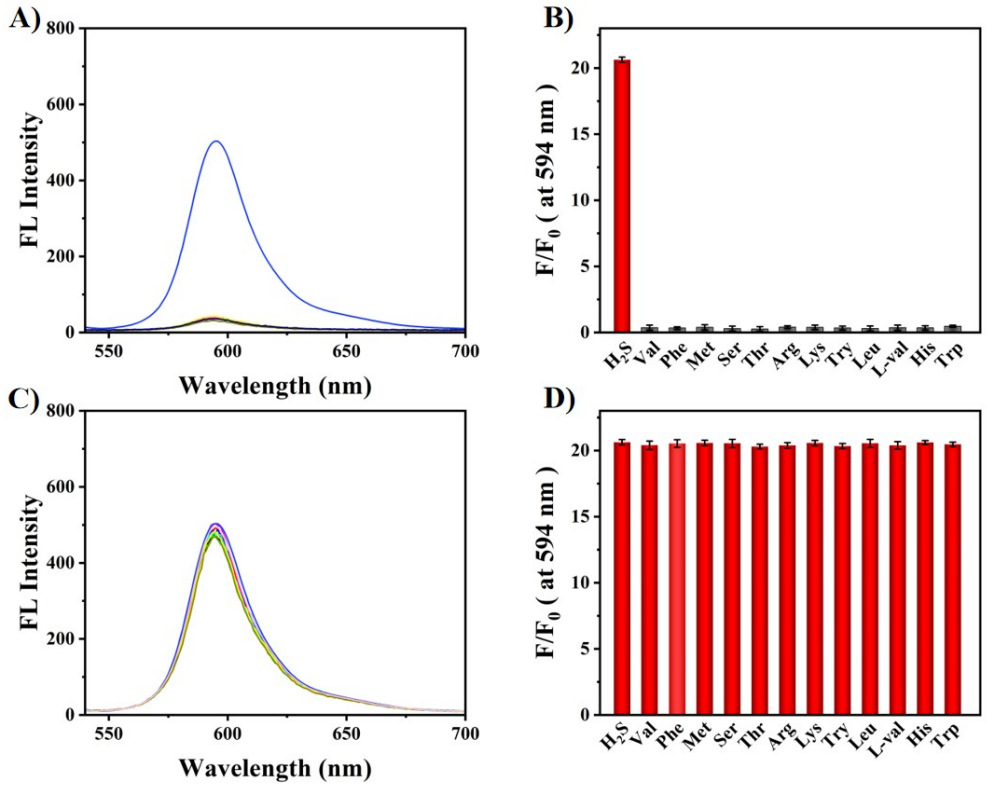


Figure S9. Selectivity and competition assays of Probe (10 μM) toward H_2S (50 μM) in aCSF solution (pH 7.4) with amino acids (A-D):Val, Phe, Met, Ser, Thr, Arg, Lys, Try, Leu, L-Val, His, Trp (200 μM His, Leu, Trp, Phe, Ser, Tyr, Met), (50 μM Lys, Arg, Thr).

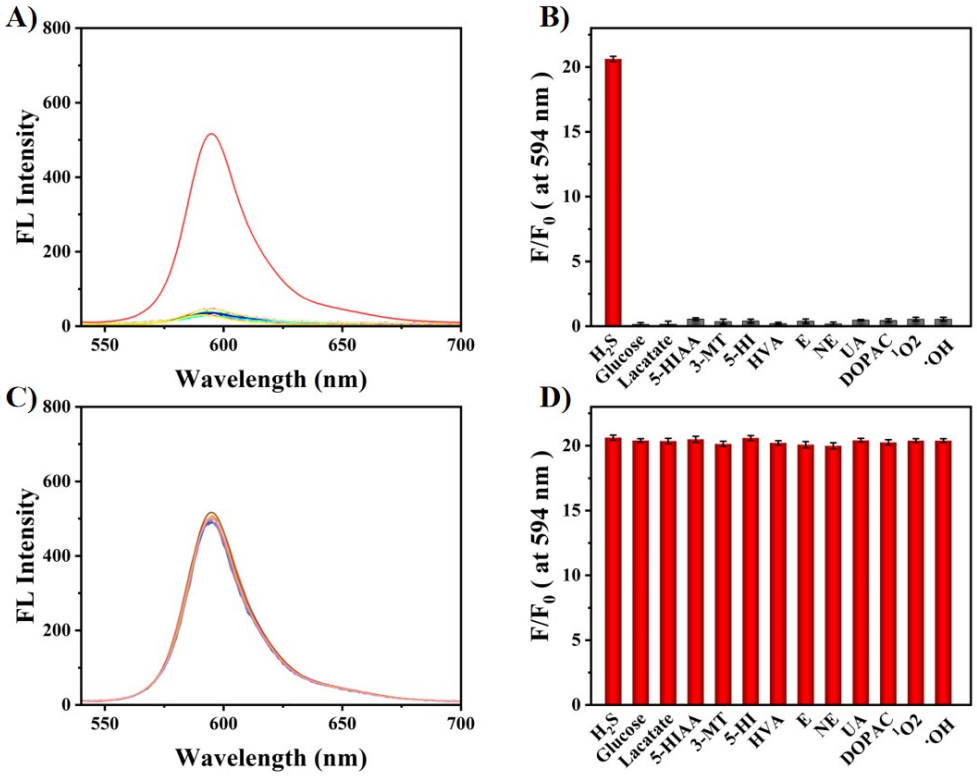


Figure S10. Selectivity and competition assays of Probe (10 μM) toward H_2S (50 μM) in aCSF solution (pH 7.4) with other possible active species in mouse brain (A-D): Glucose, Lactate, 5-HIAA, 3-MT, 5-HI, HVA, E, NE, UA, DOPAC, $^1O_2$, •OH (100 μM each, except Lactate at 1mM and Glucose at 500 μM).

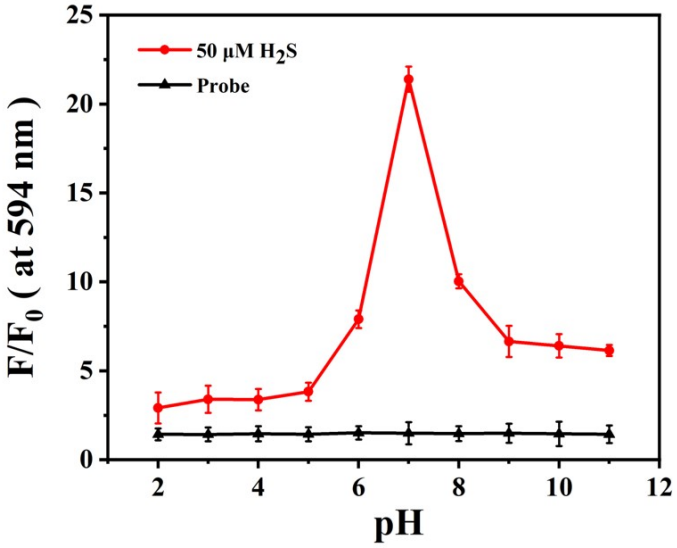
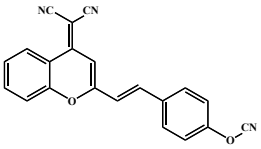
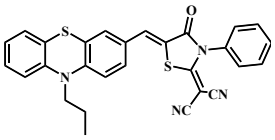
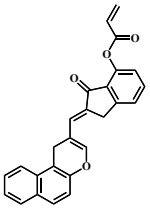
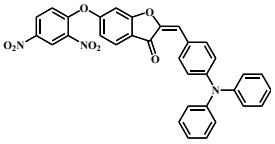
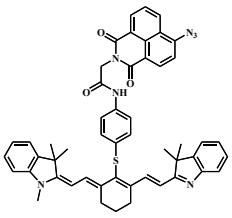
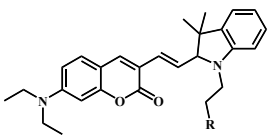
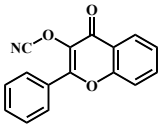
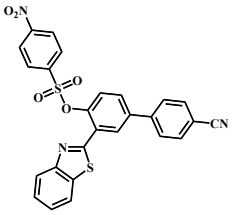
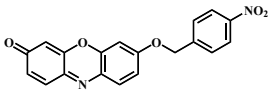
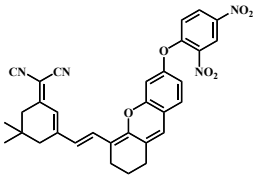
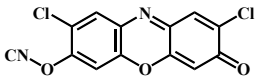


Figure S11. pH dependence. Fluorescence intensity of Probe and Probe-H_2S across pH 2.0-11.0.

Table S1. Comparison of the present H_2S sensing method with previously reported H_2S detection strategies.

Probes	Detection media	LOD (μM)	Time (min)	application	Ref.
	PBS (1% DMSO)	0.28	5	water	2
	DMF/H_2O (7/3)	1.80	10	cells/zebrafish	3
	DMSO/PBS (1/1)	-	3	mice	4
	PBS (10% DMSO)	0.42	30	cells	5
	DMF/PBS (1/1)	0.15	60	cells	6
	HEPES (10% DMSO)	10.0	3	celsl/mice	7

	PBS (3mM CTAB)	0.25	5	cells	8
	HEPES/DMSO (4/1)	0.20	25	cells/mice	9
	PBS (0.5% DMSO and 100 μM CTAB)	0.10	5	cells	10
	H_2O (10% CH_3CN)	0.35	3	cells	11
	aCSF(1‰ CH_3CN)	0.20	20	*In vivo* microdialysate	This work

References

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