

Supporting Information

A mitochondria-targeting “off-on” AIE probe with large Stokes shift for high-contrast H₂S imaging in living cells

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Fig. S19. Bioimaging of endogenous H_2S in HeLa cells with TPAS-TN (10 μM). (a) Cells stained with TPAS-TN for 1 h. (b) Cells pretreated with 50 μM Cys and then incubated with TPAS-TN. (c) Cells pretreated with 100 μM Cys and then incubated with TPAS-TN. (d) Cells pretreated with 100 μM Cys and 1 mM PAG for 1 h, and then stained with TPAS-TN. (a1-d1) Bright-field images. (a2-d2) Merged images. $\lambda_{\text{em}} = 600\text{-}700\text{ nm}$, $\lambda_{\text{ex}} = 402\text{ nm}$. (Repeated experiment)

Fig. S20. Quantification of exogenous imaging data.

Fig. S21. Quantification of endogenous imaging data.

Table S1. Comparison of fluorescence probes for sensing H_2S .

Reference

Methods and materials

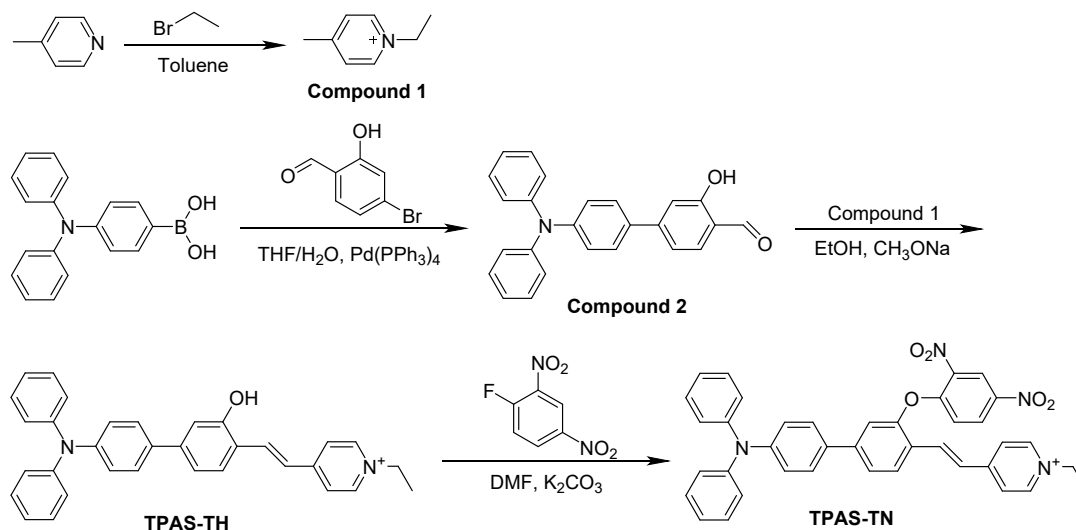
All chemicals used in the synthesis are purchased from Taitan and without further purification when applied. All solvents are purified and dried according to standard procedures. The ^1H NMR spectra and ^{13}C NMR spectra were obtained from Bruker AM 400 spectrometer by using Methanol- d_4 , CDCl_3 or $\text{DMSO-}d_6$ as solvents. High resolution mass spectrometry (HR-MS) of the products were obtained by using Waters LCT premier XE spectrometer. UV-Vis spectra of the final products were recorded on Agilent Cary 60 spectrophotometer and fluorescence spectra on F97pro fluorescence spectrophotometer, respectively. Cell imaging was performed with Laser scanning Confocal Microscopy (Nikon A1R, $\lambda_{\text{em}} = 600\text{-}700$ nm, $\lambda_{\text{ex}} = 402$ nm).

Except for the solvent effect test and AIE performance test, other spectral experiments were carried out under the condition of 1, 4-dioxane/PBS (9/1, v/v). This was mainly due to the fluorescence quenching caused by the strong TICT effect of the probe in pure PBS solution. However, subsequent cell tests showed that the probe was successfully tested inside the cell.

The limit of detection (LOD) was calculated by the equation $\text{LOD} = 3\sigma/k$ (where σ is the blank standard deviation, k is the slope between fluorescence intensity and the concentration of H_2S).

When testing the pH influence, 10% PBS solution with different pH values and 90% 1, 4-dioxane were used as the test system.

Synthesis route of the probe TPAS-TN



Synthesis of Compound 1

4-methyl pyridine (0.712 g, 7.65 mmol, 1 equiv.) and bromoethane (1g, 9.26 mmol, 1.2 equiv.) were dissolved in toluene (10 ml), and the solution was refluxed at 110 °C. After stirring for 12 h, the solution was cooled to room temperature. Then some white solid were precipitated in the solution. The solid was washed by toluene for three times, filtered, and dry by the vacuum to obtain the **Compound 1** (white solid, 1.46g, 95%). ¹H NMR (400 MHz, Methanol-d₄) δ 8.82 (d, J = 6.7 Hz, 2H), 7.93 (d, J = 6.1 Hz, 2H), 4.61 (q, J = 7.3 Hz, 2H), 2.68 (s, 3H), 1.63 (t, J = 7.4 Hz, 3H). Compound 1 has been published (Journal of Molecular Liquids, 2015, 209, 94-98).

Synthesis of Compound 2

4-bromo-2-hydroxybenzaldehyde (0.7 g, 3.5 mmol, 1 equiv.) and (4-(diphenylamino)phenyl) boronic acid (1 g, 3.46 mmol, 1 equiv.) were dissolved in the mixed solution of THF and H₂O (V (THF)/V (H₂O) = 3/1, 40 ml) in a 100ml flask, and then followed with the addition of potassium carbonate (350 mg, 0.245 mmol, 0.07equiv) and Pd (PPh₃)₄ (300 mg, 0.263 mmol, 0.075 equiv.) into the above solution. After that, the solution was stirred at 90°C in N₂ atmosphere for overnight. After the reaction bottle was cooled down to room temperature, the solution was extracted from saturated saline by using dichloromethane for three times, and the organic phase was further dried over anhydrous Na₂SO₄. After solvent removal, the obtained product was further purified by the silica-gel column chromatography using PE/DCM(v/v = 10/1) to afford the **Compound 2** (yellow solid, 1.18 g, 92%). ¹H NMR (400 MHz, Chloroform-d) δ 11.14 (s, 1H), 9.88 (s, 1H), 7.58 (d, J = 8.1 Hz, 1H), 7.51 (d, J = 8.7 Hz, 2H), 7.30 (dd, J = 8.5, 7.3 Hz, 4H), 7.23 (dd, J = 8.1, 1.7 Hz, 1H), 7.18 (d, J = 1.6 Hz, 1H), 7.15 (d, J = 8.0 Hz, 5H), 7.08 (dd, J = 13.8, 6.0 Hz, 3H). Compound 2 has been published (Angewandte Chemie - International Edition, 2019, 58(42), 14896-14900).

Synthesis of TPAS-TH

The mixture of **Compound 1** (200 mg, 1.0 mmol, 1 equiv.), **Compound 2** (365 mg, 1.0 mmol, 1 equiv.) and methanol sodium (80 mg, 1.5 mmol, 1.5 equiv.) in absolute ethanol (15ml) was heated to reflux for 8h. After the solvent cooled, the mixture removed the ethanol by using the rotary evaporator. Finally, **TPAS-TH** was isolated by neutral alumina column chromatography (DCM/MeOH = 10/1) to obtain **TPAS-TH** as a red solid (345 mg, 63%). ¹H NMR (400 MHz, DMSO-d₆) δ 10.67 (s, 1H), 8.91 (d, J = 6.6 Hz, 2H), 8.22 (d, J = 6.7 Hz, 2H), 8.11 (d, J = 16.4 Hz, 1H), 7.74 (d, J = 8.2 Hz, 1H), 7.64 - 7.50 (m, 3H), 7.35 (t, J = 7.8 Hz, 4H), 7.29 - 7.18 (m, 2H), 7.14 - 7.01 (m, 8H), 4.57 - 4.47 (q, 2H), 1.52 (t, J = 7.3 Hz, 3H). ¹³C NMR (100 MHz, DMSO-d₆) δ 157.64, 153.46, 147.30, 146.84, 143.76, 142.74, 136.48, 132.69, 129.64, 129.48, 127.47, 124.36, 123.46, 123.41, 122.82, 122.28, 120.64, 117.40, 113.55, 54.92, 16.21. HR-MS: calculated for C₃₃H₂₉N₂O⁺ [M-Br]⁺, 469.2280; found, 469.2283.

Synthesis of TPAS-TN

1-Fluoro-2,4-Dinitrobenzene (67 mg, 0.360 mmol, 1.0 equiv.) and **TPAS-TH** (200 mg, 0.365 mmol, 1.0 equiv.) were dissolved in DMF (15ml), and potassium carbonate (74 mg, 0.535 mmol, 1.5 equiv.) was added. Then the solution was stirred for overnight at 0°C. After the reaction mixture warmed up to room temperature, the solvent was removed by evaporation under reduced pressure at 65 °C. Then the crude product was diluted with dichloromethane, and washed three times with saturated saline. The organic phase was dried over anhydrous Na₂SO₄. After the solvent removal, the residue was purified by neutral alumina column chromatography with DCM/MeOH (v/v = 10/1) as eluent to afford a red-black solid (195 mg, 76%). ¹H NMR (400 MHz, DMSO-d₆) δ 8.99 - 8.93 (m, 3H), 8.46 (dd, J = 9.3, 2.8 Hz, 1H), 8.21 (d, J = 6.8 Hz, 2H), 8.13 (d, J = 8.4 Hz, 1H), 7.98 (d, J = 16.4 Hz, 1H), 7.83 (d, J = 10.1 Hz, 1H), 7.74 - 7.65 (m, 3H), 7.64 (d, J = 1.8 Hz, 1H), 7.34 (dd, J = 8.4, 7.3 Hz, 4H), 7.25 (d, J = 9.3 Hz, 1H), 7.11 (d, J = 7.4 Hz, 2H), 7.06 (d, J = 7.3 Hz, 4H), 7.01 (d, J = 8.8 Hz, 2H), 4.53 (q, J = 7.2 Hz, 2H), 1.51 (t, J = 7.3 Hz, 3H). ¹³C NMR (150 MHz, DMSO-d₆) δ 155.42, 153.11, 152.66, 148.37, 147.16, 144.71, 143.92, 142.10, 139.83, 133.04, 131.40, 130.43, 130.16, 129.93, 128.33, 126.22, 126.09, 125.02, 124.65, 124.60, 124.19, 122.89,

122.54, 120.05, 118.61, 55.78, 16.73. HR-MS: calculated for $C_{39}H_{31}N_4O_5^+$ [M-Br]⁺, 635.2294; found, 635.2306.

General procedure of solution measurements

The stock solutions of **TPAS-TN** (1 mM) and **TPAS-TH** (1 mM) were prepared in dimethyl sulfoxide (DMSO), and tested with a concentration of 10 μM. The stock solution of NaHS (10 mM) was prepared in deionized water. The measurement systems were gained by successively adding **TPAS-TN** and analytes into 1,4-dioxane/PBS (v/v, 9/1, 10 mM, pH 7.4) (3 mL). The stock solutions of various analytes (200 μM: H₂S, Al³⁺, Mg²⁺, Ca²⁺, K⁺, Na⁺, NH₄⁺, Cl⁻, F⁻, Br⁻, I⁻, ClO⁻, HCO₃⁻, SO₄²⁻, NO₃⁻, HSO₃⁻, S₂O₃²⁻, CH₃COO⁻, Cys, GSH, Hcy, DTT, H₂O₂, N₂H₄) were prepared in deionized water.

Cell incubation and imaging

HeLa cells were cultured in the condition of DMEM medium supplemented with 10 % fetal bovine serum (FBS) and antibiotics (10,000 U/mL penicillin, and 10 mg/mL streptomycin) at 37°C in a 5 % CO₂/95 % air incubator.

HeLa cells (10000/mL) were put in a petri dish with a diameter of 3 cm (1 mL per dish) and incubated for overnight. Before imaging, all cell images were washed with PBS solution three times. **TPAS-TN** was dissolved in DMSO and NaHS was soluble in PBS solution. For exogenous H₂S imaging, the cells were incubated with cell culture medium containing 10 μM and 20 μM NaHS for 30 min, and then **TPAS-TN** (10 μM) were used to culture the cells for 1h before images were taken. For quantification of imaging data., we adopted the average fluorescence intensity of the three cells. For endogenous H₂S imaging, the cells were pretreated with 50/100 μM Cys for 1 h and then incubated with **TPAS-TN** for 1 h. In order to further explore endogenous H₂S imaging, the cells were pretreated with 100 μM Cys and 1 mM PAG for 1 h, and then stained with **TPAS-TN** for 1 h. For quantification of imaging data., we adopted the average fluorescence intensity of the three cells. In colocalization experiments, cells preloaded with NaHS (20 μM) were first incubated with **TPAS-TN** (10 μM) for 1 h,

and then with Mito-tracker Green (200 nM) for 10 min.

Cytotoxicity study

Cell Counting Kit-8 (CCK-8) was used to evaluate the cytotoxicity of **TPAS-TN** and **TPAS-TH**. The cells were incubated with different concentrations of **TPAS-TN** or **TPAS-TH** (0, 5, 10, 20 μM) in the dark for 12 h and 24 h. Then, CCK-8 (10% in serum free culture medium) was added and the plate was incubated for another 1 h. The absorbance of CCK-8 at 450 nm was monitored by the microplate reader.

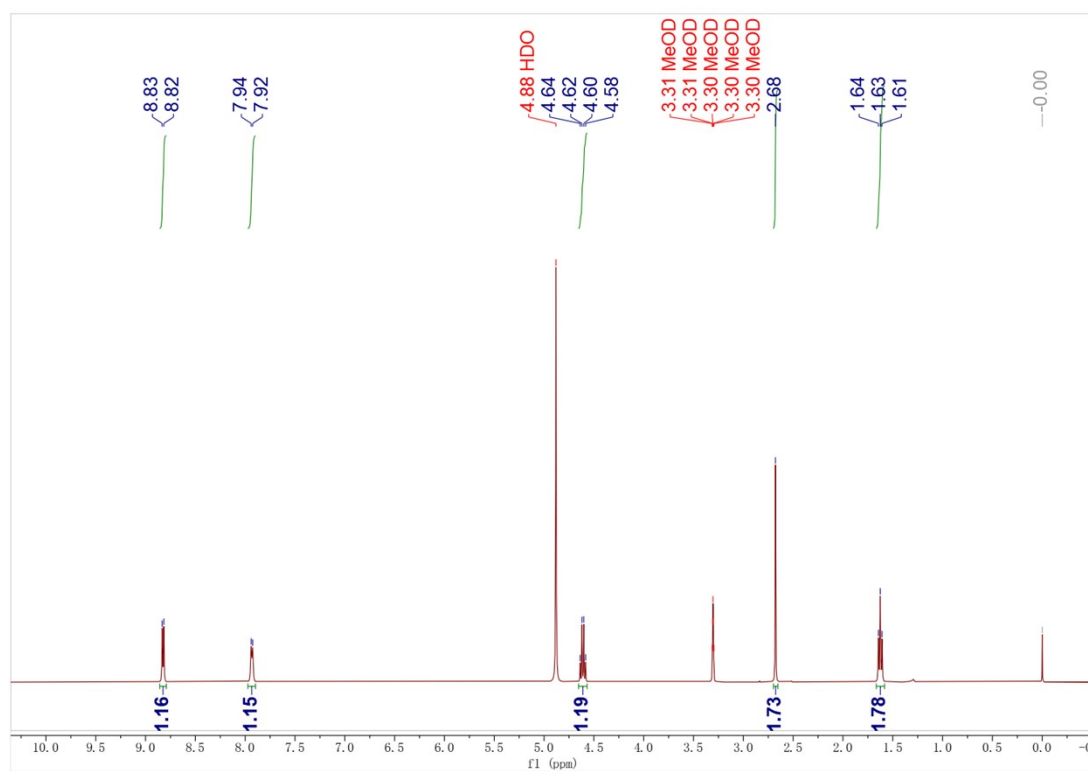


Fig. S1. ^1H NMR spectrum of **Compound 1**.

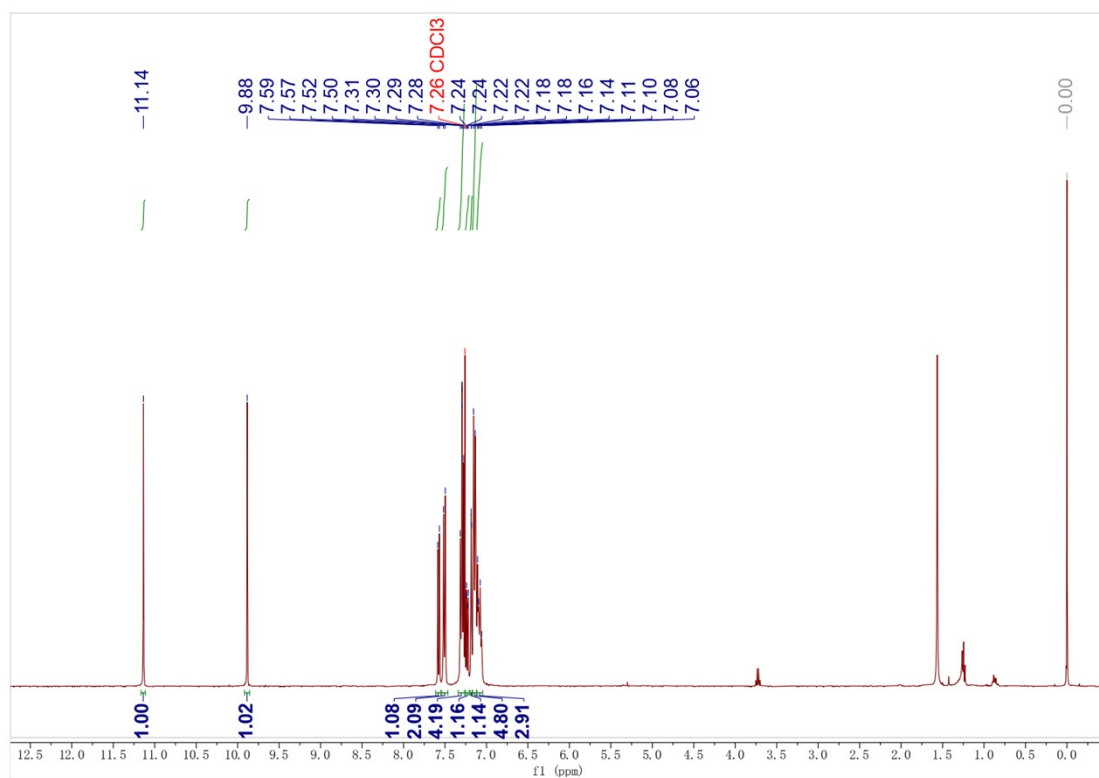


Fig. S2. ¹H NMR spectrum of Compound 2.

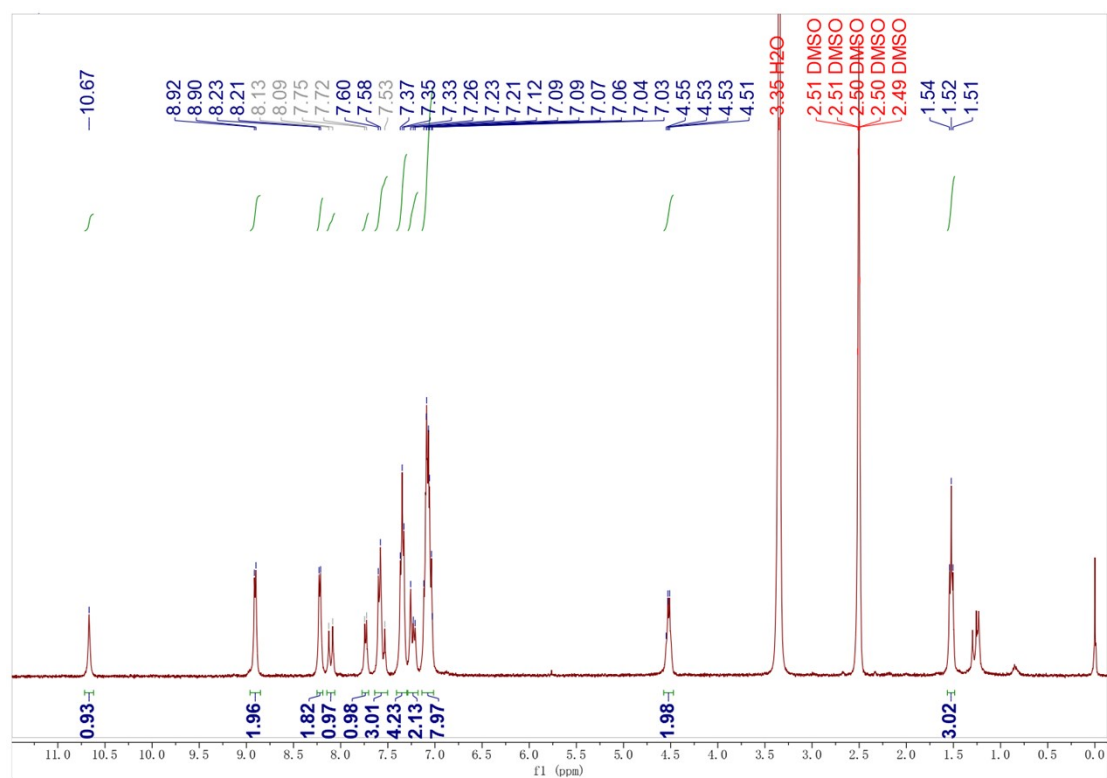


Fig. S3. ¹H NMR spectrum of TPAS-TH.

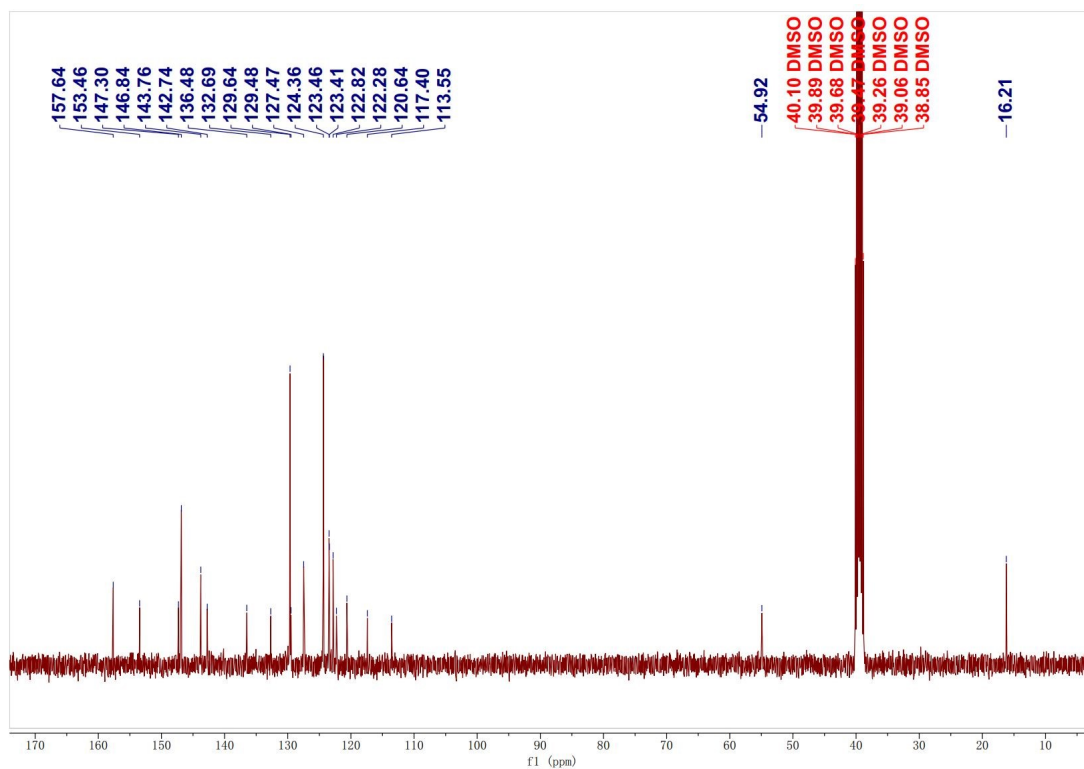


Fig. S4. ^{13}C NMR spectrum of TPAS-TH.

Elemental Composition Report

Single Mass Analysis

Tolerance = 15.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

1 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)

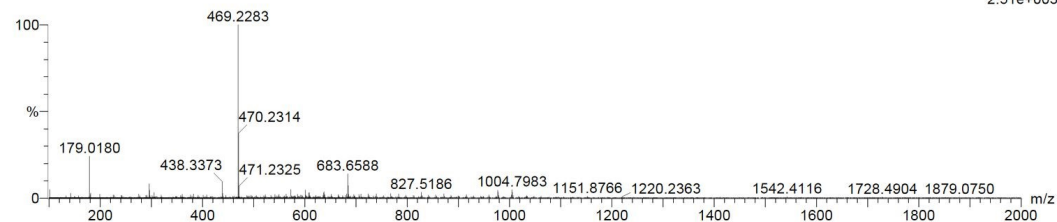
Elements Used:

C: 0-33 H: 0-29 N: 0-2 O: 0-1

CY-WANG

WC-CY-003 89 (1.033) Cm (89)

1: TOF MS ES+
2.51e+003



Minimum: -1.5
Maximum: 5.0 15.0 50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
469.2283	469.2280	0.3	0.6	20.5	90.5	0.0	C33 H29 N2 O

Fig. S5. High resolution mass spectrum of TPAS-TH.

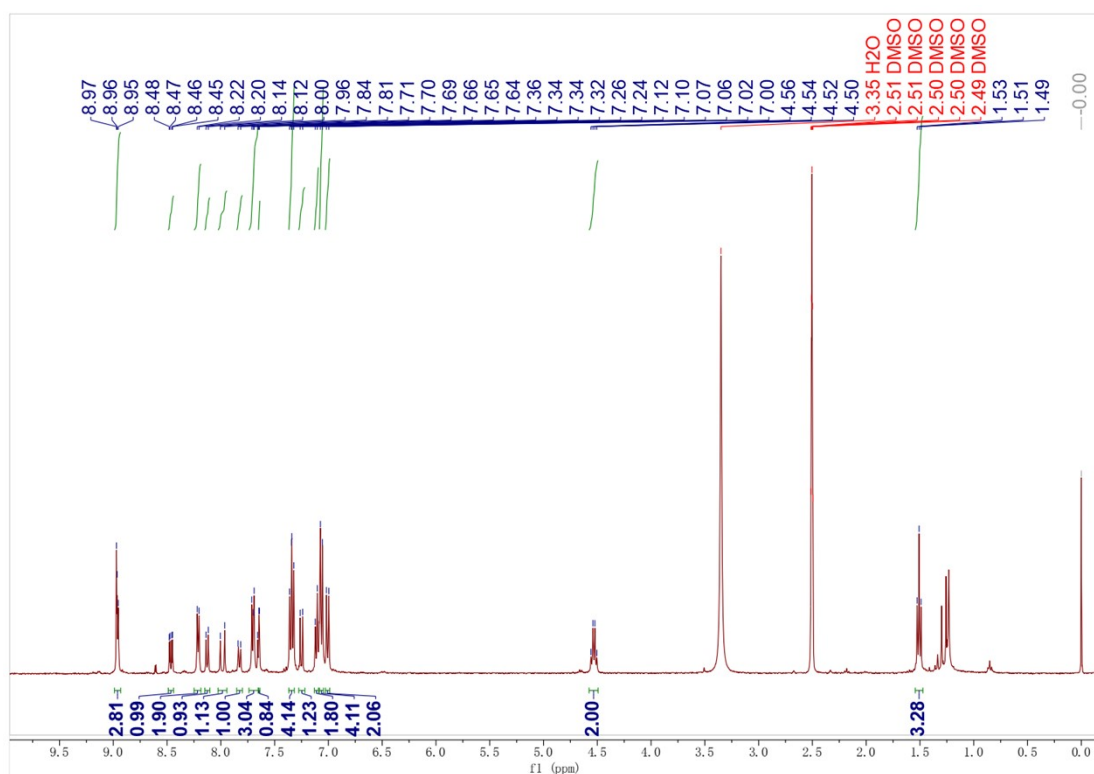


Fig. S6. ¹H NMR spectrum of TPAS-TN.

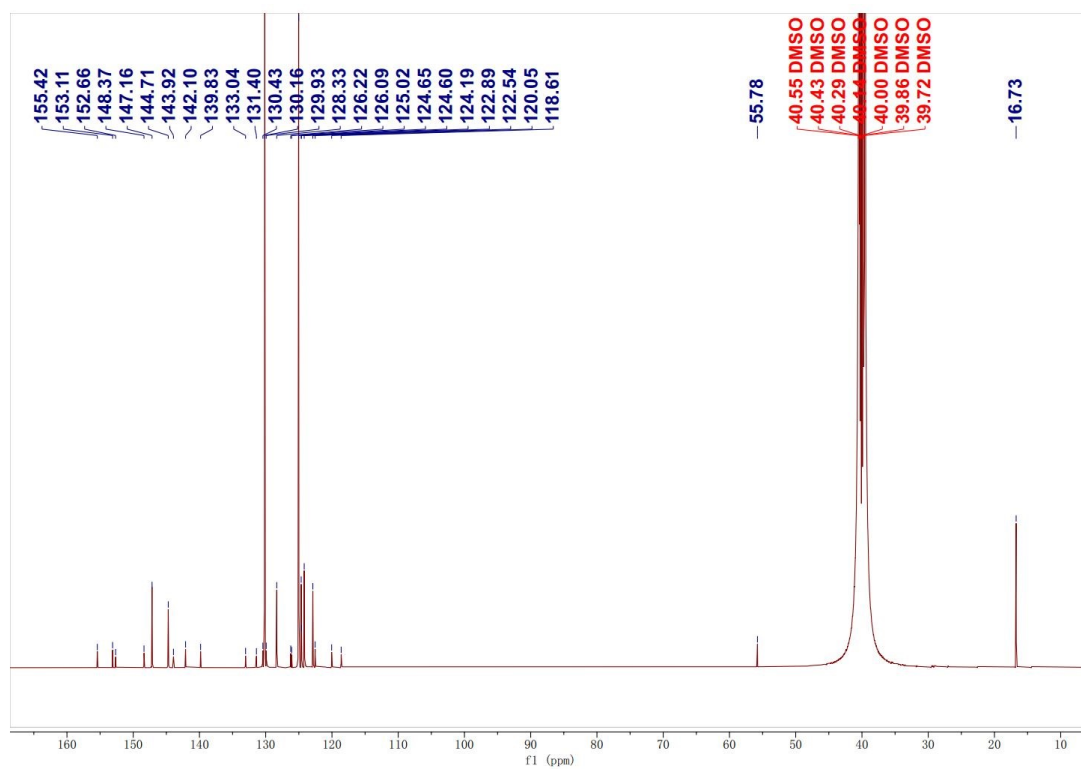


Fig. S7. ¹³C NMR spectrum of TPAS-TN.

Single Mass Analysis

Tolerance = 15.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

79 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)

Elements Used:

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CY-WANG

WC-CY-004 165 (1.881)

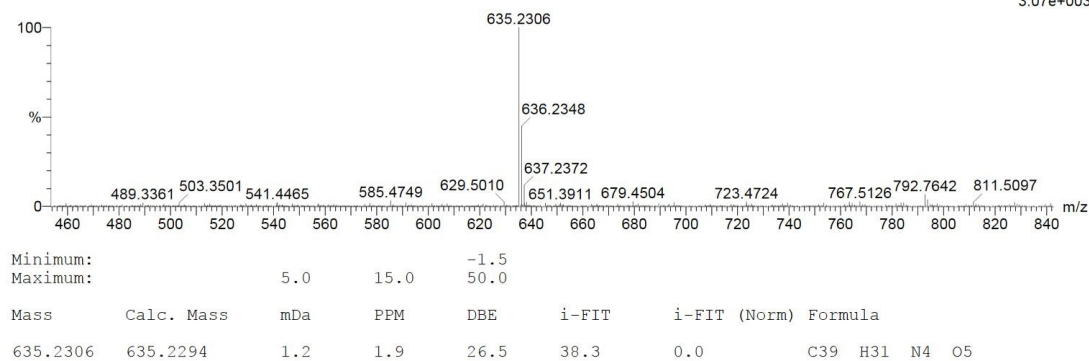
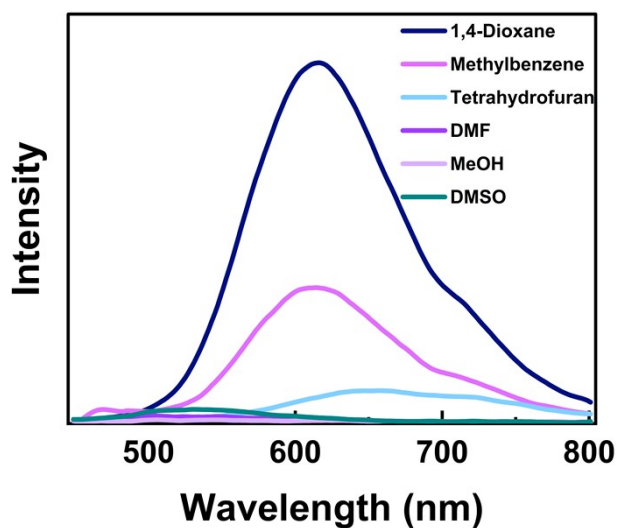
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Fig. S8. High resolution mass spectrum of TPAS-TN.

Fig. S9. Fluorescence emission spectra of TPAS-TH (10 μ M) in different organic solvent (1,4-Dioxane, Methylbenzene, Tetrahydrofuran, DMF, MeOH, DMSO). λ_{ex} = 435nm.

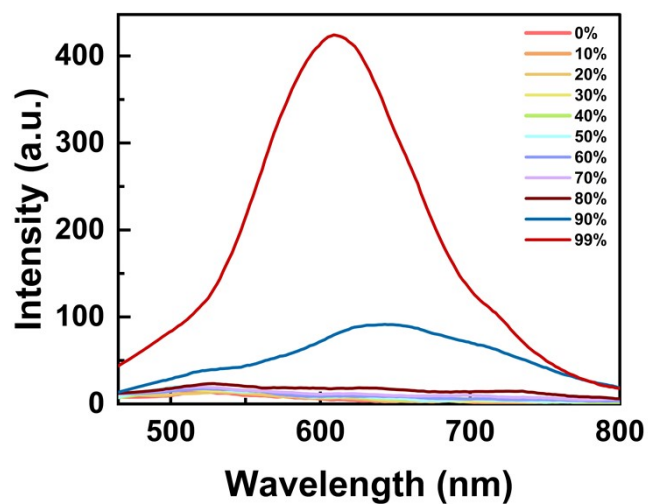


Fig. S10. Fluorescence emission spectra of TPAS-TH (10 μ M) at different concentrations of 1,4-dioxane/DMSO ranging from 0 to 99%. $\lambda_{\text{ex}} = 435\text{nm}$.

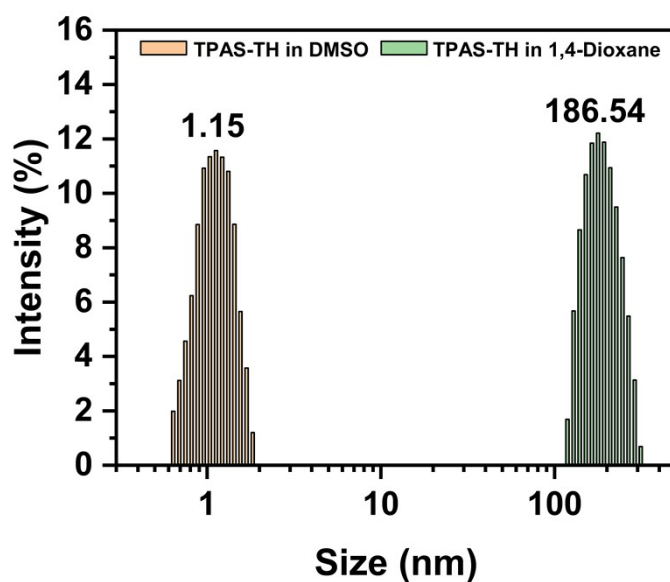


Fig. S11. DLS size distribution of TPAS-TH (10 μ M) in DMSO and 1,4-Dioxane.

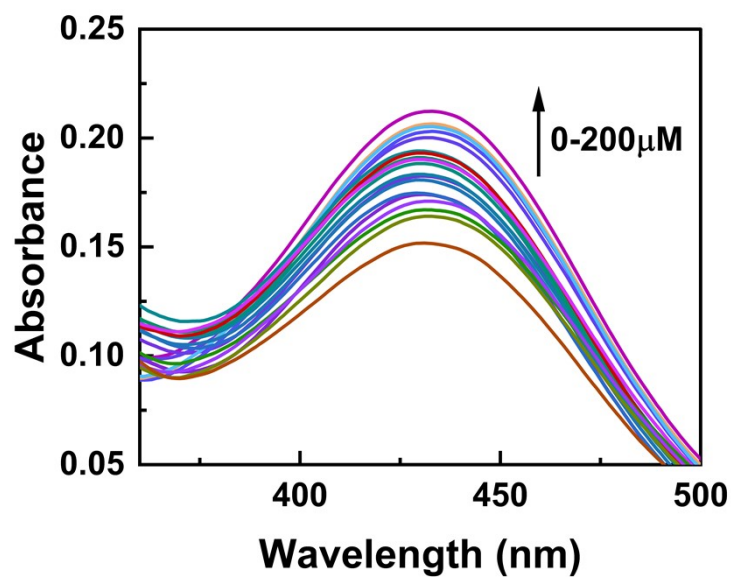


Fig. S12. The UV-vis absorption spectra of TPAS-TN (10 μM) to different concentrations of NaHS from 0-200 μM .

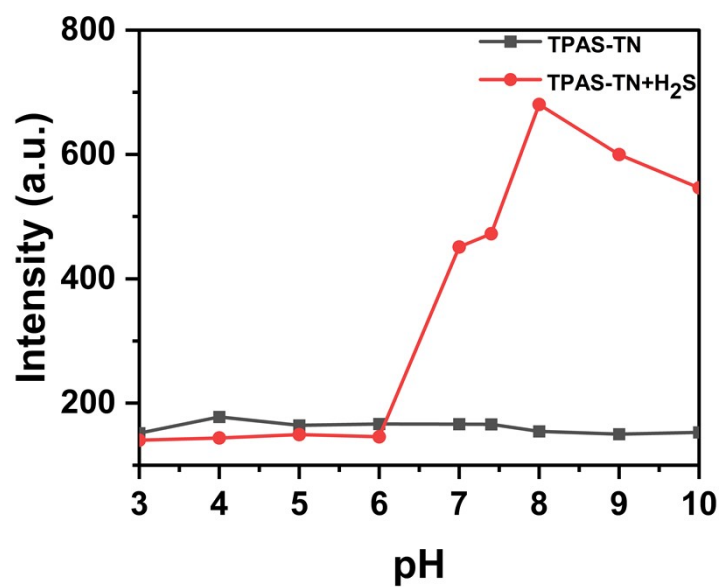


Fig. S13. pH effect of the fluorescence response of probe TPAS-TN at 643 nm in the presence and absence of NaHS. $\lambda_{\text{ex}} = 435\text{nm}$.

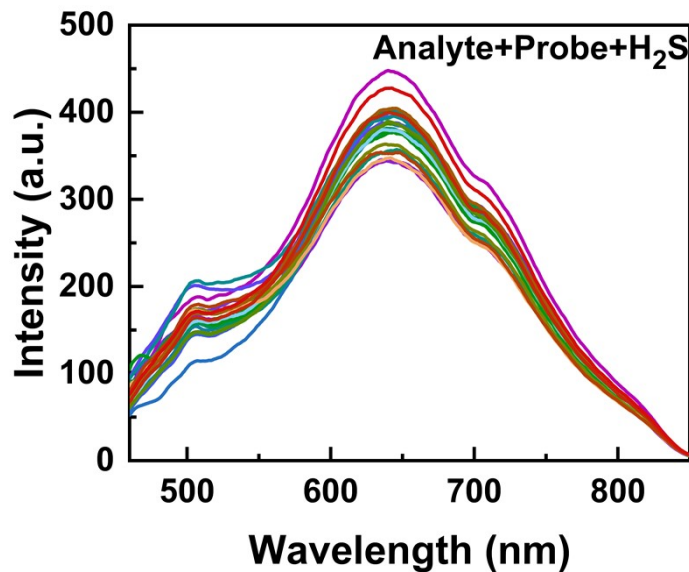


Fig. S14. Fluorescence emission spectra of TPAS-TH (10 μM) after adding H_2S (200 μM) and other analysts (200 μM), including Al^{3+} , Mg^{2+} , Ca^{2+} , K^+ , Na^+ , NH_4^+ , Cl^- , F^- , Br^- , I^- , ClO^- , HCO_3^- , SO_4^{2-} , NO_3^- , HSO_3^- , $\text{S}_2\text{O}_3^{2-}$, CH_3COO^- , Cys, GSH, Hcy, DTT, H_2O_2 , N_2H_4 . $\lambda_{\text{ex}} = 435\text{nm}$.

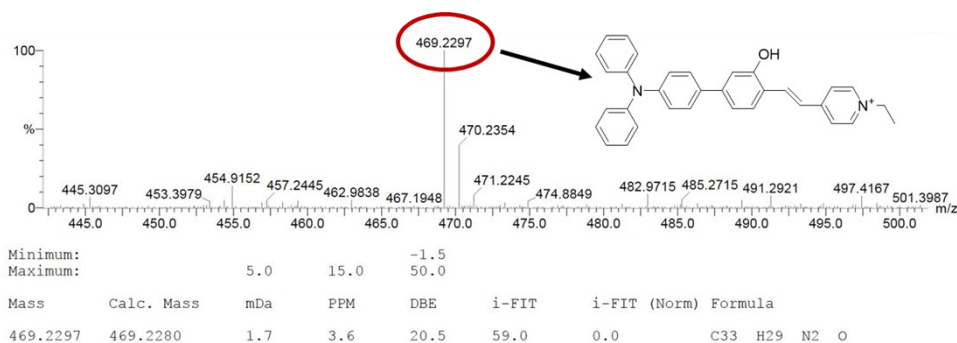


Fig. S15. High resolution mass spectrum (HR-MS) of the reaction mixture of TPAS-TN (10 μM) with H_2S (100 μM) in deionized water. The spectrum was taken after the mixture was incubated for 30 min at room temperature.

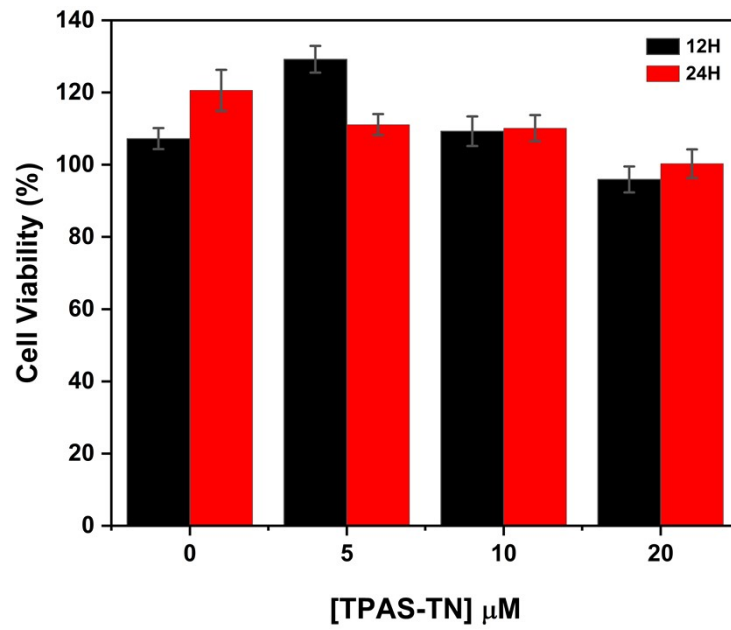


Fig. S16. Cell viability of HeLa after treatment with various concentrations of TPAS-TN (from 0-20 μM) for 12 h and 24 h.

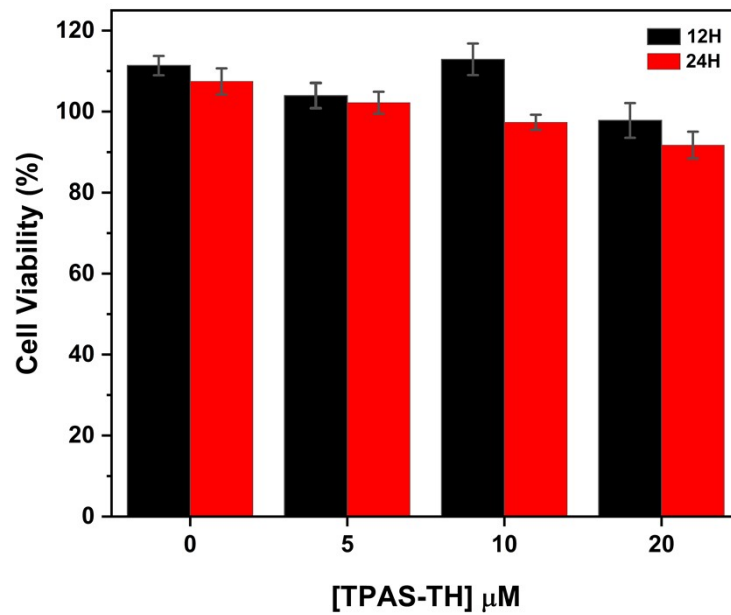


Fig. S17. Cell viability of HeLa after treatment with various concentrations of TPAS-TH (from 0-20 μM) for 12 h and 24 h.

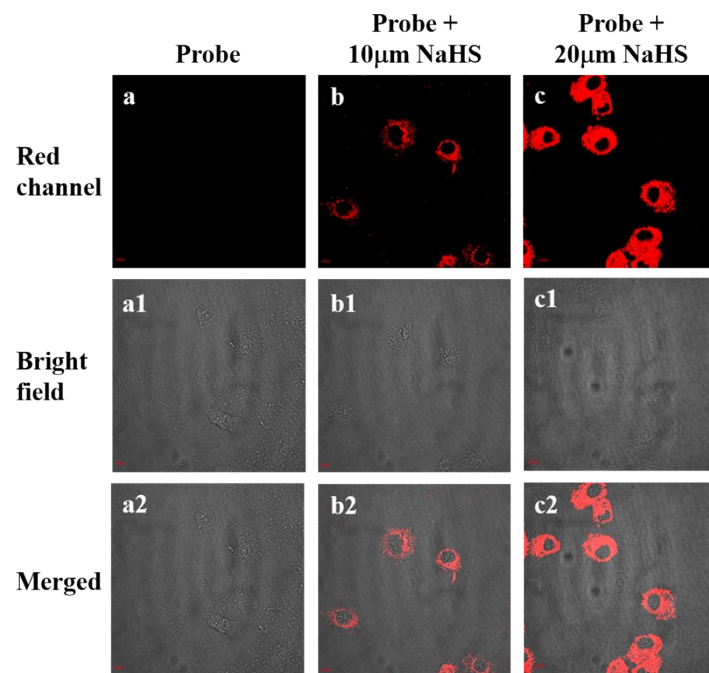


Fig. S18. Bioimaging of exogenous H₂S in HeLa cells with TPAS-TN (10 μ M). (a) Cells stained with TPAS-TN for 1 h. (b) Cells pretreated with 10 μ M NaHS and then incubated with TPAS-TN for 1 h. (c) Cells pretreated with 20 μ M NaHS and then incubated with TPAS-TN for 1 h. (a1-c1) Bright-field images. (a2-c2) Merged images. λ_{em} = 600-700 nm, λ_{ex} = 402 nm. (Repeated experiment)

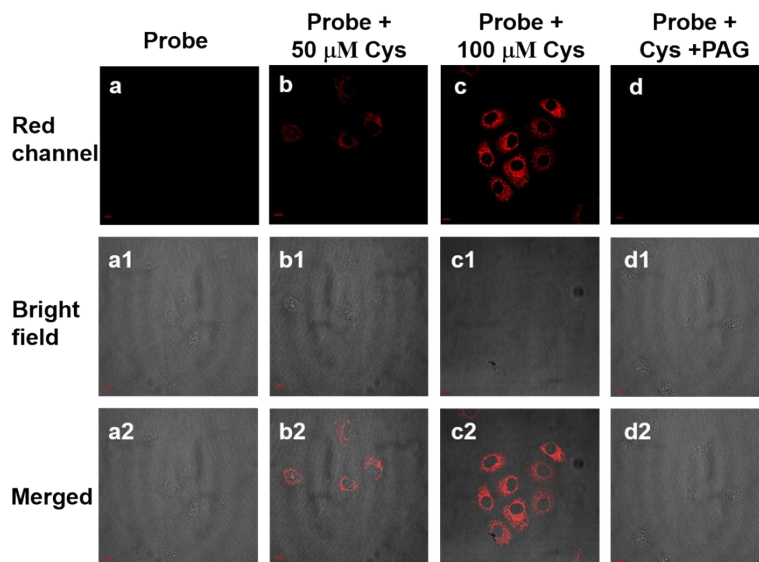


Fig. S19. Bioimaging of endogenous H₂S in HeLa cells with TPAS-TN (10 μ M). (a) Cells stained with TPAS-TN for 1 h. (b) Cells pretreated with 50 μ M Cys and then incubated with TPAS-TN. (c) Cells pretreated with 100 μ M Cys and then incubated with TPAS-TN. (d) Cells pretreated with 100 μ M Cys and 1 mM PAG for 1 h, and then stained with TPAS-TN. (a1-d1) Bright-field images. (a2-d2) Merged images. λ_{em} = 600-700 nm, λ_{ex} = 402 nm. (Repeated experiment)

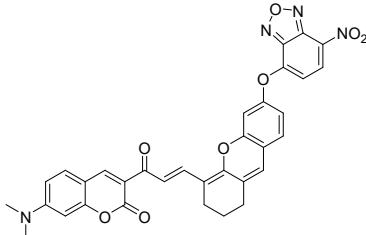
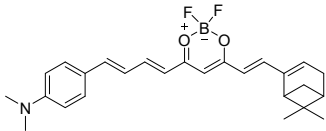
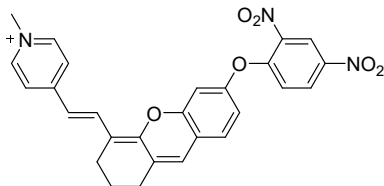
Probe		Probe + 10 μ M NaHS		Probe + 20 μ M NaHS	
Cells	Mean (intensity)	Cells	Mean (intensity)	Cells	Mean (intensity)
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2	4.637	2	78.737	2	178.362
3	3.826	3	96.299	3	173.253
4	5.297	4	99.712	4	181.746
5	4.193	5	102.331	5	162.642
6	3.324	6	89.847	6	165.876
7	3.728	7	96.068	7	179.847
8	4.156	8	88.976	8	177.738
9	3.341	9	85.917	9	169.592
		10	82.162	10	173.639
		11	77.293	11	179.726
Standard deviation	0.588		7.995		6.167
Average	4.063		89.383		173.611

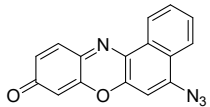
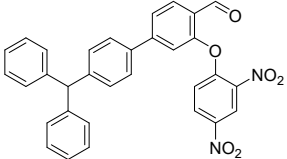
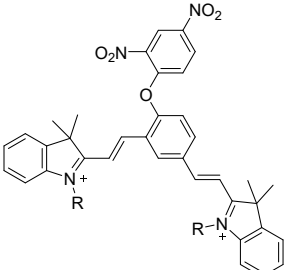
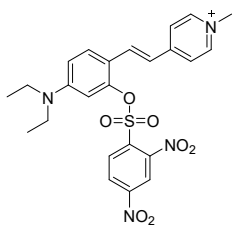
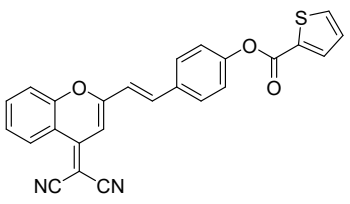
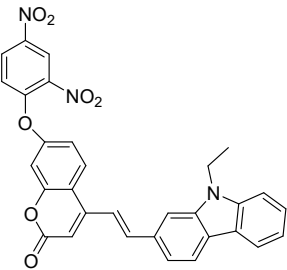
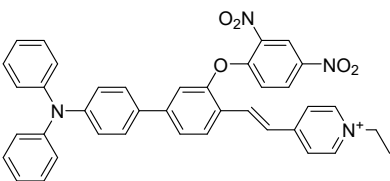
Fig. S20. Quantification of exogenous imaging data.

Probe		Probe + 50 μ M Cys		Probe + 100 μ M Cys		Probe + Cys +PAG	
Cells	Mean (intensity)	Cells	Mean (intensity)	Cells	Mean (intensity)	Cells	Mean (intensity)
1	2.683	1	24.276	1	76.683	1	2.728
2	3.162	2	25.329	2	80.372	2	3.536
3	3.427	3	24.825	3	78.281	3	2.893
4	2.192	4	26.284	4	76.752	4	4.027
5	3.718	5	22.492	5	87.273	5	3.792
6	2.367	6	25.831	6	80.227	6	3.724
7	3.211	7	23.537	7	85.271	7	2.338
8	2.489					8	3.295
9	3.314					9	3.687
Standard deviation	0.501		1.230		3.817		0.534
Average	2.951		24.653		80.694		3.336

Fig. S21. Quantification of endogenous imaging data.

Table S1. Comparison of fluorescence probes for sensing H₂S (NM= Not mentioned):

Probe	Linear detection range (μ M)	AIE performance	Stokes shift	Mitochondria -targeting	Ref
	0.5-30 (R ² = NM)	NM	155 nm	NM	1
	0-80 (R ² = 0.997)	NM	220 nm	NM	2
	0-11 (R ² = 0.996)	NM	141 nm	Yes	3

	0.1-20 (R ² = NM)	NM	85 nm	NM	4
	5-500 (R ² = 0.997)	Yes	NM	NM	5
	0-10 (R ² = 0.996)	NM	90 nm	Yes	6
	2-20 (R ² = 0.997)	NM	111 nm	NM	7
	0-14 (R ² = 0.991)	NM	125 nm	NM	8
	0-10 (R ² = 0.995)	NM	161 nm	NM	9
	0-22.5 (R ² = 0.998)	Yes	213 nm	Yes	This work

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