Supporting Information

A Fluorescent Probe Based on Aggregation-Induced Emission for Hydrogen Sulfide-Specific Assaying in Food and Biological System

Lingfeng Xu,‡ Ling Ni,‡ Lihe Sun, Fang Zeng,* and Shuizhu Wu*

State Key Laboratory of Luminescent Materials & Devices, College of Materials Science & Engineering, South China University of Technology, Guangzhou 510640, China

*Corresponding author. E-mail: mcfzeng@scut.edu.cn.

E-mail: shzhwu@scut.edu.cn

‡Equal contribution.

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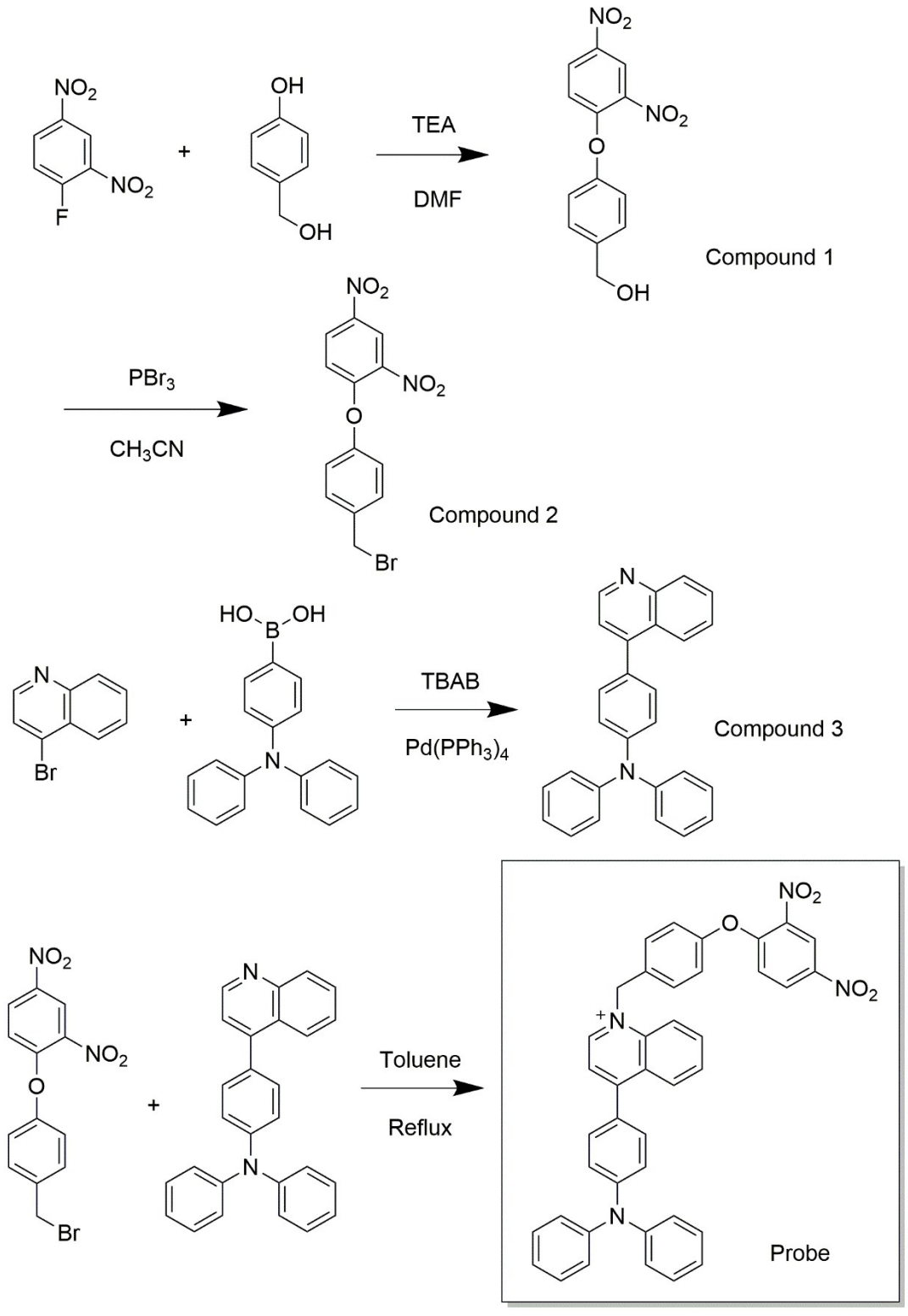


Scheme S1. The synthesis route of the probe TPANF.

1. Characterization.

The synthesized compounds were analyzed by proton nuclear magnetic resonance (^{1}H NMR) and liquid chromatography-mass spectrometrymass spectrometer (LC-MS). The ^{1}H NMR spectra were carried out by a Bruker Avance III HD 600 MHz spectrometer (Bruker Daltonics Corp., USA). MS spectra were recorded on Bruker Agilent 1290/maXis impact mass spectrometer in electrospray ionization (ESI) mode (Bruker Corp., Germany). UV-vis spectra were measured on a Hitachi U-3010 UV-vis spectrophotometer. Fluorescence spectra were obtained by a Hitachi F-4600 fluorescence spectrophotometer with a 10 mm quartz cuvette (Hitachi High-Tech Science). HPLC analyses were performed on Agilent 1260 high performance liquid chromatography (with Diode Array Detector). The particle size and distribution were determined through dynamic light scattering (DLS) through a Malvern Nano-ZS90 particle size analyzer. Imaging of living cells was performed on Olympus IX71 with a DP72 color CCD. The imaging of the xenograft tumor and main organs was performed on an AMI small animal fluorescence imaging system (Spectral Instruments Imaging Co.) with excitation filter of 430 nm and emission filter of 510 nm.

2. Synthesis.

Synthesis of (4-(2,4-dinitrophenoxy)phenyl)methanol (Compound 1).

A mixture of 2,4-dinitrofluorobenzene (186 mg, 1 mmol) and 4-hydroxybenzyl alcohol (186 mg, 1.5 mmol) in N,N-dimethylformamide (DMF) were stirred under room temperature for 60 min, then the triethylamine (Et_3N) was added dropwise, and the reaction mixture was heated at 50 °C in the dark under N_2 atmosphere for 12 h. After that, the reaction mixture was allowed to cool to ambient temperature and the solvent was removed in *vacuo*. The residue was extracted with ethyl acetate several times and dried over Na_2SO_4. The crude product was separated via silica-gel column chromatography using petroleum ether/ethyl acetate (*v/v*=1:1) as eluent to afford Compound 1 as a light yellow solid (180 mg, 62.0%). ^{1}H NMR (600 MHz, $CDCl_3$, δ ppm): 8.85 (d, J = 2.7 Hz, 1H), 8.34 (dd, J = 9.2, 2.7 Hz, 1H), 7.51 (d, J = 8.5 Hz, 2H), 7.12 (d, J = 8.6 Hz, 2H), 7.08 (d, J = 9.2 Hz, 1H), 4.52 (s, 2H). MS (ESI): *m/z* 291.3036 $[M+H]^+$.

Synthesis of 1-(bromomethyl)-4-(2,4-dinitrophenoxy)benzene (Compound 2).

Under nitrogen atmosphere, Compound 1 (290 mg, 1 mmol) was added into a 250 mL two-neck round-bottom flask and dissolved in acetonitrile (CH_3CN) (40 mL). The solution was stirred under 0 °C for 30 min, followed by the dropwise addition of phosphorus tribromide (5 equiv). After further stirring over 1 h, the mixture was reacted at ambient temperature for 12 h in the dark, then quenched with a saturated solution of $NaHCO_3$ at 0 °C, and concentrated in *vacuo*. The resulting residue was extracted with DCM. The organic layer was dried over Na_2SO_4, filtered and concentrated under vacuum. Purification by silica gel column chromatography (CH_2Cl_2: petroleum ether = 1:1) provided Compound 2 as a light yellow solid (200 mg, 56.8%). ^{1}H NMR (600 MHz, $CDCl_3$, δ ppm): 8.85 (d, J = 2.7 Hz, 1H), 8.34 (dd, J = 9.2, 2.7 Hz, 1H), 7.51 (d, J = 8.5 Hz, 2H), 7.12 (d, J = 8.6 Hz, 2H), 7.08 (d, J = 9.2 Hz, 1H), 4.52 (s, 2H). MS (ESI): *m/z* 273.0784 $[M\text{-}Br]^+$.

Synthesis of N-phenyl-N-(4-(quinolin-4-yl)phenyl)benzenamine (Compound 3).

Under nitrogen atmosphere, the 4-bromoquinoline (208 mg, 1 mmol) and 4-(diphenylamino)phenylboronic acid (435 mg, 1.5 mmol) were dissolved in the mixture of toluene (6 mL), tetrabutylammonium bromide and a few drops of tetrahydrofuran (THF) inside the Schlenk tube. The 2 M potassium carbonate aqueous solution (3 mL) was added as well. The mixture was then stirred under room temperature for 30 min, followed with the addition of tetrakis(triphenylphosphine)palladium ($Pd(PPh_3)_4$, 10 mg, 8.7×10^{-3} mmol). After that, the reaction mixture was heated to 100 °C for 24 h under N_2. After the solvent of mixture was removed under vacuum, the resultant was poured into water and extracted with ethyl acetate three times. The organic layer was dried over anhydrous Na_2SO_4 and concentrated *via* rotary evaporation. In the end, the product was purified by column chromatography on silica gel using dichloromethane/petroleum ether (1/2, v/v) as the eluent to give a dark yellow solid Compound 3 (234 mg, 65.0%). ^{1}H NMR (600 MHz, $CDCl_3$, δ ppm): 8.90 (d, J = 4.4 Hz, 1H), 8.17 (d, J = 8.4 Hz, 1H), 8.05 (d, J = 7.6 Hz, 1H), 7.70 (ddd, J = 8.3, 6.8, 1.3 Hz, 1H), 7.49 (ddd, J = 8.2, 6.8, 1.2 Hz, 1H), 7.36 (d, J = 8.6 Hz, 2H), 7.33-7.30 (m, 2H), 7.30-7.26 (m, 3H), 7.21-7.16 (m, 6H), 7.06 (t, J = 7.3 Hz, 2H). MS (ESI): *m/z* 373.1707 $[M+H]^+$.

Synthesis of the probe 1-(4-(2,4-dinitrophenoxy)benzyl)-4-(4-(di-phenylamino)phenyl)quinolin-1-ium (TPANF).

Compound 2 (35.2 mg, 0.1 mmol) and Compound 3 (37.2 mg, 0.1 mmol) were dissolved in toluene, and then the mixture was refluxed at 120 °C for 12 h. The solvent was evaporated under nitrogen atmosphere, then the obtained red product was filtered, washed with toluene and dried in *vacuo*. Purified by silica gel column chromatography (CH_2Cl_2/methanol = 10/1) as the eluent to afford dark red solid (the probe) (21.2 mg, 29.3%). ^{1}H NMR (600 MHz, CD_3OD, δ ppm): 9.40 (d, *J* = 6.2 Hz, 1H), 8.86 (d, *J* = 2.7 Hz, 1H), 8.53 (dd, *J* = 24.1, 9.2 Hz, 2H), 8.42 (dd, *J* = 9.2, 2.8 Hz, 1H), 8.23-8.16 (m, 1H), 8.12 -8.06 (m, 1H), 8.01-7.96 (m, 1H), 7.65 (d, *J* = 8.8 Hz, 2H), 7.55 (d, *J* = 8.6 Hz, 2H), 7.40 (t, *J* = 7.9 Hz, 4H), 7.28-7.16 (m, 11H), 6.33 (s, 2H). MS (ESI): *m/z* 645.2136 $[M-Br]^+$.

3. Cell culture.

The cell culture procedures were similar to the literature reports[1,2] with slight modifications. The cell lines, L929 (mouse fibroblasts cell) and HeLa cells (human cervical cancer cell) were incubated in complete medium composed of 89% DMEM medium with 10% fetal bovine serum (FBS) and 1% penicillin & streptomycin. Another cell line, HCT-116 cells (human colon cancer cell) were incubated in complete McCoy's 5A medium containing fetal bovine serum and 1% penicillin & streptomycin. All these cell lines were grown at 37 °C in a humidified incubator containing 5% CO_2. The medium was changed approximately every day.

4. Cytotoxicity Assay.

The test procedures were similar to the previous studies[3,4] with slight modification. To evaluate the cytotoxicity of the probe TPANF, the cell viability was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay according to ISO 10993-5. In detail, L929 cells and HeLa were seeded in 96-well plates, and incubated in the DMEM medium supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin and streptomycin), allowed to grow for 24 h at 37 °C with 5% CO_2. After that, the medium was removed and washed with PBS, cells were incubated with the medium with various concentrations (0, 1, 5, 10, 20, 50 μM) of the probe TPANF for additional 24 h. Then the

probe-containing medium was replaced by fresh medium containing 0.5 mg/mL MTT each well for 4 h. Then the medium was replaced by DMSO, and shaken gently to dissolve the formazan crystals for Thermo MK3 ELISA Microplate Reader analysis. The absorbance was recorded at 570 nm. The assays were performed in eight replicates, and the statistic mean and standard derivation were used for data estimation of cell viability. The viability of HCT-116 were operated in a similar program with the McCoy's 5A complete medium.

5. Flow Cytometry Analysis.

To evaluate the enhancement of intracellular fluorescence intensity, the flow cytometric analysis was performed. The HCT-116 cells were seeded into a six-well plate at the density of 1×10^5 cells/mL. After incubation in the McCoy's 5A complete medium at 37 °C in a 5% CO_2/95% air incubator for 24 h, the cell culture medium was discarded, and fresh culture medium which containing the probe TPANF (20 μM, 1% DMSO as the co-solvent) was then added. After the incubation for another 0, 30 or 60 min, cells were washed with PBS (3 × 2 mL/dish) prior to the flow cytometry analysis. Similarly, the HeLa cells were seeded into six-well cell culture plate (1×10^5 cells/mL). After incubation in the DMEM complete medium for 24 h at 37 °C in a 5% CO_2/95% air incubator, the cell culture medium was discarded. Followed with the cells stained with fresh culture medium containing TPANF (20 μM, 1% DMSO as the co-solvent) for another 0, 30 or 60 min, the cells were washed, trypsinized, centrifuged and dispersed into a single cell suspension (in PBS). Then, around 10000 cells were performed to analysis.

6. HPLC analysis.

HPLC analysis was performed on Agilent 1260 liquid chromatography (Agilent Technologies, Palo Alto, USA) with DAD detector. The mobile phase was methanol/H_2O/acetonitrile (v/v 89:10:1) at a flow rate of 1 mL/min. The UV absorption wavelength at 360 nm was set for analysis.

7. Determination of the detection limit.

The detection limit was determined by using the method similar to literature report.[5] The calibration curve was first obtained from the plot of fluorescence intensity at 468 nm (I_{468}),

the regression curve equation was then obtained for the lower concentration part. And the detection limit = $3 \times \sigma/k$. Where k is the slope of the curve equation, and σ represents the standard deviation for I_{468} in the absence of NaHS.

$I_{468} = 3.6339 + 4.9383[NaHS]$ $(R^2=0.9903)$

LOD = 3 × 0.2880 / 4.9383 = 0.17 μM.

8. Evaluation of the biosafety of the probe in mouse model.

To demonstrate the bio-safety of probe TPANF, the tissue histopathology was conducted with similar procedures[6] with slight modification. Mice were housed under SPF conditions and fed with autoclaved chow and water ad libitum, the body weights of mice were collected each day within 7 days in total. The mice were divided into two groups, and for each group, five mice were tested. Among them, one group was injected with the probe TPANF (1.6 mg probe/kg body weight, in PBS containing 1% DMSO) through the tail vein, another control group was injected into the tail vein with the same volume of saline. The mice were humanly euthanized via exposure to carbon dioxide gas in a rising concentration, and the main organs were excised through dissection operation and flushed with sterile PBS solution. After that, main organs were placed in the formalin solution for paraffin embedding, hematoxylin and eosin (H&E) staining; and the imaging was carried out to observe the histological sections.

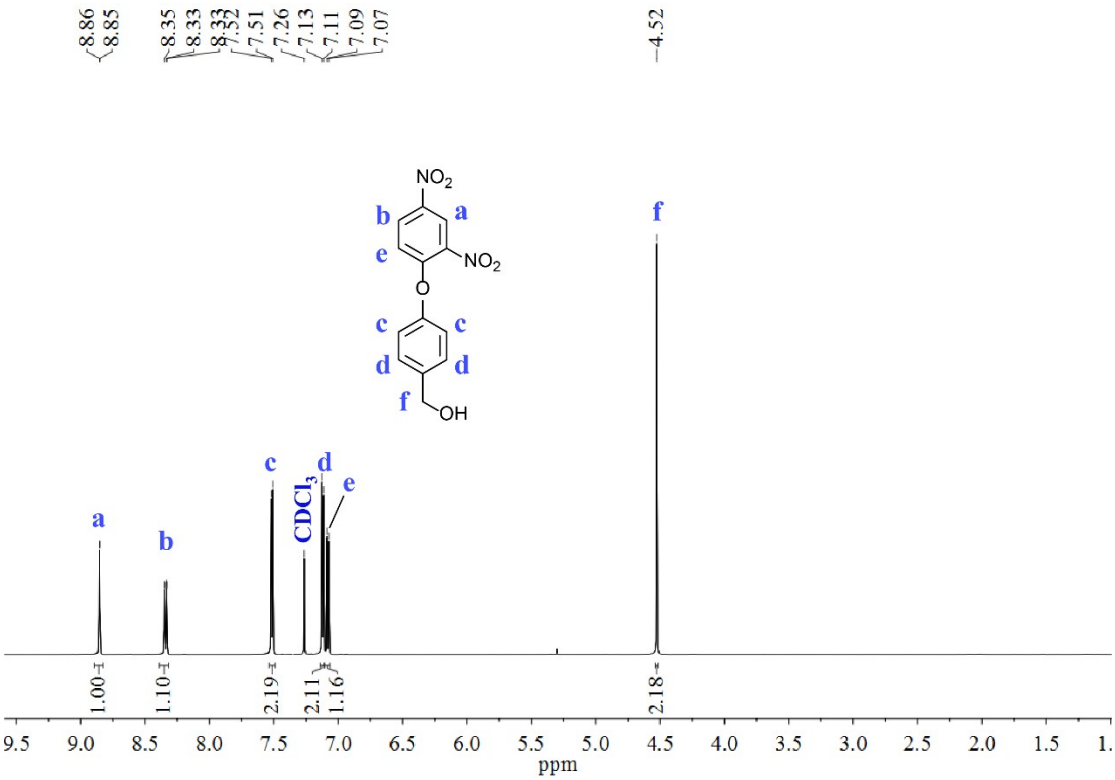


Fig. S1 ^{1}H-NMR spectrum of compound **1** in $CDCl_3$.

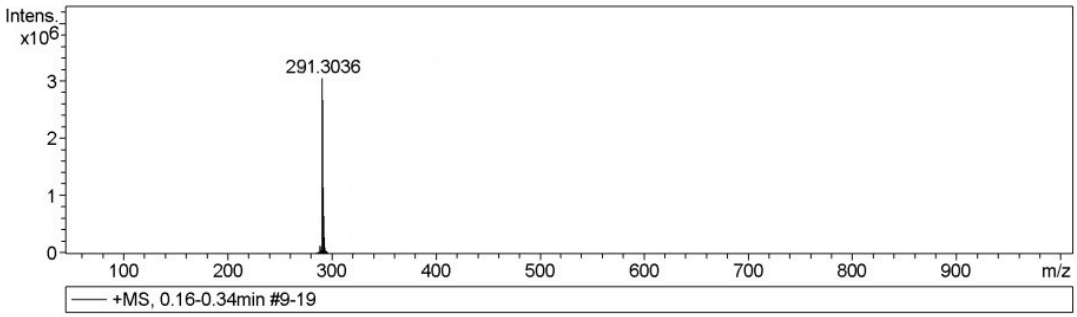


Fig. S2 Mass spectrum of compound **1**. MS (ESI): *m/z* 291.3036 $[M+H]^+$.

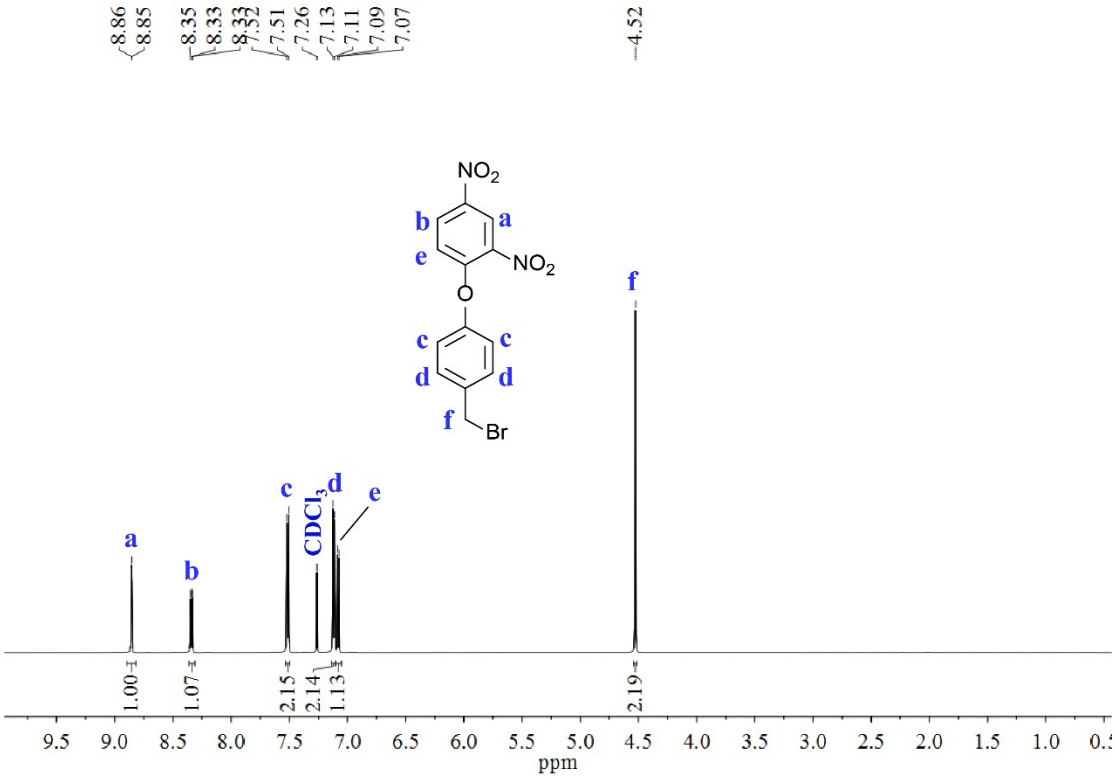


Fig. S3 ^{1}H-NMR spectrum of compound **2** in $CDCl_3$.

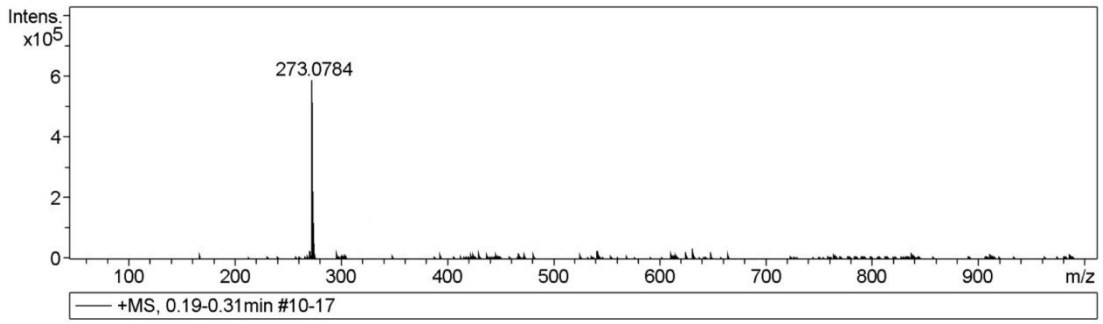


Fig. S4 Mass spectrum of compound **2**. MS (ESI): *m/z* 273.0784 $[M-Br]^{+}$.

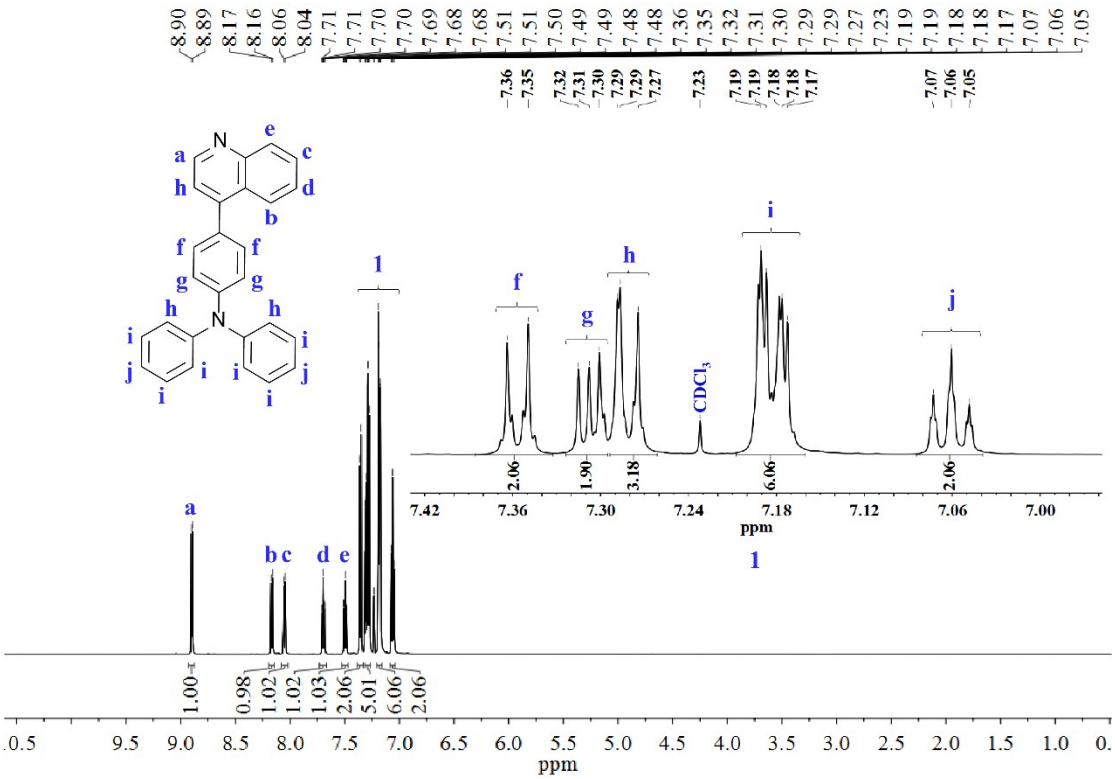


Fig. S5 ^{1}H-NMR spectrum of compound **3** in $CDCl_3$.

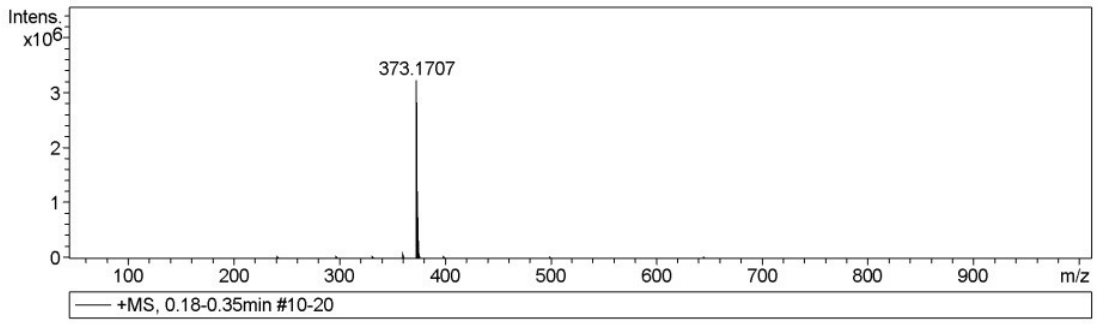


Fig. S6 Mass spectrum of compound **3**. MS(ESI): *m/z* 373.1707 $[M+H]^+$.

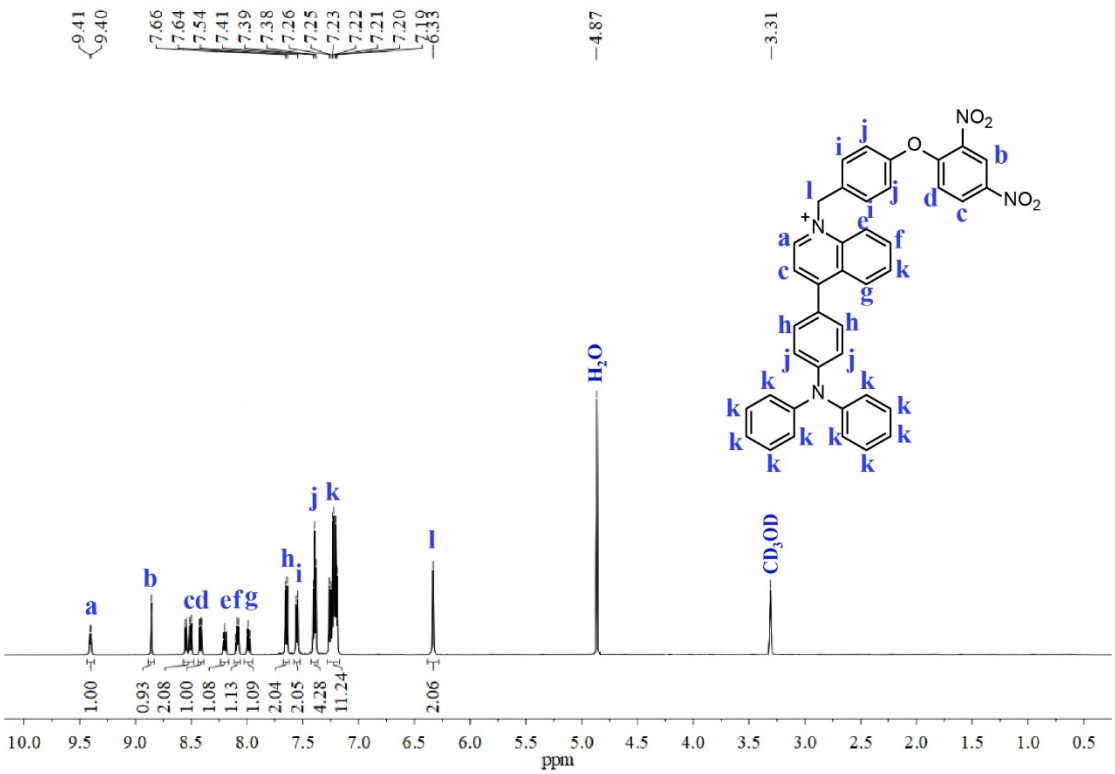


Fig. S7 ^{1}H-NMR spectrum of probe TPANF in CD_3OD.

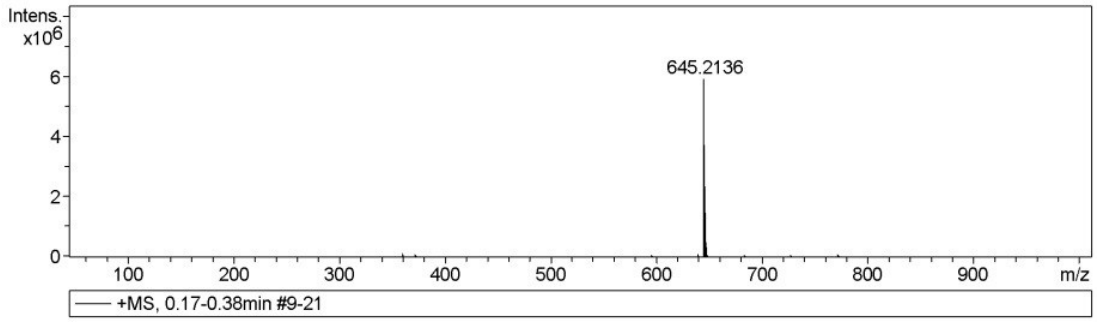


Fig. S8 Mass spectrum of probe TPANF. MS (ESI): *m/z* 645.2136 $[M\text{-}Br]^{+}$.

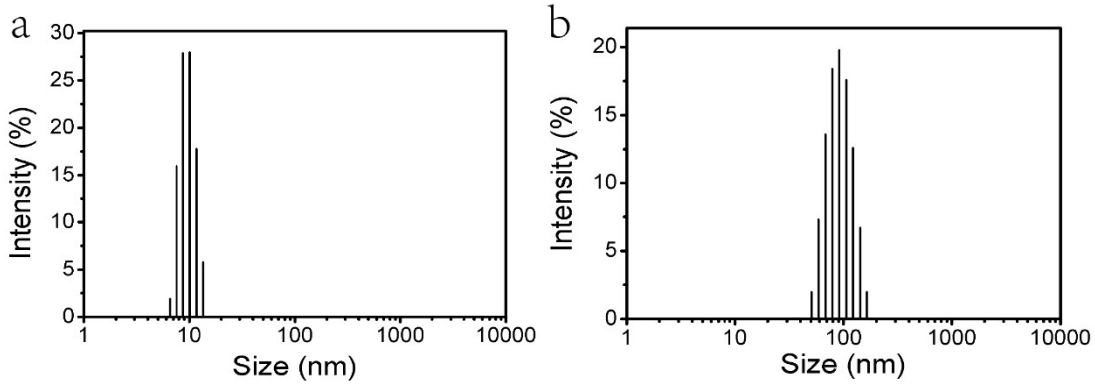


Fig. S9 Size distribution of the probe solution determined using dynamic light scattering (DLS) method without (a) and with (b) incubation with NaHS (100 μM) for 30 min.

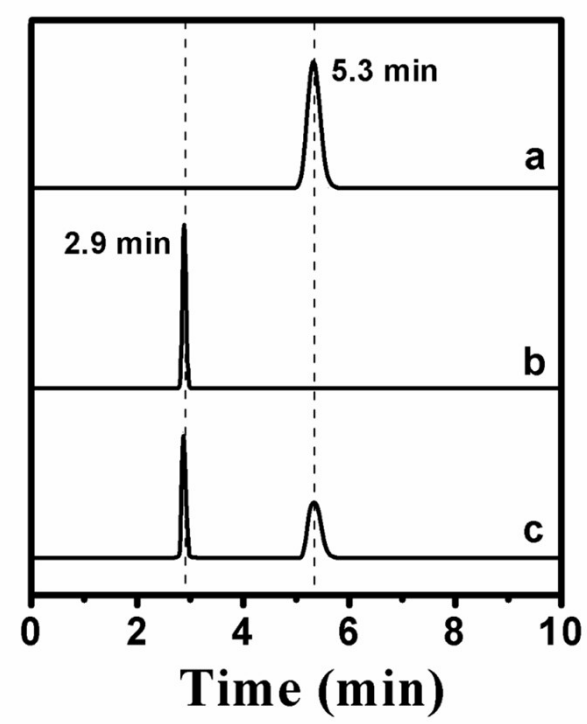


Fig. S10 Typical HPLC chromatogram of Compound 3 (a), the probe (b), and the reaction solution after the reaction between the probe and NaHS for 15 min (c) from the 360 nm channel. And the mobile phase is methanol/H_2O/acetonitrile (v/v 89/10/1).

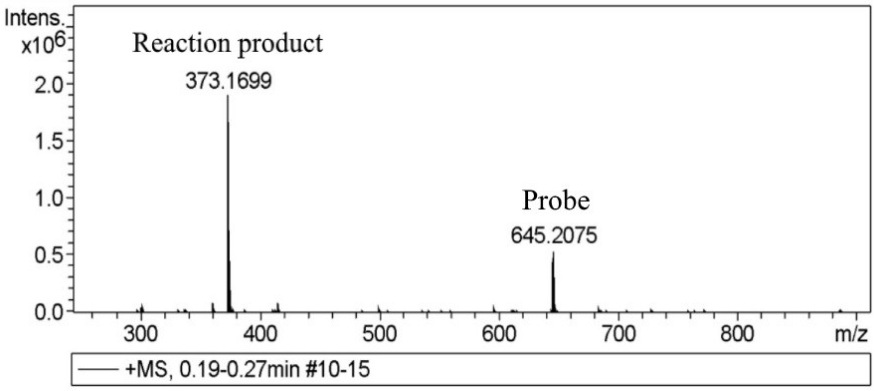


Fig. S11 Mass spectrometry analysis ($[M+H]^+$) of the assay system after the reaction between probe and NaHS in methanol. The reaction time was 15 min.

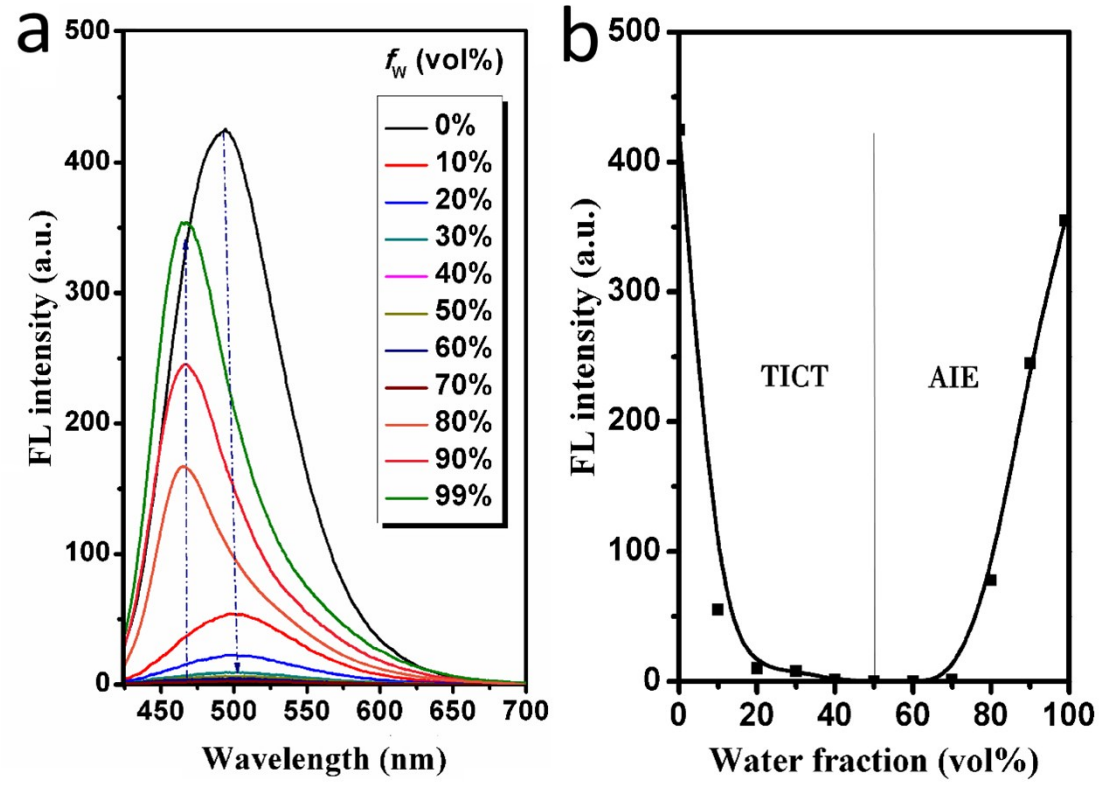


Fig. S12 Fluorescence spectra of TPAQ (10 μM) in the THF/water mixture (f_w represents the water fraction), λ_{ex} = 390 nm.

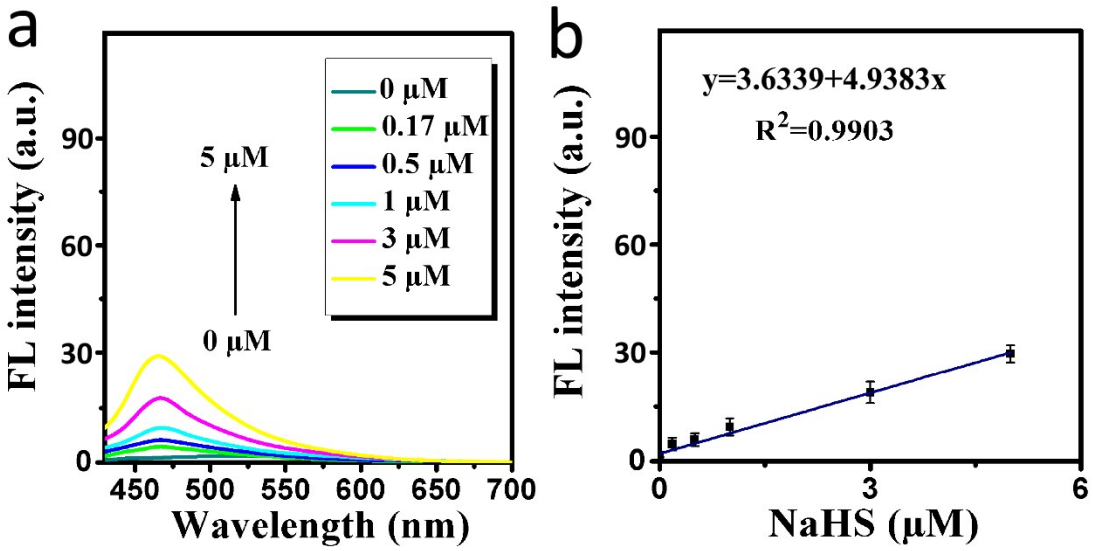


Fig. S13 (a) Fluorescence spectrum of the probe TPANF (10 μM) response to NaHS at varied concentrations (0, 0.18, 0.5, 1, 3, 5 μM). (b) A linear correlation between emission intensity and concentration of NaHS.

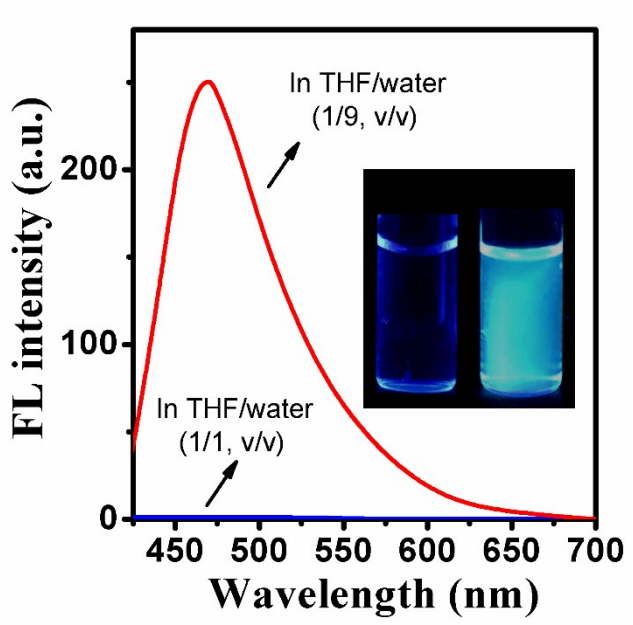


Fig. S14 Fluorescent spectra of the probe TPANF (10 μM) exposed to NaHS in the THF/water (1:1, v/v) and the THF/water (1:9, v/v) mixture solutions separately. And the corresponding fluorescent images taken under the excitation of hand-held ultraviolet lamp (365 nm).

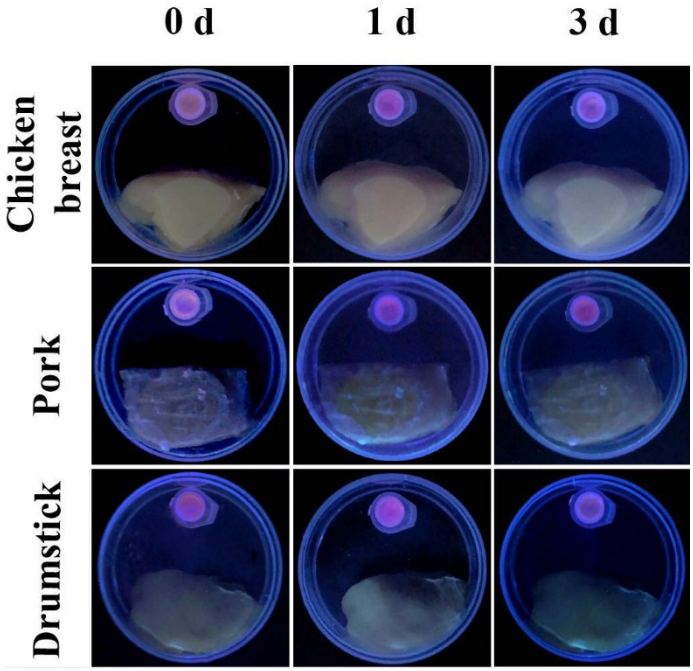


Fig. S15 Fluorescence images of the probe test solution acting as the gas indicator after the chicken breat, pork, drunstick were placed at 4 °C in the refrigerator within 0 d-3 d. A hand-held UV lamp was used to take the fluoresecence images.

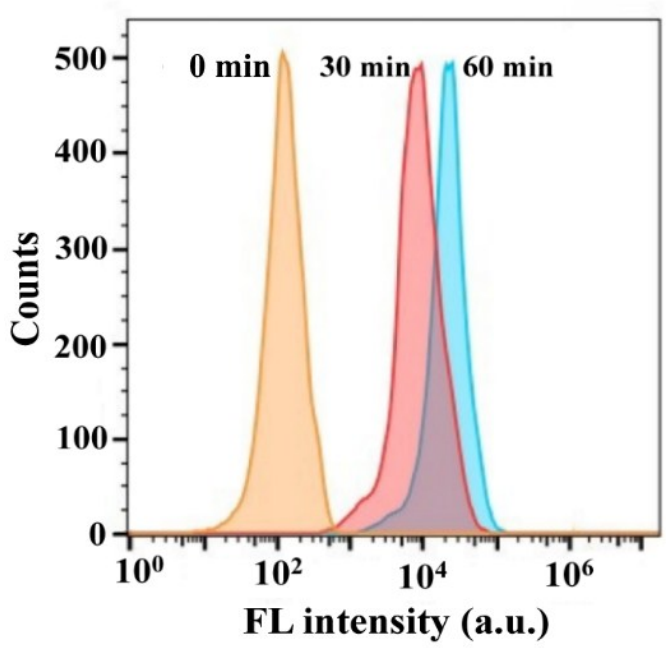


Fig. S16 Flow cytometric analysis for HeLa cells after being incubated with the probe (20 μM) for 0, 30 or 60 min.

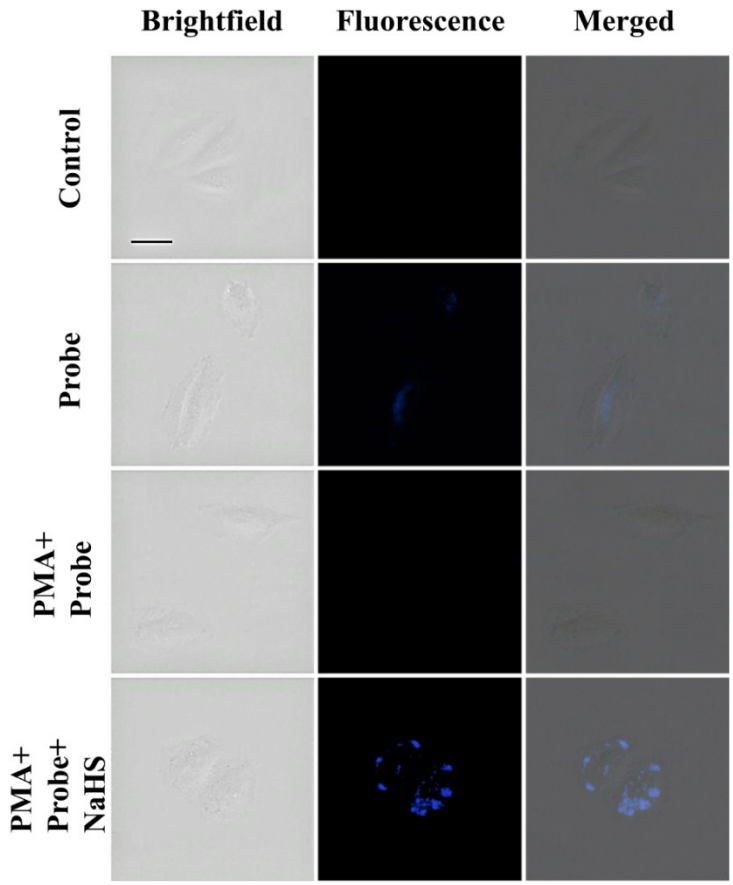


Fig. S17 Fluorescence microscopy images of HeLa cells incubated without the probe, with the probe for 30 min, with the phorbol 12-myristate 13-acetate (PMA, one kind of H_2S inhibitor) pretreated for 30 min and then the probe for 30 min, or with the inhibitor PMA for 30 min and then the probe for 30 min and afterwards with the exogenous NaHS. The probe concentration was 10 μM, the inhibitor concentration was 1 μg/mL, the addition of exogenous NaHS concentration was 50 μM. The scale bar was 20 μm.

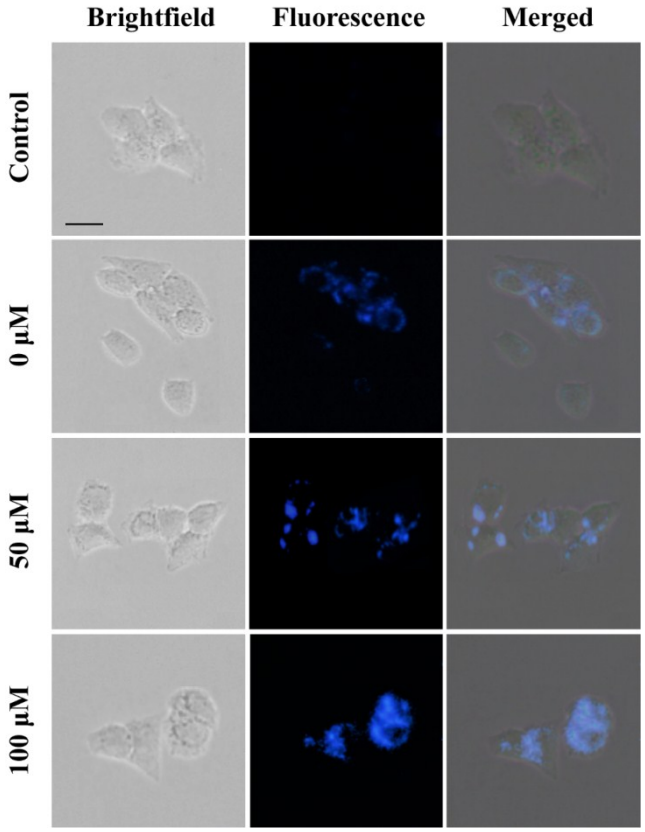


Fig. S18 Fluorescence images of untreated HCT-116 cells (control), and HCT-116 cells pretreated with the probe (10 μM) and then incubated with NaHS at concentration of 0 μM, 50 μM, and 100 μM respectively. Scale bar was 20 μm.

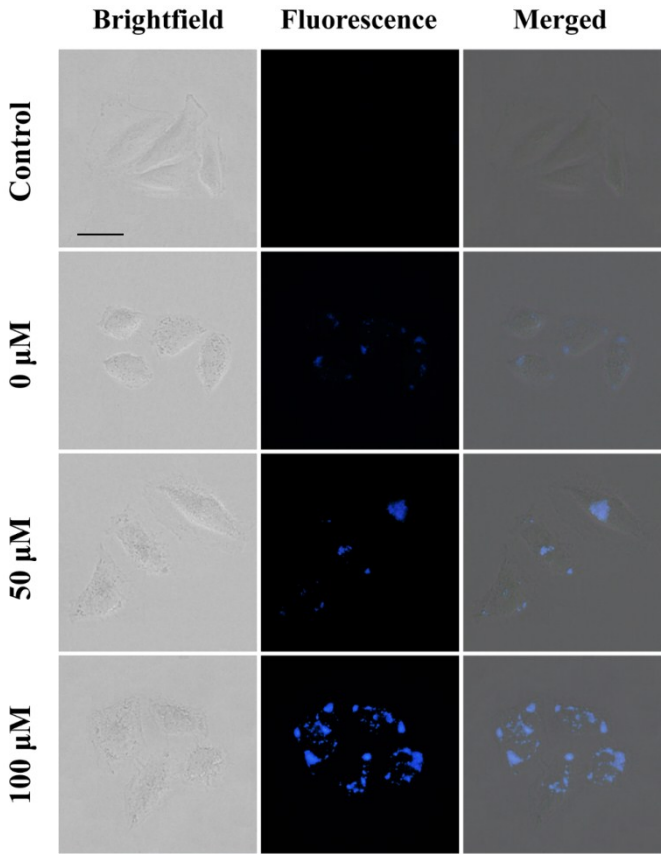


Fig. S19 Fluorescence images of untreated HeLa (control), and HeLa cells pretreated with the probe (10 μM) and then incubated with NaHS at concentration of 0 μM, 50 μM, and 100 μM respectively. Scale bar was 20 μm.

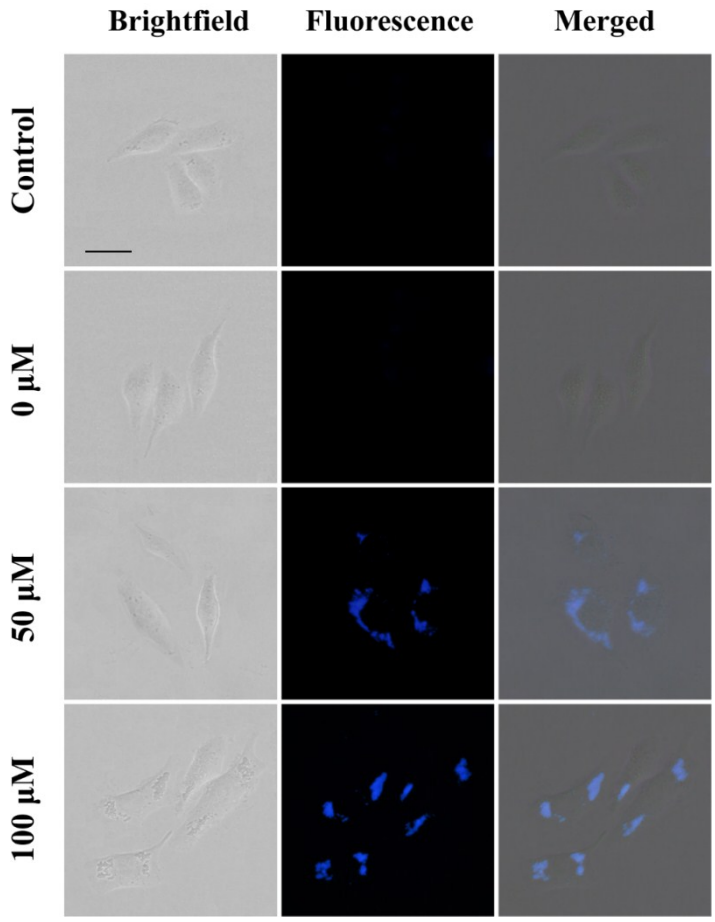


Fig. S20 Fluorescence images of untreated L929 cells (control), and L929 cells pretreated with the probe (10 μM) and then incubated with NaHS at concentration of 0 μM, 50 μM, and 100 μM respectively. Scale bar was 20 μm.

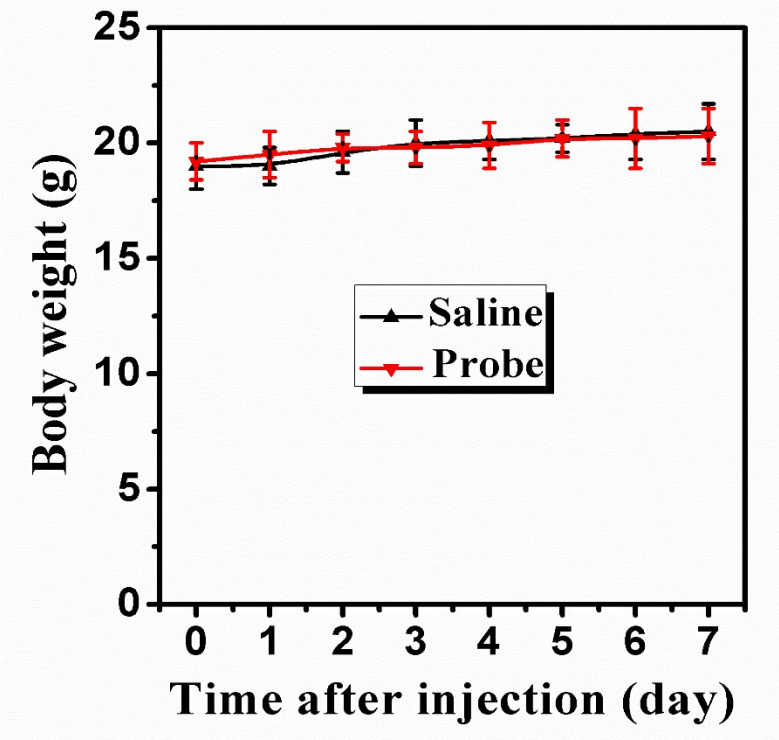


Fig. S21 Time-dependent changes in body weights of healthy mice after the mice were injected through tail vein with the probe or saline for different time (n=5 per group). Error bars represent the standard deviation (SD).

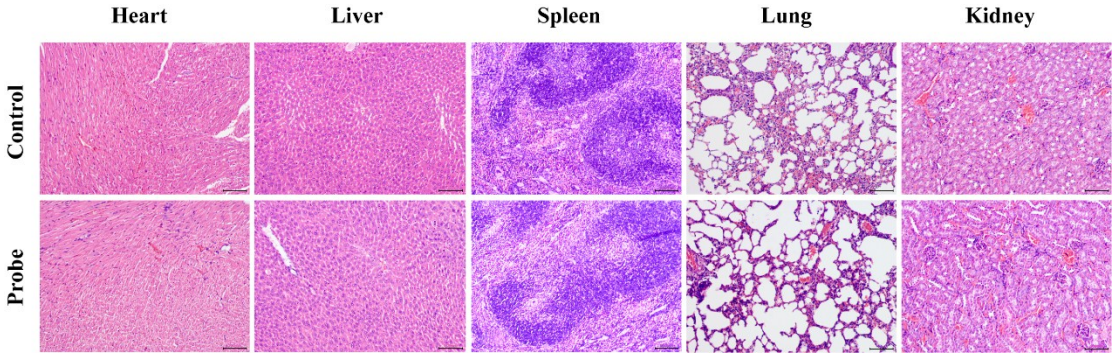
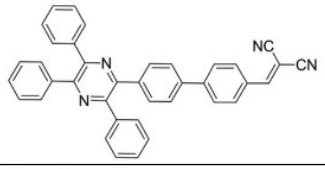
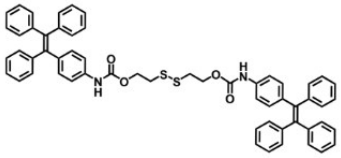
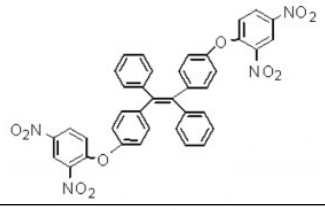
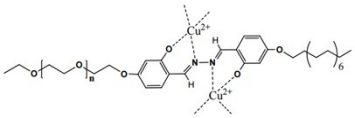
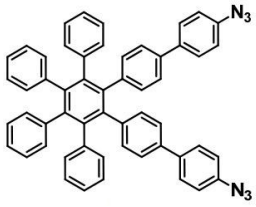
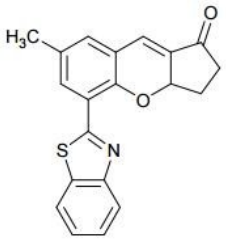
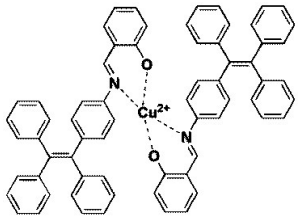
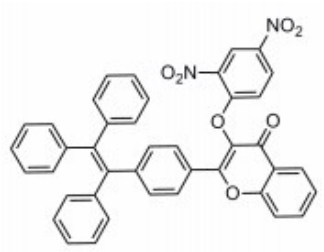
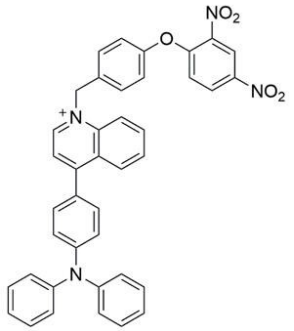


Fig. S22 Representative histological sections (H&E staining) for main organs of the healthy mice one day after intravenous injection of the saline (control) or the probe TPANF. Scale bar: 100 μm.

Table S1 Comparison of the representative AIE-based probes for H_2S assaying.

Probe	Response type	λ_{em}	Detection limit	Applica-tion	Referen-ce
	Ratiometric	429 nm	0.5 μM	Solid fluoresce-ence	7
	Turn-on	480 nm	84 nM	Living cells	8
	Turn-on	480 nm	12.8 nM	Living cells; C. elegans	9
	Turn-on	535 nm	/	Living cells	10
	Turn-on	465 nm	0.33 μM	Living cells; Blood serum	11
	Turn-on	540 nm	41 nM	Living cells	12
	Turn-on	533 nm	/	Living cells	13

	Turn-on	550 nm	0.09 μM	Living cells; Zebrafish	14
	Turn-on	470 nm	0.17 μM	Raw meat; Living cells; Tumor tissues	This work

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