Supporting Information

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

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Scheme S1. The synthesis route of the probe TPANF.

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/mAxs 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$_3$N) 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$_2$SO$_4$. 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]$^+$. 

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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$_3$CN) (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$_2$SO$_4$, filtered and concentrated under vacuum. Purification by silica gel column chromatography (CH$_2$Cl$_2$: 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-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$_2$SO$_4$ 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]$^+$. 

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Synthesis of the probe 1-(4-(2,4-dinitrophenoxy)benzyl)-4-(4-(diphenylamino)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$_2$Cl$_2$/methanol = 10/1) as the eluent to afford dark red solid (the probe) (21.2 mg, 29.3%). $^1$H NMR (600 MHz, CD$_3$OD, 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]$^+$.  


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.


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 $1 \times 10^5$ 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 ($1 \times 10^5$ 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$_2$O/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. 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 $\sigma$ represents the standard deviation for $I_{468}$ in the absence of NaHS.

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

$\text{LOD} = 3 \times 0.2880 / 4.9383 = 0.17 \mu\text{M}.$

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 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 ¹H-NMR spectrum of compound 2 in CDCl₃.

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$_3$OD.

Fig. S8 Mass spectrum of probe TPANF. MS (ESI): $m/z$ 645.2136 [M-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/H₂O/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), $\lambda_{ex} = 390$ 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 breast, pork, drumstick were placed at 4 °C in the refrigerator within 0 d-3 d. A hand-held UV lamp was used to take the fluorescence 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$_2$S 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 H₂S assaying.

<table>
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<th>$\lambda_{em}$</th>
<th>Detection limit</th>
<th>Application</th>
<th>Reference</th>
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This work
References