Electronic Supplementary Material (ESI) for Biomaterials Science.


# Supplementary Material

# A new lysosome-targetable fluorescent probe for detection of endogenous hydrogen polysulfides in living cells and inflamed mouse model

Li Liang[a‡], Weiqing Li[a‡], Jinrong Zheng[a], Ruixi Li[a], Haiyan Chen[a*], Zhenwei Yuan[a*]

[a] *Department of Biomedical Engineering, School of Engineering, China Pharmaceutical University, 24 Tongjia Lane, Gulou District, Nanjing 210009, China*

*Authors for correspondence.

Zhenwei Yuan, PhD

Email: yuanzhenwei@cpu.edu.cn

Haiyan Chen, PhD

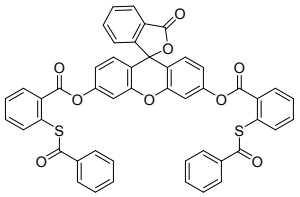
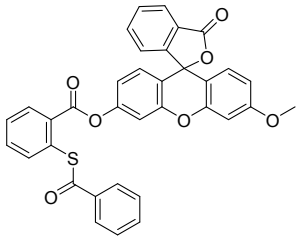
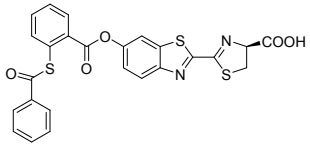
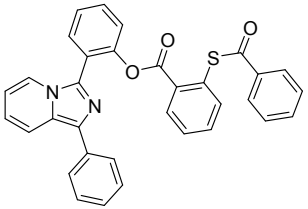
Email: chenhaiyan@cpu.edu.cn

Tel: +86-25-83271080

Fax: +86-25-83271046

‡ These authors contributed equally.

**Table S1. Comparison of fluorescent probes for $H_2S_n$.**

| Probes | Fluorescence recovery | Stokes shift (nm) | Limit of detection | Response time | Analytical applications | Reference |
|---|---|---|---|---|---|---|
|  | About 4-fold | 25 | 3 nM | 10 min | Living cells | Angew. Chem. Int. Ed. **2015** [1] |
|  | About 4.7-fold | 27 | 2 nM | 5 min | Living cells and Zebrafish | Chem. Sci., **2018** [2] |
|  | 7.3-fold | 270 | 30 nM | 180 min | Living cells and mouse | Chem. Commun., **2019**.[3] |
|  | **20.3-fold** | **178** | **1 nM** | **1min** | **Living cells and organism** | **This work** |

## 1. Materials and instruments

2-Benzoylpyridine, salicylaldehyde, ammonium acetate, glacial acetic acid, thiosalicylic acid, benzoyl chloride, $NaHCO_3$, $Na_2CO_3$, N,N′-Dicyclohexylcarbodiimide, 4-Dimethylaminopyridine, and hexadecyl trimethyl ammonium bromide (CTAB) are purchased from Bidepharm (Shanghai, China) and utilized without further purification. The commercial lysosomal dye (LysoRed, KGMP006) and the commercial mitochondria dye (MitoGreen, KGMP0072) were obtained from KeyGen Biotech (Nanjing, China). LPS are purchased from Shanghai yuanye Bio-Technology Co., Ltd (Shanghai, China). Thin-layer chromatography (TLC) on 0.25 mm silica gel plates with a fluorescent indicator (GF254) visualized under UV light was used to monitor the reactions. Distilled water was prepared through a water purification system. $^{1}H$ NMR and $^{13}C$ NMR spectra were recorded using a Bruker AV-500 instrument (Bruker, USA) with tetramethylsilane (TMS) as an internal standard. High-resolution mass spectra (HRMS) were performed on an LCMS-2010A instrument (Shimadzu, Japan). High-performance liquid chromatography (HPLC) analysis was used the Agilent 1260. UV-vis and fluorescence spectra were recorded on a Shimadzu UV-Vis spectrophotometer, UV-2550 and Shimadzu Instruments Spectrofluorometer, RF-6000, respectively.

## 2. General procedure for fluorometric analysis

All commercial chemicals and solvents are reagent grade and were used without further treatment unless otherwise noted. In *vitro* spectroscopy of the probe **PP-PS** (10 μM) was performed in phosphate buffered saline (PBS, 10 mM, pH 7.4, containing 1 mM CTAB). In this work, CTAB was employed in the detection buffer to accelerate the response. The acceleration effect of CTAB is similar with that of previous reports which may be resulted from two reasons: first, CTAB can increase the solubility of PP-PS; second, the cationic CTAB may absorb the polysulfide anion to facilitate the reaction [4,5]. After incubating the test solution for 30 minutes at room temperature, all fluorescence was measured at an excitation wavelength of 300 nm (slit width: 5 nm / 5 nm) unless otherwise specified. Besides, $Na_2S_2$ and various other analytes were dissolved in ultrapure water to prepare a standard solution.

## 3 Cell cytotoxicity evaluated by MTT assay

A549 cells (adenocarcinomic human alveolar basal epithelial cells), MCF-7 cells (human breast adenocarcinoma cells), and U87 cell (human glioma cells) were purchased from American Type Culture Collection (ATCC, USA). The cell cytotoxicity was evaluated in three cell lines (A549, MCF-7, and U87 cells) by an MTT assay. The cells were seeded into 96-well cell culture plates ($1 \times 10^5$/well) and subsequently incubated in $CO_2$ culture box for 24 h. The cells were retained at 37 °C under 5 % $CO_2$ for 24 h after incubation with **PP-PS** at different concentrations (5 μM, 10 μM, 20 μM, 50 μM, 100 μM, 250 μM, 500 μM, 750 μM and 1000 μM). And then the cells were incubated with MTT solution (20 μL, 5.0 mg $mL^{-1}$) for another 4 h. DMSO (150 μL/well) was further incubated with cells to dissolve the

precipitated formazan violet crystals at 37 °C for 20 min. The absorbance was measured at 490 nm by a multi-detection microplate reader. The following formula was used to calculate the viability of cell growth: viability (%) = (mean absorbance of test wells - mean absorbance of medium control wells) / (mean absorbance of untreated wells - mean absorbance of medium control well) × 100 %.

**4. Fluorescence quantum yield measurement**

The quantum yield of the compound was calculated according to the equation:

$$\Phi_{\mathbf{u}} = \Phi_s \frac{I_u}{I_s} \times \frac{A_s}{A_u} \times \left( \frac{n_u}{n_s} \right)^2$$

$\Phi$ denotes the quantum yield; $I$ denotes the area under the fluorescence curve; $A$ denotes the absorbance at the excitation wavelength; $n$ denotes the refractive index of the solvent. Quinine sulfate ($\Phi$ = 0.542 in 0.05 M sulfuric acid solution) was used as the reference standard.

**5. The limit of detection measurement**

The detection limit of **PP-PS** towards $Na_2S_2$ was determined through fluorescence titration, which was measured 5 times. The standard deviation of the blank solution was also measured for 20 times. After the linear slope of fluorescence intensity vs. concentrations of $Na_2S_2$ was obtained, the detection of limit (LOD) was calculated by following equation:

$$LOD = 3\sigma / \kappa$$

Where σ denotes the standard deviation of blank measurement, κ denotes the slope of the fluorescence intensity vs. $Na_2S_2$ concentrations.

## 6. $^{1}$H NMR spectrum of PP-OH

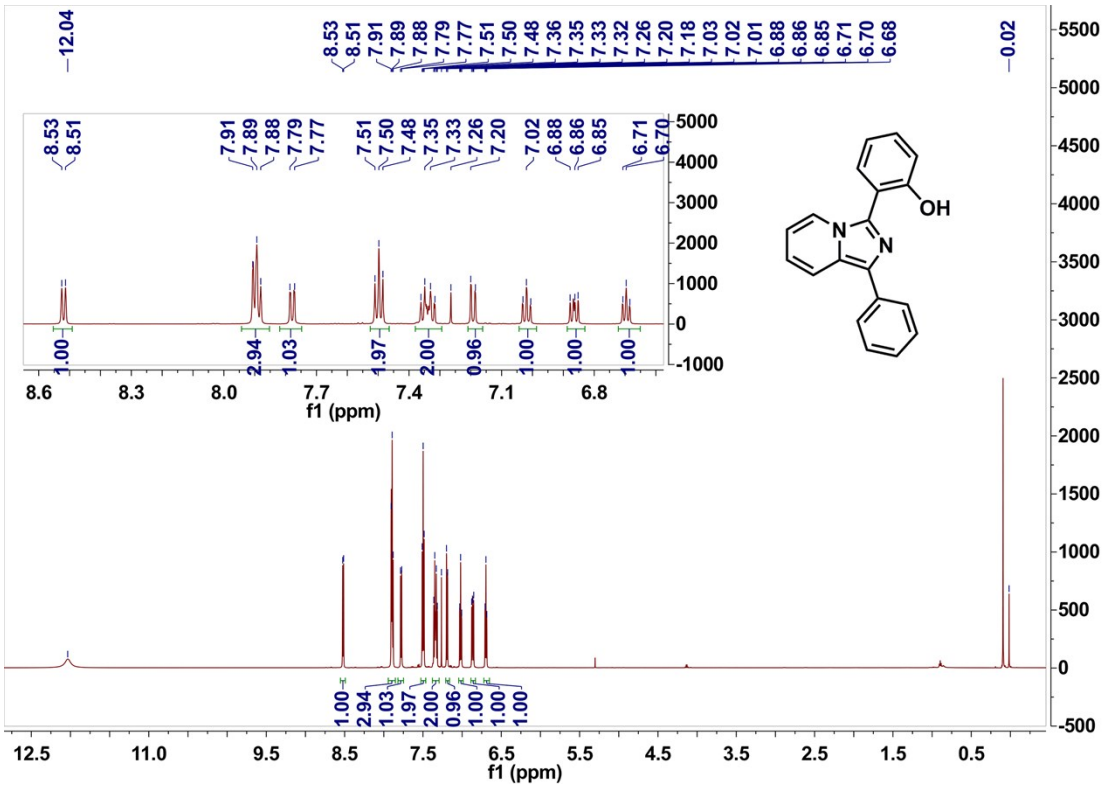



**Fig. S1** $^{1}$H NMR spectrum of **PP-OH**.

## 7. $^{13}$C NMR spectrum of PP-OH

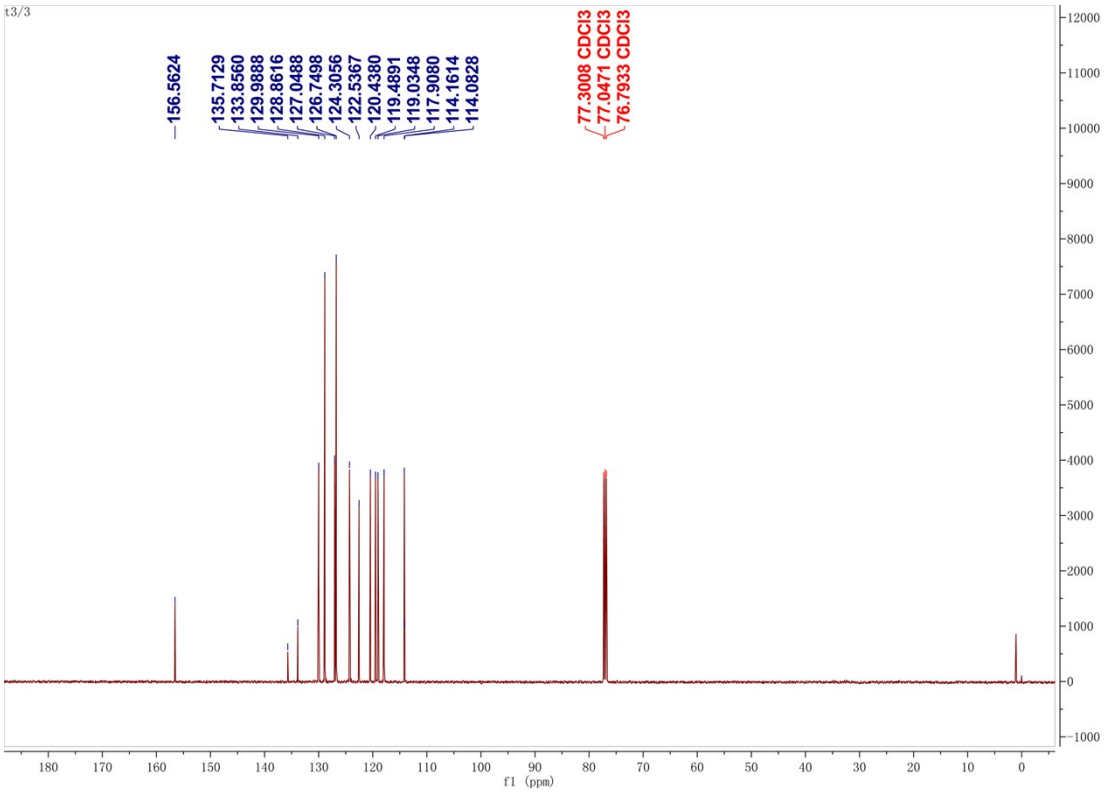



**Fig. S2** $^{13}$C NMR spectrum of **PP-OH**.

## 8. HRMS spectrum of PP-OH

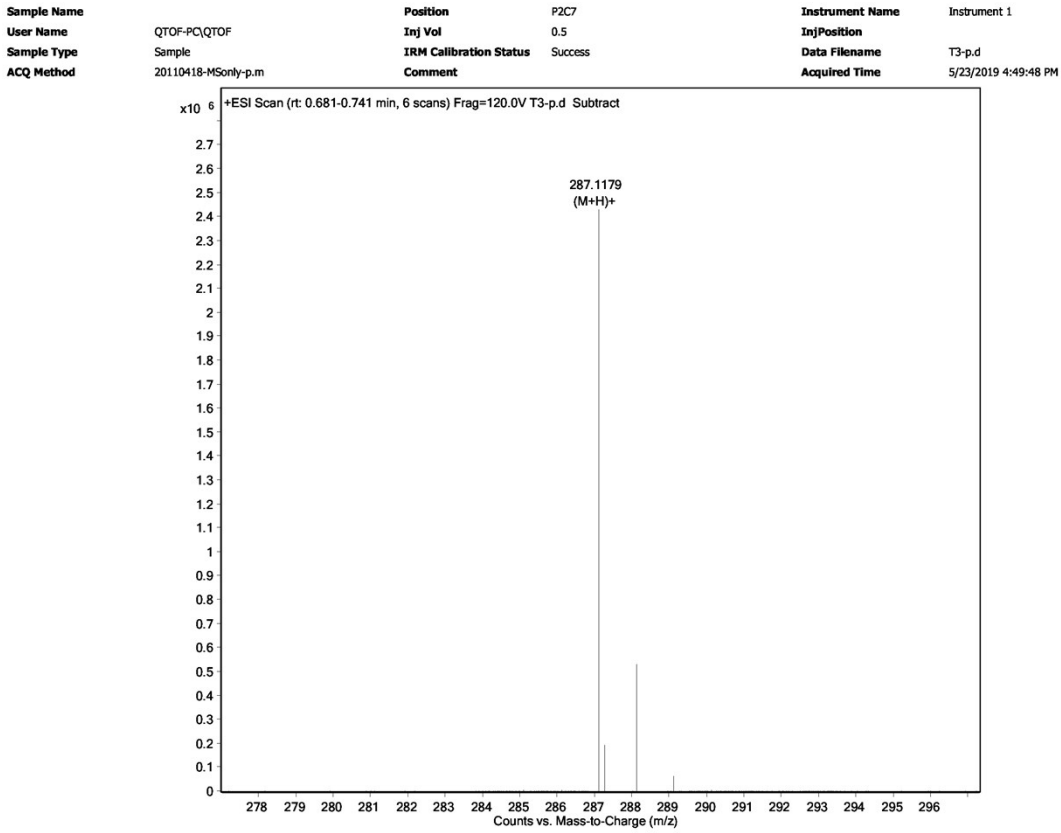



**Fig. S3** HRMS spectrum of **PP-OH**.

## 9. $^{1}$H NMR spectrum of 2-Benzoylsulfanyl-benzoic acid

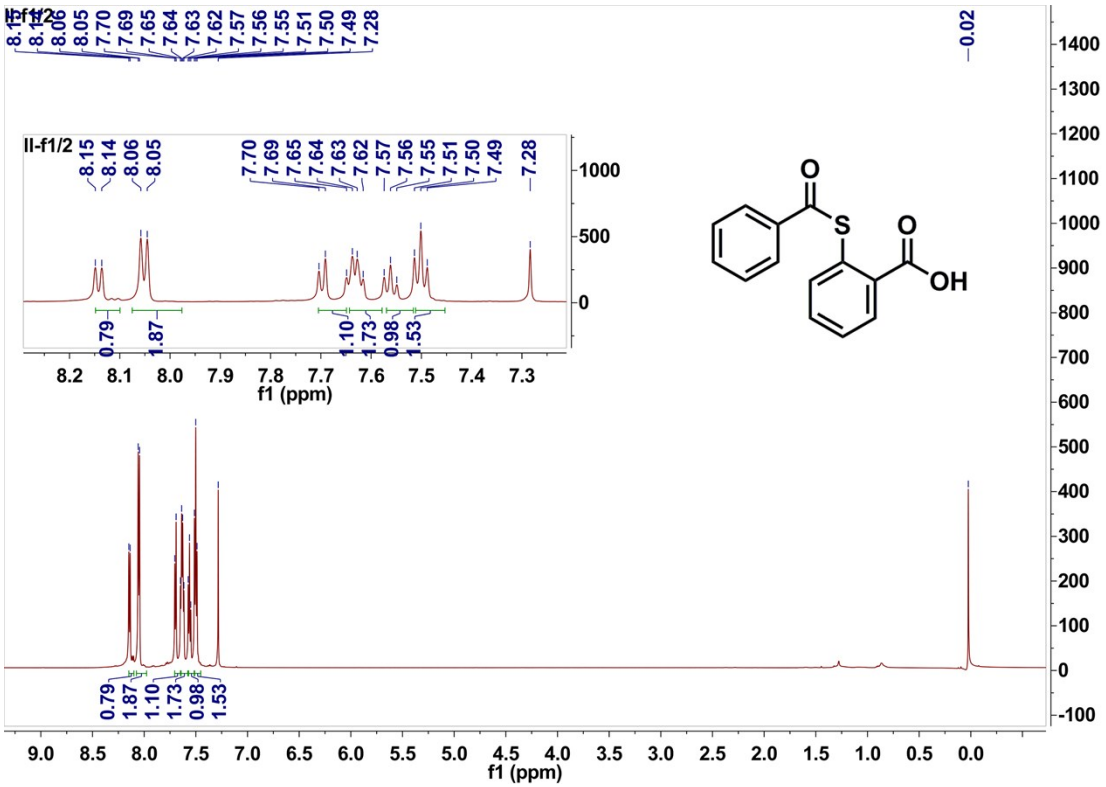



**Fig. S4** $^{1}$H NMR spectrum of **2-Benzoylsulfanyl-benzoic acid**.

## 10. $^{1}$H NMR spectrum of PP-PS

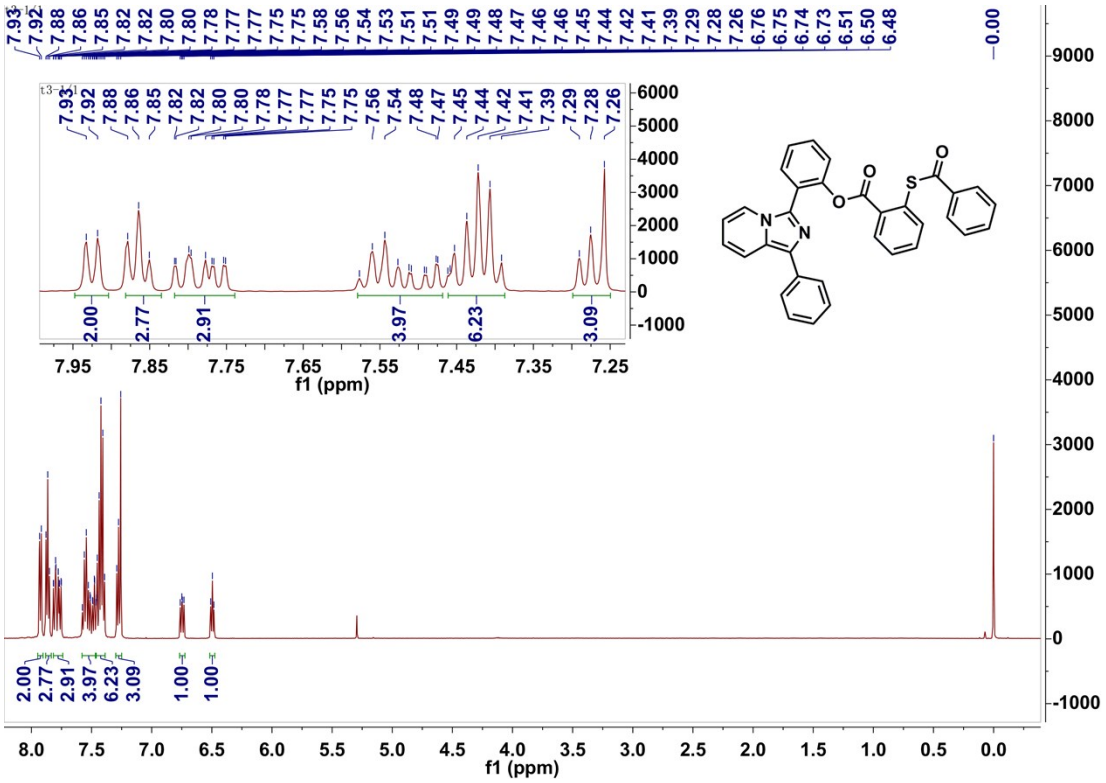



**Fig. S5** $^{1}$H NMR spectrum of **PP-PS**.

## 11. $^{13}$C NMR spectrum of PP-PS

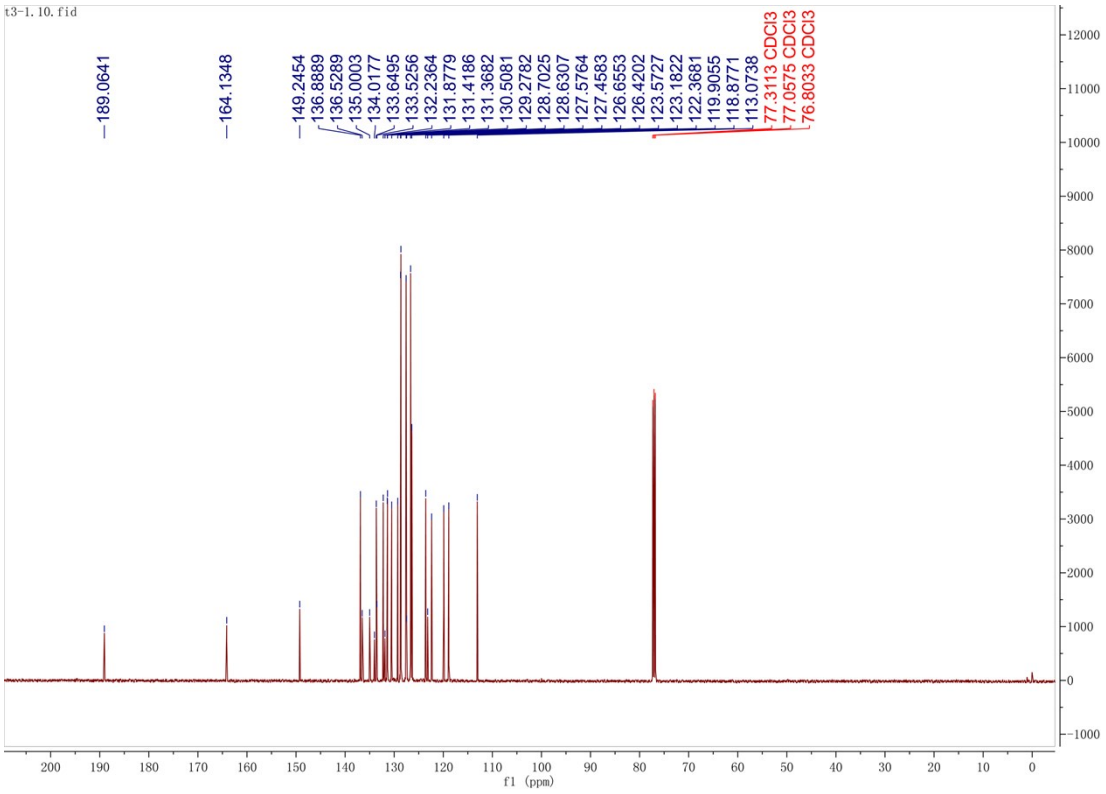



**Fig. S6** $^{13}$C NMR spectrum of **PP-PS**.

## 12. HRMS spectrum of PP-PS

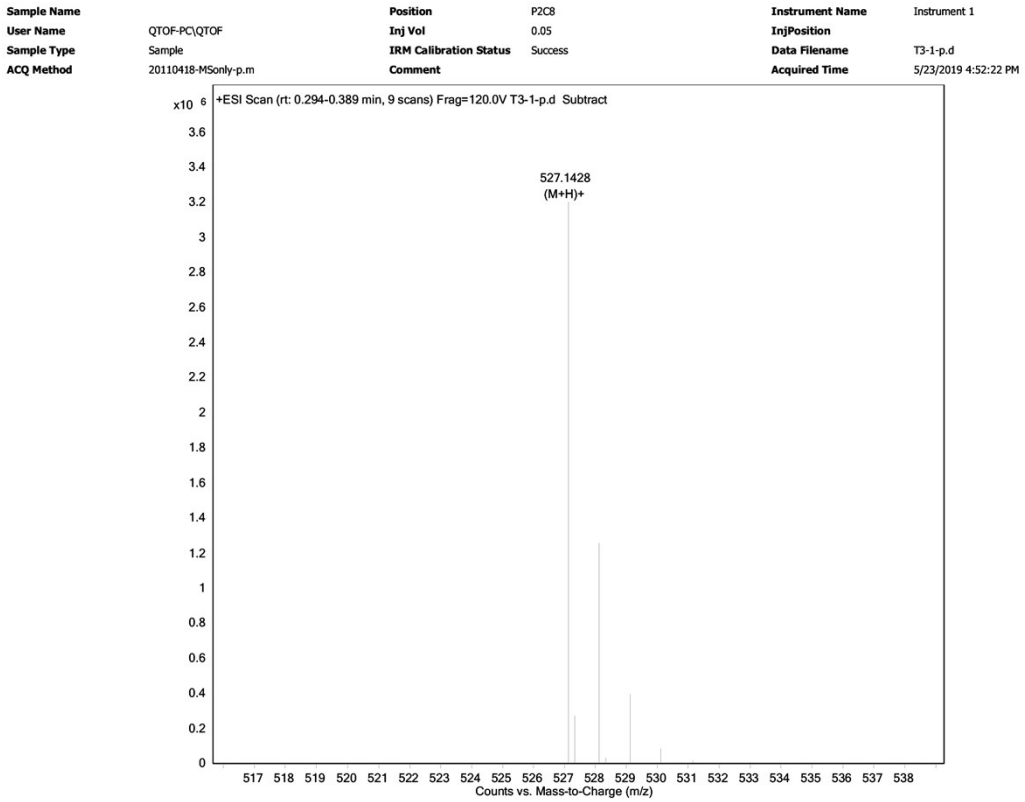
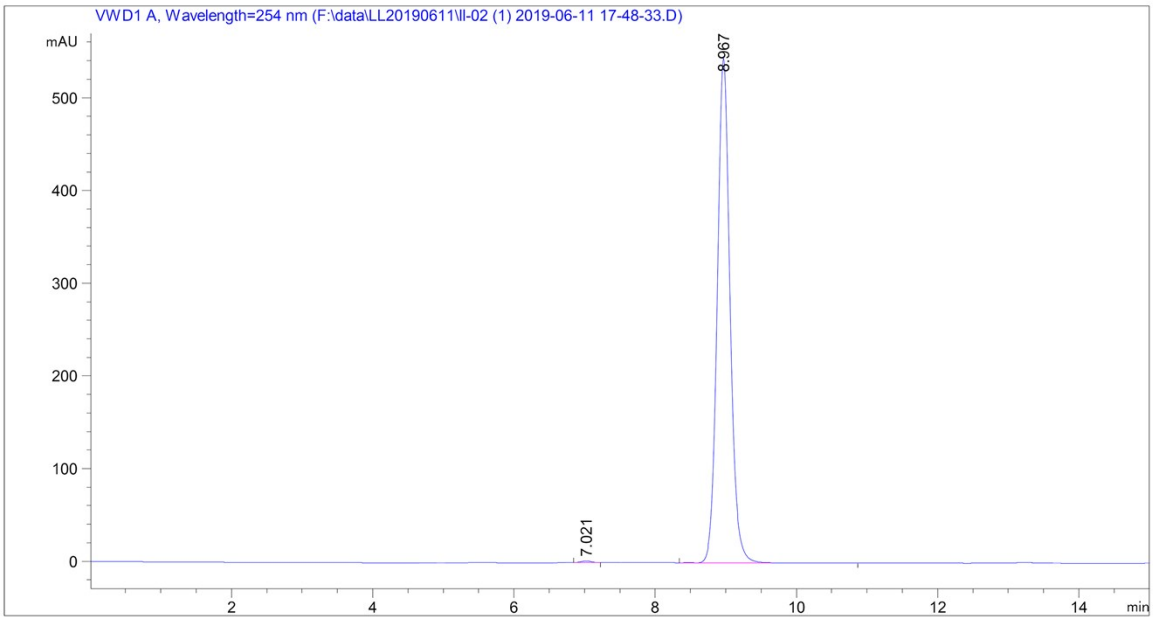



**Fig. S7** HRMS spectrum of **PP-PS**.

## 13. The HPLC purity of PP-PS.




| Peak # | Ret. Time [min] | Type | Peak With [min] | Peak Area [mAU*s] | Peak Height [mAU] | Peak Area % |
|---|---|---|---|---|---|---|
| 1 | 7.021 | BB | 0.1636 | 17.31207 | 1.71734 | 0.2497 |
| 2 | 8.967 | VV | 0.1919 | 6914.70264 | 544.37622 | 99.7503 |
| Total | | | | 6932.01471 | 546.09356 | |

Signal 1: VWD1 A, Wavelength = 254 nm

**Fig. S8** The HPLC purity of **PP-PS**.

## 14. Linear regression of the fluorescence intensity of PP-PS against the concentration of $Na_2S_2$

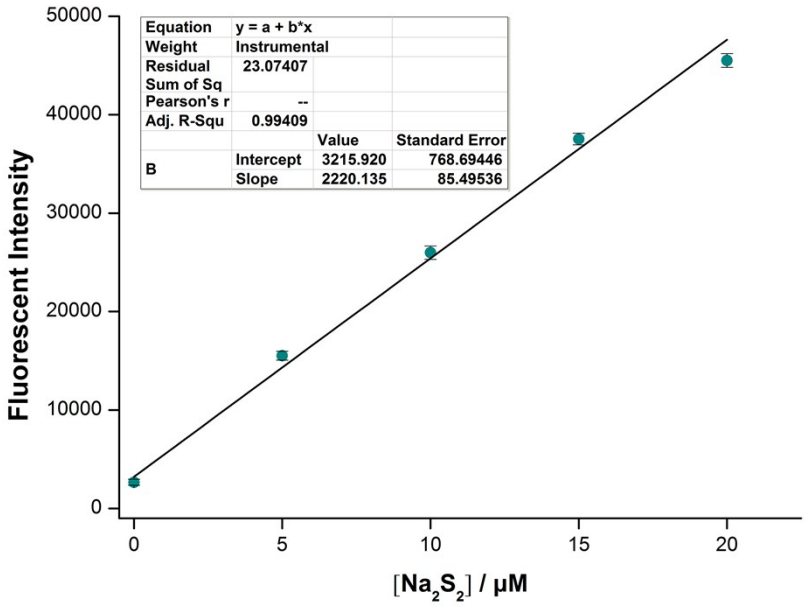



**Fig. S9** Fluorescence intensity changes of **PP-PS** (10 μM) at 478 nm against concentration of $Na_2S_2$ from 0 to 20 μM.

## 15. The effects on pH research for detection of $H_2S_n$.

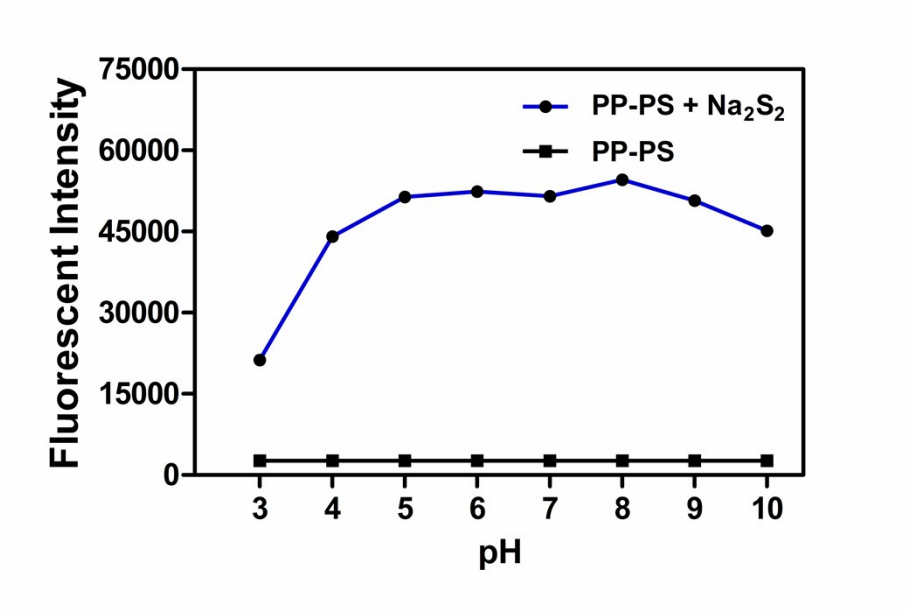



**Fig. S10** Fluorescence intensity changes of the probe **PP-PS** (10 μM) at different pH values in the absence (black) or presence (blue) of $Na_2S_2$ (50 μM).

## 16. Cell images of $H_2S_n$ in living MCF-7 cells

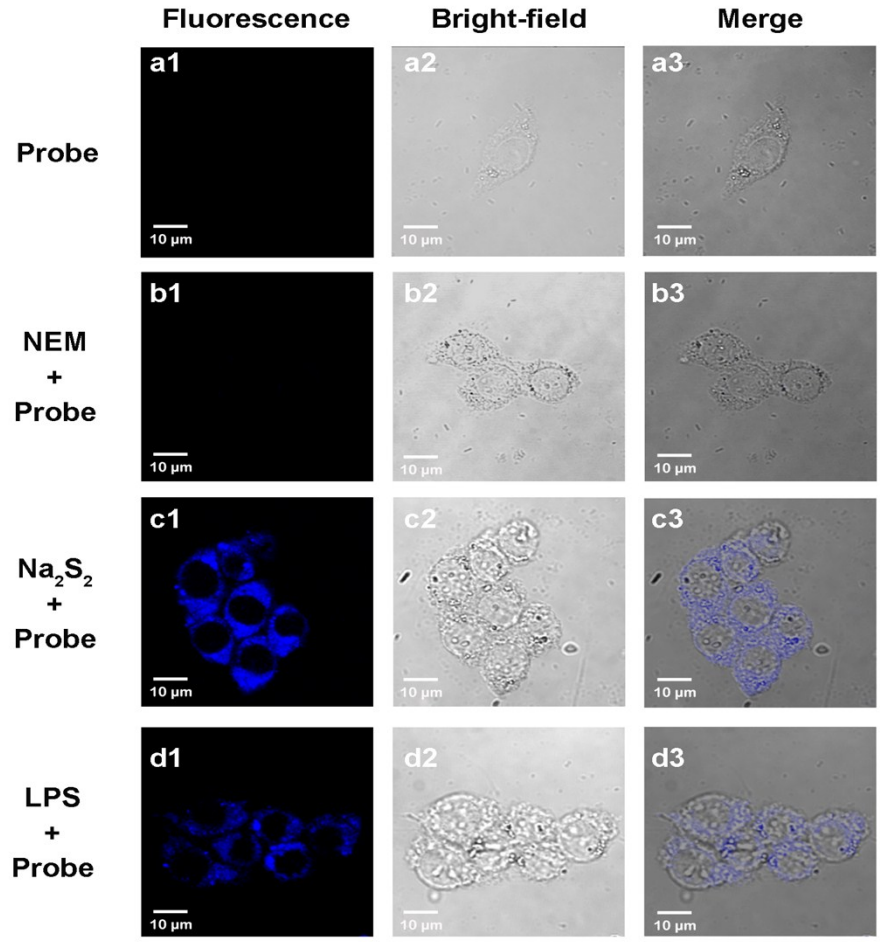



**Fig. S11** Cell images of $H_2S_n$ in living MCF-7 cells. (a1-a3) cells incubated with **PP-PS** (10 μM) for 20 min at 37 °C; (b1-b3) cells pretreated with 1mM NEM for 30 min and then incubated with **PP-PS** (10 μM) for 20 min at 37 °C; (c1-c3) cells pretreated with 15 μM $Na_2S_2$ for 20 min and then incubated with **PP-PS** (10 μM) for 20 min at 37 °C; (d1-d3) cells preincubated with 1 μg/mL LPS for 14 h and incubated with **PP-PS** (10 μM) for 20 min at 37 °C.

## 17. Cell images of $H_2S_n$ in living U87 cells

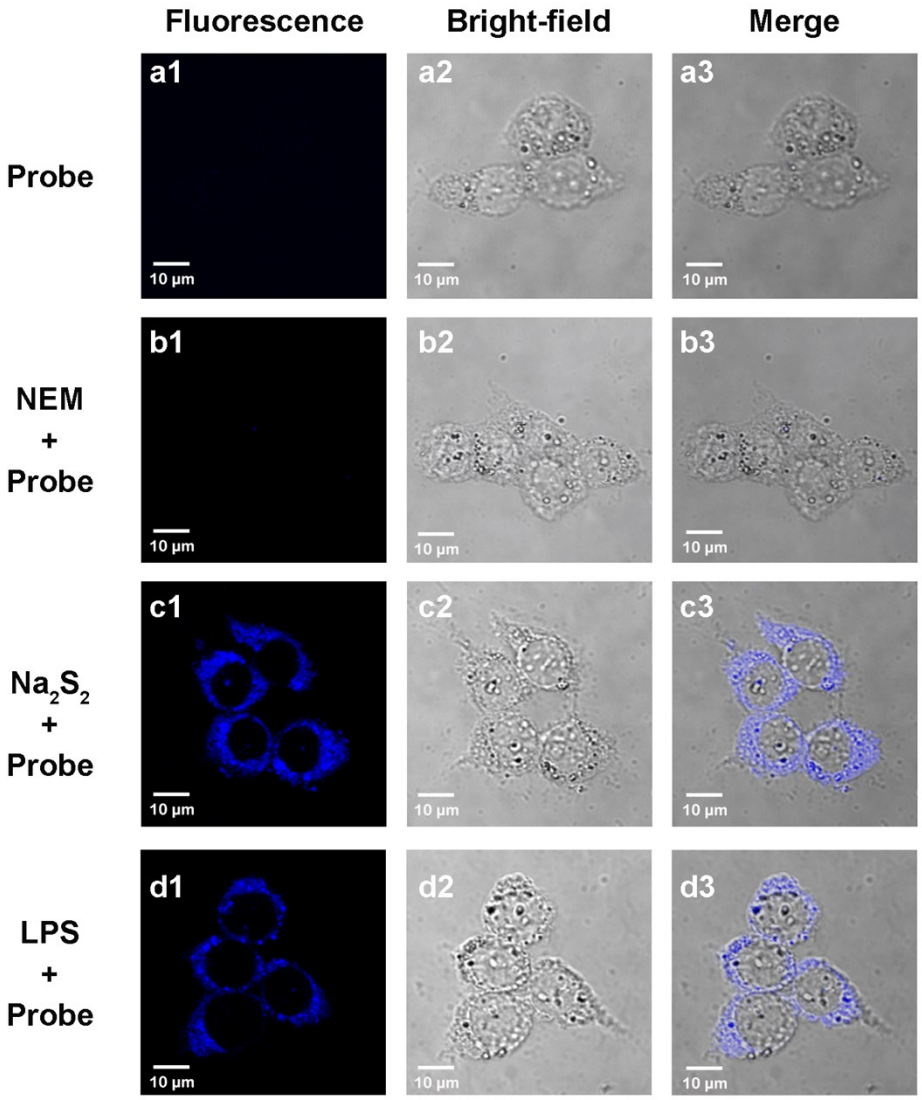



**Fig. S12** Cell images of $H_2S_n$ in living U87 cells. (a1-a3) cells incubated with **PP-PS** (10 μM) for 20 min at 37 °C; (b1-b3) cells pretreated with 1mM NEM for 30 min and then incubated with **PP-PS** (10 μM) for 20 min at 37 °C; (c1-c3) cells pretreated with 15 μM $Na_2S_2$ for 20 min and then incubated with **PP-PS** (10 μM) for 20 min at 37 °C; (d1-d3) cells preincubated with 1 μg/mL LPS for 14 h and incubated with **PP-PS** (10 μM) for 20 min at 37 °C.

## 18. Detection of $H_2S_n$ in mitochondria

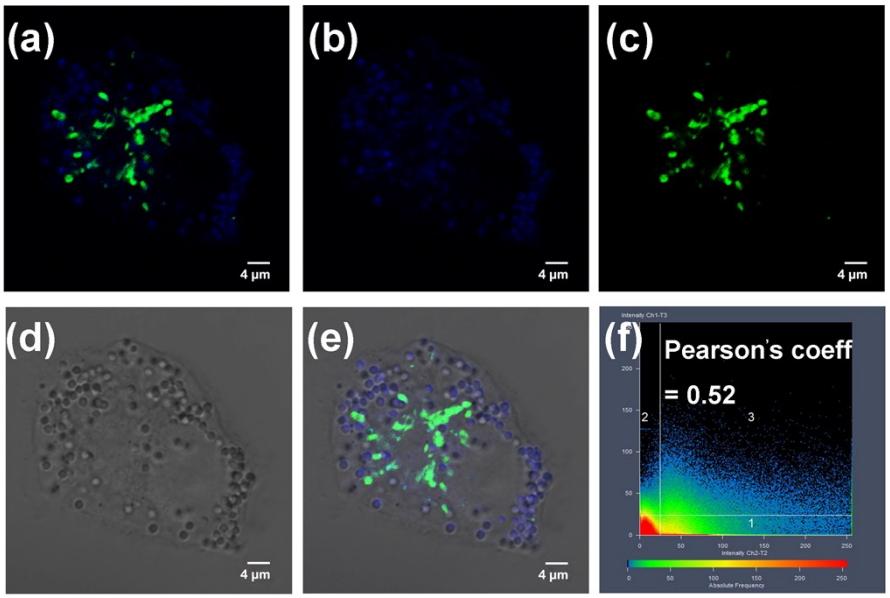



**Fig. S13** Cell images of A549 cells treated with LPS (1 μg/mL), **PP-PS** (10 μM) and MitoGreen (1 μM). The cells were pretreated with 1 μg/mL LPS for 14 h, and then incubated with MitoGreen (1 μM) for 30 min, and **PP-PS** (10 μM) for another 20 min. (a) cells imaging merged green and blue channels; (b) cells imaging of **PP-PS** with blue channel; (c) cells imaging of MitoGreen with green channel; (d) cells imaging of bright field; (e) cells imaging merged green, blue channels and bright field; (f) the colocalization areas of the images of b and c.

## Notes and references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2 F. Yang, H. Gao, S.-S. Li, R.-B. An, X.-Y. Sun, B. Kang, J.-J. Xu and H.-Y. Chen, *Chemical Science*, 2018, **9**, 5556–5563.

3 J.-B. Li, Q. Wang, H.-W. Liu, L. Yuan and X.-B. Zhang, *Chem. Commun. (Camb.)*, 2019, **55**, 4487–4490.

4 W. Li, S. Zhou, L. Zhang, Z. Yang, H. Chen, W. Chen, J. Qin, X. Shen and S. Zhao, *Sensors and Actuators B: Chemical*, 2019, **284**, 30–35.

5 W. Chen, E. W. Rosser, D. Zhang, W. Shi, Y. Li, W.-J. Dong, H. Ma, D. Hu and M. Xian, *Org. Lett.*, 2015, **17**, 2776–2779.