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Electronic Supplementary Information

A phenothiazine-based turn-on fluorescent probe for the selective detection of hydrogen sulfide in food, live cells and animals

Yaoyun Hu,[a]† Zhuye Shang,[a]† Juan Wang,[b] Min Hong,*[b] Run Zhang,[c] Qingtao Meng*[a] and Zhiqiang Zhang*[a]

[a] School of Chemical Engineering, University of Science and Technology Liaoning, Anshan, Liaoning, 114051, P. R. China.

E-mail: qtmeng@ustl.euu.cn; zzq@ustl.euu.cn, Tel.: +86-412-5929627

[b] School of Chemistry and Chemical Engineering, Liaocheng University, Liaocheng 252059, China.

E-mail: hongmin@lcu.edu.cn

[c] Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane, 4072, Australia.

† These authors contributed equally to this work and they should be regarded as co-first author.

Reagents and materials

Phenothiazine, malononitrile, phenyl isothiocyanate and 1,8-diazabicyclo[5.4.0]undec -7-ene were purchased from Aladdin reagent Co. (Shanghai, China). 1-Bromopropane, piperidine, phosphorus oxychloride, anions (sodium salts) and biothiols were obtained from Sinopharm Chemical Reagent Co., Ltd. (China). Sodium hypochlorite (NaOCl) and 3-morpholinosydnonimine (SIN-1) ($ONOO^-$ donor) were purchased from Alfa Aesar. All the experiments of live zebrafish were performed in compliance with relevant local laws and institute guidelines. Unless otherwise stated, solvents and reagents were of analytical grade from commercial suppliers and were used without further purification. Deionized water was used throughout.

Apparatus

^{1}HNMR and ^{13}CNMR spectra were recorded with an AVANCE600MHZ spectrometer (BRUKER) with chemical shifts reported as *ppm* (in $CDCl_3$, TMS as internal standard). Coupling constants (*J* values) are reported in hertz. API mass spectra were recorded on an Agilent 6530 QTOF spectrometer. Absorption spectra were measured with a Perkin Elmer Lambda 900 UV-vis spectrophotometer (USA). High-performance liquid chromatography (HPLC) analysis was performed on Agilent 1100 Series with methanol-H_2O (9/1, v/v) as the eluent. Fluorescence spectra were measured with Perkin Elmer LS55 luminescence spectrometer (USA). All pH measurements were carried out with an OHAUS Starter 3100/f meter (USA). H_2S in adult zebrafish and nude mice were imaged by a SPECTRAL Ami Imaging Systems (Spectral Instruments Imaging, LLC, Tucson, AZ) with an excitation filter 465 nm and an emission filter 610 nm. All the data were

calculated using the region of interest (ROI) function of Amiview Analysis software (Version 1.7.06).

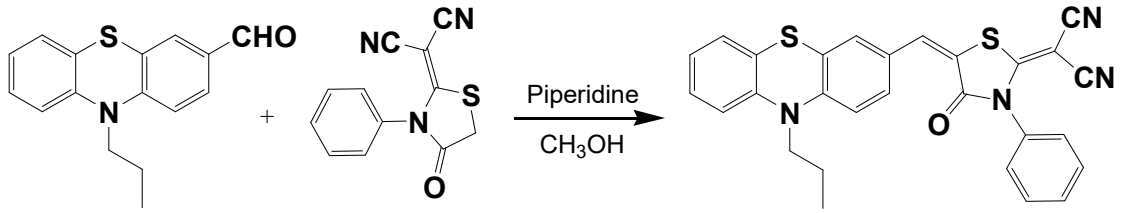
Preparation of stock solutions of PR and analytes

Stock solution of **PR** at the concentration of 0.5 mM was prepared by dissolving certain amount of **PR** in dimethylformamide (DMF). For the detection of H_2S in buffer, the stock solution of **PR** was diluted into 20 mM phosphate-buffered saline (PBS) of pH 7.4 (DMF: H_2O=7:3, v/v, 20 mM, pH=7.4) for all spectrometric measurements. For spectroscopic titration measurements of **PR** to H_2S, the spectra were recorded after mixing **PR** with analytes at room temperature. NaHS was used as H_2S source. Solutions of a series of anions and biothiols (20 mM) were freshly prepared by dissolving corresponding chemicals in deionized water. The ROS species were prepared in deionized water according to the previous literatures.[1-3] A stock solution of HOCl was prepared by diluting of the commercial NaOCl solution and stored. Hydroxylradical (·OH) was generated in the Fenton system from ferrous ammonium sulfate and hydrogen peroxide. Superoxide anion radical (O_2^-) was generated from the xanthine-xanthine oxidase system. Singlet oxygen ($^1O_2$) was generated from the Na_2MoO_4-H_2O_2 system in 0.05 M carbonate buffer of pH 10.5. $ONOO^-$ was obtained by using SIN-1 as a donor. Hydrogen peroxide (H_2O_2) was diluted immediately from a stabilized 30% solution, and was assayed using its molar absorption coefficient of 43.6 M^{-1} cm^{-1} at 240 nm.

MTT assay of cytotoxicity of PR in HeLa cells

The cytotoxicity of **PI** to live HeLa cells was investigated by MTT assays.[4] The following formula was used to calculate the viability of cell growth: Viability (%)=(mean of absorbance value of treatment group-blank)/(mean absorbance value

of control-blank)×100.



Scheme S1 Synthetic procedure of fluorescent probe **PR**.

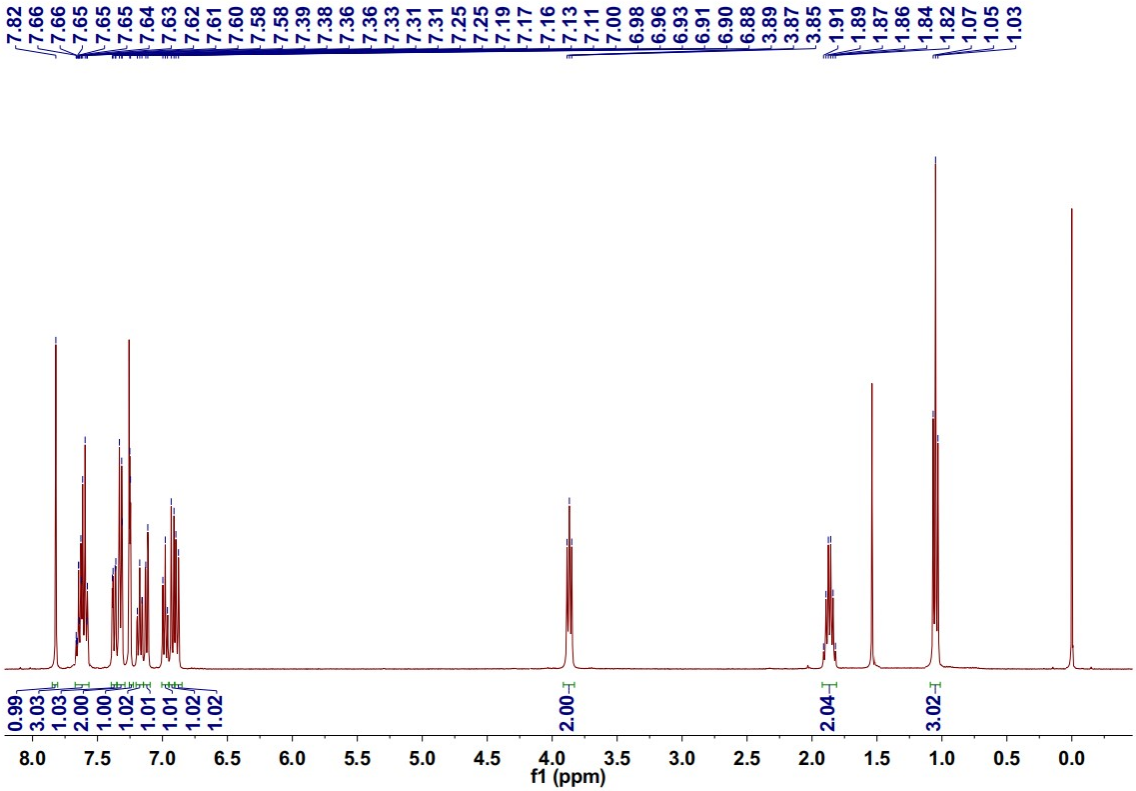


Fig. S1 ^{1}H NMR of Probe **PR** (600 MHz, $CDCl_3$).

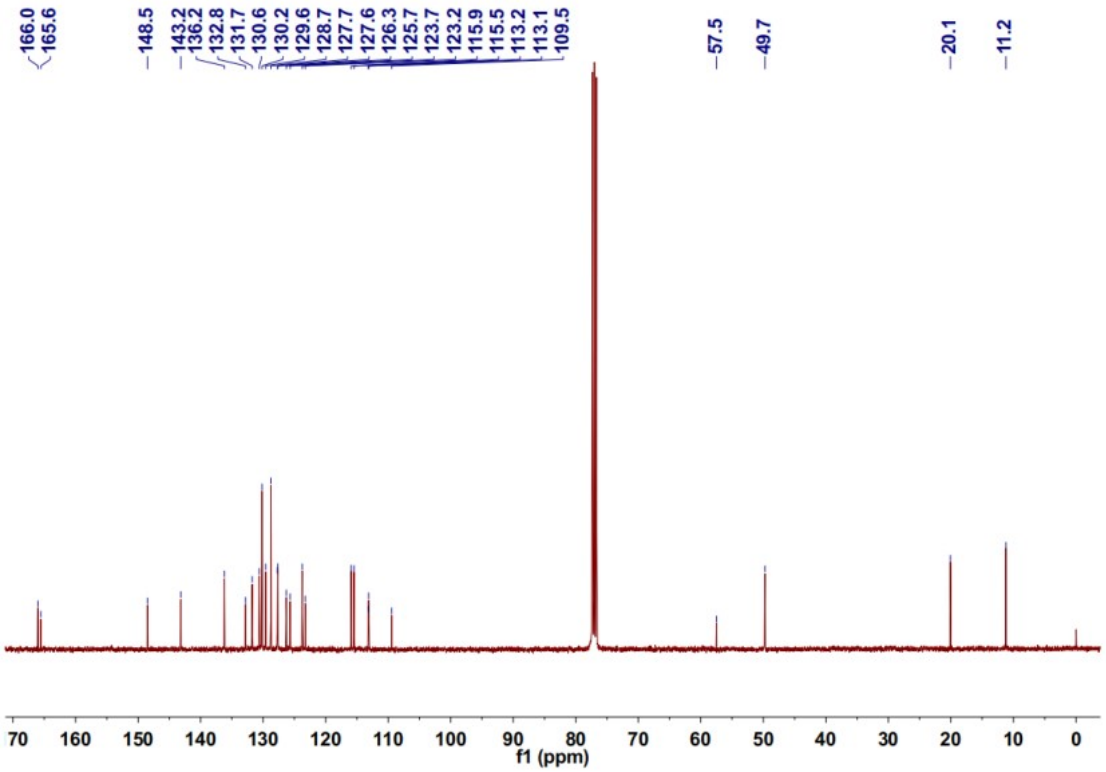


Fig. S2 ^{13}C NMR of Probe **PR** (150 MHz, $CDCl_3$).

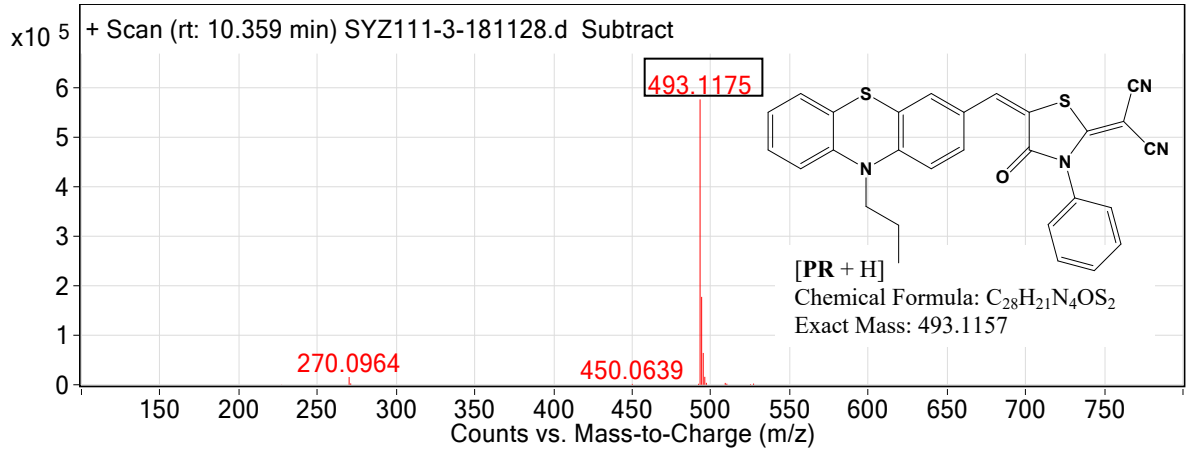


Fig. S3 HRMS of Probe **PR**.

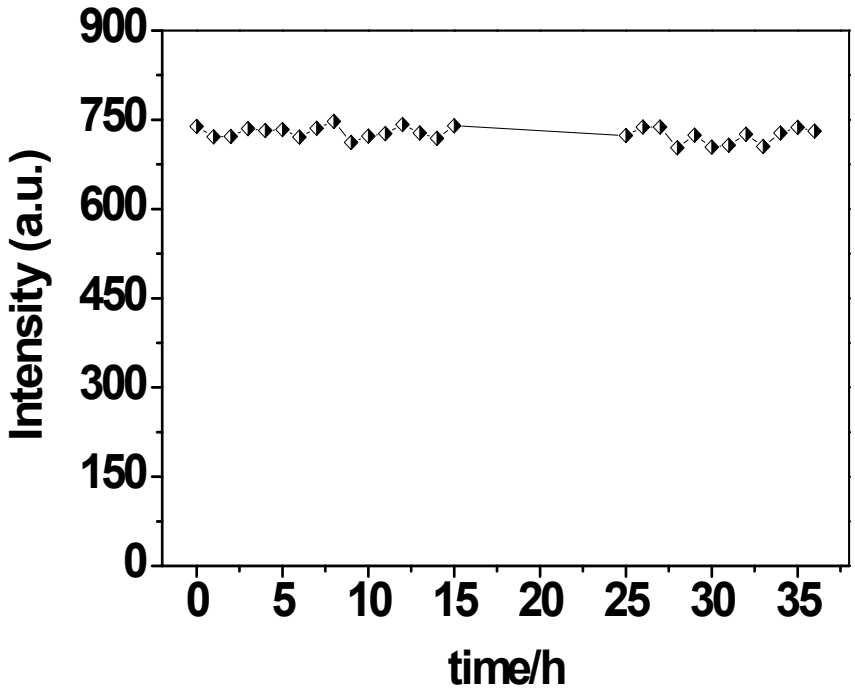


Fig. S4 Fluorescence intensities of Probe **PR** (10 μM) in PBS aqueous buffer (DMF: PBS=7:3, 20 mM, pH=7.4). The intensities were recorded at 596 nm, excitation at 425 nm.

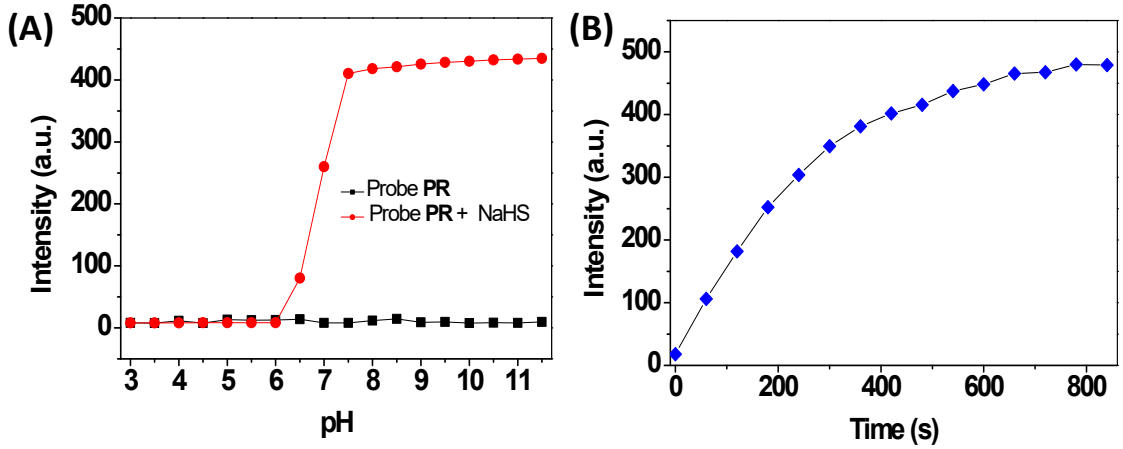


Fig. S5 (A) pH-dependent fluorescence intensity of **PR** in the absence and presence of NaHS (150 μM). (B) Time-dependent fluorescence spectra of **PR** (10 μM) upon the addition of 150 μM NaHS in PBS aqueous buffer (DMF: PBS=7:3, 20 mM, pH=7.4). The intensities were recorded at 596 nm, excitation at 425 nm.

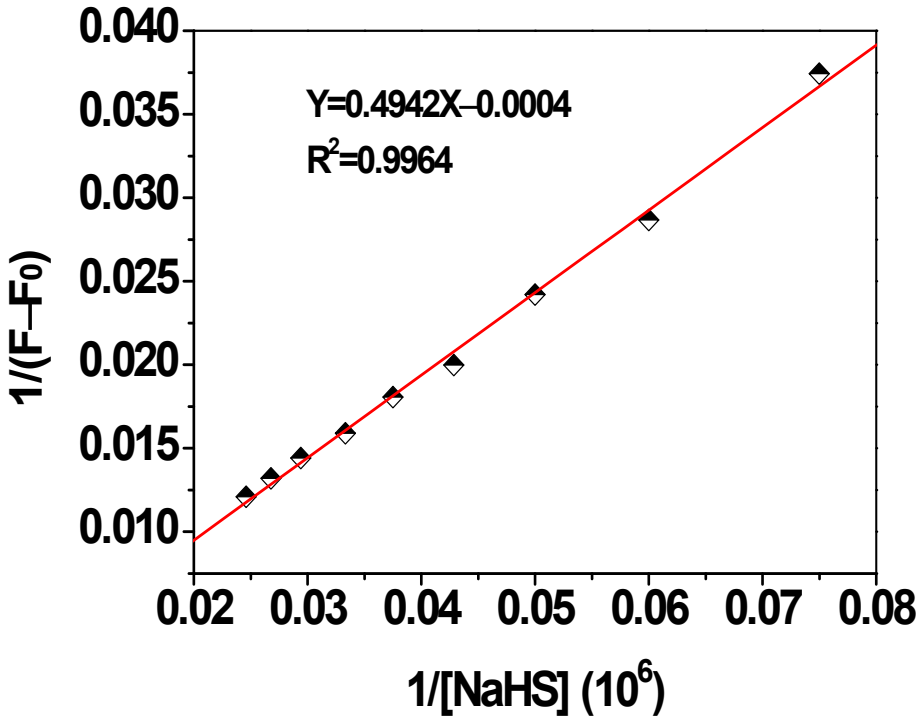


Fig. S6 Benesi-Hildebrand plot (emission at 596 nm) of Probe **PR** (10 μM) based on 1:1 binding stoichiometry with NaHS. Excitation at 425 nm.

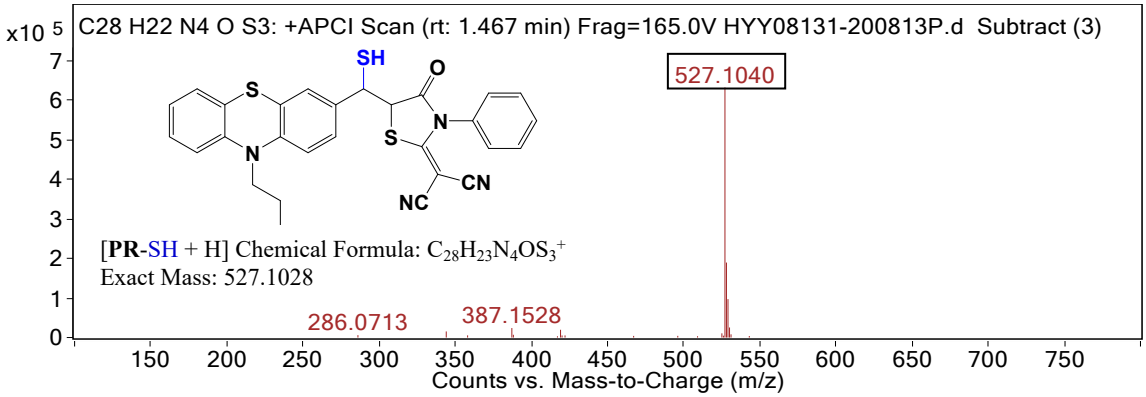


Fig. S7 HRMS of **PR** in the presence of NaHS.

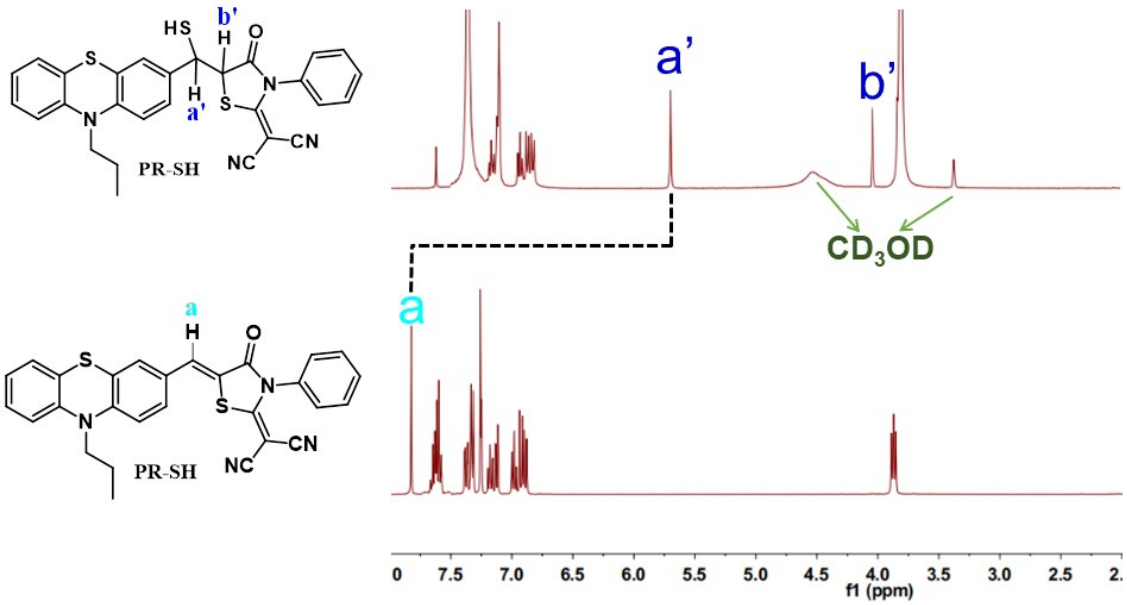


Fig. S8 ^{1}H NMR titration of **PR** in the absence and presence of NaHS in a $CDCl_3/CD_3OD/D_2O$ mixed solution (400 MHz).

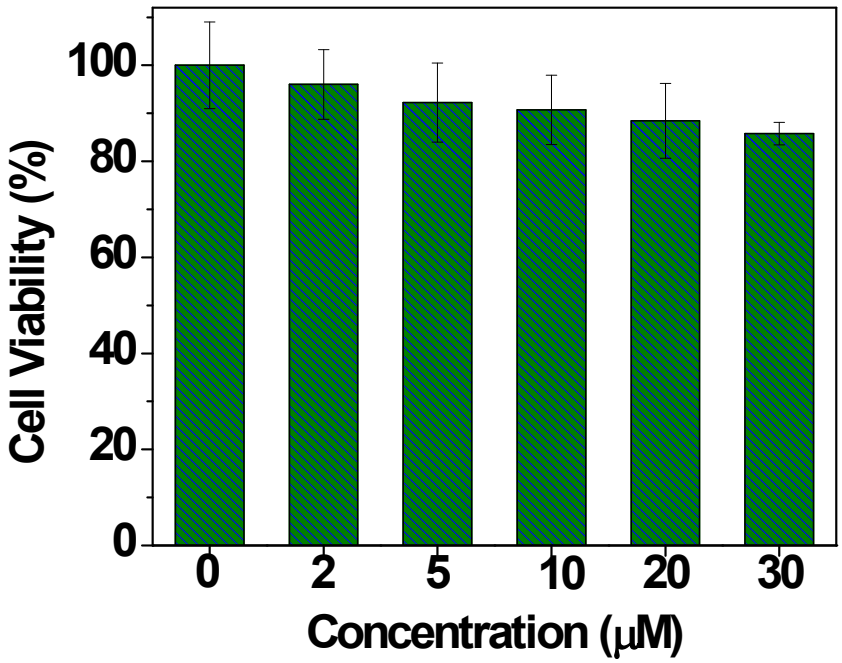


Fig. S9 Cytotoxicity of **PR** towards HeLa cells estimated by MTT assay.

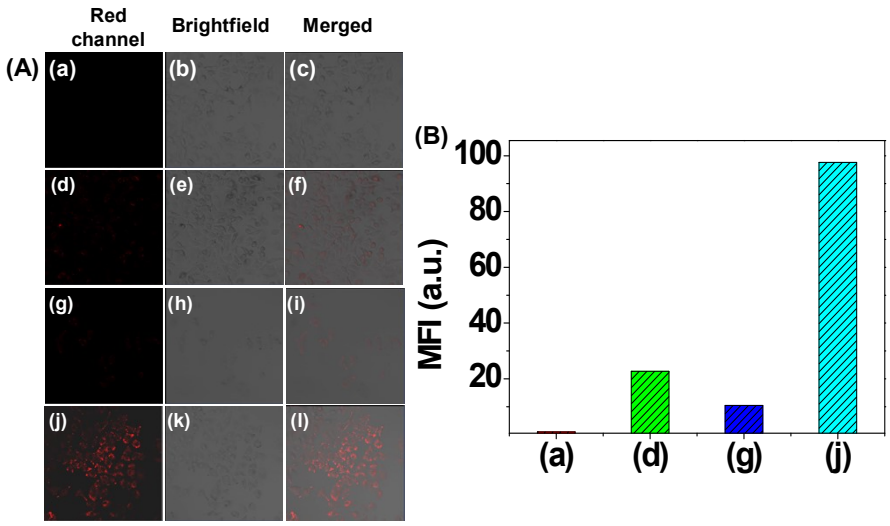


Fig. S10 Confocal imaging of exogenous H_2S with **PR** in live cells. (a-c) Control A549 cells. (d-f) A549 cells incubated with only **PR** (5 μM) for 15 min. (g-i) A549 cells treated with NEM (1 mM) for 60 min and then **PR** (5 μM) for another 15 min. (j-l) NEM (1 mM) pre-treated A549 cells incubated with **PR** (5 μM) and then NaHS (150 μM) for another 15 min. The images were acquired using a confocal microscope with 488 nm excitation and 610 nm collection. (B) Mean fluorescence intensity per A549 cell of (a), (d), (g) and (j). Scale bar, 30 μm.

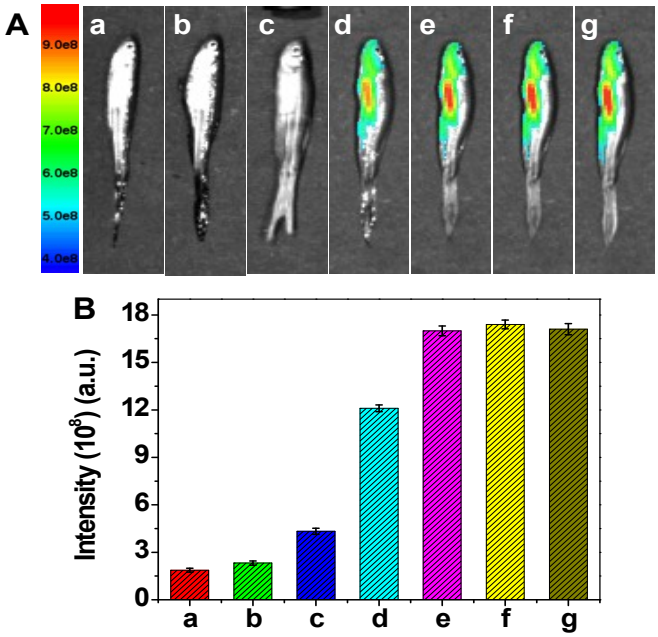


Fig. S11 (A) Fluorescence imaging of exogenous H_2S in live zebrafish with **PR** (10 μM). (a) Blank zebrafish. (b) Zebrafish treated with NaHS (150 μM) for 3 min only. (c) Zebrafish treated with **PR** (10 μM) only. (d) Zebrafish was pre-incubated with NaHS (150 μM) for 3 min and then **PR** (10 μM) for 2 min. The images were taken in the following (e) 5 min, (f) 10 min and (g) 12 min. (B) Mean fluorescence intensity of zebrafish shown in (a−g). The images of zebrafish were recorded with an excitation filter (465 nm) and an emission filter (610 nm).

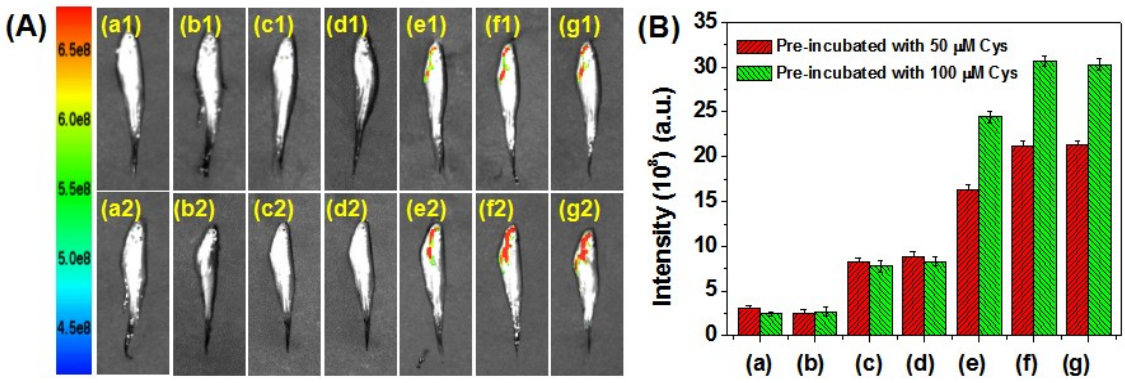


Fig. S12 (A) Fluorescence imaging of H_2S endogenously produced by Cys in live zebrafish with **PR** (10 μM). (a1, a2) Control zebrafish. Zebrafish incubated with (b1) 50 μM Cys and (b2) 100 μM Cys for 60 min. (c1,c2) Zebrafish incubated with **PR** (10 μM) for 20 min. (d1, d2) Zebrafish incubated with 100 μM NEM for 15 min and then **PR** (10 μM) for 20 min. Zebrafish incubated with 50 μM Cys for 60 min and then **PR** (10 μM) for (e1) 10 min, (f1) 20 min (g1) and 30 min. Zebrafish incubated with 100 μM Cys for 60 min and then **PR** (10 μM) for (e2) 10 min, (f2) 20 min and (g2) 30 min. (B) Mean fluorescence intensity of zebrafish shown in (a−g). The images of zebrafish were recorded with an excitation filter (465 nm) and an emission filter (610 nm).

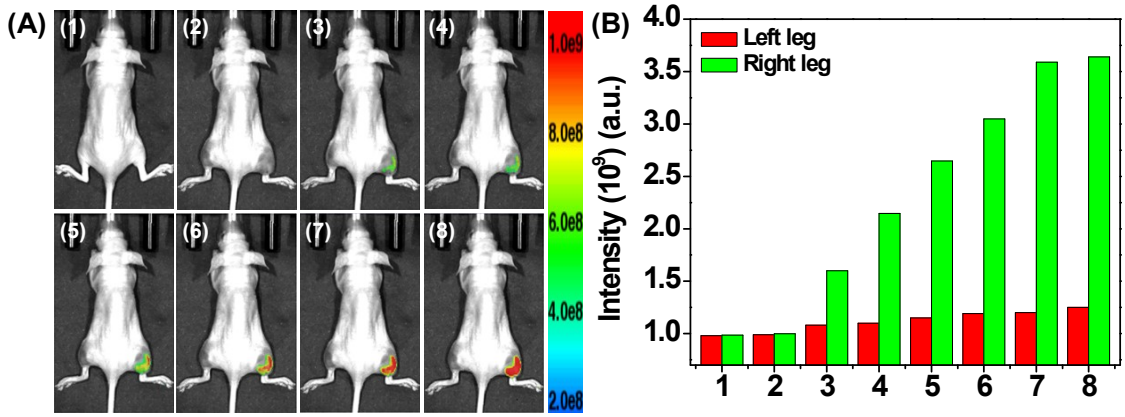


Fig. S13 (A) Fluorescence imaging of exogenous H_2S in live nude mouse with **PR**. (1) Blank nude mouse. (2) **PR** (10 μM, 125 μL) was subcutaneously injected into the left and right legs of mouse. Followed by the injection of PBS (2 mM, 10 μL) into the left leg and NaHS (2 mM, 10 μL) to the right leg areas of interest. The images were then recorded at different times: (3) 2 min, (4) 4 min, (5) 6 min, (6) 8 min, (7) 10 min and (8) 15 min, respectively. (B) Mean fluorescence intensity of interested area at the different times shown in Fig. 8A. The images of nude mouse were recorded with an excitation filter (465 nm) and an emission filter (610 nm).

Table S1. Comparison of this work with reported fluorescent probes for H_2S detection.

Probes	Red-Emission	Detection limit	Response time	Detection of H_2S intake in live animals	Detection of H_2S intake in cell	Detection of H_2S in corrupt food	Ref.
M-H_2S	560 nm/650 nm	39.1 nM	12 min	Yes	Yes	No	5
Ru-NBD	644nm	88 nM	4 min	No	Yes	No	6
KF-DNBS	500 nm	3.2 μM	40 min	No	No	No	7
H-LDS	550 nm	0.57 mM	10 min	No	Yes	No	8
CMDN	460 nm	0.207 μM	13 min	Yes	Yes	No	9
BSCN	515 nm	17 nM	60 min	No	Yes	No	10
CDs–PNBD	440 nm/543 nm	57 nM	100 min	No	No	No	11
P1	530 nm	1.06 μM	10 min	No	Yes	No	12
DCI-NCN	618 nm	50 nM	15 min	Yes	Yes	No	13
NBD-CMC	530 nm/665 nm	0.5 μM	1 min	No	Yes	No	14
Dye-H_2S-Gal	545 nm	195 nM	1 min	No	Yes	No	15
DPQI	465 nm/593 nm	3.5 μM	2 min	No	Yes	No	16
SPy-DNs	608 nm	356 nM	4 s	NO	Yes	Yes	17
DCM-OCN	690 nm	0.28 μM	2 min	No	No	No	18
FLVN−OCN	525 nm	0.25 μM	15 min	No	Yes	No	19
MI-H_2S	663 nm	19 nM	3 min	Yes	Yes	No	20
DC-NBD	744 nm	26 nM	3 min	Yes	Yes	No	21
PR	596 nm	1.8 μM	10 min	Yes	Yes	Yes	**This work**

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