

Electronic Supplementary Information

A photostable fluorescent probe based on PET off for the detection of hydrogen sulfide and its application in bioimaging

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I: Material and Methods

1.1. Materials and Physical measurements

All the reagents and solvents were commercially available. Naphthalene-1,6-diol and ethyl acetoacetate were got from Aladdin Industrial Corporation (Shanghai, China). Amino acids were got from Shanghai Experiment Reagent Co., Ltd (Shanghai, China). Fluorescence spectra were recorded by HITACHI F-7000 fluorescence spectrophotometer. Ultraviolet-visible spectra were detected by Hitachi U-3900 UV spectrophotometer. ¹H NMR and ¹³C NMR data were obtained by Bruker AVANCE-600 MHz NMR spectrometers (Bruker, Billerica, MA). HR-MS determinations were implemented on an AB SCIEX Tripple TOF5600 Instruments. Nikon Ti-S microsystem was used to evaluate the response of probes to H₂S in HeLa cells.

Figure S1: ^1H MNR (600 MHz) spectrum of **Compound 1** in CD_3OD and ^{13}C MNR (150 MHz) spectrum of **Compound 1** in $\text{DMSO}-d_6$.

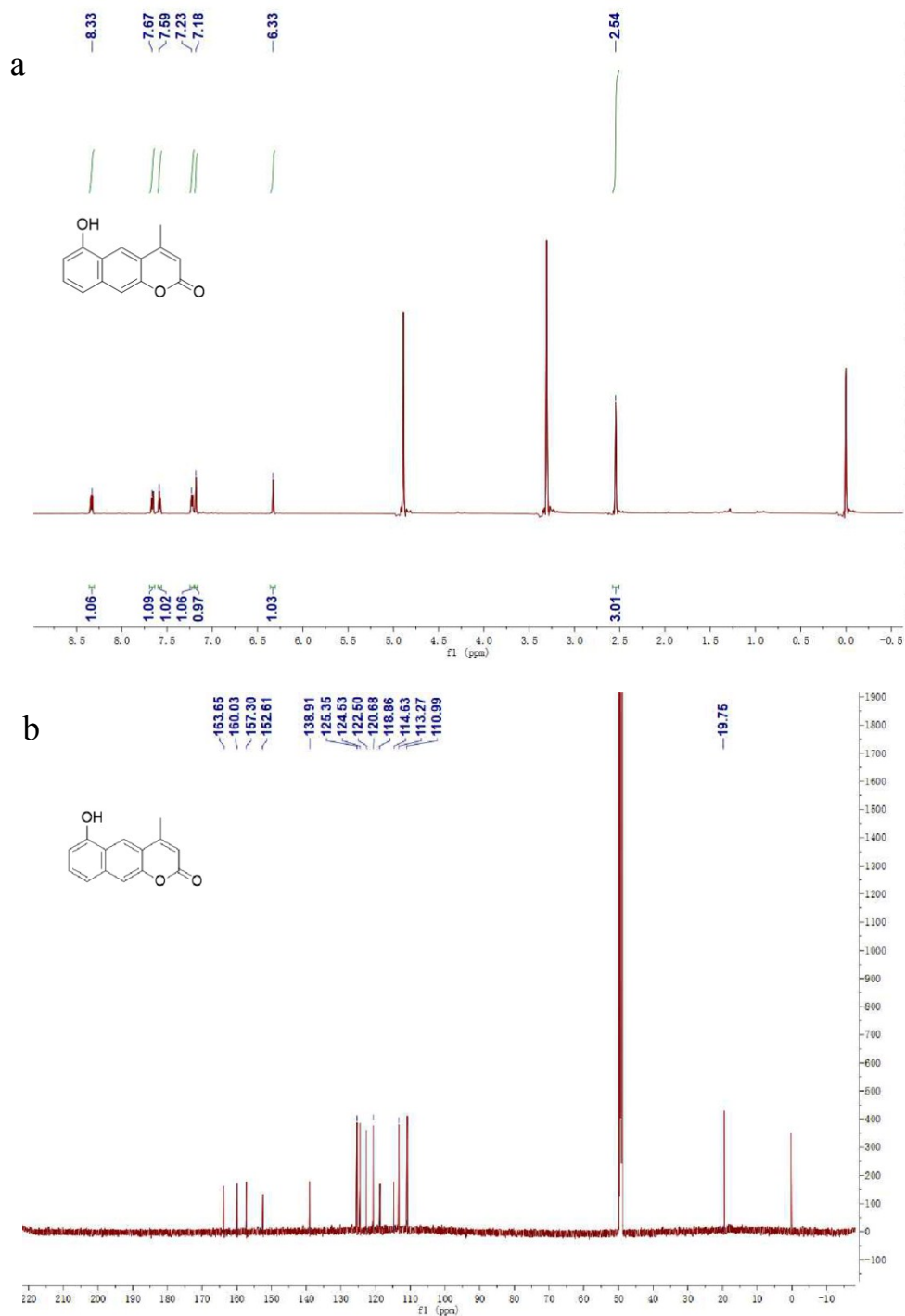
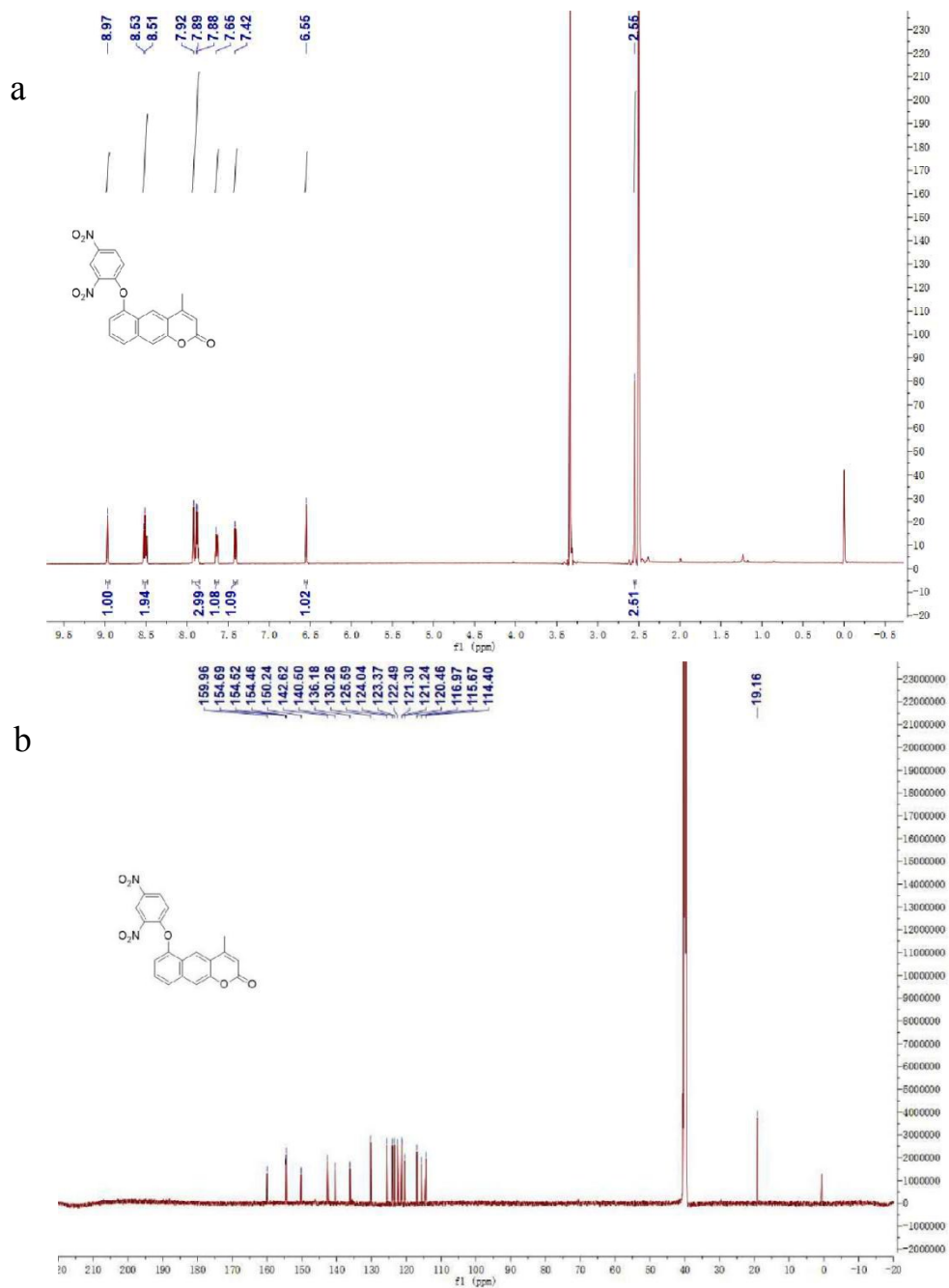
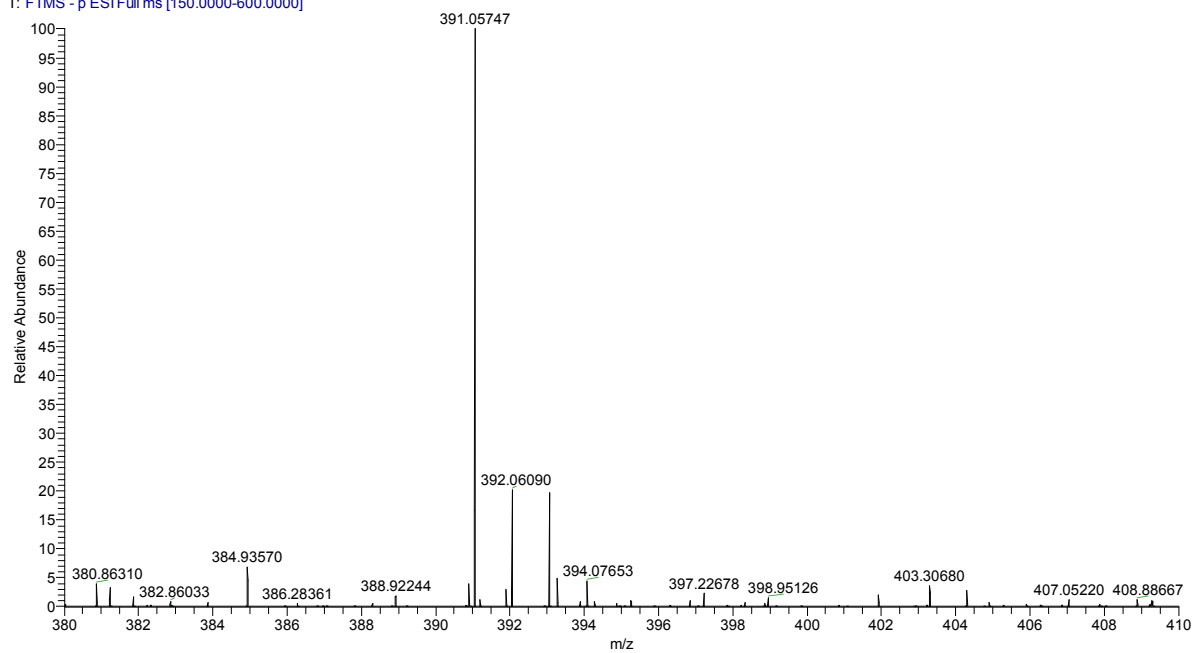


Figure S1: (a) ^1H MNR (600 MHz) spectrum of **Compound 1** in MeOD , (b) ^{13}C MNR (150 MHz) spectrum of **Compound 1** in $\text{DMSO}-d_6$.

Figure S2: ^1H MNR (600 MHz) spectrum, ^{13}C MNR (150 MHz) spectrum of **P1** in DMSO- d_6 and the HR-MS of **P1** (c)



YJL0610 #15-28 RT: 0.16-0.27 AV: 7 NL: 1.34E6
T: FTMS - p ESI Full ms [150.0000-600.0000]

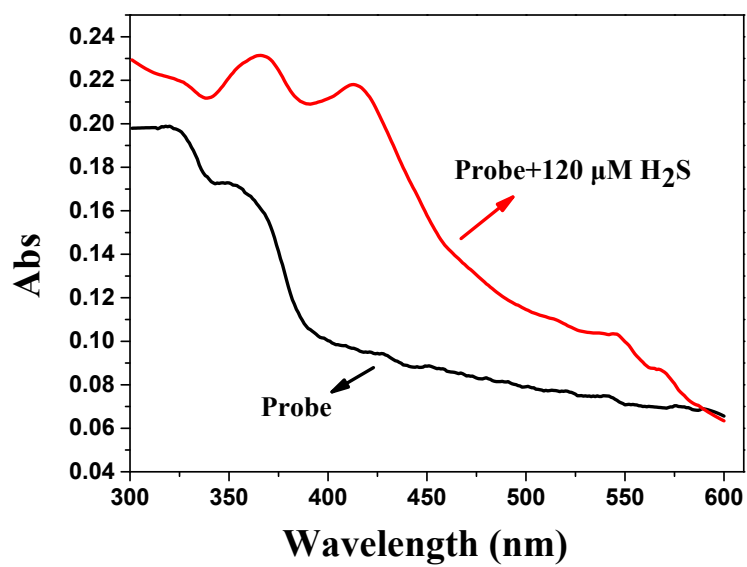


(c)

(a) ^1H MNR (600 MHz), (b) ^{13}C MNR (150 MHz) spectra of **P1** in $\text{DMSO}-d_6$ and (c)

the HR-MS of **P1** (c).

Figure S3: The UV-visible absorption spectra of H₂S (120 μM) added to the **P1**.



The UV-Vis absorption spectra of **P1** (10 μM) in the absence or presence of Na₂S (120 μM) in DMSO: PBS=1:1 (v/v, pH = 7.4).

Figure S4: Fluorescence intensity of **P1** + Na₂S (100 μM) / **P1** (10 μM) at 530 nm at different pH conditions.

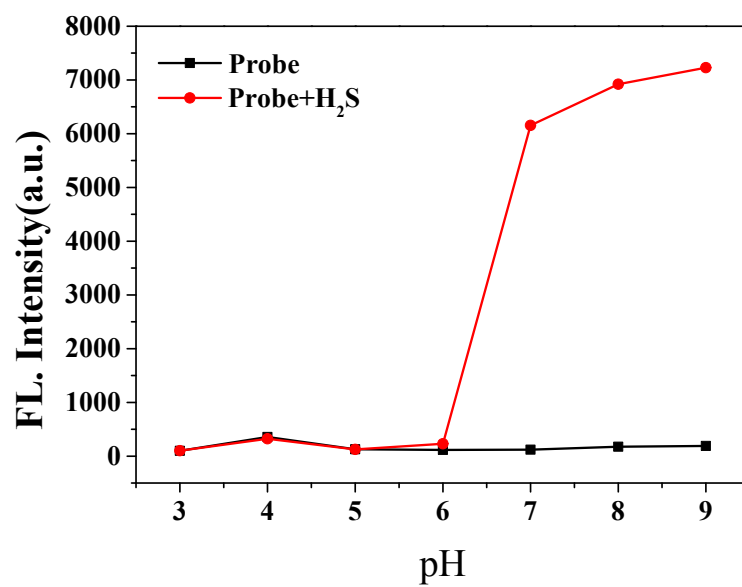


Figure S5: The HR-MS of (a) **P1** and (b) the mixture reaction product of **P1** and H₂S.

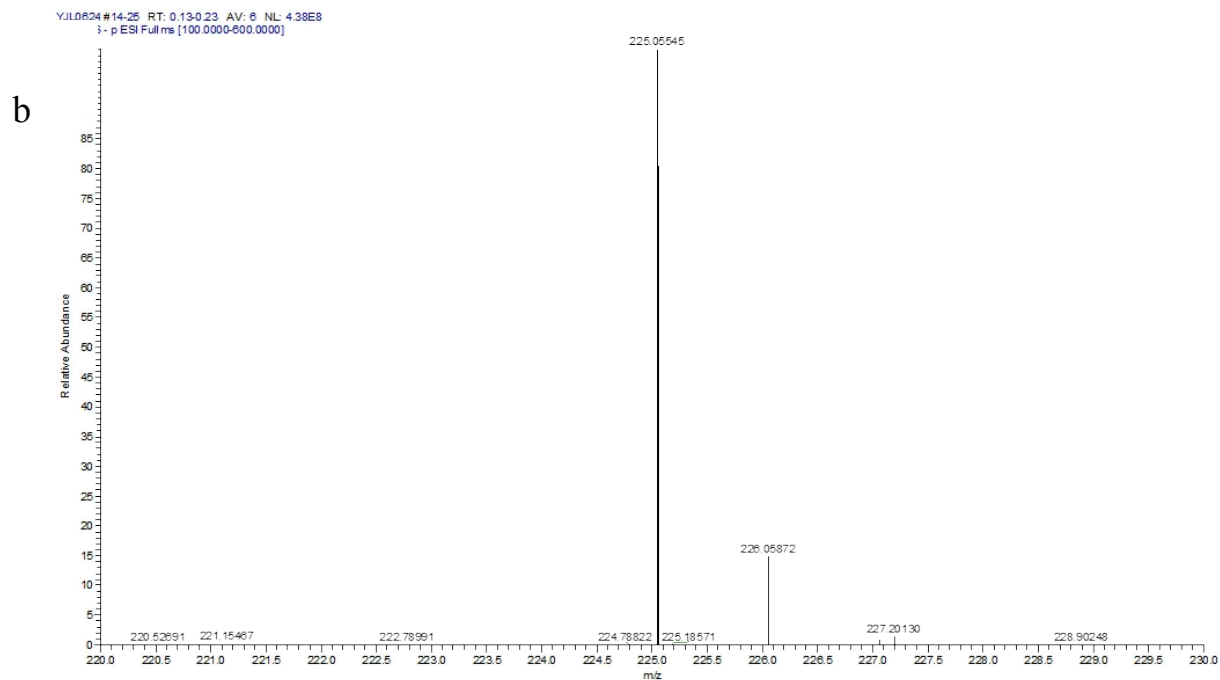


Figure S6: Cell viability estimated by CCK-8 assay with RAW 264.7 cells, which were cultured in the presence of 0-20 μM **P1** for 5 and 10 h.

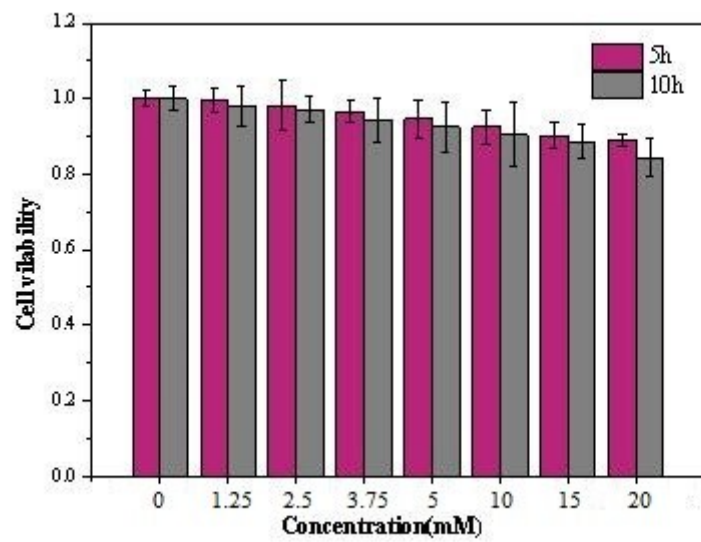
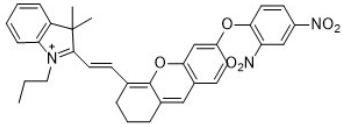
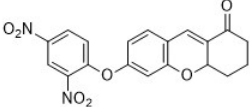
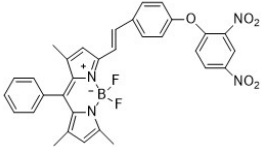
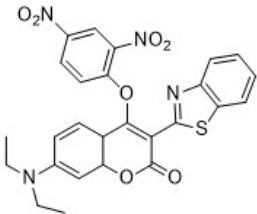
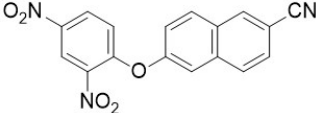
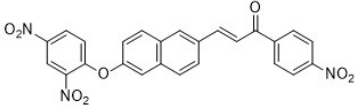
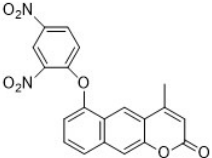


Table S1: Comparison of **P1** and previously reported response time of the same type probes to H₂S.

Probe	Structure	Excitation and Emission	Reaction time	Probe concentration	Reference
Probe 1		$\lambda_{em}=723\text{nm}$	20 min	10 μM	Ref (27)
Probe 2		$\lambda_{ex}=410\text{nm}$ $\lambda_{em}=514\text{nm}$	40 min	5 μM	Ref (28)
Probe 3		$\lambda_{ex}=574\text{nm}$ $\lambda_{em}=592\text{nm}$	20 min	10 μM	Ref (29)
Probe 4		$\lambda_{ex}=370\text{nm}$ $\lambda_{em}=424\text{nm}$	180 min	30 μM	Ref (30)
Probe 5		$\lambda_{em}=432\text{nm}$	30 min	10 μM	Ref (31)
Probe 6		$\lambda_{ex}=403\text{nm}$ $\lambda_{em}=502\text{nm}$	15 min	10 μM	Ref (32)

P1		$\lambda_{\text{ex}}=430\text{nm}$ $\lambda_{\text{em}}=530\text{nm}$	10 min	10 μM	This work
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