*Supporting Information for*

# An ESIPT based fluorescent probe for imaging hydrogen sulfide with a large turn-on fluorescence signal

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## Materials and instruments

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. Twice-distilled water was used throughout all experiments; Mass spectrometric analyses were measured on a Finnigan MAT 95 XP spectrometer; High resolution mass spectrometric (HRMS) analyses were measured on an Agilent 1100 HPLC/MSD spectrometer; NMR spectra were recorded on an AVANCE III 400 MHz Digital NMR Spectrometer, using TMS as an internal standard; Electronic absorption spectra were obtained on a Shimadzu UV-2700 power spectrometer; Photoluminescent spectra were recorded with a HITACHI F4600 fluorescence spectrophotometer with a 1 cm standard quartz cell; The fluorescence imaging of cells was performed with a Nikon A1MP confocal microscope; The pH measurements were carried out on a Mettler-Toledo Delta 320 pH meter; TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300), both of which were obtained from the Qingdao Ocean Chemicals.

## Method of spectral measurements

Unless otherwise noted, all the measurements were made according to the following procedure. A stock solution (1.0 mM) of **ESIPT-HS** was prepared by dissolving the requisite amount of it in DMSO. In a 10 mL tube the test solution of compounds **ESIPT-HS** was prepared by placing 0.1 mL of stock solution, 2.9 mL of DMSO, 3 mL of 20 mM PBS buffer (different pH) and an appropriate volume of $Na_2S$ sample solution. After adjusting the final volume to 10 mL with distilled-deionized water, standing at room temperature 1 h, 3 mL portion of it was transferred to a 1 cm quartz cell to measure absorbance or fluorescence. The stock solutions of ions for selectivity experiments were prepared respectively by dissolving KBr, KF, $NaH_2PO_4$, $Na_2HPO_4$, $Na_2S_2O_3$, $Na_2SO_3$, $NaHSO_3$, Cys, GSH, Hcy, NaClO, $H_2O_2$ and $Na_2S$ in twice-distilled water. The slight pH variations of the solutions were achieved by adding the minimum volumes of NaOH (0.1 M) or HCl (0.2 M).

## Determination of the Fluorescence Quantum Yield.

Fluorescence quantum yields for **ESIPT-HS** and the solution of **ESIPT-HS** treated with $Na_2S$ were determined by using quinine sulfate ($\Phi$ = 0.546 in 0.1 M $H_2SO_4$) as fluorescence standard.[1] The fluorescence quantum yields of the probe **ESIPT-HS** and the solution of **ESIPT-HS** treated with $Na_2S$ were calculated using the following equation (1).

$$\Phi_s = \Phi_r \left( \frac{A_r(\lambda_r)}{A_s(\lambda_s)} \right) \left( \frac{n_s^2}{n_r^2} \right) \frac{F_s}{F_r} \qquad (1)$$

In the formula, symbol $\Phi$ represents the fluorescence quantum yield, A is the absorbance at the excitation wavelength, F is the peak area of fluorescence spectrum, and n is the refractive index of the solvent used. Subscripts s and r refer to the sample and reference quinine sulfate, respectively.

## Culture and preparation of HeLa cells

HeLa cells were cultured in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% $CO_2$ and 95% air at 37 °C. Before the experiments, seed the HeLa cells in 35-mm glass-bottomed dishes at a density of $2\times10^5$ cells per dish in 2 mL of culture medium and incubate them inside an incubator containing 5% $CO_2$ and 95% air at 37 °C. Incubate the cells for 24 h. Cells will attach to the glass surface during this time.

## Cytotoxicity assay

*In vitro* cytotoxicity was measured using the colorimetric methyl thiazolyl tetrazolium (MTT) assay on HeLa cells. Cells were seeded into the 24-well tissue culture plate in the presence of 500 μL Dulbecco's modifed eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C and 5% $CO_2$ atmosphere for overnight and then incubated for 24 h in the presence of **ESIPT-HS** at different concentrations (0, 5, 10, 20, 30, 50 μM). Then cells were washed with PBS buffer and 500 μL supplemented DMEM medium was added. Subsequently, 50 μL MTT (5 mg/mL) was added

to each well and incubated for 4 h. Violet formazan was dissolved in 500 μL sodium dodecyl sulfate solution in the water-DMF mixture. Absorbance of the solution was measured at 570 nm using a microplate reader. The cell viability was determined by assuming 100% cell viability for cells without **ESIPT-HS**.

## Imaging of $H_2S$ in living cells

### *1) Imaging of exogenous $H_2S$ in HeLa cells*

Before the experiments, the well prepared cells were washed with PBS (pH=7.4) buffer three times. Subsequently, incubating with probe **ESIPT-HS** (5 μM) (containing 0.1 % DMSO as a cosolvent) for another 20 min at 37 ˚C, the HeLa cells were rinsed with PBS three times, and the cells were incubated with $H_2S$ for 30 min at 37 ˚C, and then washed with PBS three times, and the fluorescence images were acquired through a Nikon A1MP confocal microscopy inverted fluorescence microscopy equipped with a cooled CCD camera.

### *2) Imaging of endogenous $H_2S$ in HeLa cells*

Before the experiments, the well prepared cells were washed with PBS (pH=7.4) buffer three times. HeLa cells were treated with 10 μM **ESIPT-HS** for 20 min and then washed with PBS three times to remove the probe left in solution and optimize the background signal, the cells incubated with 200 μM cysteine for 2 hours in an atmosphere of 5% CO2 and 95% air. For the control experiments, the cells were treated with 10 μM **ESIPT-HS** for 20 min and then washed with PBS three times, and then cultivated for 2 hours under the same conditions. Before imaging, cells were washed with PBS three times, and the fluorescence images were acquired through a Nikon A1MP confocal microscopy inverted fluorescence microscopy equipped with a cooled CCD camera.

## Synthesis

### *Synthesis of compound ESIPT-HS*

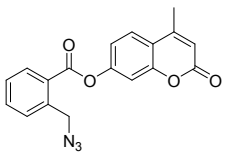
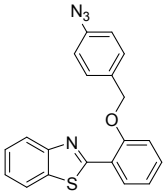
Compound **1** (94 mg, 0.53 mmol, 1.0 eq) was dissolved in anhydrous dichloromethane (20 mL), added DMAP (201 mg，1.6 mmol, 3.0 eq ) and DCC (218 mg, 1.06 mmol, 2.0 eq ) to

the solution and react at room temperature 30 min, and then added compound **HBT** (160 mg, 0.53 mmol, 1.0 eq) to the mixture. The reaction mixture was reacted at room temperature for 12 h with an inert atmosphere of nitrogen, and then the solvent was removed under reduced pressure. The resulting residue was purified by column chromatography on silica gel (petroleum ether to petroleum ether / $CH_2Cl_2$ = 1: 1, v/v) to afford the compound **ESIPT-HS** as a white powder (166 mg, yield: 81%). $^1$H-NMR (400 MHz, $CDCl_3$) δ 8.40 (d, *J* = 7.4 Hz, 1H), 8.31 (d, *J* = 7.5 Hz, 1H), 8.09 (d, *J* = 7.8 Hz, 1H), 7.83 (dd, *J* = 19.6, 7.6 Hz, 2H), 7.71 (dd, *J* = 15.9, 7.5 Hz, 3H), 7.58 (t, *J* = 7.5 Hz, 2H), 7.54 – 7.48 (m, 1H), 7.44 (t, *J* = 7.5 Hz, 1H), 4.84 (s, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ165.12 , 148.40 , 137.99 , 135.03 , 134.27 , 132.72 , 132.28 , 131.04 , 130.55 , 129.22 , 128.43 , 127.62 , 127.16 , 126.21 , 124.95 , 123.21 , 122.60 , 52.37. HRMS (ESI) m/z calcd for $C_{21}H_{14}N_4O_2S$ [M+1]: 387.0916; found 387.0912.

## References


1. G. A. Crosby and J. N. Demas *J. Phys. Chem.*, 1971, **75**, 991-1024.


**Table S1.** The property of probe **ESIPT-HS** compared with the previous reported probe.

| Probe | Signal response | Imaging exogenous $H_2S$ | Imaging endogenous $H_2S$ | Ref. |
|---|---|---|---|---|
|  | –– [a] | Yes | –– [a] | *Chem. Commun.*, 2012, **48**, 10120 |
|  | 43 fold | Yes | –– [a] | *Talanta*, 2014, **121**, 122 |
| **ESIPT-HS** | 400 fold | Yes | Yes | This Work |

[a] Not reported in the literature.

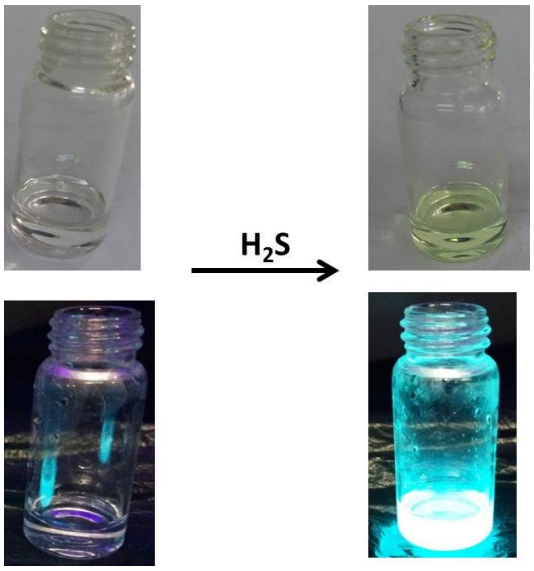



**Figure S1** The color and fluorescence changes of the probe **ESIPT-HS** solution (0.5 mM) with the addition of $Na_2S$ (10 eq) in PBS buffer solution (pH 7.4, containing 30 % DMSO as a co-solvent) with 365 nm ultraviolet light.

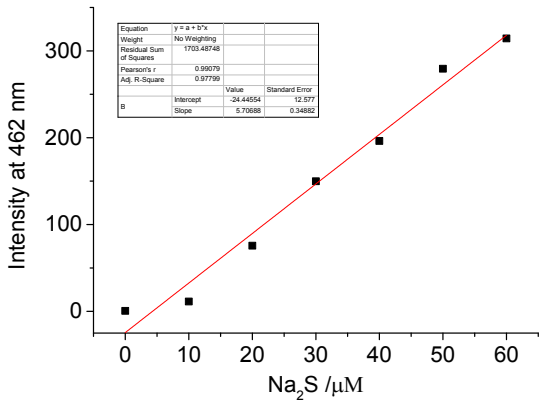



**Figure S2** Relationship between fluorescence intensity at 462 nm of **ESIPT-HS** (10 μM) and the amount of $Na_2S$ (0-60 μM).

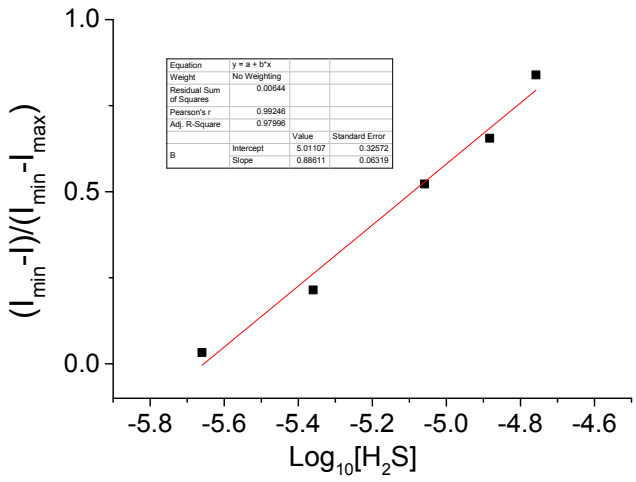



**Figure S3** Normalized response of the fluorescence signal to changing $H_2S$ concentrations.

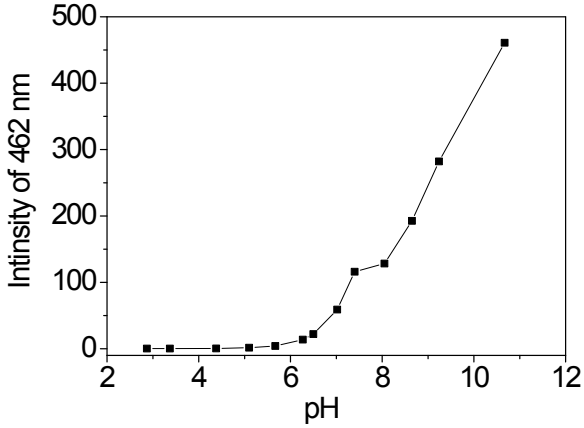



**Figure S4** The emission intensity changes (at 462 nm) of **HBT** at different pH PBS buffer, containing 30 % DMSO as a cosolvent ($\lambda_{ex}$ = 410 nm).

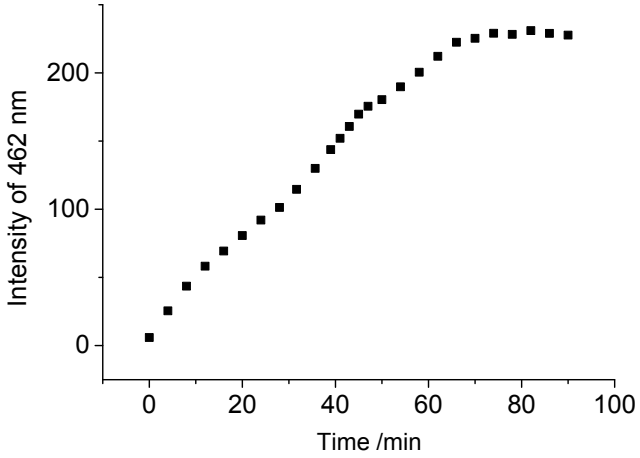



**Figure S5** The fluorescence intensities at 462 nm of **ESIPT-HS** (10 μM) in the presence of $Na_2S$ (50 μM) for continuously monitored at time intervals periods (0-90 min) in PBS buffer (pH 7.4, containing 30 % DMSO as a cosolvent).

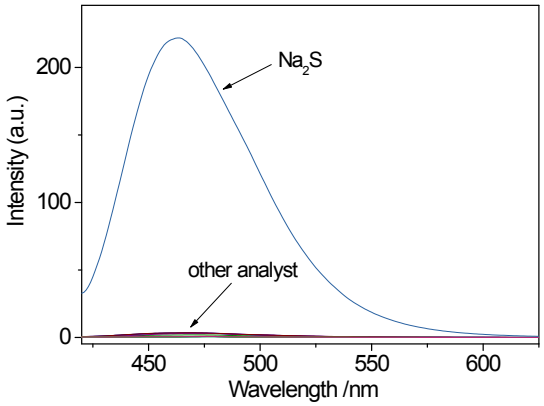



**Figure S6** The fluorescence spectra changes of probe **ESIPT-HS** (10 μM) in the presence of various analytes (30 μM) ($Br^-$; $F^-$; $H_2PO_4^-$; $HPO_4^{2-}$; $S_2O_3^{2-}$; $SO_3^{2-}$; $HSO_3^-$; Cys; GSH; Hcy; $ClO^-$; $H_2O_2$) in PBS buffer (pH 7.4, containing 30% DMSO as a cosolvent).

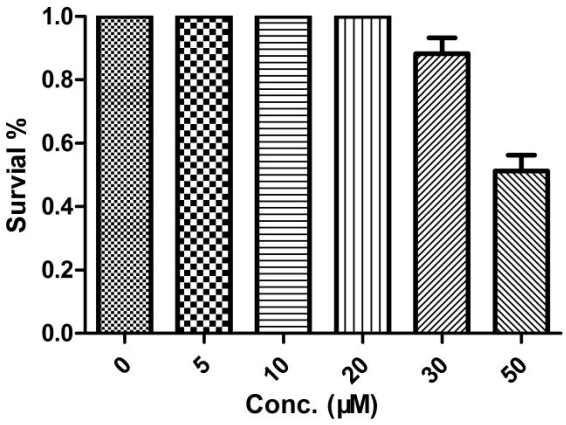



**Figure S7** Cytotoxicity assays of **ESIPT-HS** at different concentrations for HeLa cells

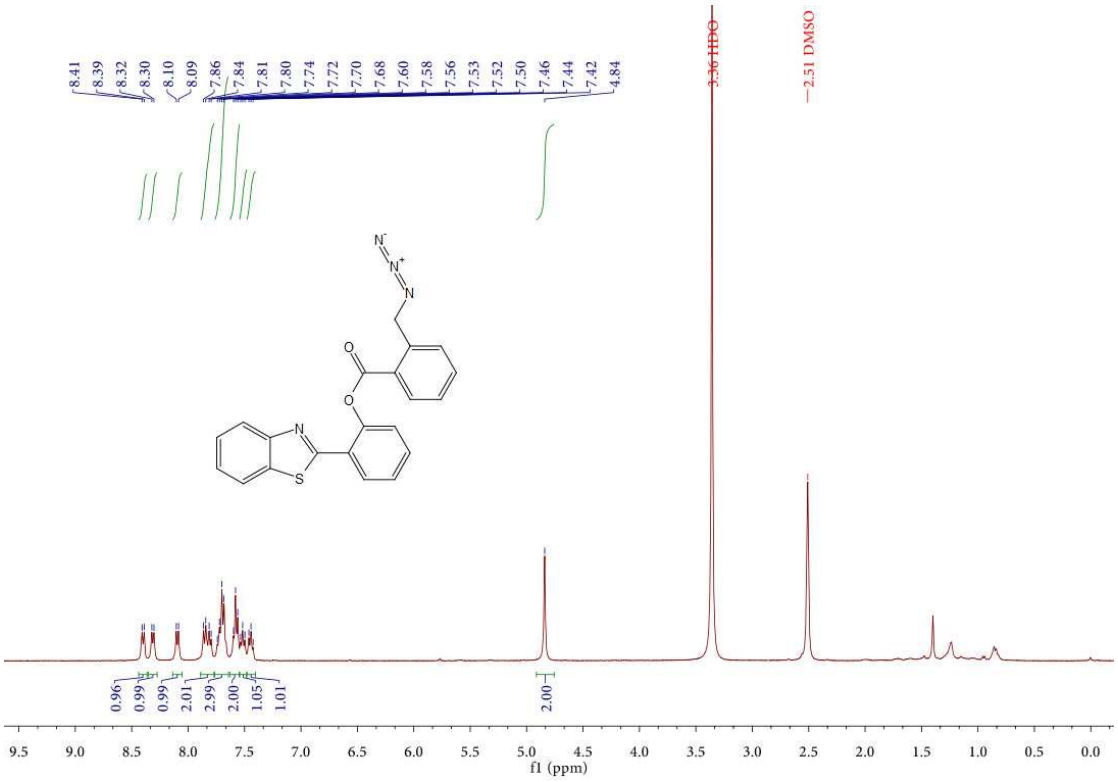



**Figure S8** $^{1}$H-NMR (DMSO-$d_6$) spectrum of **ESIPT-HS**.

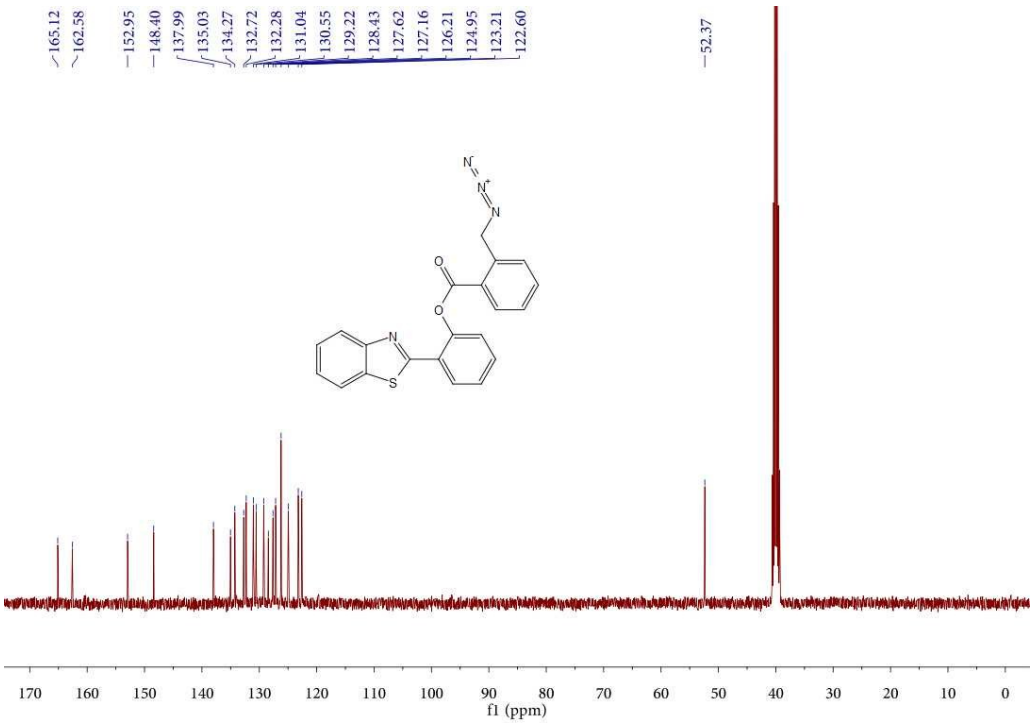



**Figure S9** $^{1}$C-NMR (DMSO-$d_6$) spectrum of **ESIPT-HS**.

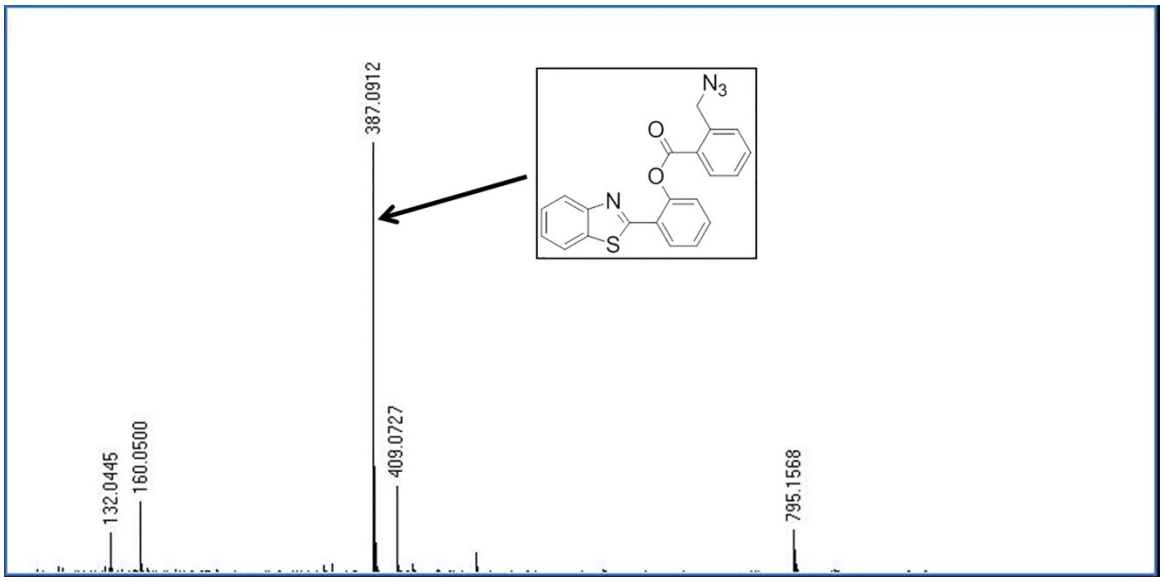



**Figure S10** HRMS (ESI) spectrum of **ESIPT-HS.**