Supporting Information

A New ESIPT-Based Fluorescent Probe for Highly Selective and Sensitive Detection of Hydrogen Sulfide and Its Application in Live-Cell Imaging

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### Photophysical properties of PHS1

**Table S1** Photophysical properties of the probe.

<table>
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<tr>
<th>entry</th>
<th>λ&lt;sub&gt;em&lt;/sub&gt; (nm)</th>
<th>Φ&lt;sup&gt;a&lt;/sup&gt;</th>
<th>ε / M&lt;sup&gt;-1&lt;/sup&gt; cm&lt;sup&gt;-1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHS1</td>
<td>483</td>
<td>0.009</td>
<td>3277</td>
</tr>
<tr>
<td>PHS1+H&lt;sub&gt;2&lt;/sub&gt;S</td>
<td>483</td>
<td>0.104&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4014</td>
</tr>
</tbody>
</table>

(a) The quantum yield (Φ) of PHS1 and PHS1-H<sub>2</sub>S system were determined according to the literature.<sup>1</sup> (b) Φ was determined in the present of 2.0 equiv. of H<sub>2</sub>S.

\[
Φ_{\text{Sample}} = \frac{Φ_{\text{ref}} \cdot A_{\text{ref}} \cdot F_{\text{Sample}} \cdot λ_{\text{ex}}^{2} \cdot η^{2}_{\text{sample}}}{A_{\text{Sample}} \cdot F_{\text{ref}} \cdot λ_{\text{ex}}^{2} \cdot η^{2}_{\text{ref}}}
\]

Where Φ is quantum yield; A is absorbance at the excitation wavelength; F is integrated area under the corrected emission spectra; λ<sub>ex</sub> is the excitation wavelength; η is the refractive index of the solution; the Sample and QS refer to the sample and the standard, respectively. We chose fluorescein in 0.1 M NaOH as standard, which has the quantum yield of 0.95.<sup>2</sup>
Additional spectroscopic data

Scheme S1 ESIPT process of 3-aminophthalimide (3).
Fig. S1 The UV-vis absorption (unsmoothed curves) of PHS1 (10.0 μM) and compound 3 (10.0 μM) in PBS buffer solution (10 mM, pH 7.4, containing 50% EtOH). (Data were collected after incubation of PHS1 with H₂S for 1 h).
**Fig. S2** Fluorescence intensity of **PHS1** (10.0 μM) at 486 nm as a function of H$_2$S concentration (0-80.0 μM) in PBS buffer (10.0 mM, pH 7.4, containing 50% EtOH). Inset: fluorescence intensity of **PHS1** (10.0 μM) at 486 nm as a function of H$_2$S concentration (0-2.0 μM) in PBS buffer (10.0 mM, pH 7.4, containing 50% EtOH). (Data were collected after incubation of **PHS1** with H$_2$S for 1 h).

The detection limit (DL) of H$_2$S using **PHS1** was determined from the following equation: $^3$

\[
DL = 3\sigma/K
\]

Where $\sigma$ is the standard deviation of the blank solution; K is the slope of the calibration curve.
Scheme S2 The proposed mechanism of PHS1-H$_2$S interactions.
Fig. S3 The comparison of fluorescence spectra of the probe-H$_2$S mixture solution (PHS1-Na$_2$S mixture solution) and control (compound 3) in PBS buffer solution (10 mM, pH 7.4, containing 50% EtOH) ($\lambda_{ex} = 393$ nm).
Fig. S4 Comparison of the TLC analysis of PHS1, PHS1-Na$_2$S system, and compound 3 (control).

The pictures of the thin layer chromatography TLC plates under different light used to compare probe PHS1, the reference sample of compound 3 and the reaction mixture of probe PHS1 with Na$_2$S in 1:1 PBS-EtOH (v/v). (A) Under light of 254 nm, and (B) under light of 365 nm. Spots on the TLC plate are: (a) compound 3, (b) the reaction mixture of probe PHS1 and Na$_2$S, (c) probe PHS1. The eluent for TLC: hexane:EtOAc = 3:1 (v/v). This indicates that the reaction of probe PHS1 with Na$_2$S produced compound 3.
Fig. S5 Kinetics of PHS1 (10.0 μM) in the presence of 2.0 equiv. of H₂S in PBS buffer solution (10 mM, pH 7.4, containing 50% EtOH) (λₑₓ = 393 nm).
Fig. S6 Fluorescence responses of PHS1 (10.0 μM) to various reactive sulfur species and coexisting ions (H₂S at 20.0 μM, GSH at 1.0 mM, and Cys, HSO₃⁻, S₂O₃²⁻, S₂O₅²⁻, S₂O₄²⁻, ClO⁻, I⁻, Fe³⁺, F⁻, Cl⁻, Br⁻, H₂PO₄⁻, NO₃⁻ and CO₃²⁻ at 100.0 μM, respectively) in PBS buffer solution (10 mM, pH 7.4, containing 50% EtOH) (λex = 393 nm). (Data were collected after incubation of PHS1 with each analytes for 1 h).
Fig. S7 Fluorescence responses of PHS1 (10.0 μM) to H₂S (20.0 μM) in the presence of various reactive sulfur species and coexisting ions (GSH at 1.0 mM, and Cys, HSO₃⁻, S₂O₃²⁻, S₂O₅²⁻, SO₃²⁻, ClO⁻, I⁻, Fe³⁺, F⁻, Cl⁻, Br⁻, H₂PO₄⁻, NO₃⁻ and CO₃²⁻ at 100.0 μM, respectively) in PBS buffer solution (10 mM, pH 7.4, containing 50% EtOH) (λex = 393 nm). (Data were collected after incubation of PHS1 with each analytes for 1 h).
**Fig. S8** Effect of the pH on the fluorescence emission of PHS1 (10.0 μM) in buffer solution (λex = 393 nm). (Data were collected after incubation of PHS1 with H2S for 1 h).
Fig. S9 Effect of the pH on the fluorescence emission of PHS1-H$_2$S system (10.0 μM of PHS1 and 2.0 equiv. of H$_2$S) in buffer solution (λex = 393 nm). (Data were collected after incubation of PHS1 with H$_2$S for 1 h).
Fig. S10 Effect of the pH on the fluorescence emission of PHS1 (10.0 μM) and PHS1-H$_2$S system (10.0 μM of PHS1 and 2.0 equiv. of H$_2$S) in buffer solution (λ$_{ex}$ = 393 nm). (Data were collected after incubation of PHS1 with H$_2$S for 1 h).
Fig. S11 Effect of different contents of EtOH in PBS solution on the fluorescence emission of PHS1 (10.0 μM) in the presence of 2.0 equiv. of H₂S. (λex = 393 nm). (Data were collected after incubation of PHS1 with H₂S for 1 h).
Fig. S12 Cell viability of HeLa cells treated with different concentration of PHS1 for different time periods. No cytotoxic effect was observed for the cells incubated with PHS1 at 10 μM even for 24 h.
The characterization data of PHS1

$^1$H NMR of 1

$^1$H NMR of 2
$^{13}$C NMR of 2

$^1$H NMR of 3
$^{13}$C NMR of 3

![Chemical structure and NMR spectrum](image-url)
References

