Supporting Information

A mitochondria-targeted red-emitting probe for imaging of hydrogen sulfide in living cells and zebrafish

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Table of Contents

1. Supporting Figures and Table 2-5
2. Supporting spectra 6-11
1. Supporting Figures and Table

**Figure S1.** HPLC trances of the probe 1 (0.25 mM) and H₂S (5 mM) at different reaction time (inset). Conditions: ANGELA TECHNOLOGIES HPLC LC-10F; C18 column with 4.6 mm x 250 mm; wavelength: 273 nm; flow 1.0 mL / min; buffer A: 0.1% (v / v) trifluoroacetic acid in water; buffer B: MeOH; elution condition: 0-3 min, B: 5-50%; 3-13 min, B: 50-80%; 13-25 min, B: 80-95%; 25-30 min, B: 95-5%; 30-32 min, B: 5%.

**Figure S2.** The titration of the probe 1 (5 µM) with variable concentrations of H₂S (0-200 µM). The emission spectra were excited with 565 nm.
Figure S3. The emission intensity at 585 nm of 1 (5 µM) at the indicated pH values in the absence or presence of H₂S (100 µM).

Table S1. The properties of several NBD-based H₂S probes.

<table>
<thead>
<tr>
<th>Probe</th>
<th>λex/λem (nm)</th>
<th>Fluor. enhancement</th>
<th>φ</th>
<th>LOD/µM</th>
<th>Rate/k₂</th>
<th>Ref</th>
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<tr>
<td>1</td>
<td>565/585</td>
<td>~19</td>
<td>0.77</td>
<td>0.36</td>
<td>27.8 M⁻¹s⁻¹</td>
<td>This work</td>
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<td></td>
<td>567/589</td>
<td>~4.5</td>
<td>0.36</td>
<td>0.58</td>
<td>113 M⁻¹s⁻¹</td>
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<td>502/530</td>
<td>~65</td>
<td>0.64</td>
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<td>28 M⁻¹s⁻¹</td>
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<tr>
<td></td>
<td>449/496</td>
<td>~200</td>
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<td>0.9</td>
<td>7.6 M⁻¹s⁻¹</td>
<td>2</td>
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Figure S4. Evaluation on the cytotoxicity of the probe using xCELLigence RTCA system. Real-time monitoring of the density-dependent growth and proliferation of HEK293 cells with different probe concentration. The cell index values with time can reflect the adhesion number of cells inside the well.
Figure S5. Fluorescence imaging for the L-Cys-induced H₂S in living cells. Cells were incubated with L-Cys (100 μM) for 30 min, then washed cells and incubated with probe 1 (5 μM) at different time intervals (0-30 min). The average fluorescence of the time-dependent images is shown below.

Supporting references:
2. Supporting spectra
Chemical Formula: C₄₂H₁₃FeO₄S₂
Exact Mass: 595.9670

Chemical Formula: C₆H₁₃N₂O₄⁺
Exact Mass: 410.1630
Chemical Formula: C_{35}H_{52}N_{7}O_{5}^+
Exact Mass: 642.2459

probe 1 + Na_{2}S

Chemical Formula: C_{30}H_{32}N_{4}O_{2}^+
Exact Mass: 479.2442