Supporting Information

A Near-Infrared Water-Soluble Fluorescent Probe for the Detection of Biothiols in Living Cells and Escherichia Coli

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1. Synthesis of Compounds S1
Scheme S1. Synthetic route of HXPIS.

Compound 1 and HXPI were prepared referring to the literature reports.\textsuperscript{1-2}

**HXPIS**: To a solution of HXPI (500.0 mg, 0.93 mmol) in dry CH\textsubscript{2}Cl\textsubscript{2} (10 mL), NaH (110 mg, 2.79 mmol) was added. The mixture was stirred at room temperature for 30 min. Then 2,4-dinitrobenzenesulfonyl chloride (740 mg, 2.79 mmol) was diluted in 10 mL CH\textsubscript{2}Cl\textsubscript{2} and added dropwise to the solution. The mixture was stirred at room temperature for another 5 hours. The color of the reaction changed from deep blue to purple. The solvent was removed under reduced pressure and the crude product was purified by column chromatography over silica gel eluting with CH\textsubscript{2}Cl\textsubscript{2}/CH\textsubscript{3}OH = 80:1 to afford purple solid (463 mg, yield 56.0%). \textsuperscript{1}H-NMR (400 MHz, DMSO-d\textsubscript{6}) \(\delta\) 0.93–0.97 (t, \(J_1=8.0\) Hz, \(J_2=8.0\) Hz, 3H), 1.71 (s, 6H), 1.79–1.85 (m, 4H), 2.64–2.67 (m, 4H), 4.43–4.46 (t, \(J_1=8.0\) Hz, \(J_2=4.0\) Hz, 2H), 6.69–6.73 (d, \(J=16.0\) Hz, 1H), 6.99–7.02 (d, \(J=12.0\) Hz, 1H), 7.30 (s, 1H), 7.50–7.57 (m, 3H), 7.42 (s, 1H), 7.76–7.79 (t, \(J_1=4.0\) Hz, \(J_2=8.0\) Hz, 2H), 8.04–8.06 (d, \(J=8.0\) Hz, 1H), 8.33–8.37 (m, 2H), 8.48–8.51 (m, 2H), 8.60–8.63 (d, \(J=8.0\) Hz, 1H), \(\delta\) 9.14 (s, 1H). \textsuperscript{13}C-NMR (101 MHz, DMSO-d\textsubscript{6}) \(\delta\) 11.4, 20.1, 21.7, 23.9, 27.5, 29.1, 47.1, 51.5, 107.8, 110.6, 114.6, 115.1, 118.7, 121.6, 121.9, 123.2, 126.1, 128.0, 128.5, 129.4, 129.5, 131.1, 131.2, 131.5, 134.2, 141.7, 143.1, 145.1, 145.9, 147.8, 148.6, 149.6, 152.0, 152.9, 158.2, 174.3, 179.7. HRMS (ESI) m/z calcd. for C\textsubscript{34}H\textsubscript{32}N\textsubscript{3}O\textsubscript{8}S\textsuperscript{+} (M\textsuperscript{+}): 642.1905. Found: 642.1903.

# 2. Molar Extinction Coefficient
Figure S1. (a) UV spectra of HXPIS at different concentrations (0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 μM) (b) Absorption-concentration curve of HXPIS.

3. UV spectra

Figure S2. Absorption spectra of HXPIS (5 μM), HXPIS (5 μM) with GSH (100 μM), and HXPI (5 μM) in PBS buffer solution (10 mM, pH 7.4).

4. Time-dependent fluorescence changes of HXPIS with biothiols.
5. Fluorometric titration experiment

Figure S3. Time-dependent fluorescence changes of HXPI (5 µM) to (a) GSH, (b) Cys and (c) Hcy; Fluorescence intensity changes at 703 nm of HXPI with (d) GSH, (e) Cys and (f) Hcy at different indicated time.

5. Fluorometric titration experiment
Figure S4. Fluorescence changes of HXPI (5 μM) on the incremental addition of (a) Cys, (c) Hcy; The tendency chart of the fluorescence intensity of HXPI with the increased concentration of (b) Cys, (d) Hcy.

6. Limit of detection

The limit of detection, expressed as the concentration, CL, $CL = 3\sigma/m$

$$\sigma = \sqrt{\frac{\sum(x - \bar{x})^2}{n-1}}$$

$x$ is the mean of the blank measures (probe only), $x_i$ is the values of blank measures, $n$ is the tested number of blank measures, $m$ is the slope of the linear regression equation.

Figure S5. (a) The fluorescence response of the probe HXPI to GSH at the varied concentrations (0-1 μM). (b) A linear correlation between fluorescent response and concentration of GSH. The
spectra were recorded in PBS buffer (pH 7.4, 10 mM). $\lambda_{\text{ex/em}} = 654/703$ nm.

Figure S6. (a) The fluorescence response of the probe HXPIS to Cys at the varied concentrations (0-1 $\mu$M). (b) A linear correlation between fluorescent response and concentration of Cys. The spectra were recorded in PBS buffer (pH 7.4, 10 mM). $\lambda_{\text{ex/em}} = 654/703$ nm.

Figure S7. (a) The fluorescence response of the probe HXPIS to Hcy at the varied concentrations (0-1 $\mu$M). (b) A linear correlation between fluorescent response and concentration of Hcy. The spectra were recorded in PBS buffer (pH 7.4, 10 mM). $\lambda_{\text{ex/em}} = 654/703$ nm.

7. pH influence
**Figure S8.** Effects of pH on the fluorescence of HXPIS (5 μM) + GSH (100 μM), HXPI (5 μM) and HXPIS (5 μM). Conditions: 10 mM PBS, pH 7.4. \( \lambda_{ex}/\lambda_{em} = 654/703 \) nm.

8. Selectivity of the probe HXPIS towards biothiols.
Figure S9. (a) The fluorescence spectra of the probe HXPIs (5 μM) in the presence of various relevant analytes (20 equiv. of NaHS, Na₂SO₃, NaHSO₃, Cystine, D-Met, L-Phe, L-Pro, L-Asp, D-Glu, L-Lys, Cys, Hcy and GSH). (b) The fluorescence spectra of HxpiS (5 μM) upon the addition of different interferents (NaHS, Na₂SO₃, NaHSO₃, Cystine, D-Met, L-Phe, L-Pro, L-Asp, D-Glu, L-Lys, 100 μM) in the presence of GSH (100 μM) in PBS (pH = 7.4, λₑₓ/λₑ𝐦 = 654 /703 nm). (c) The histogram of (b). The data were obtained after the incubation of the probe HXPIs with the analytes at 37 °C for 30 min.

![HRMS result of the reaction product of HXPIS with GSH.](image)

**Figure S10.** HRMS result of the reaction product of HXPIS with GSH.

10. Cytotoxicity experiments

![Cytotoxicity of different concentrations of HXPIS and HXPI to Hela cells by a standard MTS assay, the experiment was repeated three times and the data are shown as mean (±S.D.).](image)

**Figure S11.** Cytotoxicity of different concentrations of HXPIS and HXPI to Hela cells by a standard MTS assay, the experiment was repeated three times and the data are shown as mean (±S.D.).

11. Table S1. Summary of the optical properties of representative near-infrared fluorescent probes for distinguishing biothiols.

<table>
<thead>
<tr>
<th>NIR probe</th>
<th>( \lambda_{ex} / \lambda_{em} ) (nm)</th>
<th>Solubility</th>
<th>References</th>
</tr>
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<tr>
<td><img src="image" alt="NIR probe" /></td>
<td>600/737</td>
<td>EtOH/PBS (v/v, 1:1, pH = 7.4)</td>
<td>J. Mater. Chem. B, 2018, 6, 1791-1798</td>
</tr>
<tr>
<td>Compound</td>
<td>Excitation/Emission</td>
<td>Solvent</td>
<td>Source</td>
</tr>
<tr>
<td>----------</td>
<td>---------------------</td>
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<tr>
<td><img src="image1.png" alt="Compound 1" /></td>
<td>600/746</td>
<td>EtOH/PBS (v/v, 3:2, pH = 7.4)</td>
<td>J. Mater. Chem. B, 2018, 6, 7486-7494</td>
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<tr>
<td><img src="image2.png" alt="Compound 2" /></td>
<td>470/540 &amp; 600/670</td>
<td>THF/PBS (v/v, 1:1, pH = 7.4)</td>
<td>Analyst, 2018, 143, 5218-5224</td>
</tr>
<tr>
<td><img src="image3.png" alt="Compound 3" /></td>
<td>646/661</td>
<td>THF/PBS (v/v, 1:1, pH = 7.4)</td>
<td>Dyes and Pigments, 2018, 152, 85-92</td>
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<tr>
<td><img src="image4.png" alt="Compound 4" /></td>
<td>620/688</td>
<td>DMSO/PBS (v/v, 1:1, pH = 7.4)</td>
<td>Sensor and Actuators B, 2017, 246, 988-993</td>
</tr>
<tr>
<td>Chemical Structure</td>
<td>Wavelength (nm)</td>
<td>Solvent/Buffer</td>
<td>Source</td>
</tr>
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<td><img src="image1.png" alt="Chemical Structure 1" /></td>
<td>620 / 685</td>
<td>DMSO/PBS (v/v, 1: 1, pH = 7.4)</td>
<td>Sensor and Actuators B, 2018, 257, 1076-1082</td>
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<td><img src="image2.png" alt="Chemical Structure 2" /></td>
<td>560 / 690</td>
<td>DMSO/H_2O (v/v, 1: 1, pH = 7.4)</td>
<td>Chem. Commun., 2014, 50, 1751-1753</td>
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<tr>
<td><img src="image3.png" alt="Chemical Structure 3" /></td>
<td>530 / 650</td>
<td>DMSO/PBS (v/v, 1: 1, pH = 7.4)</td>
<td>Chem. Sci., 2016, 7, 4958-4965</td>
</tr>
<tr>
<td><img src="image4.png" alt="Chemical Structure 4" /></td>
<td>575 / 655</td>
<td>CH_3CN/HEPES (v/v, 1: 1, pH = 9)</td>
<td>RSC Adv., 2015, 5, 28713-28716</td>
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<tr>
<td><img src="image5.png" alt="Chemical Structure 5" /></td>
<td>650 / 748</td>
<td>DMSO/HEPES (v/v, 1: 1, pH = 7.4)</td>
<td>RSC Adv., 2014, 4, 8360-8364</td>
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<tr>
<td>Chemical Structure</td>
<td>Solvent</td>
<td>pH</td>
<td>Journal and Volume</td>
</tr>
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<tr>
<td><img src="image1.png" alt="Chemical Structure" /></td>
<td>PBS, pH=7.4</td>
<td>600/ 698</td>
<td>Org. Biomol. Chem., 2013, 11, 2098-</td>
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<td><img src="image2.png" alt="Chemical Structure" /></td>
<td>DMSO/PBS (v/v, 1: 1, pH = 7.4)</td>
<td>550 / 680</td>
<td>Chem. Sci. 2016, 7, 1896-1903</td>
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<td><img src="image3.png" alt="Chemical Structure" /></td>
<td>PBS, pH=7.4</td>
<td>600/ 661</td>
<td>Chem. Nano. Mat., 2016, 2, 396-399</td>
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<tr>
<td><img src="image4.png" alt="Chemical Structure" /></td>
<td>HEPES, pH=7.4</td>
<td>600 / 810</td>
<td>J. Am. Chem. Soc., 2014, 136, 7018-7025</td>
</tr>
</tbody>
</table>
12. NMR and HRMS spectra of HXPI

Figure S12. $^1$H-NMR spectrum of HXPI in DMSO-d$_6$. 

Table: 

<table>
<thead>
<tr>
<th>681 / 703</th>
<th>PBS, pH=7.4</th>
<th>This Work</th>
</tr>
</thead>
<tbody>
<tr>
<td>681 / 703</td>
<td>PBS, pH=7.4</td>
<td>This Work</td>
</tr>
</tbody>
</table>
Figure S13. $^{13}$C-NMR spectrum of HXPIS in DMSO-$d_6$.

MS(E+)

Figure S14. HRMS result of HXPIS.

Reference
