Electronic Supplementary Information for

A two-photon ratiometric fluorescent probe for highly selective sensing mitochondrial cysteine in live cells

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Fig. S7  Fluorescence spectral changes of DNEPI (10 μM) against time in the presence of 100 μM of (a) Cys, (b) Hcy, (c) GSH in DMSO/PBS (1/99, v/v, pH 7.4). (d) Fluorescence responses of DNEPI toward thiols after 30 min in DMSO/PBS (1/99, v/v, pH 7.4).
Fig. S8  Fluorescence intensity ratio ($F_{583\text{nm}}/F_{485\text{nm}}$) of DNEPI with Cys concentration in the range of 0-300 μM.

Table S1. Photophysical data for DNEPI and compound 1

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$\lambda_{ex}$ (nm)</th>
<th>$\lambda_{em}$ (nm) $^a$</th>
<th>$F_{max}$ $^b$</th>
<th>$\Phi_F$ (DNEPI)</th>
<th>$\Phi_F$ (compound 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS(7.4)</td>
<td>350</td>
<td>472</td>
<td>$3.91 \times 10^3$</td>
<td>0.0021</td>
<td>0.0126</td>
</tr>
<tr>
<td>MeOH</td>
<td>350</td>
<td>460</td>
<td>$2.44 \times 10^4$</td>
<td>0.0114</td>
<td>0.0825</td>
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<tr>
<td>DMSO</td>
<td>354</td>
<td>462</td>
<td>$2.73 \times 10^4$</td>
<td>0.0201</td>
<td>0.0620</td>
</tr>
<tr>
<td>DCM</td>
<td>356</td>
<td>460</td>
<td>$1.49 \times 10^4$</td>
<td>0.0091</td>
<td>--</td>
</tr>
</tbody>
</table>

$a$) $\lambda_{em}$ is the maximum emission wavelength. $b$) $F_{max}$ is the maximum fluorescence intensity of DNEPI. $c$) $\Phi_F$ is the fluorescence quantum yield. “--” represents that compound 1 is insoluble in DCM.

Fig. S9  Fluorescence decay as a function of lifetime of DNEPI in DMSO/PBS (1/1, v/v, pH 7.4).
Fig. S10  UPLC-MS spectral changes for DNEPI upon addition of Cys. The interactions were monitored at 0 min, 5 min, 10 min and 15 min, respectively. The integral area of DNEPI around 3.0 min decreased markedly from 2.06×10^6 to 5.78×10^4, concomitant with a new peak of compound 1 at 2.2 min appeared and over time, and its integral area increased gradually from 2.86×10^8 to 3.91×10^8.

![Graph showing UPLC-MS spectral changes for DNEPI upon addition of Cys.]

Fig. S11  The pH effect on the fluorescence ratio \((F_{583nm}/F_{485nm})\) changes of DNEPI (10 μM) in the absence and presence of Cys, Hcy, GSH and Na₂S (100 μM) in DMSO/PBS (1/1, v/v, pH 7.4) at different pH, respectively.

![Graph showing the pH effect on the fluorescence ratio \((F_{583nm}/F_{485nm})\) changes of DNEPI (10 μM) at different pH.]

Figure S12  Changes in fluorescence intensity of DNEPI (10 μM) against time in DMSO/PBS (1/1, v/v, pH 7.4) \((λ_{ex} = 370 \text{ nm}, λ_{em} = 485 \text{ nm})\).
Fig. S13  Cell viability of SMMC7721 cells treated with different concentration of DNEPI (0, 1, 5, 10, 15 and 20 μM) in the absence (black bars) and presence (red bars) of 100 μM Cys for 24 h in fresh medium.

Fig. S14  Two-photon action cross-section excited spectra of DNEPI and its released fluorophore compound 1 in DMSO:H₂O (1:1), respectively.