Electronic Supplementary Information

Fast and sensitive fluorescent probe for hydrogen sulfide ratiometric detection in mitochondria

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Synthetic procedures of dye and intermediates

Scheme S1. Synthesis of intermediates and Mito-BT.

(1) 2, the synthesis of 4-(diphenylamino)benzaldehyde.11

POCl₃ (4.0 mL) was dropped slowly into DMF (16 mL) at 0 °C and stirred for another 2 h at room temperature. To the above solution was added a dichloromethane (CH₂Cl₂)
solution of triphenylamine (7.5 g, 31 mmol). After the mixture was refluxed for 10 h \(\text{CH}_2\text{Cl}_2\) was removed. The residue was poured into water (500 mL) and the yellow solid was collected by suction filtration. Product was obtained by column chromatography (7.2 g, yield 86%).

(2) 5, the synthesis of 3-benzyl-2-methylbenzothiazolium bromide salt.

Benzyl bromide (0.85 g, 5 mmol) was added under nitrogen to 2-methyl benzothiazole (0.90 g, 6 mmol) with stirring at 60-70 °C for 3 h. The mixture was then cooled, the precipitate filtered off, and washed with ether to give 3-benzyl-2-methylbenzothiazolium bromide, 3-benzyl-2-methylbenzothiazolium bromide salt 1a was obtained as white solid as crude product (2.6 g, yield 81%).

(3) the synthesis of Mito-BT.

Mito-BT was conveniently synthesized via the condensation of compound 5 with 2 (Scheme 1). Compound 5 (1.4 g, 4.4 mmol), compound 2 (1.0 g, 3.6 mmol), triethylamine (3 drops) were mixed in an absolute ethanol solution (20 mL). The solution was refluxed under nitrogen for 24 h, and then cooled down. The precipitate was collected, washed with diethyl ether, then dried, giving Mito-BT as a violet solid (1.49 g, 82.4%). \(^1\text{H} \text{NMR} \text{ (400 MHz, DMSO)} \delta 8.43 \text{ (d, } J = 7.7 \text{ Hz, } 1\text{H}), 8.24 \text{ (d, } J = 15.4 \text{ Hz, } 1\text{H}), 8.14 \text{ (d, } J = 8.2 \text{ Hz, } 1\text{H}), 7.95 \text{ (dd, } J = 21.5, 12.1 \text{ Hz, } 3\text{H}), 7.75 \text{ (dt, } J = 15.2, 7.2 \text{ Hz, } 2\text{H}), 7.43 \text{ (t, } J = 7.8 \text{ Hz, } 4\text{H}), 7.36 \text{ (dd, } J = 14.7, 6.6 \text{ Hz, } 5\text{H}), 7.30 - 7.17 \text{ (m, } 8\text{H}), 6.88 \text{ (d, } J = 8.8 \text{ Hz, } 2\text{H}), 6.24 \text{ (s, } 2\text{H}). \text{HR-MS (ESI): } m/z, \text{ calcd for } \text{C}_{34}\text{H}_{27}\text{N}_{2}\text{S}^+ \text{495.1889, found 495.1885.}
Table S1. Spectral properties of Mito-BT in various solvents.

<table>
<thead>
<tr>
<th>Solvents</th>
<th>$\lambda_{\text{abs}}$</th>
<th>$\lambda_{\text{em}}$</th>
<th>$\Delta\lambda$</th>
<th>$\Phi$</th>
<th>$\varepsilon$</th>
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<tbody>
<tr>
<td>C$_6$H$_5$CH$_3$</td>
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<td>654</td>
<td>122</td>
<td>0.011</td>
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<tr>
<td>Dioxane</td>
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<td>651</td>
<td>124</td>
<td>0.239</td>
<td>14300, 39000</td>
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<tr>
<td>CH$_3$CN</td>
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<td>620</td>
<td>98</td>
<td>0.061</td>
<td>14500, 45700</td>
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<tr>
<td>CH$_3$CH$_2$OH</td>
<td>295, 531</td>
<td>649</td>
<td>118</td>
<td>0.058</td>
<td>13700, 48500</td>
</tr>
<tr>
<td>CH$_3$OH</td>
<td>292, 527</td>
<td>643</td>
<td>116</td>
<td>0.065</td>
<td>14200, 50600</td>
</tr>
<tr>
<td>DMF</td>
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<td>635</td>
<td>115</td>
<td>0.108</td>
<td>15400, 40200</td>
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<tr>
<td>DMSO</td>
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<td>645</td>
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<td>0.073</td>
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<tr>
<td>H$_2$O</td>
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<td>663</td>
<td>148</td>
<td>0.014</td>
<td>12900, 40600</td>
</tr>
</tbody>
</table>


**Fig. S1.** Absorption spectra of Mito-BT (30 μM) in water-ethanol (v/v=1:4) upon titration with HS$^-$ (0–60 μM).

**Fig. S2.** Proposed H$_2$S sensing mechanism of Mito-BT.
Fig. S3. The sensing mechanism of Mito-BT for HS\(^{-}\). \(^1\)H NMR spectral change of Mito-BT (10 mM) in the absence and presence (2, 3, 4, 5) of HS\(^{-}\), the gradient of HS\(^{-}\) is 2 mM. The \(^1\)H NMR titration experiment was performed for examining the sensing mechanism of Mito-BT to HS\(^{-}\). Mito-BT was dissolved in DMSO to get a mixed solution of 10 mM. Then, a solution of HS\(^{-}\) in D\(_2\)O was added into the above solution in a gradient of 2 mM.

Fig. S4. The photofading experiment of Mito-BT (30 µM) in DMSO under 1000 W iodine–tungsten lamp.
$^1$H NMR (400 MHz) spectra of Mito-BT in d$_6$-DMSO.

References: