Electronic Supplementary Information

A mitochondria-targeted ratiometric fluorescent probe for hypochlorite and its applications in bioimaging

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Fig. S6 $^{13}$C NMR spectrum of RCP.
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Fig. S8 High resolution mass spectrum of RCP.
**Fig. S9** Fluorescence intensity ratio ($I_{570}/I_{483}$) of RCP versus pH values in the absence (■) or presence (●) of $\cdot$OCl (8 equivalent). Condition: [RCP] = 5 µM, [·OCl] = 40 µM, PBS buffer (pH 4.0-10.0, containing 0.5% EtOH), incubation time = 30 min. $\lambda_{ex} = 420$ nm.

**Fig. S10** Time-dependent fluorescence intensity ratio ($I_{483}/I_{570}$) changes of RCP upon addition of $\cdot$OCl (6 equivalent). Condition: [RCP] = 5 µM, [·OCl] = 30 µM, PBS buffer (pH 7.4, containing 0.5% EtOH), $\lambda_{ex} = 420$ nm.
Table S1. The performances of RCP and other °OCl probes

<table>
<thead>
<tr>
<th>Probes</th>
<th>Probe concentration (µM)</th>
<th>Solvent</th>
<th>λex/λem (nm)</th>
<th>Detection limit (nM)</th>
<th>Reaction time</th>
<th>Ref.</th>
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<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td>5</td>
<td>EtOH:PBS (pH 7.4) = 0.5:99.5 (v:v)</td>
<td>420/483,570</td>
<td>70</td>
<td>within 1 min</td>
<td>This work</td>
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<tr>
<td><img src="image2.png" alt="Image" /></td>
<td>5</td>
<td>DMF:potassium phosphate buffer (pH 8.5) = 4:6 (v:v)</td>
<td>410,554/501,578</td>
<td>24</td>
<td>within 1 min</td>
<td>4</td>
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<tr>
<td><img src="image3.png" alt="Image" /></td>
<td>10</td>
<td>THF:Na₂BaO₃/NaOH (pH 12) = 3:7 (v:v)</td>
<td>520/578</td>
<td>27</td>
<td>20 min</td>
<td>26</td>
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<tr>
<td><img src="image4.png" alt="Image" /></td>
<td>10</td>
<td>DMF:NaH₂PO₄ (pH 5) = 4:6 (v:v)</td>
<td>410/470,580</td>
<td>–</td>
<td>within 100 s</td>
<td>46</td>
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<tr>
<td><img src="image5.png" alt="Image" /></td>
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<td>DMF:PBS (pH 7.4) = 1:1 (v:v)</td>
<td>414/473,594</td>
<td>52</td>
<td>–</td>
<td>33</td>
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<tr>
<td><img src="image6.png" alt="Image" /></td>
<td>10</td>
<td>DMSO:PBS (pH 7.4) = 1:99 (v:v)</td>
<td>553/558</td>
<td>9</td>
<td>2 min</td>
<td>30</td>
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<td>EtOH:Na₂HPO₄ (pH 6) = 3:7 (v:v)</td>
<td>350/440,585</td>
<td>100</td>
<td>2 min</td>
<td>38</td>
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<tr>
<td><img src="image8.png" alt="Image" /></td>
<td>5</td>
<td>MeCN:PBS (pH 7.4) = 3:7 (v:v)</td>
<td>550/575</td>
<td>1.06</td>
<td>40 min</td>
<td>47</td>
</tr>
</tbody>
</table>
**Fig. S11** HRMS spectra of the crude product after treatment of RCP with ‘OCl.

**Fig. S12** Fluorescence images of RAW264.7 cells co-stained with RCP (5 μM) and Lyso Tracker Deep Red (0.1 μM). (a) Blue fluorescence of RCP (405-555 nm), λex = 405 nm. (b) Red fluorescence of Lyso Tracker Deep Red, λex = 640 nm. (c) Merge images of (a) and (b). (d) Bright field images. (e) Quantitation of co-localization coefficient (Pearson’s coefficient): 0.59. Scale bar = 10 μm.
**Fig. S13** Fluorescence images of RAW264.7 cells co-stained with RCP (5 μM) and Mito Tracker Deep Red (0.3 μM). (a) Red fluorescence of RCP (560-700 nm), \( \lambda_{ex} = 405 \text{ nm} \). The red fluorescence was coloured as green for discrimination. The signal has been amplified to emphasize the probe’s location. (b) Red fluorescence of Mito Tracker Deep Red, \( \lambda_{ex} = 644 \text{ nm} \). (c) Merge images of (a) and (b). (d) Bright field images. (e) Quantitation of co-localization coefficient (Pearson’s coefficient): 0.91. Scale bar = 20 μm.
**Fig. S14** Photostability of RCP (5 µM) in RAW264.7 cells. (a) Fluorescence images of RAW264.7 cells after 0, 30, 60, 90 and 120 s of continuous irradiation. λ<sub>ex</sub> = 405 nm. First line: fluorescence images at blue channel (405-555 nm), second line: fluorescence images at red channel (560-700 nm), third line: bright field images, fourth line: merge images of first, second and third line. (b) The relative ratio of red fluorescence intensity (rhodamine moiety) in cells at different periods of time. (c) The relative ratio of blue fluorescence intensity (coumarin moiety) in cells at different periods of time. (d) The corresponding relative ratio of red/blue fluorescence intensity in cells at different periods of time [the initial red/blue fluorescence intensity ratio (i.e., at about 0 s) was defined as 1.0]. Fluorescence intensity quantitation was analyzed by the Image J. The results were presented as means ± SE with replicates n = 3. Scale bar = 20 µm.