Supporting Information (SI)

Promoted fluorescent sensing strategy for hypochlorous acid by using serum albumin as a signal amplifier

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Contents

1. Synthesis of compound TPP........................................................................................................S-2
2. Generation of various ROSs..................................................................................................S-2
3. Spectral profiles (Figures S1-S16)..........................................................................................S-3
4. $^1$H, $^{13}$C NMR and HRMS spectra (Figures S17-S21)..............................................................S-12
5. References..................................................................................................................................S-14
1. Synthesis of compound TPP.

Scheme 1. Structure and synthesis of TPP.

To the solution of 4-(N, N-diphenylamino)benzaldehyde (0.54 g, 2.0 mmol) in acetic anhydride (10 mL) was added 4-methylpyridine (0.23 g, 2.5 mmol) at r.t.. The mixture was refluxed for 24 h., after completion of the reaction the reaction mixture was cooled to r.t. and the solvent was removed by rotary evaporation under vacuum. The resulting crude product was then subjected to column purification (silica gel, 40-50 % EtOAc in hexanes) to afford the pure TPP (yield: 30 %). $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ (ppm) 8.51 (d, $J = 4.7$ Hz, 2H), 7.56 (d, $J = 8.6$ Hz, 2H), 7.52 (d, $J = 5.7$ Hz, 2H), 7.47 (d, $J = 11.6$ Hz, 1H), 7.33 (m, 4H), 7.10 (d, $J = 4.9$ Hz, 2H), 7.07 (m, 5H), 6.96 (d, $J = 8.6$ Hz, 2H); $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ (ppm) 150.34, 148.10, 147.16, 144.97, 132.92, 130.37, 130.06, 129.83, 128.70, 124.94, 124.38, 124.04, 123.66, 122.67, 121.05. ESI-MS m/z: [M+H]$^+$ Calcd for C$_{25}$H$_{21}$N$_2^+$ 349.1699; Found 349.1703.

2. Generation of various ROSs

(1) ClO$^-$ was prepared by dilution of commercial NaClO solution in deionized water and the concentration of the ClO$^-$ stock solution was determined by measuring the absorbance at 209 nm with a molar extinction coefficient of 350 M$^{-1}$cm$^{-1}$.

(2) OONO$^-$ stock solution was prepared by mixing the following three kinds of solutions simultaneously, the mixture of hydrogen peroxide (0.7 M, 1.5 mL) and hydrochloric acid (0.6 M, 1.5 mL), solution of sodium nitrite (0.6 M, 3 mL) and solution of sodium hydroxide (1.5 M, 3 mL).$^{[1]}$ The concentration of the OONO$^-$ stock solution was determined in 0.1 M NaOH by measuring the absorbance at 302 nm with a molar extinction coefficient of 1670 M$^{-1}$ cm$^{-1}$.

(3) •OH was generated in the Fenton system from ferrous ammonium sulfate and
hydrogen peroxide. [2]

(4) H$_2$O$_2$ solution was purchased from Aladdin reagent. The concentration of the H$_2$O$_2$ stock solution was determined by measuring the absorbance at 240 nm with a molar extinction coefficient of 43.6 M$^{-1}$cm$^{-1}$.

(5) O$_2^-$ was obtained by the water solution of potassium superoxide.

(6) t-BuOO$^-$ was prepared by dilution of commercial t-BuOOK in water.

(7) $^1$O$_2$ was obtained by adding NaClO solution (1.0 M, 2 mL) to H$_2$O$_2$ solution (1.0 M, 2 mL).

3. Spectral titration profiles

![Graph showing the linear relationship between ratio of A$_{484}$ nm/A$_{388}$ nm and ClO$^-$ concentrations with in 0–100 μM in ethanol][3]

Figure S1 The linear relationship between ratio of A$_{484}$ nm/A$_{388}$ nm and ClO$^-$ concentrations with in 0–100 μM in ethanol.[3]
Figure S2 Partial $^1$H NMR (500 MHz) spectra of TPM (a), TPM+ClO$^-$ (b) in MeOD and TPP in DMSO-$d_6$ (c). [TPM]=[TPP]=2.0 mM.

Figure S3 HRMS spectrum of TPM with 1 equivalent ClO$^-$ in EtOH. [TPM]=50 μM. Signal at m/z 349.1706 (calcd. 349.1705) for the oxidation product [TPP+H]$^+$ ([C$_{25}$H$_{21}$N$_2$]$^+$) in the mixture of TPM/ClO$^-$ in EtOH matched well with the theoretical simulation.
**Figure S4** (A) Absorption and (B) fluorescence spectra of 50 μM TPM in the presence of 15 equivalents of ClO⁻ and 50 μM TPP in ethanol.

**Figure S5** (A) Absorption and (C) fluorescence spectra of 50 μM TPM in the presence of ClO⁻ of increasing concentration in PBS buffer (10 mM, pH 7.4). Excitation wavelength: 462 nm. Plot of (B) absorption (A₄₆₆ nm) and (D) fluorescence intensity (Int. ₆₃₈ nm) changes as a function of ratio [ClO⁻]/[TPM]. Inset shows the color changes upon the addition of 2.0 eq. ClO⁻ under ambient light or 365 nm UV light.
Figure S6 The linear relationship between fluorescence intensity and ClO⁻ concentrations within 0–25 μM in PBS buffer solution.

![Graph showing the linear relationship between fluorescence intensity and ClO⁻ concentrations](image)

<table>
<thead>
<tr>
<th>Equation</th>
<th>y = a + b'x</th>
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<tr>
<td>Adj. R-Square</td>
<td>0.99746</td>
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<table>
<thead>
<tr>
<th>Value</th>
<th>Standard Error</th>
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</thead>
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<tr>
<td>170.64319</td>
<td>2.2638</td>
</tr>
<tr>
<td>-3.85745</td>
<td>0.16794</td>
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Figure S7 (A) Absorption and (B) fluorescence spectra of 50 μM TPM in the presence of 1.0 equivalents of ClO⁻ and 50 μM TPP in PBS buffer.

![Absorption and fluorescence spectra](image)
Figure S8 Partial $^1$H NMR (500 MHz) spectra of TPM (a), TPM+ClO$^-$ (b) in the mixture of DMSO-$d_6$ and D$_2$O (v/v: 4/6) and TPP in DMSO-$d_6$ (c). [TPM]=[TPP]=2.0 mM.

Figure S9 HRMS spectrum of TPM with 1 equivalent ClO$^-$ in PBS buffer. [TPM]=50 μM.
Figure S10 Time-dependent fluorescence intensity at 638 nm upon the addition of 20 μM BSA to 50 μM TPM in PBS buffer solution (10 mM, pH 7.4).

Figure S11. Absorption of 50 μM TPM in the presence and absence of 20 μM BSA in 10 mM PBS solution at pH=7.4.
Figure S12  (A) Absorption and (B) fluorescence spectra of 50 μM TPM in the presence of 1.0 equivalents of ClO⁻ and 50 μM TPP in PBS buffer with 20 μM BSA.

Figure S13  The linear relationship between fluorescence intensity and ClO⁻ concentrations within 0–50 μM in the presence of 20 μM BSA in PBS buffer solution.

Table S1. Photophysical properties of TPM without or with ClO⁻ in different solvents.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Entry</th>
<th>Ex/Em (nm)</th>
<th>Stokes shift (nm)</th>
<th>ε/10⁴ (L·mol⁻¹·cm⁻¹)</th>
<th>QY/%</th>
<th>LOD for ClO⁻</th>
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<tbody>
<tr>
<td>EtOH</td>
<td>TPM</td>
<td>--</td>
<td>--</td>
<td>1.32</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>TPM+ClO⁻</td>
<td>416/514</td>
<td>98</td>
<td>1.01</td>
<td>45.1</td>
<td>22 nM</td>
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<tr>
<td>PBS</td>
<td>TPM</td>
<td>462/638</td>
<td>176</td>
<td>1.62</td>
<td>0.27</td>
<td>--</td>
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<tr>
<td></td>
<td>TPM+ClO⁻</td>
<td>--</td>
<td>--</td>
<td>0.36</td>
<td>--</td>
<td>680 nM</td>
</tr>
<tr>
<td>PBS+BSA</td>
<td>TPM</td>
<td>390/600</td>
<td>210</td>
<td>0.83</td>
<td>9.9</td>
<td>--</td>
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<tr>
<td></td>
<td>TPM+ClO⁻</td>
<td>390/468</td>
<td>178</td>
<td>0.37</td>
<td>37.8</td>
<td>1.5 nM</td>
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Table S2. Properties of representative fluorescent HOCl probes
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<tr>
<th>Entry</th>
<th>Probe</th>
<th>λ\text{abs}/λ\text{em} nm</th>
<th>LOD nM</th>
<th>Target Imaging</th>
<th>Reaction Time</th>
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<tbody>
<tr>
<td>1(^4)</td>
<td>BClO</td>
<td>500/505</td>
<td>0.56</td>
<td>-</td>
<td>within 1 s</td>
</tr>
<tr>
<td>2(^5)</td>
<td>SeCy7</td>
<td>--/786</td>
<td>310</td>
<td>-</td>
<td>within dozens of seconds</td>
</tr>
<tr>
<td>3(^6)</td>
<td>PZ–Py</td>
<td>400/562</td>
<td>17.9</td>
<td>mitochondria</td>
<td>within seconds</td>
</tr>
<tr>
<td>4(^7)</td>
<td>Ir2</td>
<td>405/565</td>
<td>-</td>
<td>mitochondria</td>
<td>within seconds</td>
</tr>
<tr>
<td>5(^8)</td>
<td>Rh-Py</td>
<td>544/577</td>
<td>24</td>
<td>mitochondria</td>
<td>Within seconds</td>
</tr>
<tr>
<td>6(^9)</td>
<td>MITO-TP</td>
<td>375/500</td>
<td>17.2</td>
<td>mitochondria</td>
<td>within seconds</td>
</tr>
<tr>
<td>7(^9)</td>
<td>LYSO-TP</td>
<td>375/500</td>
<td>19.6</td>
<td>lysosome</td>
<td>within seconds</td>
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<tr>
<td>8(^10)</td>
<td>HBP</td>
<td>480/508</td>
<td>2.4</td>
<td>-</td>
<td>within 30 min</td>
</tr>
<tr>
<td>9(^11)</td>
<td>BRT</td>
<td>525/580&amp;540</td>
<td>38</td>
<td>-</td>
<td>within 15 seconds</td>
</tr>
<tr>
<td>10(^12)</td>
<td>HES-BODIPY</td>
<td>480/532&amp;562</td>
<td>430</td>
<td>-</td>
<td>within seconds</td>
</tr>
<tr>
<td>11(^13)</td>
<td>Lyso-1</td>
<td>500/563</td>
<td>60</td>
<td>lysosome</td>
<td>ca. 5 min</td>
</tr>
<tr>
<td>12(^14)</td>
<td>PNIS</td>
<td>325/447</td>
<td>210</td>
<td>mitochondrial</td>
<td>-</td>
</tr>
<tr>
<td>13(^15)</td>
<td>YDN</td>
<td>485/516</td>
<td>8.7</td>
<td>-</td>
<td>2 min</td>
</tr>
<tr>
<td>14(^16)</td>
<td>HKOCl-3</td>
<td>490/527</td>
<td>0.33</td>
<td>-</td>
<td>within 1 min</td>
</tr>
<tr>
<td>15(^17)</td>
<td>meso-(4-pyridinyl)-substituted BODIPY</td>
<td>495/515</td>
<td>600</td>
<td>mitochondria</td>
<td>within 5 min</td>
</tr>
<tr>
<td>16 this wok</td>
<td>TPM</td>
<td>390/468&amp;600</td>
<td>1.5</td>
<td>mitochondria</td>
<td>within seconds</td>
</tr>
</tbody>
</table>

**Figure S14** Ratiometric fluorescence responses of 50 μM TPM toward various analytes in (A) ethanol and (B) PBS buffer solution respectively.
Figure S15 I/I₀ at 468 nm of TPM (50 μM) in the present of BSA (20 μM) in response to ClO⁻ in the presence of various ROSs (2-6: ^1O₂, H₂O₂, t-BuOO⁻, O₂⁻, and •OH), RNSs (7-8:NO₂⁻ and ONOO⁻), RSSs (9-14: S²⁻, HSO₃⁻, SO₃²⁻, Cys, Hcy and GSH), anions (15-21: F⁻, Cl⁻, I⁻, AcO⁻, SO₄²⁻, NO₃⁻ and CO₃²⁻) and cations (22-29: Na⁺, K⁺, Mg²⁺, Ca²⁺, Ni²⁺, Fe³⁺, Al³⁺ and Cu²⁺).

Figure S16 Fluorescence images of mitochondria in HeLa cells. HeLa cells were incubated with Mito Tracker Green (100 nM) and TPM (10 μM) for 30 min respectively. (A) Emission from the green channel (Mito Tracker Green, λ_ex = 488 nm, λ_em = 500–540 nm), (B) emission from the red channel (TPM, λ_ex = 458 nm, λ_em = 580–680 nm), (C) merged image of images (A) and (B), (D) intensity correlation plot of TPM and Mito Tracker Green, and (E) intensity profile of ROIs across HeLa cells.
Scale bar = 20 μm.

4. $^1$H, $^{13}$C NMR and HRMS spectra

Figure S17 $^1$H NMR spectrum of TPM in DMSO-$d_6$.

Figure S18 $^{13}$C NMR spectrum of TPM in DMSO-$d_6$. 
Figure S19 HRMS spectrum of TPM in MeOH.

Figure S20 $^1$H NMR spectrum of TPP in DMSO-$d_6$. 
**Figure S21** $^{13}$C NMR spectrum of TPP in DMSO-$d_6$.

5. References


5-14
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(2016) 77-83.

BODIPY-based ratiometric fluorescent probe for hypochlorous acid and its

probe for hypochlorous acid in water and its applications for highly lysosome-

