Electronic Supplementary Information

A novel ratiometric fluorescent probe for selectively determining HClO based on ESIPT mechanism and its application in real samples

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The content

1. ROS preparation .................................................................................................................. 2
2. HPLC analysis for detection mechanism .......................................................................... 2
3. Optical properties of the probe ....................................................................................... 2
4. Purification of the product generated from the probe ...................................................... 5
5. Comparison the \textsuperscript{1}HNMR spectra of the product and the probe ..................... 6
6. Characterization of the compounds .................................................................................. 6
7. Reference ............................................................................................................................ 9
1. ROS preparation

The other analytes with oxidizing properties including ·OH, $^{1}$O$_{2}$, Fe$^{3+}$, H$_{2}$O$_{2}$, ONOO$^{-}$, $t$-BuOOH, NO, and HClO were prepared according to the following methods in literature. The species of ·OH was generated in the Fenton system consisting of ferrous ammonium sulfate and hydrogen peroxide$^{1}$. The species of $^{1}$O$_{2}$ was prepared through adding NaOCl into the solution of H$_{2}$O$_{2}$$^{2}$. H$_{2}$O$_{2}$ solution was prepared through diluting the commercial H$_{2}$O$_{2}$ solution. The exact concentration of H$_{2}$O$_{2}$ was determined based on the molar extinction coefficient of H$_{2}$O$_{2}$ at 240 nm (43.6 M$^{-1}$ cm$^{-1}$). The species of ONOO$^{-}$ was obtained by using 3-morpholinosydnonimine as a donor$^{3}$. $t$-BuOOH was obtained commercially from Alfa Aesar. NO was generated by using sodium nitroferricyanide(III)dihydrate as a donor. The source of Fe$^{3+}$ was obtained from the solution of FeCl$_{3}$. The stock solution of HClO was prepared by diluting a commercial NaOCl solution. The concentration of HClO was determined based on the molar extinction coefficient of HClO at 292 nm (350 M$^{-1}$ cm$^{-1}$).

2. HPLC analysis for detection mechanism

HPLC analysis was performed on a Shimadzu UFLC system (Shimadzu, Kyoto, Japan) consisting of two LC-20AD pumps, an SPD-M20A diode-array detector, a CTO-20A oven, and an SIL-20A auto sampler. The detection wavelengths were 345 nm. The mobile phase was water-methanol (gradient from 5% to 90% in 12 min). The flow rate was 1.0 mL/min. A Dikma Diamonsil C$_{18}$ column (250 mm×4.6 mm, 5 µm, Dikma Technologies Inc, Beijing, China) was used throughout.

3. Optical properties of the probe

Table S1. Linear equation of the probe toward to HClO

<table>
<thead>
<tr>
<th>Entry</th>
<th>Equation</th>
<th>R$^{2}$</th>
<th>Detection limit/nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>The probe</td>
<td>Y=0.109 X + 0.086</td>
<td>0.996</td>
<td>14.6</td>
</tr>
</tbody>
</table>

Table S2. Fluorescent properties of the compounds and the probe

<table>
<thead>
<tr>
<th>Entry</th>
<th>QY (%)</th>
<th>$\lambda_{em}$ nm</th>
<th>$\lambda_{ex}$ nm</th>
<th>ε $^{10^{4}M^{-1}cm^{-1}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>The probe</td>
<td>0.11</td>
<td>600</td>
<td>340</td>
<td>2.71/1.89</td>
</tr>
<tr>
<td>The probe +HClO</td>
<td>0.34</td>
<td>485/600</td>
<td>340</td>
<td>3.52/2.43</td>
</tr>
</tbody>
</table>
Figure S1. Effects of pH on the fluorescence of the probe (20 μM) reacting with HClO (10μM) for 30 s in PBS/MeOH(v/v, 4:1, pH=7.4, 10mM). λ_{ex} =340, slit width: 2/8 nm.

Figure S2. Fluorescent spectrum of the probe (20 μM) in PBS/MeOH(v/v, 4:1, pH=7.4, 10mM) with different pH value. λ_{ex} =340, slit width: 2/8 nm.
Figure S3. The HPLC chromatography of the probe (a, 30μM, marked with red arrow) treated with HClO (b, 15μM; c, 45μM; new product was marked with blue arrow)

Figure S4. The mass spectrum of the new species at retention time 7.25 min
4. **Purification of the product generated from the probe**

Scheme S1. Synthesis of the product generated from the probe

To a solution of the probe (50 mg) in 20 mL of PBS buffer (10mM, pH7.4, containing 10% ethanol) was added the solution of HClO. TLC monitored the reaction process. The mixture was stirred at room temperature for 10 min and poured into 20 mL of water. Then, 10 mL of CH$_2$Cl$_2$ was added to extract the final products. Repeat the above procedure twice to collect the organic phase. The obtained organic phase of CH$_2$Cl$_2$ was isolated and concentrated to 1mL. The residue was purified by preparative SiO$_2$ plates using petroleum ether-ethyl acetate (4:1, v/v) as an eluent to give a yellow solid (5.2 mg). The obtained products were characterized by $^1$H NMR.
5. Comparison the $^1$HNMR spectra of the product and the probe

![HNMR spectra](image)

Figure S6 the $^1$HNMR spectra of the product (top, in DMSO-$d_6$, 300 MHz) and the probe (down, in CDCl$_3$, 300 MHz)

6. Characterization of the compounds

![MS spectra](image)

Figure S7. MS spectra of the probe 1
Figure S8. MS spectra of the 5-CH$_3$-HBI

Figure S9. MS spectra of the 5-CH$_3$-HBI-CHO
Figure S10. $^1$HNMR spectra of the 5-CH$_3$-HBI

Figure S11. $^1$HNMR spectra of the 5-CH$_3$-HBI-CHO
7. Reference

