Electronic Supplementary Information (ESI)

for

Real-time detection of hypochlorite in tap water and biological samples by a colorimetric, ratiometric and near-infrared fluorescent turn-on probe

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1. Structure characterizations of NIR-1 and probe 1

\[ \text{NIR-1} \]

\[ \text{DMSO} \]

\[ \text{H}_2\text{O} \]

\[ ^1\text{H-NMR spectrum of NIR-1 in DMSO-}d_6 \]

\[ ^{13}\text{C-NMR spectrum of NIR-1 in DMSO-}d_6 \]
IR spectrum of NIR-1

MS (EI) spectrum of NIR-1
HR-MS spectrum of NIR-1

C_{33}H_{33}N_2O_5^+

Exact Mass: 537.2384

1H-NMR spectrum of probe 1 in CDCl₃
$^{13}$C-NMR spectrum of probe 1 in CDCl$_3$

IR spectrum of probe 1
MS (EI) spectrum of probe 1

HR-MS spectrum of probe 1
2. Additional data for the sensing property of probe 1

Fig. S1 Selective color changes of probe 1 (10 μM) for ClO$^-\$ upon addition of various representative analytes (100 μM). (a) Color changes. (b) Fluorescence color changes under a 365 nm UV light. Analytes from left to right: 1. none, 2. F$^-\$, 3. Cl$^-\$, 3. SO$_4^{2-}\$, 4. NO$_3^-\$, 5. AcO$^-\$, 6. NO$_2^-\$, 7. H$_2$O$_2\$, 10. O$_2^-\$, 11. 'OH, 12. NO, 13. 'BuOO$', 14. ONOO$', 15. ClO$^-\$, 16. SO$_3^{2-}\$, 17. GSH, 18.Cys, 19. HS$^-\$, 20. CN$^-\$) in PBS buffer (20 mM, pH 7.4) with 30% CH$_3$CN at room temperature.

Fig. S2 (a) The ratio of absorption intensity changes at 651 nm and 427 nm (A$_{651}$/A$_{427}$) and (b) the ratio of fluorescence intensity changes at 707 nm and 486 nm (I$_{707}$/I$_{486}$) of probe 1 (5 μM) for ClO$^-\$ detection in the presence of various analytes. Black bars represent the addition of a single analyte (100 μM except 1mM GSH) including: 1. none, 2. F$^-\$, 3. Cl$^-\$, 4. Br$^-\$, 5. I$^-\$, 6. NO$_3^-\$, 7. NO$_2^-\$, 8. AcO$^-\$, 9. SCN$^-\$, 10. CO$_3^{2-}\$, 11. SO$_4^{2-}\$, 12. SO$_3^{2-}\$, 13. S$_2$O$_3^{2-}\$, 14. CN$^-\$, 15. HS$^-\$, 16. ONOO$^-\$, 17. 'BuOO$', 18. 'OH, 19. NO, 20, HNO, 21. H$_2$O$_2\$, 22. H$_2$NCH$_2$CH$_2$NH$_2\$, 23. HOCH$_2$CH$_2$NH$_2\$, 24. C$_2$H$_3$NH$_2\$, 25. C$_6$H$_5$CH$_2$NH$_2\$, 26. Cys, 27, Hcy, 28. GSH (1mM), 29. ROO$', 30. O$_2^-\$, 31. Phe, 32. Gly. Red bars represent the subsequent addition of NaClO (30 μM) to the mixture. All experiment was performed in PBS buffer (20 mM, pH 7.4) with 30% CH$_3$CN at room temperature and each spectrum was obtained 1 min after addition of various analytes. For fluorescence, λ$_{ex}$ was used at 430 nm and 650 nm, slit width: (10,10).
**Fig. S3** Kinetics of probe 1 (5 µM) upon addition of NaClO (30 µM) in PBS buffer (20 mM, pH 7.4) with 30% CH₃CN. (a) Monitored by absorbance changes at 651 nm. (b) monitored by fluorescence changes at 707 nm with λ_ex = 650 nm.

**Fig. S4** Fluorescence spectra changes of probe 1 (5 µM) toward addition of NaClO (0-50 µM) in a in PBS buffer (20 mM, pH 7.4) with 30% CH₃CN solution. (a) λ_ex = 430 nm. (b) λ_ex = 650 nm. Slit: 10 nm, 10 nm. (c) Fluorescence intensity changes of probe 1 (5 µM) at 486 nm and 707 nm toward addition of NaOCl (0-50 µM).
**Fig. S5** Linear relationship of fluorescent intensity changes of probe 1 (5 μM) at 707 nm as a function of NaClO concentration from 0 to 15 μM.

\[
y = 10.631 + 21.064\text{[NaClO]} \\
R^2 = 0.997
\]

**Fig. S6** The effect of pH on the absorption intensity changes of probe 1 (5 μM) at (a) 426 nm and (b) 652 nm, and the effect of pH on the fluorescence intensity changes of probe 1 (5 μM) at (c) 486 nm and (d) 707 nm in absence and presence of NaClO (30 μM) in PBS buffer (20 mM, pH 7.4, containing 30% CH₃CN, v/v) at 25 °C. All data were obtained 5 min after mixing. Excitation wavelength for (c) and (d) were set at 430 nm and 650 nm, respectively.
Fig. S7 (a) Fluorescence spectra changes of probe 1 (5 μM) upon addition of NaClO (0-8 equiv) with λ_{ex} = 430 nm. (b) Fluorescence spectra changes of probe 1 (5 μM) upon addition of NaClO (0-8 equiv) with λ_{ex} = 650 nm. (c) Fluorescence intensity changes of probe 1 (5 μM) at 486 nm and 707 nm upon addition of NaClO (0-8 equiv). (d) Linear relationship of fluorescence intensity changes at 707 nm as a function of NaClO concentration (0-12.5 μM). All data were collected in PBS buffer (20 mM) with 30% CH₃CN solution at pH = 5.0 and each spectrum was obtained 1 min after NaClO addition.

3. Sensing mechanism of probe 1 for sensing of ClO⁻

NaClO (6 mM in water, 30 mL) was added to probe 1 (22 mg, 0.04mmol) solution in CH₃CN (30 mL) slowly under stirring. After the solvents were removed under reduced pressure, the residue was purified on silica gel column chromatography using CH₂Cl₂/CH₃OH (20/1, v:v) as eluent and a blue solid product A was obtained.
Fig. S8 TLC analysis of the isolated product A from the reaction of probe 1 and NaClO. (A) under room light, (B) under light of 254 nm, (C) under light of 365 nm. Spots on the TLC plate are: a. probe 1, b. the reaction, c. mixture of the product and the reference sample of NIR-1, d. the reference sample of NIR-1. The eluent for TLC: petroleum ether: ethyl acetate 2:1 (v/v). The results show that product A is identical to NIR-1.

Fig. S9 ¹H NMR spectrum of product A in DMSO-<i>d</i><sub>6</sub>, which is identical to the ¹H NMR spectrum of NIR-1 (see above).
Fig. S10 MS spectrum of the isolated product A from the reaction of probe 1 and NaClO, which shows the right mass of NIR-1.

4. Comparison of fluorescent probes for ClO⁻

Table S1. Comparison of fluorescent probes for ClO⁻

<table>
<thead>
<tr>
<th>Ref</th>
<th>Ref</th>
<th>probe</th>
<th>λ&lt;sub&gt;ex&lt;/sub&gt; / λ&lt;sub&gt;em&lt;/sub&gt;</th>
<th>Detection medium and [probe]</th>
<th>Detection limit</th>
<th>NIR Fluorescence</th>
<th>Ratio metric</th>
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<td>6a</td>
<td>Org. Lett.,</td>
<td></td>
<td>λ&lt;sub&gt;em&lt;/sub&gt;=525 nm, λ&lt;sub&gt;em&lt;/sub&gt;=545 nm</td>
<td>0.1M phosphate buffer (0.1% DMF, pH 7.4), 10 µM</td>
<td>a: 42 nM, b: 18 nM, c: 37 nM</td>
<td>NO</td>
<td>NO</td>
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<td></td>
<td>2014, 16,</td>
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<tr>
<td>6b</td>
<td>Org. Lett.,</td>
<td></td>
<td>λ&lt;sub&gt;em&lt;/sub&gt;=510 nm, λ&lt;sub&gt;em&lt;/sub&gt;=526 nm</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;O-CH₃CN (v/v = 99/1), 0.1 M PBS, pH 7.4, 10 µM</td>
<td>7.98 nM</td>
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<td>6c</td>
<td>Chem. Commun.,</td>
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<td>λ&lt;sub&gt;em&lt;/sub&gt;=460 nm, λ&lt;sub&gt;em&lt;/sub&gt;=510 nm</td>
<td>20 mM pH 7.4 PBS containing 30% acetonitrile, 10 µM</td>
<td>0.98 µM</td>
<td>NO</td>
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<td>Org. Lett.,</td>
<td></td>
<td>λ&lt;sub&gt;em&lt;/sub&gt;=405 nm, λ&lt;sub&gt;em&lt;/sub&gt;=480 nm</td>
<td>PBS buffer, 4 µM</td>
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<td>2002–2005</td>
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<td>Journal, Year, Pages</td>
<td>Compound</td>
<td>λ&lt;sub&gt;ex&lt;/sub&gt;, nm</td>
<td>λ&lt;sub&gt;em&lt;/sub&gt;, nm</td>
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<td>Cytotoxicity</td>
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<td>6e</td>
<td>Chem. Commun., 2013, 49, 1862–1864</td>
<td>CM2</td>
<td>340</td>
<td>570</td>
<td>0.1 M phosphate buffer (pH 7.4, with 1% DMF), 20 µM</td>
<td>0.7 µM</td>
<td>NO</td>
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<td>6f</td>
<td>Chem. Commun., 2013, 49, 7836–7838</td>
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<td>470</td>
<td>514</td>
<td>pH 7.2 buffer/DMF (v/v, 4:1), 5 µM</td>
<td>0.5 µM</td>
<td>NO</td>
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<td>6g</td>
<td>Analyst, 2013, 138, 6091–6096</td>
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<td>488</td>
<td>529</td>
<td>PBS buffer (pH 7.4, 10 mM) and EtOH (1/9, v/v), 5 µM</td>
<td>0.52 µM</td>
<td>NO</td>
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<td>6h</td>
<td>Chem. Commun., 2013, 49, 2445-2447</td>
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<td>450</td>
<td>523</td>
<td>20 mM PBS, pH 7.4, 3 µM</td>
<td>586 nM</td>
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<td>6i</td>
<td>Org. Lett., 2014, 16, 520–523</td>
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<td>572</td>
<td>597</td>
<td>water–ethanol: v/v = 99:1, 0.1 M PBS, pH 7.5, 45 µM</td>
<td>3.7 µM</td>
<td>NO</td>
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<td>6j</td>
<td>Chem. Commun., 2014, 50, 11911-11914</td>
<td></td>
<td>450</td>
<td>556</td>
<td>H₂O-CH₃CN (99.5 : 0.5, v/v), buffered with HEPES (50 mM), pH = 7.4, 5 µM</td>
<td>163 nM</td>
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<td>6k</td>
<td>J. Am. Chem. Soc., 2014, 136, 12820–12823</td>
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<td>480</td>
<td>505</td>
<td>PBS-ethanol (0.01 M, pH 7.4, 9:1, v/v), 1 µM</td>
<td>0.56 nM</td>
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<td>7a</td>
<td>J. Am. Chem. Soc., 2007, 129, 7313–7318</td>
<td></td>
<td>552</td>
<td>575</td>
<td>0.10 M sodium phosphate buffer at pH 7.4 and 0.10% DMF, 2 µM</td>
<td>-</td>
<td>NO</td>
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<td>7b</td>
<td>Org. Lett., 2009, 11, 859–861</td>
<td></td>
<td>500</td>
<td>546</td>
<td>PBS buffer-DMF (0.1%), at pH 7.4, 1 µM</td>
<td>25 nM</td>
<td>NO</td>
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<td>7c</td>
<td>J. Am. Chem. Soc., 2013, 135, 13365–13370</td>
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<td>570</td>
<td>606</td>
<td>pH 7.4 phosphate buffer solution and 0.5% DMF, 5 µM</td>
<td>-</td>
<td>NO</td>
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<td>7d</td>
<td>Chem. Sci., 2013, 4, 460–467</td>
<td>silica</td>
<td>488</td>
<td>525/590</td>
<td>Na₂HPO₄-citrate buffer (100 mM, pH 5.0), 1 mg/ ml</td>
<td>5 µM</td>
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| 8a | *Chem.–Eur. J.*, 2008, 14, 4719–4724 | λ<sub>ex</sub>=520 nm  
λ<sub>em</sub>=578 nm  
0.03 m Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>/NaOH buffer (pH 12) and 30 % (v/v) THF, 10 μM | 27 nM  
NO  
NO |
| 8b | *Sens. Actuators, B*, 2010, 158, 774–780 | λ<sub>ex</sub>=525 nm  
λ<sub>em</sub>=579 nm  
Na<sub>2</sub>HPO<sub>4</sub>–NaH<sub>2</sub>PO<sub>4</sub> buffer (pH 7.4, DMSO:H<sub>2</sub>O = 1:99, v/v), 10 μM | 0.3 μM  
NO  
NO |
| 8c | *Chem. Commun.*, 2011, 47, 4373–4375 | λ<sub>ex</sub>=515 nm  
λ<sub>em</sub>=550 nm  
PBS buffer (0.1 M, pH 5.5, 1% CH<sub>3</sub>CN), 2 μM | -  
NO  
NO |
| 8d | *J. Am. Chem. Soc.*, 2013, 135, 9944–9949 | λ<sub>ex</sub>=466 nm  
λ<sub>em</sub>=520/629 nm  
PBS buffer (10 mM, 0.1% Triton X-100, 1% EtOH, pH = 7.4), 2.5 μM | 0.5 μM  
NO  
Yes |
| 8e | *Chem. Commun.*, 2014, 50, 8640–8643 | λ<sub>ex</sub>=540 nm  
λ<sub>em</sub>=566/780 nm  
20.0 mM PBS, pH 7.40 containing 0.5% DMF, 5 μM | 19.5 nM  
Yes  
(Turn-Off NIR F.)  
Yes |
| 9a | *Analyst*, 2013, 138, 3368–3371 | λ<sub>ex</sub>=305 nm  
λ<sub>em</sub>=354/430 nm  
pH 7.4 in CH<sub>3</sub>CN:H<sub>2</sub>O (pH 7.4, 4 : 1), 5 μM | 56 nM  
NO  
Yes |
| 9b | *Analyst*, 2013, 138, 6291–6295 | λ<sub>ex</sub>=540 nm  
λ<sub>em</sub>=566/780 nm  
20.0 mM PBS, pH 7.40 containing 0.5% DMF, 5 μM | 19.5 nM  
Yes  
(Turn-Off NIR F.)  
Yes |
| 9d | *Chem. Commun.*, 2013, 49, 11656–11658 | λ<sub>ex</sub>=50 nm  
λ<sub>em</sub>=550 nm  
PBS/DMF (pH 7.4, 1:1)  
containing 0.5% DMF, 5 μM | 52 nM  
NO  
Yes |
| 9e | *Chem. Commun.*, 2011, 47, 12691–12693 | λ<sub>ex</sub>=410 nm  
λ<sub>em</sub>=470/580 nm  
DMF:NaH<sub>2</sub>PO<sub>4</sub> (4:6, v/v, pH = 5.0, 0.1 M)  
10 μM | -  
Yes  
(Turn-Off NIR F.)  
Yes |
| 10a | *Chem.–Eur. J.*, 2012, 18, 2700–2706 | λ<sub>ex</sub>=414 nm  
λ<sub>em</sub>=473/594 nm  
PBS/DMF (pH 7.4, 1:1)  
5 μM | 52 nM  
NO  
Yes |
| 10b | *Chem. Commun.*, 2012, 48, 2949–2951 | λ<sub>ex</sub>=550 nm  
λ<sub>em</sub>=750/575 nm  
H<sub>2</sub>O:ethanol (4:1, v/v)  
10 mg mL<sup>−1</sup>.  
-  
Yes  
(Turn-Off NIR F.)  
Yes |
| 10c | *Chem. Commun.*, 2014, 50, 14141–14244 | λ<sub>ex</sub>=410 nm  
λ<sub>em</sub>=470/580 nm  
DMF:NaH<sub>2</sub>PO<sub>4</sub> (4:6, v/v, pH = 5.0, 0.1 M)  
10 μM | -  
NO  
Yes |
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<tr>
<th>Reference</th>
<th>Compound</th>
<th>λ&lt;sub&gt;ex&lt;/sub&gt;, nm</th>
<th>λ&lt;sub&gt;em&lt;/sub&gt;, nm</th>
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<td>Chem. Commun., 2015, 51, 6781-6784</td>
<td>λ&lt;sub&gt;ex&lt;/sub&gt; 415 nm, 481 nm and λ&lt;sub&gt;em&lt;/sub&gt; 540 nm, 570 nm</td>
<td>PBS (pH 7.4, 10 mM, containing 0.1% DMSO)</td>
<td>5 μM</td>
<td>0.12 nM</td>
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<td>Yes</td>
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<td>11a</td>
<td>Chem. Biol., 2007, 14, 1221-1231</td>
<td>λ&lt;sub&gt;ex&lt;/sub&gt; 625 nm, λ&lt;sub&gt;em&lt;/sub&gt; 676 nm</td>
<td>PBS (pH 7.4) at room temperature</td>
<td>10 μM</td>
<td>-</td>
<td>Yes</td>
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<td>11b</td>
<td>J. Am. Chem. Soc., 2011, 133, 5680-5682</td>
<td>λ&lt;sub&gt;ex&lt;/sub&gt; 620 nm, λ&lt;sub&gt;em&lt;/sub&gt; 670 nm</td>
<td>PBS at pH 7.4, containing 0.1% DMF</td>
<td>5 μM</td>
<td>-</td>
<td>Yes</td>
<td>NO</td>
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<td>11d</td>
<td>Chem. Commun., 2014, 50, 1018-1020</td>
<td>λ&lt;sub&gt;ex&lt;/sub&gt; 690 nm, λ&lt;sub&gt;em&lt;/sub&gt; 786 nm</td>
<td>PBS buffer (pH 7.4, 20 mM)</td>
<td>30 μM</td>
<td>0.31 μM</td>
<td>Yes</td>
<td>NO</td>
</tr>
<tr>
<td>This work</td>
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<td>λ&lt;sub&gt;ex&lt;/sub&gt; 430 nm, λ&lt;sub&gt;em&lt;/sub&gt; 486 nm and λ&lt;sub&gt;em&lt;/sub&gt; 650 nm, 707 nm</td>
<td>20 mM PBS containing 30% CH&lt;sub&gt;3&lt;/sub&gt;CN, 25 °C</td>
<td>5 μM</td>
<td>40 nM (pH 7.4); 21 nM (pH 5.0)</td>
<td>Yes</td>
<td>(Turn-ON NIR F.)</td>
</tr>
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</table>

5. Additional data for applications of probe 1 for sensing of ClO⁻

![Fluorescence response of probe 1](attachment:fig_s11.png)

Fig. S11 Fluorescence response of probe 1 (5 μM) to tap water, boiled tap water, and double distilled water. All spectra were obtained after 15 min of incubation at 25 °C, λ<sub>ex</sub> = 650 nm, slit width: d<sub>ex</sub> = 10 nm, d<sub>em</sub> = 10 nm.
Fig. S12 Detection of NaClO in aqueous solution by test strips containing probe 1. NaClO concentrations from 1 to 8: 1. 0 μM, 2. 50 μM, 3. 80 μM, 4. 100 μM, 5. 300 μM, 6. 500 μM, 7. 800 μM, 8. 1 mM. Test strips were prepared by wetting a neutral filter paper with a 200 μM of probe 1 solution (in CH₃CN) and then drying it in the air.

Fig. S13 Detection of NaClO in fetal bovine serum (FBS) samples by probe 1. (a) Fluorescence intensity changes of probe 1 (5 μM) at 707 nm against time in FBS sample. (b) Fluorescence spectra changes of probe 1 (5 μM) in the presence and absence NaClO (30 μM) in FBS sample. (c) Fluorescence spectra changes of probe 1 (5 μM) in FBS sample upon addition of NaClO (0, 3, 6, 9, 12, and 15 μM, respectively). (d) Linear relationship of fluorescence intensity changes at 707 nm as a function of NaClO concentration (0-15 μM). All data were collected in CH₃CN-PBS buffer (20 mM, pH 7.4, containing 30% CH₃CN and 10% FBS, v/v, respectively) at 25 °C. λₑx = 650 nm, slit
width: $d_{ex} = d_{em} = 10$ nm.

**Fig. S14** Viable HeLa cells after treatment with indicated concentrations of probe 1 after 12 hours. The cell viability was observed via MTT assay.