Electronic Supporting Information

A two-photon fluorescent probe for ratiometric imaging of endogenous hypochlorous acid in live cells and tissues

Yong Woong Jun,‡a Sourav Sarkar,‡a Subhankar Singha,⁎a Ye Jin Reo,⁎a Hye Rim Kim,⁎a Jong-Jin Kim, b Young-Tae Chang⁎ab and Kyo Han Ahn⁎a

aDepartment of Chemistry, Pohang University of Science and Technology, 77 Cheongam-Ro, Nam-Gu, Pohang, Gyeongbuk 37673, Republic of Korea
bLaboratory of Bioimaging Probe Development, Singapore Bioimaging Consortium, Agency for Science, Technology and Research (A*STAR), Biopolis 138667, Singapore
⁎Corresponding authors. E-mail address: subhankar@postech.ac.kr; ytchang@postech.ac.kr; ahn@postech.ac.kr
‡ These authors contributed equally to this work and are considered co-first authors.

Contents
1. General information on materials and methods
2. Fluorescence assays with the probe rTP-HOCl 1
3. Preparation of cell samples and their two-photon imaging
4. Preparation of mouse brain tissue samples and their TPM imaging
5. Synthesis of the probe rTP-HOCl 1
6. Supporting figures (including HPLC analysis, cell viability assay)
7. Supporting tables (including two-photon photophysical properties of probe and product)
8. NMR (1H & 13C) spectra
1. General information on materials and methods

Unless otherwise noted, the chemical reagents were purchased from Sigma-Aldrich or Alfa-Aesar and used as received. LysoTracker® Deep Red was purchased from Invitrogen. All solvents were purified and dried by standard methods prior to use. Deionized water was used to prepare all aqueous solutions. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker 500 MHz spectrometer using tetramethylsilane as the internal reference. All chemical shifts are reported in the standard notation of parts per million (ppm) using residual solvent protons as the internal standard. Mass spectroscopic data were obtained from the Korea Basic Science Institute (Daegu) with a JEOL JMS 700 high resolution mass spectrometer.

2. Fluorescence assays with the probe rTP-HOCl 1

All of the solvents used were of analytical grade. NaOCl solution was used as source of HOCl ($pK_a = 7.46$). A stock solution of NaOCl (300–600 µM) was prepared by dilution of commercial NaOCl solution (Aldrich) in 0.1 M NaOH solution. The concentration of NaOCl solution was determined from the absorption at 292 nm ($\varepsilon = 350 \text{ M}^{-1}\text{cm}^{-1}$) in 0.1 M NaOH solution. Finally, the required volumes of NaOCl stock solution were gradually added to the probe solutions in cuvette to make the desired concentrations of HOCl. Stock solutions of the probe rTP-HOCl 1 (1.0 mM) were made by dissolving the probe in dimethylsulfoxide (DMSO). Stock solution of the probes (15 µL) was added to 3 mL of PBS buffer (pH 7.4) solution to make the final probes concentration of 5 µM. UV–vis absorption spectra were obtained using an UV–vis spectrophotometer (HP 8453). Fluorescence spectra were recorded on a fluorimeter (Photon Technology International) with a 10 mm cuvette. The excitation and emission wavelength band paths were both set at 2 nm.

3. Preparation of cell samples and their two-photon imaging

HeLa cells were obtained from Korean Cell Line Bank. The cells were incubated in DMEM supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) penicillin-streptomycin (PS) at 37 °C in a humidified atmosphere of 5% of CO$_2$ in the air. Cells were passaged when they reached approximately 80% confluence. Cells were seeded onto a cell culture dish at a density of $1.0 \times 10^5$ cells, which was incubated at 37 °C overnight under 5% CO$_2$ in the air. For imaging experiments, cells were incubated in three conditions: For the detection of endogenous HOCl, cells were incubated in DMEM containing the probe rTP-HOCl 1 (10 µM) for 10 min and washed with PBS (phosphate buffered saline) three times to remove the remaining probe. In the negative control experiment with N-acetylcysteine (NAC), cells were
co-incubated in DMEM containing the probe (10 μM) and NAC (1 mM or 0.5 mM) for 10 min and washed with PBS (phosphate buffered saline) three times to remove the remaining probe. In the positive control experiment with an exogenous HOCl source, the cells were co-incubated in DMEM containing the probe (10 μM) and HOCl (5 μM or 10 μM) for 10 min and washed with PBS (phosphate buffered saline) three times to remove the remaining probe. Fluorescence images of the cell samples were recorded by two-photon microscopy (TPM). TPM imaging was performed using a Ti-Sapphire laser (Chameleon Vision II, Coherent) at 140 fs pulse width and 80 MHz pulse repetition rate (TCS SP5 II, Leica, Germany) through a 40x objective lens (obj. HCX PL APO 40×/ 1.10 W CORR CS, 506341, Leica, Germany). The two-photon excitation wavelength for the probe was tuned to 900 nm. Each emission light was spectrally resolved into two channels (λ_{em, Green} = 415–520 nm, λ_{em, Red} = 540–665 nm). The cell samples prepared as above were mounted on a tight-fitting holder. The excitation laser power was approximately 9.3 mW. The images were consisted of 1024 × 1024 pixels, and the scanning speed was maintained as 100 MHz during the entire imaging. Acquired images were processed using LAS AF Lite (Leica, Germany), and all the images were converted into the corresponding pixel-to-pixel ratiometric images based on the intensity ratio of I_{Red}/I_{Green}.

4. Preparation of mouse brain tissue samples and their TPM imaging

The experimental procedures regarding mouse tissues herein were performed in accordance with protocols approved by the Pohang University of Science and Technology Committee on Animal Research and followed the guidelines for the use of experimental animals established by The Korean Academy of Medical Science. We made every effort to minimize animal suffering and reduce the number of animals used to prepare samples for imaging. Balb/C type mice (6 weeks) were used for this experiment. Basically experiment was done under light protected conditions (in a dark-room and using aluminum foil). The mouse was dissected after dislocation of the cervical vertebra. The mouse brain was dissected within 2 min and sliced to the thicknesses of 300 μm under PBS buffer solution by using Vibratome (Leica vt1000s model). The tissues were incubated with the probe rTP-HOCl 1 (10 μM; 30 min) only or probe followed by HOCl (10 μM; 30 min) treatment and washed three times with PBS buffer. The prepared tissue samples were mounted on the microscope slides by using buffer solution and subjected to two-photon microscopic imaging. Fluorescence images of the tissue samples were recorded by two-photon microscopy (TPM). TPM imaging was performed using a Ti-Sapphire laser (Chameleon Vision II, Coherent) at
140 fs pulse width and 80 MHz pulse repetition rate (TCS SP5 II, Leica, Germany) and a 40× objective lens (obj. HCX PL APO 40×/ 1.10 W CORR CS, 506341, Leica, Germany). The two-photon excitation wavelength was tuned to 900 nm for the probe. Each emission light was spectrally resolved into two channels (λem, Green = 415–520 nm, λem, Red = 540–665 nm). The tissue samples prepared as above were mounted on a tight-fitting holder. The excitation laser power was approximately 9.3 mW. The images were consisted of 1024 × 1024 pixels, and the scanning speed was maintained as 100 MHz during the entire imaging. Acquired images were processed by using LAS AF Lite (Leica, Germany).

5. Synthesis of the probe rTP-HOCl 1

Scheme 1.

3-Hydroxy-6-(2-(hydroxymethyl)pyrrolidin-1-yl)-2-naphthaldehyde (3)

This compound was prepared by following the reported procedure (Ref: D. Kim, Q. P. Xuan, H. Moon, Y. W. Jun, and K. H. Ahn, Asian J. Org. Chem., 2014, 3, 1089), starting from 2,7-dihydroxynaphthalene and DL-prolinol, and obtained as a yellow solid. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 10.51 (s, 1H), 9.86 (s, 1H), 7.87 (s, 1H), 7.65 (d, $J = 9.0$ Hz, 1H), 6.95 (dd, $J = 9.0$, 2.5 Hz, 1H), 6.94 (s, 1H), 6.22 (d, $J = 2$ Hz, 1H), 4.06–4.08 (m, 1H), 3.74–3.78 (m, 1H), 3.66–3.70 (m, 1H), 3.59–3.62 (m, 1H), 3.32–3.37 (m, 1H), 2.04–2.14 (m, 4H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 195.4, 157.1, 149.1, 140.9, 138.1, 131.3, 121.0, 119.2, 115.0, 108.8, 103.7, 63.6, 60.1, 49.2, 28.7, 23.6.

Acetyl-benzocoumarin 2 (AcBC 2)

Compound 3 (200 mg, 0.737 mmol) was taken in a round-bottom flask and 10 mL of ethanol was added to it. Following the addition of ethyl acetoacetate (186 µL, 1.474 mmol) and piperidine (3 drop, cat. amount) at room temperature, the reaction mixture was stirred for 2 h. After the completion of the reaction (checked by TLC, eluent: ethyl acetate/hexane = 1/1), the reaction mixture was diluted with dichloromethane (30 mL) and washed with water (10 mL). The aqueous layer was further extracted with dichloromethane (10 mL). The combined organic layer was concentrated by rotary-evaporator. The residue was subjected to column
chromatography (eluent: starting from only CH$_2$Cl$_2$ to 10% EtOAc in CH$_2$Cl$_2$) to collect the pure acetyl-benzocoumarin 2 (226 mg, 91%) as red solid. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 8.57 (s, 1H), 7.95 (s, 1H), 7.76 (d, $J = 9.0$ Hz, 1H), 7.38 (s, 1H), 7.12 (dd, $J = 9.0$, 2.5 Hz, 1H), 6.79 (d, $J = 1.5$ Hz, 1H), 4.10–4.12 (m, 1H), 3.78–3.80 (m, 1H), 3.71–3.72 (m, 1H), 3.63–3.66 (m, 1H), 3.35–3.40 (m, 4H), 2.73 (s, 3H), 2.14 (m, 4H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 195.9, 160.4, 152.1, 148.6, 148.4, 139.0, 132.1, 130.9, 124.0, 121.5, 117.0, 114.7, 109.4, 104.4, 63.5, 60.3, 49.3, 30.9, 28.7, 23.6; HRMS: $m/z$ calcd for C$_{20}$H$_{19}$NO$_4$ [M$^+$] 337.1314; found 337.1316 [M$^+$].

**rTP-HOCI 1 (Probe)**

Acetyl-benzocoumarin 2 (34 mg, 0.1 mmol), methanesulfonic acid (25 μL) and 2-mercaptoethanol (8 μL, 0.11 mmol) were mixed in 10 mL dichloromethane solution and refluxed for 3 h under Ar atmosphere. After the reaction was completed, the mixture was cooled to room temperature and purified by column chromatography (eluent: starting from only CH$_2$Cl$_2$ to 10% EtOAc in CH$_2$Cl$_2$). The pure probe rTP-HOCI 1 was obtained as orange solid (31 mg, 78%). $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.80 (s, 1H), 7.79 (s, 1H), 7.71 (d, $J = 8.5$ Hz, 1H), 7.41 (d, $J = 1.5$ Hz, 1H), 7.10 (d, $J = 9.0$ Hz, 1H), 6.80 (s, 1H), 4.41–4.45 (m, 1H), 4.16–4.21 (m, 1H), 4.07–4.08 (m, 1H), 3.76–3.79 (m, 1H), 3.69–3.72 (m, 1H), 3.61–3.65 (m, 1H), 3.31–3.35 (m, 1H), 3.14–3.19 (m, 1H), 3.01 (br, m, 1H), 2.08–2.14 (m, 4H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 160.8, 151.0, 147.4, 136.9, 134.8, 130.5, 129.8, 128.4, 123.9, 116.6, 115.5, 109.7, 104.4, 91.9, 71.5, 63.7, 60.3, 49.4, 33.7, 29.9, 28.8, 23.7; HRMS: $m/z$ calcd for C$_{22}$H$_{23}$NO$_4$S [M$^+$] 397.1348; found 397.1349 [M$^+$].
6. Supporting figures

**Fig. S1** Absorption spectra of rTP-HOCl 1 (5 μM) and acetyl-benzocoumarin 2 (5 μM) in PBS (pH 7.4) containing 1% DMSO, recorded at 25 °C.

**Fig. S2** The emission intensity ratio \((I_{633 \text{ nm}}/I_{598 \text{ nm}})\) changes of rTP-HOCl 1 (5 μM) depending on [HOCl] in a low concentration region (0–2000 nM) in PBS (pH 7.4) containing 1% DMSO. The spectra were recorded at 25 °C under excitation at 460 nm. On the basis of this plot, the detection limit was calculated by following the equation,

\[
\text{Detection limit (LOD)} = 3\sigma/k = 34.8 \text{ nM},
\]

Where \(\sigma\) is the standard deviation of three blank measurements = \(8.53232 \times 10^{-4}\), \(k\) is the slope of the linear plot of the fluorescence intensity ratios changes \((I_{633 \text{ nm}}/I_{598 \text{ nm}})\) in the lower HOCl concentration region (0–2000 nM) = \(7.35544 \times 10^{-5}\).
**Fig. S3** Emission changes of **rTP-HOCl 1** (5 µM) in the presence of various biologically relevant analytes (50 µM) [ATP, NAD, glutathione (GSH), cysteine (Cys), Fe(III), Zn(II), Cu(II)] and reactive oxygen species (100 µM) [nitric oxide (NO•), singlet oxygen (¹⁰O₂), superoxide (O₂⁻), ⁵-butyl hydrodroperoxide (⁵BuOOH), hydrogen peroxide (H₂O₂), ⁵-butyl peroxide radical (⁵BuOO•), hydroxyl radical (HO•) and hypochlorous acid (HOCl; 15 µM)], dissolved in PBS (pH 7.4) buffer containing 1% DMSO. The spectra were recorded at 25 °C under excitation at 460 nm.

**Fig. S4** Reversed-phase HPLC chromatograms (with absorption at 450 nm) of the solution of various substrates in PBS buffer (pH 7.4). (a) **rTP-HOCl 1** (5 µM) only; (b) a mixture of **rTP-HOCl 1** (5 µM) and HOCl (10 µM), after 1 min; (c) a mixture of **rTP-HOCl 1** (5 µM) and HOCl (20 µM), after 1 min; (d) acetyl-benzocoumarin 2 (5 µM) only.

**Analytical HPLC analysis methods:** To examine the sensing mechanism of **rTP-HOCl 1** responding to HOCl, high pressure liquid chromatography (HPLC) and LC/MS were performed on Agilent system with C18 reversed phase HPLC column (Eclipse XDB, 3.5 µm, 4.6 mm × 150 mm). The signals were recorded at 450 nm as a function of retention time. H₂O (eluent A) / acetonitrile (eluent B) with a linear gradient elution profile: 0 min, 90% A; 7 min, 0% A; 13 min, 0% A was used as the mobile phase. The temperature of the column was maintained at 25 °C and the flow rate of the mobile phase was 0.7 mL/min.
**Fig. S5** The proposed HOCl sensing mechanism of the probe rTP-HOCl 1, based on the HPLC analysis for the reaction mixtures. Acetyl-benzocoumarin AcBC 2 is formed as the only product, while chlorinated by-product was not observed (in contrast to the case of the acedan-based probe, see Fig. S6).

**Fig. S6** The previously reported HOCl sensing mechanism of the probe TP-HOCl. Besides the formation of acedan as product, a chlorinated by-product was also formed. (Ref: L. Yuan, L. Wang, B. K. Agrawalla, S.-J. Park, H. Zhu, B. Sivaraman, J. Peng, Q.-H. Xu and Y.-T. Chang, *J. Am. Chem. Soc.*, 2015, **137**, 5930)
**Fig. S7** Cell viability assay of the probe toward HeLa cells. Cell viability was assessed by measuring their ability to metabolize CCK-8 (Cell Counting Kit-8) in HeLa cell line (Ref: Ishiyama, M.; Miyazono, Y.; Sasamoto, K.; Ohkura, Y.; Ueno, K. *Talanta* 1997, 44, 1299; Tominaga, H. *et al.* *Anal. Commun.* 1999, 36, 47). Cells were seeded onto 96-well plates at a density of about 5 × 10^3 cells per well in the growth medium and incubated until about 70–80% confluency. Following the probe (10 µM) treatment, 10 µL of the CCK-8 solution (from Dojindo Molecular Technologies, Inc.) was added to each well and cells were maintained for 1 h at 37 °C. Absorbance at 450 nm was measured after incubation of various times as indicated.

**Fig. S8** The two-photon emission spectra of *rTP-HOCl* 1 and its reaction product with HOCl, acetyl-benzocoumarin AcBC 2, in Hela cells. The cells were incubated with only *rTP-HOCl* 1 (10 µM) and only AcBC 2 (10 µM) separately for 30 min. The spectra were recorded under excitation at 900 nm.
Fig. S9 Co-localization experiments of rTP-HOCl 1 (10 µM) with LysoTracker® Deep Red (200 nM) in HeLa cells. Hela cell was cultured with rTP-HOCl 1 and LysoTracker® Deep-Red for 10 min. The cells were excited with 900 nm of two-photon laser and the emission was collected from 410 nm to 610 nm for rTP-HOCl 1. The cells were excited with 633 nm laser line and the emission was collected from 640 to 800 nm for LysoTracker. POC value was calculated as 0.834 from ROI.

Fig. S10 Co-localization experiments of rTP-HOCl 1 (10 µM) with MitoTracker® Red (400 nM) in HeLa cells. Hela cell was cultured with rTP-HOCl 1 and MitoTracker® Red for 15 min. The cells were excited with 900 nm of two-photon laser and the emission was collected from 500 nm to 605 nm for rTP-HOCl 1. The cells were excited with 633 nm laser line and the emission was collected from 640 to 800 nm for MitoTracker. POC value was calculated as 0.443 from ROI.
**Fig. S11** Co-localization experiments of **rTP-HOCl 1** (10 µM) with ERTracker® Red (1 µM) in HeLa cells. Hela cell was cultured with the probe and ERTracker® Red for 15 min. The cells were excited with 900 nm of two-photon laser and the emission was collected from 410 nm to 610 nm for **rTP-HOCl 1**. The cells were excited with 594 nm laser line and the emission was collected from 615 to 800 nm for ERTracker. POC value was calculated as 0.129 from ROI.

**Fig. S12** Co-localization experiments of **rTP-HOCl 1** (10 µM) with SYTO 60 (200 nM) in HeLa cells. Hela cell was cultured with the probe and SYTO 60 for 15 min. The cells were excited with 900 nm of two-photon laser and the emission was collected from 500 nm to 605 nm for **rTP-HOCl 1**. The cells were excited with 633 nm laser line and the emission was collected from 648 to 800 nm for SYTO 60. POC value was calculated as 0.224 from ROI.
7. Supporting tables

Table S1. One-photon and two-photon photophysical properties of the probe rTP-HOCl 1 and the product acetyl-benzocoumarin 2 (AcBC 2).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>λ_{abs} (nm)^a</th>
<th>ε (L mol^{-1} cm^{-1})^b</th>
<th>λ_{em} (nm)^c</th>
<th>Φ_F^d</th>
<th>λ_{TP-ex} (nm)^e</th>
<th>σ (GM)^f</th>
<th>σΦ_F (GM)^g</th>
</tr>
</thead>
<tbody>
<tr>
<td>rTP-HOCl 1</td>
<td>424</td>
<td>18700</td>
<td>598</td>
<td>0.29</td>
<td>900</td>
<td>142</td>
<td>41</td>
</tr>
<tr>
<td>AcBC 2</td>
<td>487</td>
<td>12000</td>
<td>633</td>
<td>0.12</td>
<td>900</td>
<td>439</td>
<td>53</td>
</tr>
</tbody>
</table>

^a maximum one-photon absorption wavelength (λ_{abs}), ^b molar extinction coefficient (ε), and ^c maximum one-photon emission wavelength (λ_{em}) measured in PBS buffer (pH = 7.4). ^d Fluorescence quantum yield (Φ_F) measured in ethanol using coumarin 343 (Φ_F = 0.63 in EtOH) as reference; G. A. Reynolds and K. H. Drexhage, *Optics Commun.*, 1975, 13, 222. ^e Two-photon excitation wavelength (λ_{TP-ex}). ^f Two-photon absorption cross-section (σ) and ^g two-photon action cross-section (σΦ_F) values measured in EtOH using rhodamine B in MeOH (11.2 GM at 900 nm) as reference, (GM: 10^{-50} cm^4 s photon^{-1}).
Table 2. Properties of the reported two-photon probes for HOCl.

<table>
<thead>
<tr>
<th>Probes and references</th>
<th>Signaling mode</th>
<th>Product</th>
<th>Sensing moiety</th>
<th>LOD (nM)</th>
<th>Reaction time</th>
<th>Working solution</th>
<th>Imaging application</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( \lambda_{em} ) (nm)</td>
<td>( \Phi_f )</td>
<td>( \lambda_{TP-ex} ) (nm)</td>
<td>( \sigma \Phi_F ) (GM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J. Am. Chem. Soc. 2015, 137, 5930</td>
<td>Turn-on</td>
<td>500</td>
<td>0.40</td>
<td>750</td>
<td>82</td>
<td>1,3-oxathiolane</td>
<td>16.6</td>
</tr>
<tr>
<td>J. Am. Chem. Soc. 2015, 137, 5930</td>
<td>Turn-on</td>
<td>500</td>
<td>0.35</td>
<td>750</td>
<td>74</td>
<td>1,3-oxathiolane</td>
<td>17.2</td>
</tr>
<tr>
<td>J. Am. Chem. Soc. 2015, 137, 5930</td>
<td>Turn-on</td>
<td>500</td>
<td>0.41</td>
<td>750</td>
<td>83</td>
<td>1,3-oxathiolane</td>
<td>19.6</td>
</tr>
<tr>
<td>Angew. Chem. Int. Ed. 2015, 54, 4890</td>
<td>Turn-on</td>
<td>442</td>
<td>0.49</td>
<td>750</td>
<td>0.50</td>
<td>imidazo line-2-thiones</td>
<td>---</td>
</tr>
<tr>
<td>Angew. Chem. Int. Ed. 2015, 54, 4890</td>
<td>Turn-on</td>
<td>505</td>
<td>0.28</td>
<td>800</td>
<td>8.4</td>
<td>imidazo line-2-thiones</td>
<td>71</td>
</tr>
<tr>
<td>Chem. Commun., 2015, 51, 10150</td>
<td>Turn-on</td>
<td>520</td>
<td>0.1</td>
<td>800</td>
<td>7.8</td>
<td>selenide</td>
<td>350</td>
</tr>
<tr>
<td>Dalton Trans., 2015, 44, 6613</td>
<td>Turn-on</td>
<td>495</td>
<td>0.28</td>
<td>740</td>
<td>65</td>
<td>imine</td>
<td>310</td>
</tr>
<tr>
<td>Sensors and Actuators B, 2016, 222, 483</td>
<td>Turn-on</td>
<td>546</td>
<td>0.14</td>
<td>860</td>
<td>230</td>
<td>oxime</td>
<td>580</td>
</tr>
<tr>
<td>Compound</td>
<td>Type</td>
<td>τ1/τ2 (ns)</td>
<td>λ1/λ2 (nm)</td>
<td>λ3 (nm)</td>
<td>Reaction</td>
<td>Buffer</td>
<td>Cells/Compartments</td>
</tr>
<tr>
<td>----------</td>
<td>------</td>
<td>------------</td>
<td>-------------</td>
<td>---------</td>
<td>----------</td>
<td>--------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Turn-on 508</td>
<td>---</td>
<td>720</td>
<td>---</td>
<td>4-amino-phenol ether</td>
<td>7.6</td>
<td>5 minute</td>
<td>PBS (pH 7.4)</td>
</tr>
<tr>
<td>Turn-on 547</td>
<td>0.30</td>
<td>800</td>
<td>21.3</td>
<td>thiocarb amate</td>
<td>0.007</td>
<td>2 minute</td>
<td>PBS/EtOH (1:1, pH 7.4)</td>
</tr>
<tr>
<td>Turn-on 447</td>
<td>---</td>
<td>700</td>
<td>0.18</td>
<td>imidazoline-2-thiones</td>
<td>210</td>
<td>3 minute</td>
<td>PBS (pH 7.4)</td>
</tr>
<tr>
<td>Turn-on 534</td>
<td>0.56</td>
<td>740</td>
<td>1131.5</td>
<td>1,3-oxathiolane</td>
<td>---</td>
<td>---</td>
<td>PBS/EtOH (1/1, pH 7.4)</td>
</tr>
<tr>
<td>Ratiometric 656</td>
<td>↓ 0.44</td>
<td>800</td>
<td>---</td>
<td>thiosemicarbazide</td>
<td>1150</td>
<td>10 minute</td>
<td>PBS/THF (1/1, pH 7.4)</td>
</tr>
<tr>
<td>Ratiometric 470</td>
<td>↓ 0.29</td>
<td>810</td>
<td>234.1</td>
<td>oxime</td>
<td>---</td>
<td>1 minute</td>
<td>PBS (pH 7.4)</td>
</tr>
<tr>
<td>Ratiometric 598</td>
<td>↓ 0.29</td>
<td>900</td>
<td>41.2</td>
<td>1,3-oxathiolane</td>
<td>34.8</td>
<td>within seconds</td>
<td>PBS (pH 7.4)</td>
</tr>
</tbody>
</table>

8. NMR ($^1$H & $^{13}$C) spectra

$^1$H NMR (CD$_3$OD, 500 MHz, 298 K) of compound 3

$^{13}$C NMR (CDCl$_3$, 125 MHz, 298 K) of compound 3
$^1$H NMR (CDCl$_3$, 500 MHz, 298 K) of acetyl-benzocoumarin 2

$^{13}$C NMR (CDCl$_3$, 125 MHz, 298 K) of acetyl-benzocoumarin 2
$^1$H NMR (CDCl$_3$, 500 MHz, 298 K) of probe rTP-HOCl 1

$^{13}$C NMR (CDCl$_3$, 125 MHz, 298 K) of probe rTP-HOCl 1