Supporting Information

A highly specific and ultrasensitive fluorescent probe for basal lysosomal
HOCl detection based on the chlorination induced by chlorinium ion (Cl⁺)

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1. Determination of the detection limit

The detection limit was calculated based on the fluorescence titration. The fluorescence emission spectrum of probe Lyso-HOCl was measured by five times and the standard deviation of blank measurement was obtained. To gain the slope, the fluorescence intensity at 587 nm was plotted as a concentration of HOCl. So the detection limit was calculated with the following equation (1):

\[
\text{Detection limit} = \frac{3\sigma}{k}
\]  

Where \(\sigma\) is the standard deviation of blank measurement, \(k\) is the slope between the fluorescence intensity versus HOCl concentration.

2. Determination of quantum yield

The quantum yield of probe Lyso-HOCl was determined according to the following equation:

\[
\phi_1 = \frac{\phi_B I_1 A_{\lambda_{ex}} \eta_1}{I_B A_{\lambda_{ex}} \eta_B}
\]  

Where \(\Phi\) is quantum yield; \(I\) is integrated area under the uncorrected emission spectra; \(A\) is absorbance at the excitation wavelength; \(\lambda_{ex}\) is the excitation wavelength; \(\eta\) is the refractive index of the solution; the subscripts 1 and B refer to probe Lyso-HOCl and the standard, respectively. \(N\)-butyl-4-butylamino-1,8-naphthalimide in absolute ethanol was used as the standard, which has a quantum yield of 0.810.\(^1\)

3. Time courses of probe Lyso-HOCl and HOCl system

The time required for the reaction of Lyso-HOCl with HOCl in the completed aqueous solution containing PBS (10 mM, pH = 5.0) was investigated.
Figure S1. Time-course of fluorescence intensity (at 587 nm) of Lyso-HOCl (5 μM) with HOCl (5 μM) in the aqueous solution containing PBS (10 mM, pH = 5.0). Excitation wavelength was 520 nm, excitation and emission slit widths were 3 nm.

4. Effects of pH on probe Lyso-HOCl in the absence and presence of HOCl

Figure S2. The fluorescence spectra of probe Lyso-HOCl (5 μM) in the absence and presence of HOCl (5 μM) under the completed aqueous solution containing PBS (10 mM).
5. HRMS of the reaction of probe Lyso-HOCl with HOCl

![HRMS graph]

**Figure S3.** HRMS of the reaction of probe Lyso-HOCl with HOCl

6. The absorption spectra of probe Lyso-HOCl in the absence and presence of HOCl

![Absorption spectra graph]

**Figure S4.** The absorption spectra of Lyso-HOCl (5 μM) in the absence and presence of HOCl (5 μM) under the aqueous solution containing PBS (10 mM, pH = 5.0).

7. Cell culture and bioimaging

RAW264.7 macrophage cells were grown on glass-bottom culture dishes using DMEM supplemented with 10% (v/v) fetal bovine serum (FBS) and 50 μg mL⁻¹ penicillin-streptomycin in a humidified 37 °C, 5% CO₂ incubator. Before use, the adherent cells were washed three times with FBS-free DMEM. Cells were incubated with 10 μM Lyso-HOCl in culture media for 20 min at 37 °C, and washed with PBS
(pH 7.4) three times. Then the fluorescence imaging of cells was carried out. On the other hand, cells pretreated with PMA (1.0 μg mL⁻¹) or NAC (200.0 μM) for 1 h were incubated with 10 μM Lyso-HOCI in culture media for another 20 min at 37 °C, and washed with PBS three times. Then the fluorescence imaging of cells was carried out. Fluorescence imaging of RAW264.7 macrophage cells was observed under an Olympus FV1000-IX81 confocal fluorescence microscope. The excitation wavelength is 488 nm, and the emission wavelength is 500-600 nm. Similarly, the exogenous HOCl of imaging in living RAW264.7 macrophage cells were carried out by the preincubation of NaOCl (10 μM) for 20 min. Referring to the above-mentioned experimental procedures, the co-location of probe Lyso-HOCI and Lyso-Tracker Blue (λex = 405 nm and λem = 420-460 nm) in living RAW264.7 macrophage cells were also performed. The green channel for probe fluorescence, and the blue channel for Lyso-Tracker Blue fluorescence.

8. Cytotoxicity assays

RAW 264.7 cells (10⁶ cell mL⁻¹) were dispersed within replicate 96-well microtiter plates to a total volume of 200 μL well⁻¹. Plates were maintained at 37 °C in a 5% CO₂ / 95% air incubator for 24 h. Then RAW 264.7 cells were incubated for 12 h upon different probe concentrations of 5, 10, 20, 30, 50, and 100 μM. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg mL⁻¹, HEPES) was then added to each well. After 4 h, the remaining MTT solution was removed, and 150 μL of DMSO was added to each well to dissolve the formazan crystals. Absorbance was measured at 490 nm in a Triturus microplate reader.
9. Preparation of reactive oxygen species (ROS) and reactive nitrogen species (RNS)²

Sodium hypochlorite (NaOCl), H₂O₂ and tert-butylhydroperoxide (TBHP) were diluted from the commercially available solution to 0.1 M in ultrapure water. Hydroxyl radical (·OH) and tert-butoxy radical (·O'Bu) were generated by Fenton reactions. Nitric oxide (NO) was generated from potassium nitroprusside dihydrate. Superoxide (O₂⁻) was prepared from KO₂ in DMSO. Singlet oxygen (¹O₂) was generated from HOCl and H₂O₂, and peroxynitrite (ONOO⁻) was synthesized from sodium nitrite and hydrogen peroxide in the presence of hydrochloric acid. The concentration of H₂O₂ was determined from the absorption at 240 nm (ε = 43.6 M⁻¹ cm⁻¹). The concentration of ONOO⁻ was determined from the absorption at 302 nm (ε = 1670 M⁻¹ cm⁻¹) in 0.1 N NaOH aqueous solution. The concentration of ·OCl was determined from the absorption at 292 nm (ε = 350 M⁻¹ cm⁻¹).

Figure S5. Cytotoxicity assays of Lyso-HOCl at different concentrations for RAW264.7 cells.
10. Bioimaging of basal and endogenous HOCl in live RAW264.7 macrophage cells

**Figure S6.** Confocal fluorescence images of live Raw 264.7 macrophages cells: (a-c) control Raw 264.7 macrophages cells incubated with Lyso-HOCl (10 μM) for 20 min; (d-f) Raw 264.7 macrophages cells pretreated with NAC (100 μM) for 60 min, and then with Lyso-HOCl (10 μM) for another 20 min; (g-i) Raw 264.7 macrophages cells pretreated with PMA (1.0 μg mL⁻¹) for 60 min, and then with Lyso-HOCl (10 μM) for another 20 min. (a, d, g) bright-field images; (b, e, h) green channel; (c, f, i) merged images of the corresponding bright-field and green channel. (j) relative fluorescence intensity of cells in panels (b, e and h). The provided images of Raw
264.7 macrophages cells are representative ones (n = 10 fields of cells). Incubation was performed at 37 °C under a humidified atmosphere containing 5% CO₂. Fluorescence images were acquired using a 488 nm excitation, and fluorescence emission windows: 500–600 nm. Scale bar is 50 µm.

11. Bioimaging of exogenous HOCl in live RAW264.7 macrophage cells

Figure S7. Confocal fluorescence images of live Raw 264.7 macrophages cells: (a-c) control Raw 264.7 macrophages cells incubated with Lyso-HOCl (10 µM) for 20 min; (d-f) Raw 264.7 macrophages cells pretreated with HOCl (10 µM) for 60 min, and then with Lyso-HOCl (10 µM) for another 20 min; (a, d) bright-field images; (b, e) green channel; (c, f) merged images of the corresponding bright-field and green channel. (g) relative fluorescence intensity of cells in panels (b and e). The provided images of Raw 264.7 macrophages cells are representative ones (n = 10 fields of cells). Incubation was performed at 37 °C under a humidified atmosphere containing 5% CO₂. Fluorescence images were acquired using a 488 nm excitation, and fluorescence emission windows: 500–600 nm. Scale bar is 50 µm.
12. Spectra of $^1$H NMR and $^{13}$C NMR for probe Lyso-HOCl

**Figure S8.** Spectrum of $^1$H NMR for probe Lyso-HOCl

**Figure S9.** Spectrum of $^{13}$C NMR for probe Lyso-HOCl
13. References
