Supporting Information

A simple pyridine-based highly specific fluorescent probe for tracing hypochlorous acid in lysosomes of living cells

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1. Materials and instruments

Unless otherwise stated, chemical reagents were purchased from commercial vendor and were used as received. Absorption spectra were carried out using a UV-3101PC spectrophotometer. Fluorescence emission spectra were performed using a Horiba FluoroMax-4 spectrophotometer. The slit width was 5.0 nm for both excitation and emission. High resolution mass spectra (HRMS) were carried out using a LC-MS2010A instrument. Fluorescence imaging of HOCl in live cells was carried out on an Olympus FV1000-IX81 confocal fluorescence microscope.

2. Determination of the detection limit

The detection limit was calculated based on the fluorescence titration. The limit of detection (LOD) was calculated as follows:

$$LOD = 3\sigma/k,$$

$$\sigma = \sqrt{\frac{\sum(\bar{x} - x_i)^2}{n - 1}}$$

where σ is the standard deviation of the blank solution, $\bar{x}$ is the mean of the blank measures, x_i is the value of blank measures, n is the number of tested blank measures (n = 5), and k is the slope between the fluorescence intensities versus the concentrations of HOCl.

3. Synthesis of compound 2

4-Chloro-1,8-naphthalic anhydride (2.32 g, 10 mmol) and 4-Aminopyridine (1.41 g, 15 mmol) were dissolved in ethanol (50 mL) and stirred under reflux for 10 h.

After cooling to room temperature, the light pink crude product (1.95 g) is obtained by vacuum filtration. This product was not further purification.

4. Spectral experiments of probe Lyso-PHE

In the pH-dependent experiment, the PBS buffer solutions with different pH in the range of 3 to 9 were prepared. Subsequently, the response of probe **Lyso-PHE** (5 μM) to HOCl (5 μM) was tested in PBS solution (10 mM) with different pH (3-9). The excitation wavelength is 468 nm and the fluorescence intensity at 560 nm was recorded.

In the time-dependent fluorescence experiment, we first prepared a probe solution with probe **Lyso-PHE** concentration of 5 μM, and then the spectral data of the solution were collected at 560 nm. When 5 μM HOCl was added at 50 s, a significant fluorescence enhancement was observed, and then the spectral data were recorded and saved.

In the fluorescence titration experiment, the probe solution with probe **Lyso-PHE** concentration of 5 μM was prepared and divided into several groups. According to a certain concentration gradient, HOCl (0-5 μM) was added to the solution. When the reaction between HOCl and probe **Lyso-PHE** was complete, we recorded and saved all spectral data.

In the selective experiment, we first prepared a series of analyte solutions, such as K^+, Ca^{2+}, Na^+, Mg^{2+}, Zn^{2+}, Fe^{3+}, Fe^{2+}, Cu^{2+}, NO_3^-, NO_2^-, I^-, CO_3^{2-}, Mn^{2+}, Br^-, SO_4^{2-}, Cl^-, cysteine (Cys), homocysteine (Hcy), glutathione (GSH), S^{2-}, hydroxyl radical ($^{\bullet}OH$), *tert*-butoxy radical ($^{\bullet}O^tBu$), NO, $^1O_2$, *tert*-butylhydroperoxide (TBHP), O_2^-,

H_2O_2, $ONOO^-$, HOCl. Then we prepared a probe solution with probe **Lyso-PHE** concentration of 5 μM, and the solution was divided into several groups. The above analytes were added to the probe solution. After that we collect the spectral data at 560 nm.

5. UV-vis absorption spectra

In the UV-vis absorption spectra, we prepared a probe solution with probe **Lyso-PHE** concentration of 20 μM, and the solution was divided into two groups: A and B. A as the control group, after adding 30 μM HOCl to B, we collected the absorption spectra of A and B.

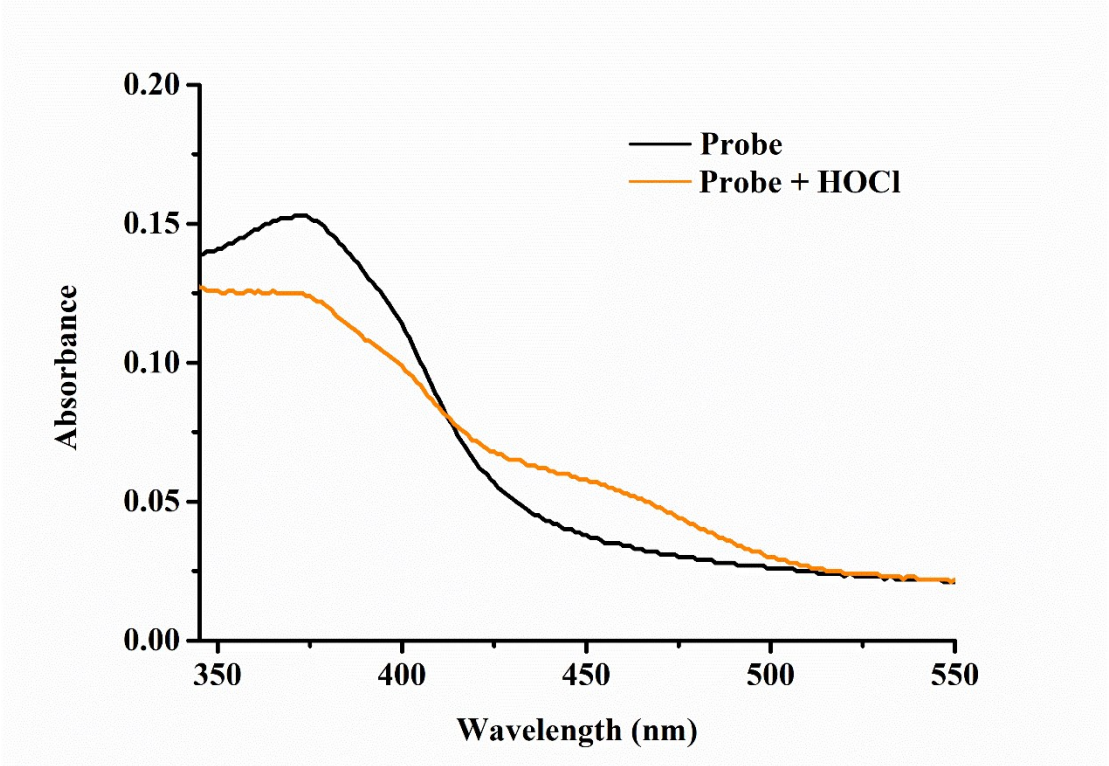


Figure S1. Absorption responses of probe **Lyso-PHE** (20 μM) in the presence of HOCl (30 μM) in PBS solution (10 mM, pH = 5.0) at 25 °C.

6. Cytotoxicity assays

The cell viability of HeLa cells, treated with probe **Lyso-PHE**, was assessed by a cell counting kit-8 (CCK-8; Dojindo Molecular Technologies, Tokyo, Japan). Briefly, HeLa, seeded at a density of 1×10^6 cells·mL^{-1} on a 96-well plate, were maintained at 37 °C in a 5% CO_2 / 95% air incubator for 12 h. Then, the live HeLa cells were incubated with various concentrations (0, 5, 10, 20 and 30 μM) of probe **Lyso-PHE** suspended in culture medium for 10 h. Subsequently, CCK-8 solution was added into each well for 2 h, and absorbance at 450 nm was measured.

7. Cell culture conditions

The HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM) and incubated under an atmosphere containing 5% CO_2 at 37 °C humidified air for 24 h. DMEM contains 10% fetal bovine serum and 1% penicillin-streptomycin.

8. Preparation of reactive oxygen species (ROS) and reactive nitrogen species (RNS)

TBHP

tert-butylhydroperoxide (TBHP) were diluted from the commercially available solution to 0.1 M in ultrapure water.

•OH

Hydroxyl radical (•OH) was generated by Fenton reactions. To prepare •OH solution, hydrogen peroxide (H_2O_2, 2 eq) was added to $FeSO_4$ in deionised water.

•O^{t}Bu

tert-butoxy radical (•O^{t}Bu) was generated by Fenton reactions.

O_2^-

Superoxide (O_2^-) was generated from KO_2 in DMSO.

$^1O_2$

Singlet oxygen ($^1O_2$) was generated from HOCl and H_2O_2.

NO

Nitric oxide (NO) was generated from potassium nitroprusside dihydrate.

H_2O_2

The concentration of hydrogen peroxide (H_2O_2) was determined from the absorption at 240 nm (ε = 43.6 M^{-1} cm^{-1}).

NaOCl

The concentration of sodium hypochlorite (NaOCl) was determined from the absorbance at 292 nm (ε = 350 M^{-1} cm^{-1}).

$ONOO^-$

Simultaneously, 0.6 M KNO_2, 0.6 M HC1 and 0.7 M H_2O_2 was added to a 3 M NaOH solution at 0 ºC. The concentration of peroxynitrite was estimated by using the extinction co-efficient of 1670 cm^{-1} M^{-1} at 302 nm in 0.1 M sodium hydroxide aqueous solutions.

9. Characterization data of probe Lyso-PHE

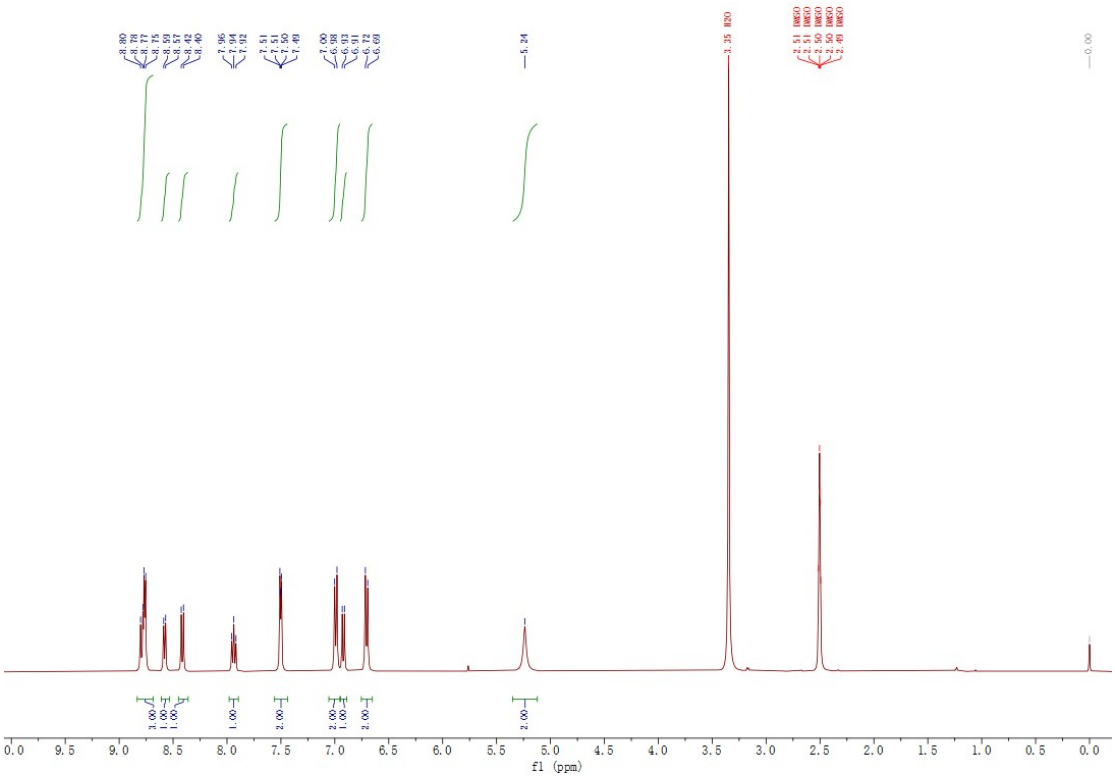


Figure S1. [1]H-NMR data of probe **Lyso-PHE**.

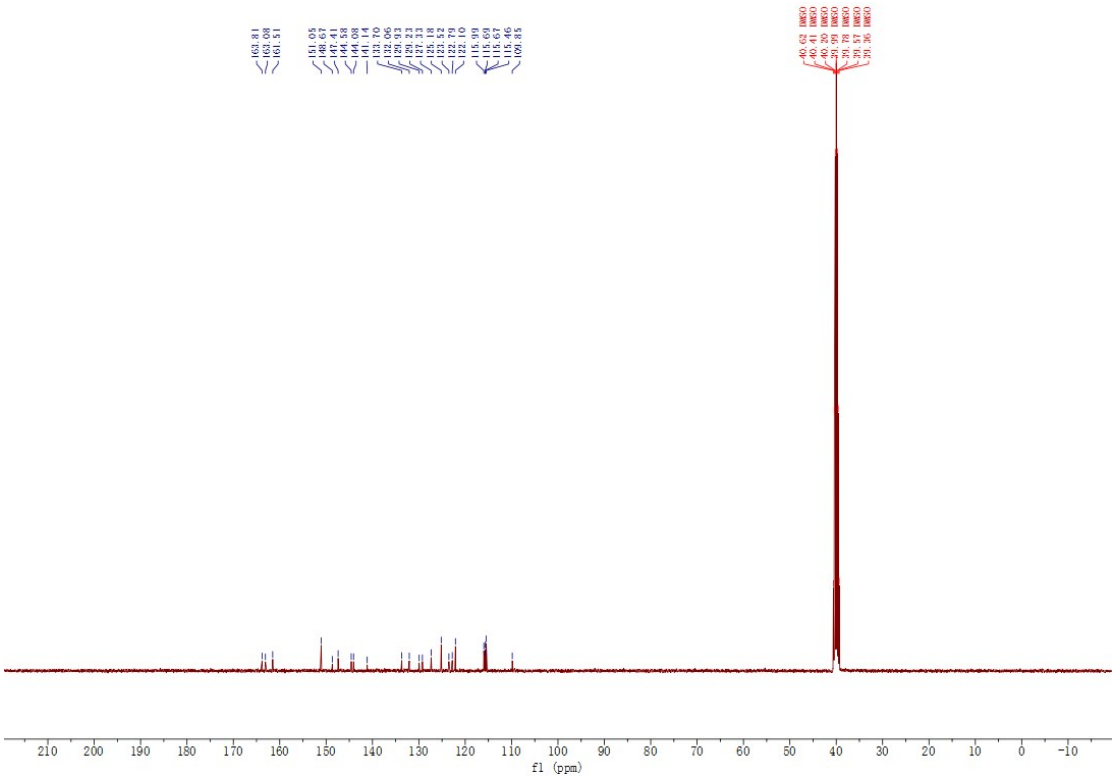


Figure S2. [13]C-NMR data of probe **Lyso-PHE.**

10. Recognition mechanism of probe Lyso-PHE for HOCl

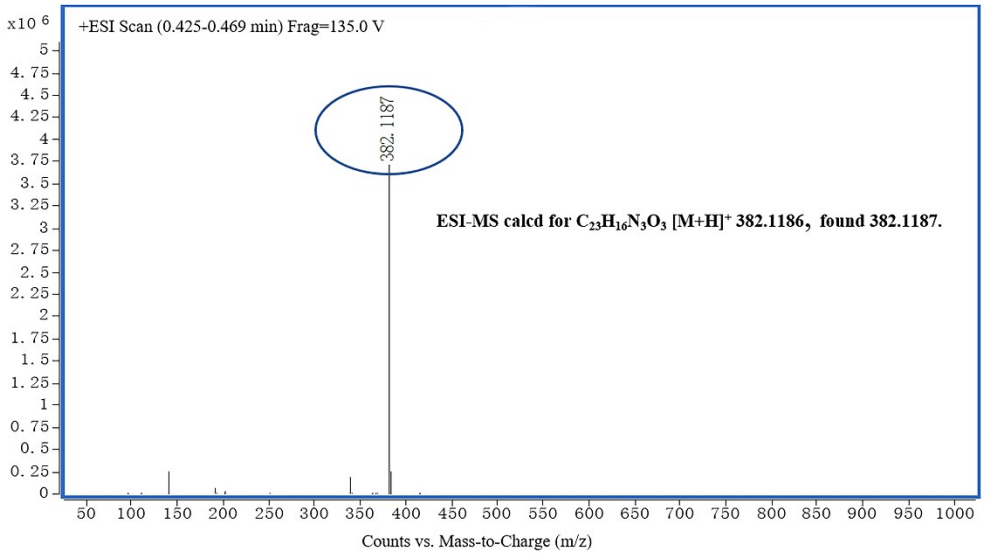


Figure S3. HRMS data of probe **Lyso-PHE**.

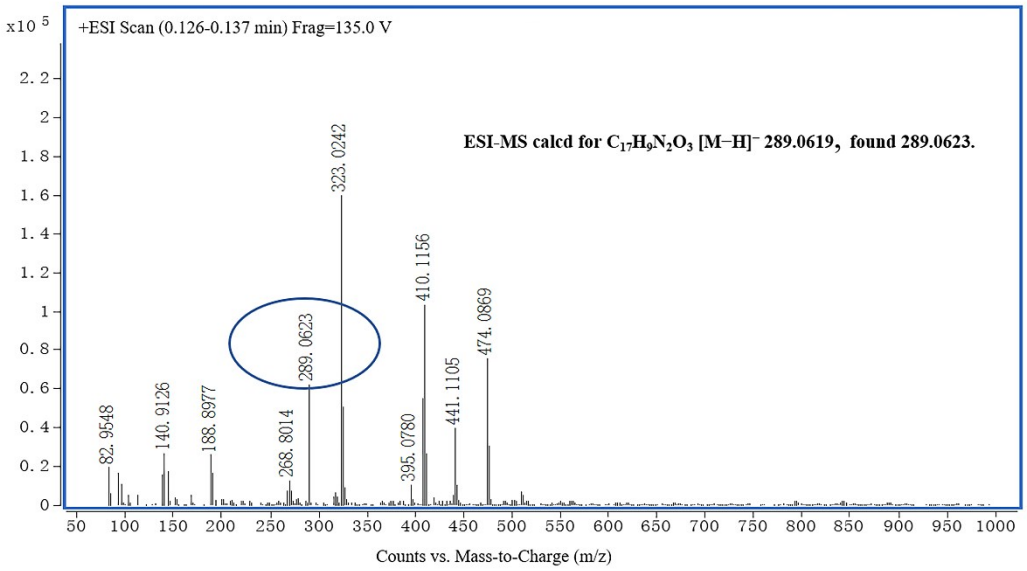
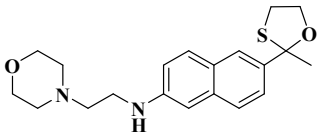
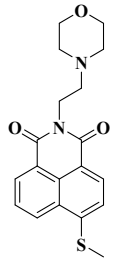
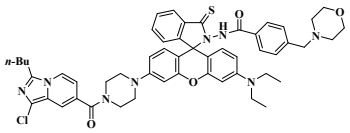
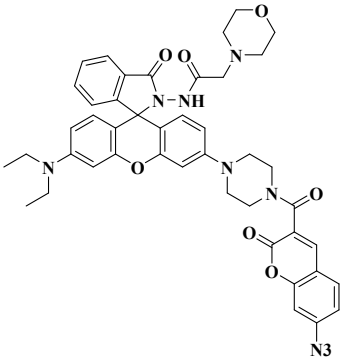
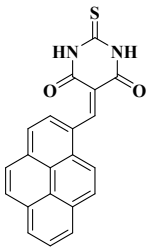
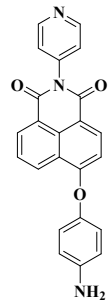


Figure S4. HRMS data of the reaction products of probe **Lyso-PHE** with HOCl.

11. Additional table of comparison between reported HOCl probes and Lyso-PHE

Table S1. Comparison of fluorescent probes for HOCl

Probe	Solution	Detection limit	Response time	Co-localization Imaging	References
	PBS/EtOH, 1:1	16.6 nM	Within seconds	HeLa and RAW 264.7 cells	*J. Am. Chem. Soc*., 2015, **137**, 11654-11657.
	aqueous solution	674 nM	2.5 min	4T1 cells (Overlap Coefficient: 0.89)	*Anal. Chem*., 2017, **89**, 10348-10390.
	PBS/EtOH, 7:3	27 nM	~0.2 s	RAW264.7 cells (Overlap Coefficient: 0.99)	*Sens. Actuators B Chem.*, 2018 **263**, 252-257.
	PBS/DMF, 8:2	73 nM	~18 s	HeLa cells (Overlap Coefficient: 0.86)	*Anal. Chem.*, 2019, **91**, 2932-2938
	0.5 % DMSO	53 nM	within 5 s	-	*Chem. Commun.,* 2020, **56**, 2598-2601

	aqueous solution	16 nM	< 5 s	HeLa, HepG2 and RAW 264.7 cells (Overlap Coefficient: 0.96,0.95 and 0.92)	This work