

*Supporting Information for*

## A Photocaged Fluorescent Probe for Imaging Hypochlorous Acid in Lysosome

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## Materials and instruments

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. Twice-distilled water was used throughout all experiments; Mass spectrometric analyses were measured on a Finnigan MAT 95 XP spectrometer; High resolution mass spectrometric (HRMS) analyses were measured on an Agilent 1100 HPLC/MSD spectrometer; NMR spectra were recorded on an AVANCE III 400 MHz Digital NMR Spectrometer, using TMS as an internal standard; Electronic absorption spectra were obtained on a Shimadzu UV-2700 power spectrometer; Photoluminescent spectra were recorded with a HITACHI F4600 fluorescence spectrophotometer with a 1 cm standard quartz cell; The fluorescence imaging of cells was performed with a Nikon A1MP confocal microscope; The pH measurements were carried out on a Mettler-Toledo Delta 320 pH meter; TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300), both of which were obtained from the Qingdao Ocean Chemicals.

## Cells culture

HeLa cells and RAW 264.7 cells were cultured in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO<sub>2</sub> and 95% air at 37 °C.

## Cytotoxicity assay

*In vitro* cytotoxicity was measured using the colorimetric methyl thiazolyl tetrazolium (MTT) assay on HeLa cells. Cells were seeded into the 24-well tissue culture plate in the presence of 500 µL Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C and 5% CO<sub>2</sub> atmosphere for overnight and then incubated for 24 h in the presence of **PL-HA** at different concentrations (0, 5, 10, 20, 30, 50 µM). Then cells were washed with PBS buffer and 500 µL supplemented DMEM medium was added. Subsequently, 50 µL MTT (5 mg/mL) was added to each well and incubated for 4 h. Violet formazan was dissolved in 500 µL sodium dodecyl

sulfate solution in the water-DMF mixture. Absorbance of the solution was measured at 570 nm using a microplate reader. The cell viability was determined by assuming 100% cell viability for cells without **PL-HA**.

## **Imaging in living cells**

### **Imaging of exogenous HOCl in HeLa cells**

Before the experiments, the HeLa cells were seeded on two 35-mm glass-bottomed dishes and allowed to adhere for 24 h. the cells were washed with PBS (pH=7.4) buffer three times. Subsequently, the first group was incubating with probe **PL-HA** (5  $\mu$ M) (containing 0.1 % DMSO as a cosolvent) for 30 min at 37 °C, the HeLa cells were rinsed with PBS three times. The second group was incubating with probe **PL-HA** (5  $\mu$ M) (containing 0.1 % DMSO as a cosolvent) for 30 min at 37 °C, the HeLa cells were rinsed with PBS three times and the cells were incubated with NaOCl (20  $\mu$ M) for 20 min at 37 °C, and then washed with PBS three times, and the fluorescence images were acquired through a Nikon A1MP confocal microscopy inverted fluorescence microscopy equipped with a cooled CCD camera.

### **Imaging of endogenous HOCl in RAW 264.7 cells**

Before the experiments, the RAW 264.7 cells were plated on 6-well plates and allowed to adhere for 24 h and then incubated with probe **PL-HA** (5  $\mu$ M) for 30 min at 37 °C, washed by PBS buffer and subsequently incubated with 2  $\mu$ g/ml PMA (phorbol 12-myristate13-acetate) and 2  $\mu$ g/ml LPS (lipopolysaccharides) for 2 h. For the control experiments, the cells without treated with PMA/LPS were incubated with probe **PL-HA** (5  $\mu$ M) for 2 hours under the same conditions. For negative control group, the RAW 264.7 cells incubated with probe **PL-HA** (5  $\mu$ M) for 30 min at 37 °C, washed by PBS buffer and subsequently incubated with 2  $\mu$ g/ml PMA, 2  $\mu$ g/ml LPS and 4-aminobenzoic acid hydrazide (ABH, 200  $\mu$ M) for 2 h prior to imaging. The cells were washed with PBS (pH=7.4) buffer. The fluorescence images were acquired through a Nikon A1MP confocal microscopy inverted fluorescence microscopy equipped with a cooled CCD camera.

### Colocation experiment in HeLa cells

HeLa cells were seeded on two 35-mm glass-bottomed dishes and allowed to adhere for 24 h. the cells were washed with PBS (pH=7.4) buffer three times. Subsequently, the cells incubating with probe **PL-HA** (5  $\mu$ M) (containing 0.1 % DMSO as a cosolvent) and 25 nM LysoTracker Green for 30 min at 37 °C, the cells were rinsed with PBS three times and the cells were incubated with NaOCl (20  $\mu$ M) for 20 min at 37 °C, and then washed with PBS three times, and the fluorescence images were acquired through a Nikon A1MP confocal microscopy inverted fluorescence microscopy equipped with a cooled CCD camera.

### Synthesis

*Compound 1 and 2 were synthesized according to the reported method.<sup>[1]</sup>*

#### *Synthesis of compound PL-HA*

Compound **2** (100 mg, 0.16 mmol, 1.0 eq) was dissolved in  $\text{CHCl}_3$  (15 mL), under nitrogen atmosphere, triethyleamine (45  $\mu$ l, 0.32 mmol, 2.0 eq) and bromoacetyl bromide (30  $\mu$ L, 0.32 mmol, 2.0 eq) were added to the solution and reacted at 0°C for 1h. Then, the mixture was warmed to the room temperature and reacted for 3 h. The reaction mixture was poured into water and was extracted with dichloromethane (3×30 mL). The combined organic phase was dried over anhydrous  $\text{Na}_2\text{SO}_4$ , and concentrated under reduced pressure, obtaining product **3**, which was used directly in next step.

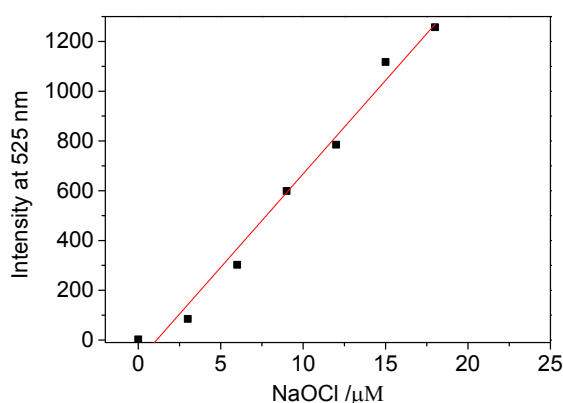
Compound **3** (82.0 mg, 0.10 mmol, 1.0 eq) in dry DMF (3 mL) was added  $\text{K}_2\text{CO}_3$  (41.4 mg, 0.3 mmol, 3.0 eq) and KI (16.6 mg, 0.10 mmol, 1.0 eq), the reaction solution was stirred for 10 min. Morpholine (44 mg, 0.50 mmol, 5.0 eq) was added into the solution. The reaction was stirred for 6 h at the room temperature. Then, the reaction was poured into water and extracted with ethyl acetate (3×20 mL). The combined extracts were washed with water three times, dried over hydrous  $\text{Na}_2\text{SO}_4$ , and concentrated under reduced pressure. The oil residue was purified by column chromatography on silica gel using ethanol/dichloromethane (v/v 1 : 10) to afford a white solid as compound **PL-HA** (50 mg, two step yield: 42%).

$^1\text{H-NMR}$  (400 MHz,  $\text{DMSO-}d_6$ )  $\delta$ . 9.62 (s, 1H), 8.15 (d,  $J = 7.9$  Hz, 2H), 7.91 – 7.88 (m, 1H), 7.83 – 7.76 (m, 4H), 7.68 – 7.62 (m, 4H), 7.14 (dd,  $J = 5.5, 2.9$  Hz, 1H), 6.88 (d,  $J = 2.5$  Hz, 2H), 6.73 (dd,  $J = 8.8, 2.5$  Hz, 2H), 6.67 (d,  $J = 8.8$  Hz, 2H), 5.49 (s, 4H), 3.44 – 3.38 (m,

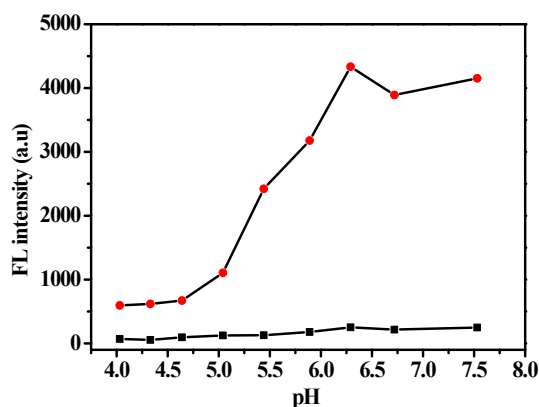
4H), 2.83 (s, 2H), 2.13 (d,  $J = 4.2$  Hz, 4H).;  $^{13}\text{C}$  NMR (101 MHz,  $\text{DMSO}-d_6$ )  $\delta$  167.52, 163.75, 159.36, 152.99, 150.88, 148.03, 134.45, 134.11, 132.40, 130.75, 130.10, 129.82, 129.77, 129.60, 129.39, 125.32, 124.54, 123.41, 112.27, 111.22, 101.76, 67.20, 66.45, 64.97, 59.85, 55.36, 52.87. HRMS (ESI)  $m/z$  calcd for  $\text{C}_{40}\text{H}_{33}\text{N}_5\text{O}_{10}$   $[\text{M}+1]^+$ : 744.2306; found 744.2303.

## References:

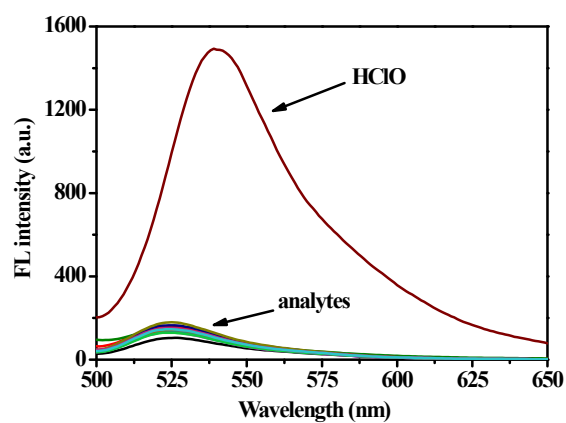
[1] L. Yuan, W. Lin, Z. Cao, L. Long and J. Song, *Chem.–Eur. J.*, 2011, **17**, 689-696.



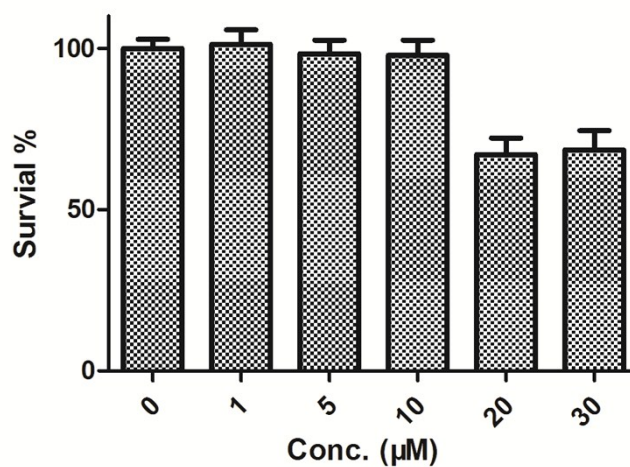
**Figure S1** The relationship between fluorescence intensity at 525 nm of **PL-HA** (5  $\mu\text{M}$ ) and the amount of NaClO (0-25  $\mu\text{M}$ ) with the fixed UV illumination time (100 s).



**Figure S2** The emission intensity changes (at 525 nm) of **PL-HA** before (■) and after (●) upon addition of HClO (10 equiv) at different pH PBS buffer with the fixed UV illumination time (100 s), containing 20 % DMF as a cosolvent.



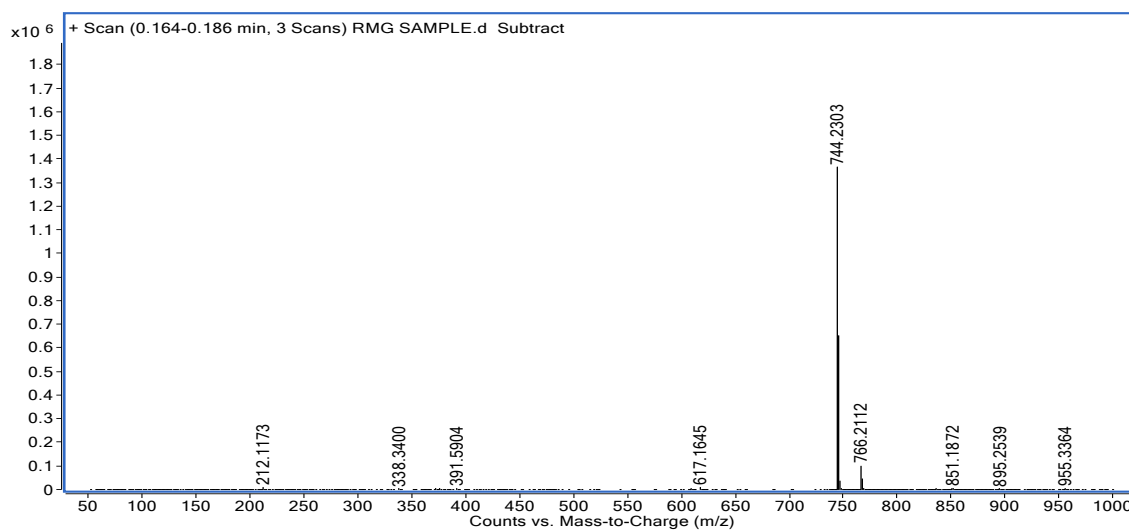
**Figure S3** The fluorescence spectra changes of probe **PL-HA** (10  $\mu\text{M}$ ) in the presence of various analytes (100  $\mu\text{M}$ ) in PBS buffer (containing 20% DMF as a cosolvent).



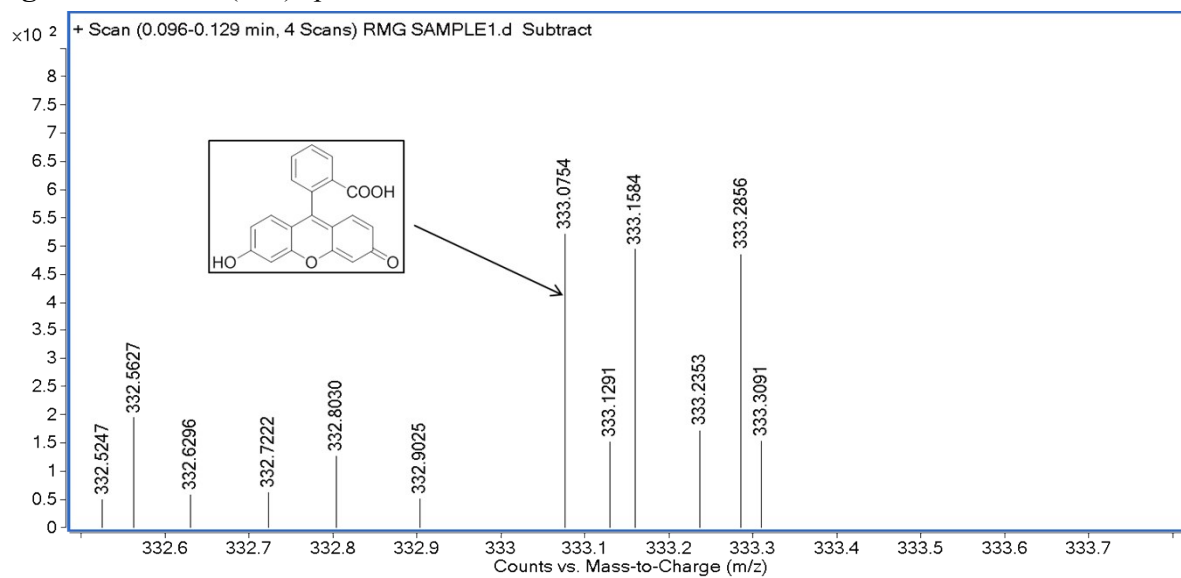
**Figure S4** Cytotoxicity assays of **PL-HA** at different concentrations in HeLa cells.







**Figure S7** HRMS (ESI) spectrum of **PL-HA**.



**Figure S8** HRMS (ESI) spectrum of the product of **PL-HA** reacted with HOCl.