Supporting Information

A Mitochondria-Targeted Near-Infrared Fluorescent Probe with Large Stokes Shift for Real-Time Detection of Hypochlorous Acid

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General

Materials, AR grade or dry grade solvents and reagents mentioned in this paper were purchased from Tokyo Kasei Kogyo (TCI: Tokyo, Japan) and Sigma-Aldrich Chemical Company, and used as received without further purification. Fetal bovine serum (FBS) was purchased from Invitrogen and Life technologies. The reactions were conducted in oven-dried glass wares with a magnetic stirring. NMR spectra was recorded on a Bruker spectrometer at 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR).

Chemical shifts (δ values) were reported in ppm down field from internal Me_4Si. High resolution mass spectra (HRMS) was acquired on an Agilent 6510 Q-TOF LC/MS instrument (Agilent Technologies, Palo Alto, CA) equipped with an electro-spray ionization (ESI) source. All absorption and fluorescence spectra were recorded using Shimadzu UV-2550 UV/Vis and Hitachi F-4600 spectrophotometer respectively with 1cm quartz cell. Melting points were recorded on a Boethius Block apparatus. Cells imaging experiments were conducted on a laser confocal microscope (Olympus FV1000-IX81). All cell images were analyzed with Olympus FV1000-ASW and Image J software.

Calculation of the relative emission quantum yield

The relative emission quantum yield (Φ_l) was evaluated with rhodamine B (Φ = 0.89, in ethanol) [1] used as the fluorescence standard. The quantum yield was calculated using the equation as follows.

$$\Phi_1 = \Phi_B \times \frac{Abs_B \times F_1 \times \lambda \mathrm{ex}_B \times \eta_1^{\,2}}{A\mathrm{bs}_1 \times F_B \times \lambda \mathrm{ex}_1 \times \eta_B^{\,2}}$$

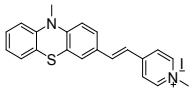
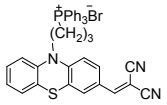
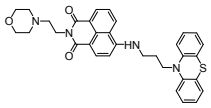
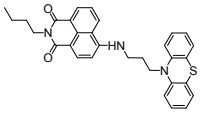
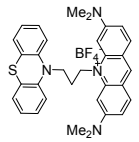
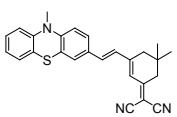
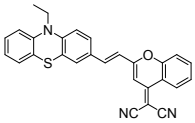
Where Φ is the fluorescence quantum yield, Abs is the absorbance at the excitation wavelength, F is the area under the corrected emission curve, and η is the refractive index of the solvent used. Subscripts 1 and B refer to the probe **L** and the standard rhodamine B, respectively.

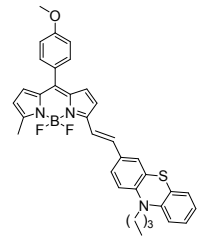
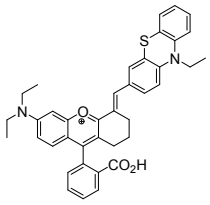
Cell culture and image experiments

L929, HeLa and RAW264.7 cells were cultured in Roswell Park Memorial Institute medium (RPMI1640) or dulbecco's modified eagle's medium (DMEM) supplemented

with 10% FBS in a 5% CO_2 and 37 °C atmosphere, then plated on 10 mm glass cover slips and allowed to adhere for another 12 h. For live cell imaging experiments, cells were incubated with 5 μM **L** for 30 min, and then incubated with 25 μM HOCl for 30 min before imaging on CLSM. To analyze the cytotoxicity, cellular morphology observation and MTT (3-(4,5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay were performed according to the reported procedures in our earlier work.[2]

Table S1. Comparison of the probes with phenothiazine unit for HOCl detection in the literature

Structrure of probes	λ_{abs}	λ_{em}	Response time	Solution	Reference
	407 nm	562 nm	few seconds	PBS buffer (pH 7.3, 10 mM, containing 0.5% DMSO)	Chem. Commun., 2015, 51, 1442-1445
	468 nm	640 nm / 522 nm	few seconds	PBS buffer (pH 7.3, 10 mM, containing 0.5% DMSO and 1 mM triton X-100).	J. Mat. Chem. B, 2015, 3, 1633-1638.
	--	535 nm	10 s	H_2O-EtOH (1:1, v/v)	Talanta 2017, 174, 234-242
	--	543 nm	10 s	H_2O-EtOH (1:1, v/v)	Talanta 2017, 174, 234-242
	--	540 nm	5 s	H_2O	Chem. Commun., 2016, 52, 7982-7985
	447 nm	618 nm	< 10 s	PBS/EtOH=1/1 (10 mM, pH=7.4, v/v)	New J. Chem., 2018, 42, 5135-5141
	506 nm	605 nm	fast responsive	PBS/EtOH=1/1 (10 mM, pH=7.4, v/v)	Sens. Actuators, B, 2018, 255, 963-969

	587 nm	610 nm	--	Methanol-water (6:4, v/v, 20 mM PBS)	Sens. Actuators, B, 2018, 263, 137-142
	575 nm	672 nm	< 3 s	PBS/EtOH=1/1 (10 mM, pH=7.4, v/v)	In this paper

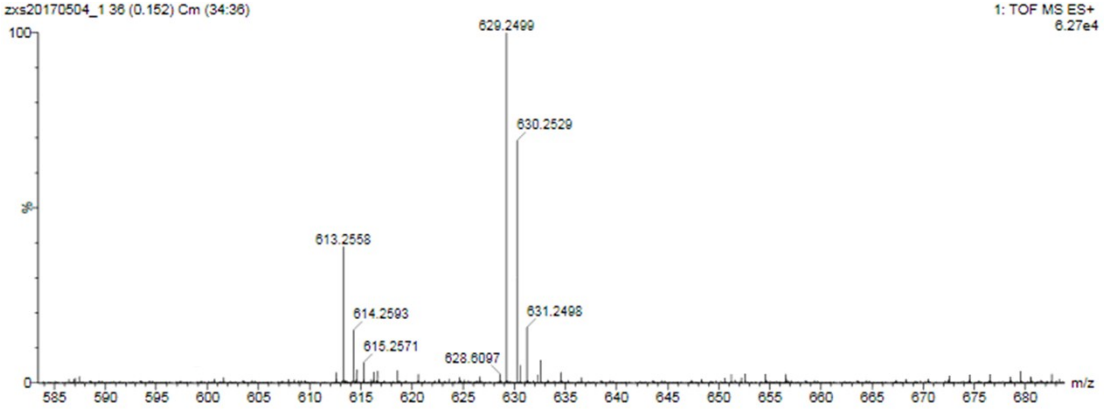


Fig. S1. The HRMS spectra of **L** upon addition of ClO^- (1.0 equiv.). The peaks (m/z) at 613.2558 and 629.2499 correspond to those of **L** (Calcd: 613.2519) and **L'** (Calcd: 629.2469), respectively.

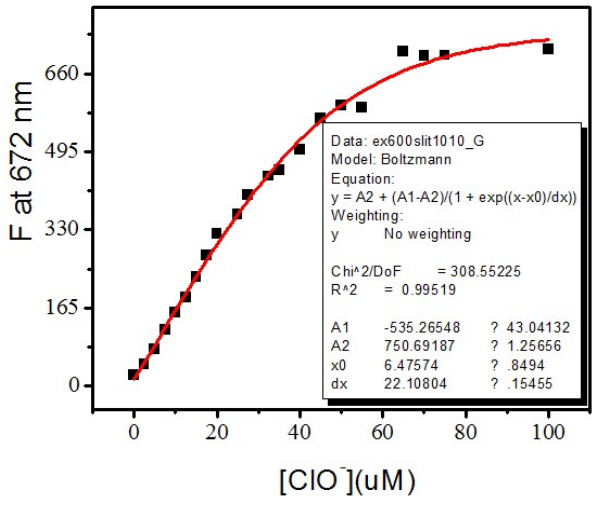


Fig. S2. The fluorescence at 672 nm of **L** (10 μM) as a function of the OCl^- concentration. λ_{ex} = 600 nm, slit = 10 nm, 10 nm

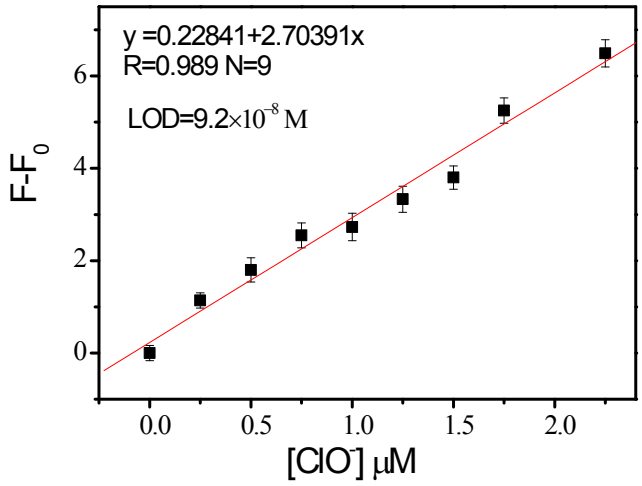


Fig. S3. Emission (at 672 nm) of **L** at different concentrations of ClO^- added. A linear relationship between the fluorescence intensity and the ClO^- concentration could be obtained in the 0-2.5 μM concentration range (R = 0.9986). The detection limit was then calculated with the equation: detection limit = 3σ/k, where k is the slope between intensity versus sample concentration, σ is the standard deviation of blank measurements (σ = 0.083). The limit of detection was measured to be 9.2 $\times 10^{-8}$ M.

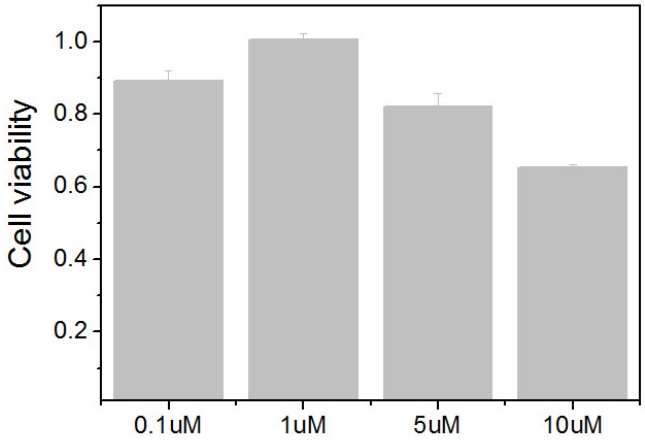
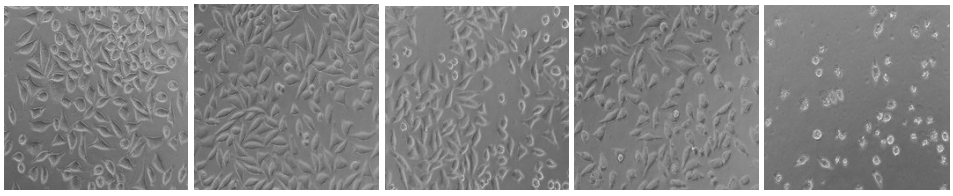


Fig. S4. MTT assays of **L** (0.1 μM to10 μM, 1% DMSO) on L929 cells after incubation time of 24 h at 37 °C.



0 μM 0.1 μM 1.0 μM 5 μM 10 μM

Fig. S5. Images of L929 cells after incubation with **L** (0.1 μM to 10 μM, 1% DMSO) at 37 °C for 24 h.

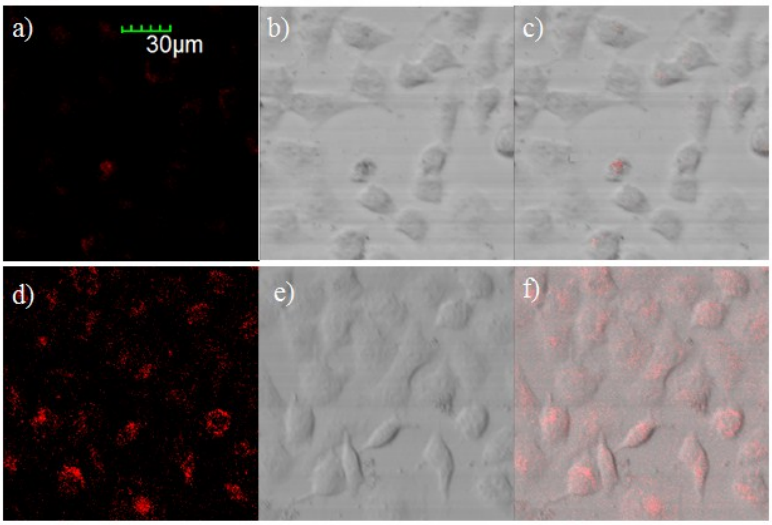


Fig. S6. Fluorescence images of HeLa cells incubated with the probe **L** (5 μM). (a-c) Brightfield, fluorescence and overlay images after staining **L** for 30 min; (d-f) Brightfield, fluorescence and overlay images after incubated with ClO^- (25 μM) for 30 min.

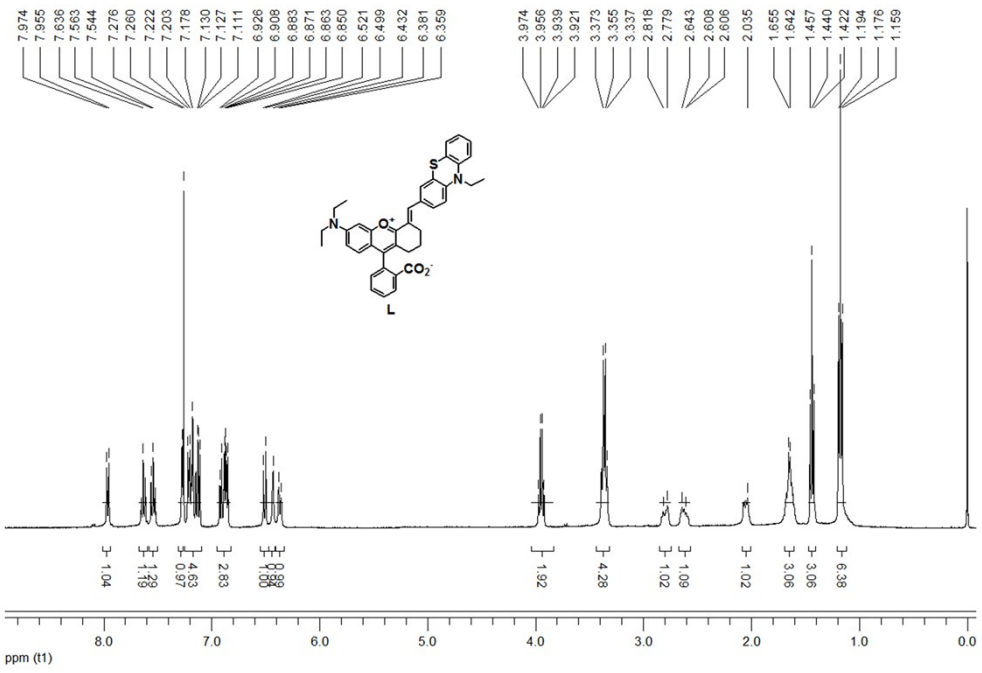


Fig. S7. ^1H NMR of compound **L** (400 MHz, $CDCl_3$).

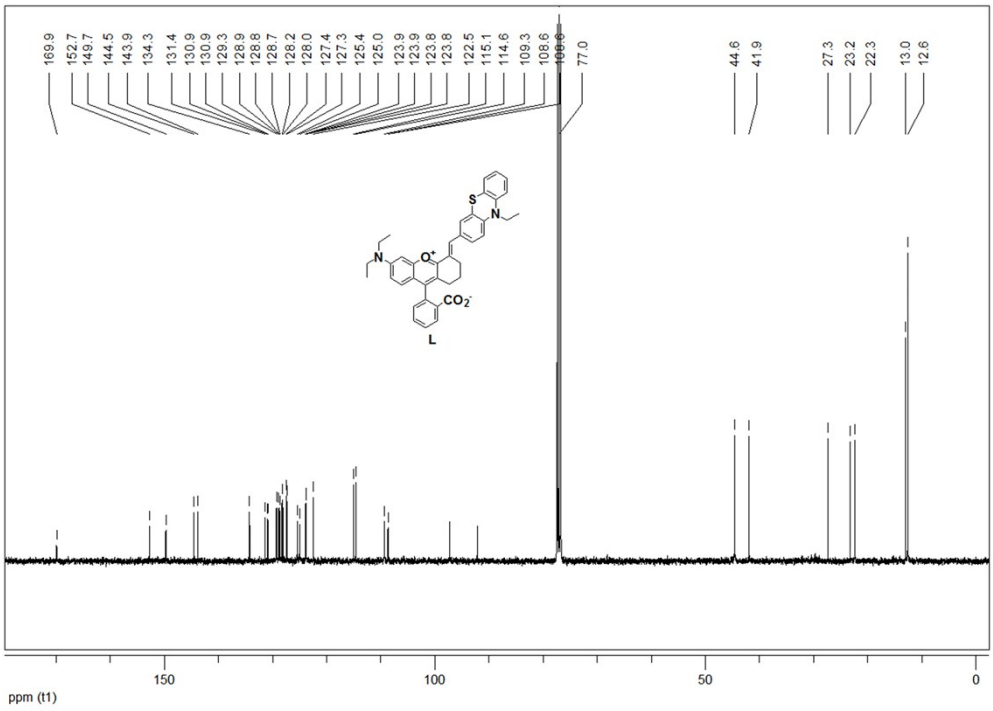


Fig. S8. ^{13}C NMR of compound **L** (100 MHz, $CDCl_3$).

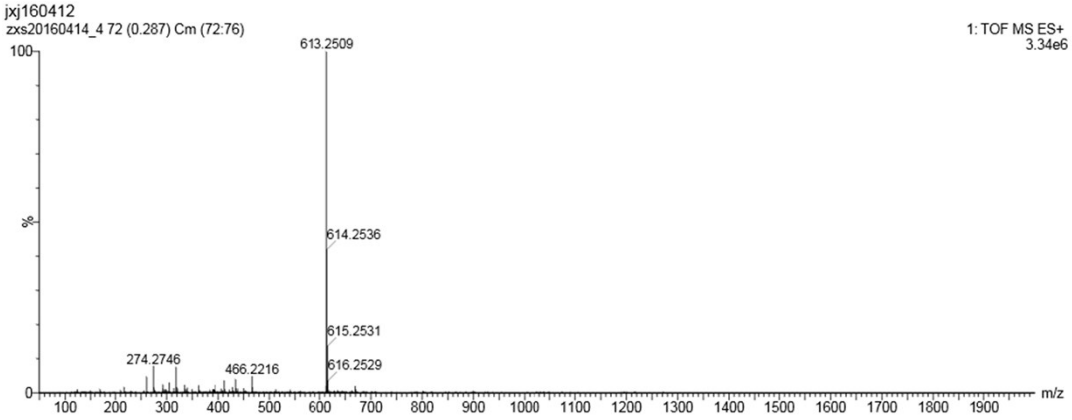


Fig. S9. HRMS of compound **L**.

References

[1] Jiao XJ, Liu C, Huang K, Zhang SW, He S, Zhao LC, Zeng XS. Molecular design and synthesis of a pH independent and cell permeant fluorescent dye and its applications. Org Biomol Chem 2015; 13(23):6647-53.

[2] Jiao XJ, Liu C, Wang Q, Huang K, He S, Zhao LC, Zeng XS. Fluorescence probe for hypochlorous acid in water and its applications for highly lysosome-targetable live cell imaging. Anal Chim Acta 2017; 969:49-56.