Supporting Information

Detection of hypochlorous acid fluctuation via a selective near-infrared fluorescent probe in living cells and in vivo under hypoxia stress

Yan Huang,‡[a,b] Na He,‡[b,c] Yude Wang,[b,c] Liangwei Zhang,[b] Qi Kang,*[a] Yunqing Wang,[b] Dazhong Shen,[a] Jaebum Choo,*[d] Lingxin Chen*[b,e]

[a] *College of Chemistry, Chemical Engineering and Materials Science, Key Laboratory of Molecular and Nano Probes, Ministry of Education, Shandong Normal University, Jinan 250014, China. *E-mail: kangqi@sdnu.edu.cn (Q. Kang)*

[b] *CAS Key Laboratory of Coastal Environmental Processes and Ecological Remediation, The Research Center for Coastal Environmental Engineering and Technology, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, China. *E-mail: lxchen@yic.ac.cn (L. Chen)*

[c] *Medicine Research Center, Institute of Molecular Medicine, Binzhou Medical University, Yantai 264003, China.*

[d] *Department of Chemistry, Chung-Ang University, Seoul 06974, South Korea. *E-mail: jbchoo@cau.ac.kr (J. Choo)*

[e] *College of Chemistry and Chemical Engineering, Qufu Normal University, Qufu 273165, China*

‡ These authors contributed equally.

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1. General Experimental Section.

Instruments. Mice imaging was performed on Perkinelmer IVIS Lumina XRMS Series III In Vivo Imaging System. Fluorescence spectra were determined using a HORIBA Scientific Fluoromax-4 spectro fluorometer with a Xenon lamp and 1.0-cm quartz cells. Absorption spectra were measured on Thermo Scientific NanoDrop 2000/2000C spectrophotometer. All pH measurements were performed with a basic pH-Meter PH-3C digital pH-meter (Lei Ci Device Works, Shanghai) with a combined glass-calomel electrode. Mass spectra were taken on LCQ Fleet LC-MS System (Thermo Fisher Scientific). ^{1}H NMR, ^{13}C NMR spectra were recorded on a Bruker spectrometer. MTT Assay was carried out by a microplate reader (Tecan, Austria). The fluorescence images of cells, zebrafish and tissue sections were taken using a confocal laser scanning microscope (Japan Olympus Co., Ltd) with an objective lens (×60). Intracellular fluorescence detection was carried out on flow cytometry (Aria, BD) with excitation at 543 nm and emission at 600-700 nm.

Materials. All reactions were performed under argon protection and dark, monitored by TLC (Hailang, Yantai). Flash chromatography was carried out using silica gel (300-400 mesh). The purity of Cy-HOCl was separated on a Shimadzu LC-20AT HPLC system equipped with fluorescence and UV-vis absorption detectors. When it was used for imaging, the purity of Cy-HOCl was greater than 95%. All chemicals used in synthesis were analytical reagent grade, and were used as received. Ultrapure water was used throughout. HEPES was obtained from Aladdin and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma-Aldrich. Human normal liver cells (LO2 cells) were obtained from the Committee on Type Culture Collection of the Chinese Academy of Sciences.

Spectrophotometric Experiments. Absorption spectra were obtained with 1.0-cm cuvette cells. The probes Cy-HOCl was added to a 10.0-mL color comparison tube. After dilution to 0.5 μM with 5 mM HEPES buffer, different concentrations of HOCl were added. The mixture was incubated at 37 ^{o}C for 5 min before measurement. Fluorescence spectra were obtained with a 1.0-cm quartz cells by Xenon lamp. The probe Cy-HOCl was added to a 10.0-mL color comparison tube, respectively. After diluted to 10 μM with 10 mM HEPES buffer, different concentrations of HOCl were added. The mixture was incubated at 37 ^{o}C for 2 min before measurement.

Preparation of analytes. Cy-HOCl (1 mM) was prepared in DMSO and stored at 4°C in darkness. S-nitrosoglutathione (GSNO) was synthesized from GSH. Peroxynitrite ($ONOO^-$) solution was synthesized. NO was generated in form of 3-(Aminopropyl)-1-hydroxy-3-isopropyl-2-oxo-1-triazene (NOC-5, 100 μM/ml). NO_2^- was generated from $NaNO_2$. O_2^- was created by the enzymatic reaction of xanthine/xanthineoxidase (XA/XO; 6.0 μM/3 mU) at 25 °C for 5 min. Methyl linoleate (MeLH) and 2,2'-azobis-(2,4-dimethyl) valeronitrile (AMVN) were used to produce MeLOOH. ClO^- was generated from NaClO. All other reagents and chemicals were all from commercial sources and used without further purification. Water used in all experiments was doubly distilled and purified by a Milli-Q system (Millipore, Bedford, MA, USA).

Cell Cultures. Human normal liver cells (LO2 cells) were obtained from the Committee on Type Culture Collection of the Chinese Academy of Sciences. LO2 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO_2 and 95% air at 37 °C.

Flow cytometry. FCM assay was carried out for the detection of the intramolecular generation of HOCl with probe Cy-HOCl. The cells were cultured at 2.0×10^5 cells/well in 6-well plates, and treated with 1 μM Cy-HOCl for 20 min, and then the cells were further incubated with different concentration of HOCl for 20 min at 37 °C. After harvest, cells were washed, and resuspended in PBS and analyzed by flow cytometry.

Confocal Imaging: The fluorescence images of LO2 cells were taken by a confocal laser scanning microscope (Japan Olympus Co., Ltd) with an objective lens (× 60). Excitation wavelength was 543 nm, and the emission was collected from 600 nm to 700 nm.

Hypoxic Conditions in Cell and Zebrafish Incubation. Firstly, 1 mM HOCl was added to cells and zebrafish. 0.1% O_2 concentration was generated with an AnaeroPackTM (Mitsubishi Gas Chemical Campany, Co. Inc., Japan). 1 - 20% O_2 concentration was generated with a multi gas incubator (Sanyo) by means of N_2 substitution.

H&E staining: Heart, liver, spleen, lung and kidney of liver ischemia mice in each group were all excised and fixed in 10% formaldehyde and embedded in paraffin and stained with hematoxylin and eosin (H&E) to confirm histology. Then the treated liver tissue of liver ischemia mice model were prepared to frozen sections and stained with Cy-HOCl to confirm the amount of HOCl.

2. Selectivity of probr for HOCl.

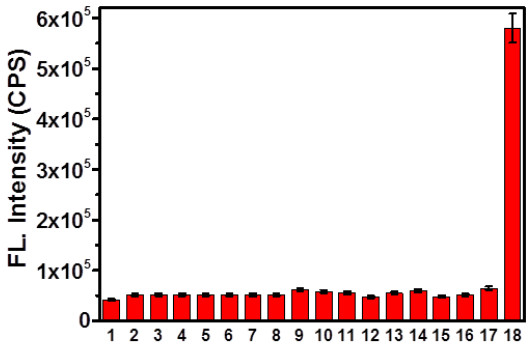


Figure S1. Fluorescence response of 10 μM Cy-HOCl to HOCl and various biospecies. 1. Blank, 2. S-nitrosoglutathione (GSNO) (100 μM), 3. $ONOO^-$ (100 μM), 4. NO (NOC-5) (100 μM), 5. NO_2^- (100 μM), 6. Tocopherols (100 μM), 7. H_2O_2 (100 μM), 8. $O_2^{\bullet-}$ (100 μM), 9. methyl linoleate hydroperoxide (100 μM), 10. Na_2S_4 (a donor of H_2S_n) (100 μM), 11. L-cysteine (L-cys) (100 μM), 12. Glutathione (GSH) (100 μM), 13. NaHS (100 μM), 14. ascorbic acid (100 μM), 15. L-arginine (L-arg) (100 μM), 16. Tyrosine (100 μM), 17. hydroxylamine (HA) (100 μM),18. NaClO (45 μM). All data were acquired in 10 mM HEPES (pH 7.4) at 37 °C after maintained 20 min (λ_{ex} = 543 nm, λ_{em} = 625 nm.).

3. Cytotoxicity of Cy-HOCl.

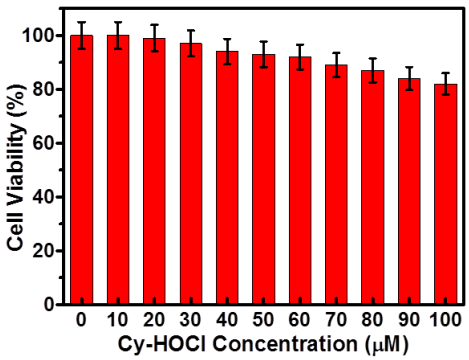


Figure S2. The 24 h cell viability for Cy-HOCl, the concentration of Cy-HOCl was 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 μM. Data are presented as mean ± SD (n = 5).

4. Bright-field Images of Figure 2.

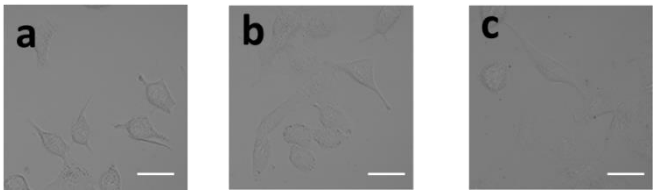


Figure S3. Bright-field images of Figure 2a) – 2c). Scale bar: 20 μm.

5. Bright-field Images of Figure 3.

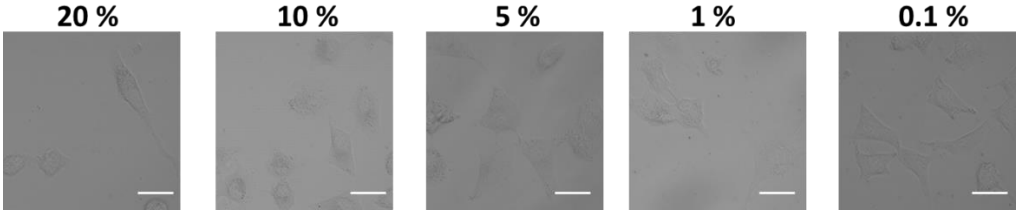


Figure S4. Bright-field images of Figure 3. Scale bar: 20 μm.

6. The Mean Fluorescence Intensity for Figure 4.

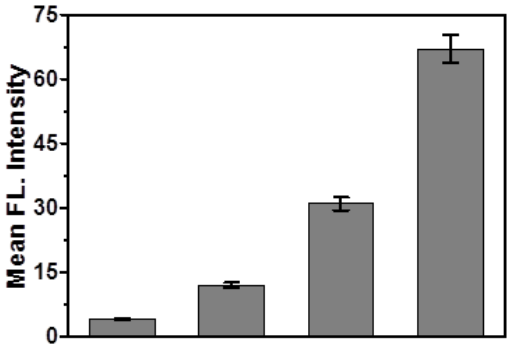
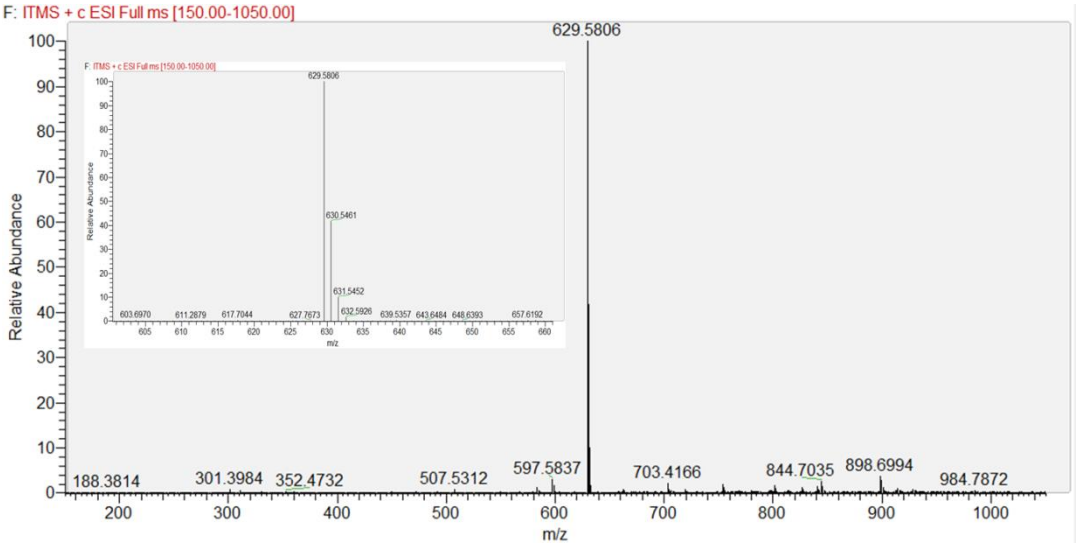


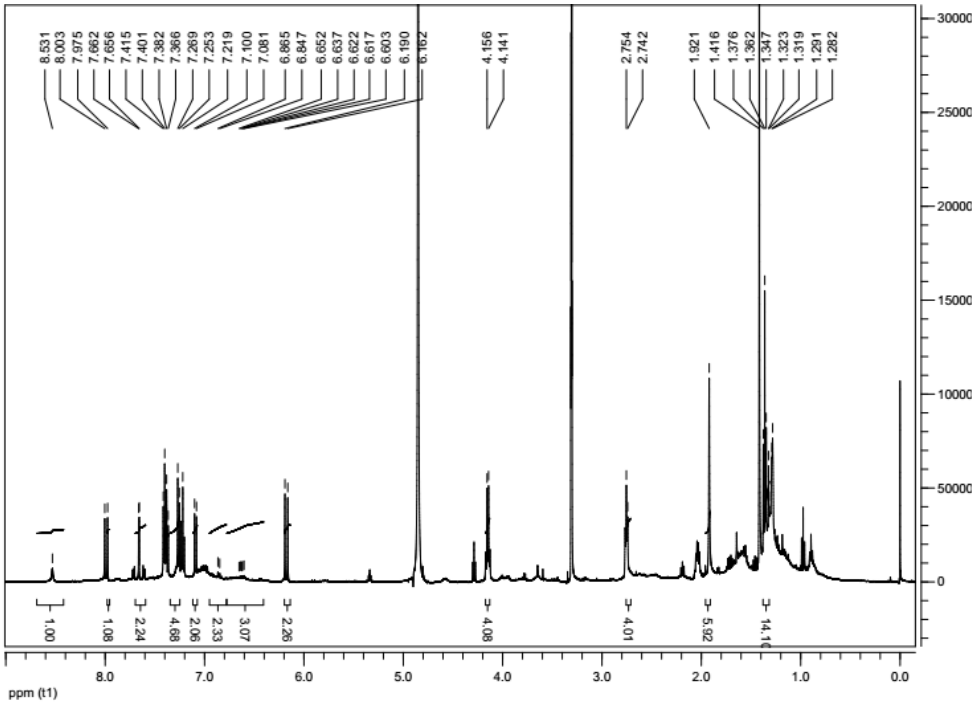
Figure S5. The mean fluorescence intensity of zebrafish imaging in Figure 4.

7. HRMS, ^{1}H NMR and ^{13}C NMR of Cy-HOCl.

HRMS



^{1}H NMR



^{13}C NMR