Supporting Information

Ultra-sensitive fluorescent probes for hypochlorite acid

detection and exogenous/endogenous imaging of living cells

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1. Experimental Section

1.1 Materials and chemicals

All chemicals used for synthesis were purchased from commercial suppliers and applied directly without purification. 6-hydroxy-2-naphthaldehyde (Naph-Oh-1), 1-(6methoxynaphthalen-2-yl)ethenone (Naph-OCH₃-2) and dimethylcarbamothioic chloride were purchased from J&K Chemical. Anhydrous dichloromethane (DCM) and methanol were purchased from Adamas-beta. Dulbecco's modified Eagle's medium (DMEM), PBS, fetal bovine serum (FBS), trypsin-EDTA and penicillin/streptomycin were purchased from GBICO. All reactions that utilize air- or moisture sensitive reagents were performed in dried glassware under dry Ar. Milli-Q water was used in all experiments. All spectroscopic measurements were performed in phosphate-buffered saline (PBS, 10 mM, pH 7.4). The progress of the reaction was monitored by thin-layer chromatography (TLC; Merck 60F-254). Merck silica gel 60 (70-200 mesh) was used for general column chromatography purification purpose. ¹H NMR and ¹³C NMR spectra were taken on a Bruker 400 MHz NMR spectrometer. For ¹H NMR, coupling constants (J) are reported in Hertz (Hz), and multiplicity is indicated as follows: s (singlet), d (doublet), t (triplet), m (multiplet), dd (doublet of doublets), and bs (broad singlet). Mass spectra were obtained using AB Sciex 3200 QTrap ESI-MS system or Bruker ultrafleXtreme MALDI-TOF/TOF. UV absorption spectra were obtained on Shimadzu 1700 UV/vis Spectrometer. Fluorescence spectra were acquired with a FluoroMax-4 fluorescence photometer. Fluorescence images were captured using a Leica SPE Confocal Scanning Microscope.

1.2 Synthesis and characterization

1.2.1 Synthesis of Naph-1



Naph-OH-1 (2 mmol, 348 mg) and N,N-diisopropylethylamine (DIEA, 4 mmol, 697 μ L) were dissolved in dry DCM (10 mL) under argon atmosphere. Then,

dimethylcarbamothioic chloride (4 mmol, 488 mg) was added into the reaction solvent. The mixture was stirred under room temperature overnight. After the reaction complete by TLC monitor, another portion of DCM (20 mL) was added and washed with water and brine. The organic layer was separated and dried with Na₂SO₄. The crude product was purified by silica gel column chromatography with pure DCM to obtain Naph-1 as a white colour powder (321 mg, 62% yield); ¹H NMR (400 MHz, CDCl₃): $\delta = 10.15$ (s, 1H), 8.34 (s, 1H), 8.02 (d, *J* = 8 Hz, 1H), 7.96 (dd, *J* = 8 Hz, 1.2 Hz, 1H), 7.89 (d, *J* = 8 Hz, 1H), 7.56 (d, *J* = 2 Hz, 1H), 7.37 (dd, *J* = 8 Hz, 2 Hz, 1H), 3.49 (s, 3H), 3.42 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): $\delta = 192.06$, 187.23, 154.05, 136.99, 134.25, 134.02, 130.67, 130.59, 128.86, 123.99, 123.46, 119.98, 43.35, 38.93; ESI-MS: calcd. for C₁₄H₁₄NO₂S [M+H]⁺ 260.067, found 260.00.

1.2.2 Synthesis of Naph-OH-2



Naph-OH-2 was synthesized following our reported method.¹

H. Zhang, P. Xiao, Y. T. Wong, W. Shen, M. Chhabra, R. Peltier, Y. Jiang, Y. He, J. He, Y. Tan, Y. Xie,
 D. Ho, Y.-W. Lam, J. Sun, H. Sun, Construction of an alkaline phosphatase-specific two-photon probe and its imaging application in living cells and tissues, Biomaterials, 2017, 140, 220–229

1.2.3 Synthesis of Naph-2



Naph-OH-2 (0.5 mmol, 93 mg) and N,N-diisopropylethylamine (DIEA, 1 mmol, 174 μ L) were dissolved in dry DCM (10 mL) under argon atmosphere. Then, dimethylcarbamothioic chloride (1 mmol, 122 mg) was added into the reaction solvent. The mixture was stirred under room temperature overnight. After the reaction complete by TLC monitor, another portion of DCM (20 mL) was added and washed with water and brine. The organic layer was separated and dried with Na₂SO₄. The crude product was purified by silica gel column chromatography with DCM/Hexane =

5/1 to obtain Naph-2 as a white colour powder (72 mg, 53% yield); ¹H NMR (400 MHz, CDCl₃): δ = 8.44 (s, 1H), 8.02 (d, *J* = 8 Hz, 1H), 7.95 (d, *J* = 8 Hz, 1H), 7.83 (d, *J* = 8 Hz, 1H), 7.52 (s, 1H), 7.32 (d, *J* = 8 Hz, 1H), 3.47 (s, 3H), 3.39 (s, 3H), 2.70 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ = 197.90, 187.46, 153.66, 136.14, 134.51, 130.65, 130.06, 128.27, 124.64, 123.70, 119.67, 43.38, 38.93, 26.74; ESI-MS: calcd. for C₁₅H₁₆NO₂S [M+H]⁺ 274.082, found 274.005.

1.2.4 Synthesis of Naph-Cl-1



Naph-OH-1 (0.23 mmol, 40 mg) was dissolved in methanol (5 mL). Then, NaOCI (300 μ L) was added into the reaction solvent at room temperature. After 5 min later, the solvent was removed under vacuum. The solid product was purified by column chromatography using DCM/Hexane = 20:1 to obtain Naph-Cl-1 as a light yellow solid (40.7 mg, 86% yield); ¹H NMR (400 MHz, *d*⁶-DMSO): 11.06 (s, 1H), 10.10 (s, 1H), 8.52 (s, 1H), 8.15 (d, *J* = 8 Hz, 1H), 8.04 (d, *J* = 8 Hz, 1H), 7.97 (dd, *J* = 8 Hz, 1.2 Hz, 1H), 7.42 (d, *J* = 8 Hz, 1H); ESI-MS: calcd. for C₁₁H₇ClO₂ [M-H]⁻ 205.0, found 205.1.

1.2.5 Synthesis of Naph-Cl-2



Naph-OH-2 (0.27 mmol, 50 mg) was dissolved in methanol (5 mL). Then, NaOCI (400 μ L) was added into the reaction solvent at room temperature. After 5 min later, the solvent was removed under vacuum. The solid product was purified by column chromatography using DCM/Hexane = 10:1 to obtain Naph-Cl-2 as a white solid (47.5 mg, 80% yield); ¹H NMR (400 MHz, *d*⁶-DMSO): 10.91 (s, 1H), 8.62 (s, 1H), 8.08 (d, *J* = 8 Hz, 1H), 8.04 (dd, *J* = 8 Hz, 1.2 Hz, 1H), 8.0- (d, *J* = 8 Hz, 1H), 7.39 (d, *J* = 8 Hz, 1H), 2.67 (s, 3H); ESI-MS: calcd. for C₁₂H₉ClO₂ [M-H]⁻ 219.0, found 219.2.

1.3 Determination of detection limit

Detection limit or limit of detection was calculated based on the fluorescence titration curve of Naph-1 or Naph-2 in the presence of HOCl and mean of the blank. In detailed, the fluorescence intensity of Naph-1 or Naph-2 was measured and the standard deviation of blank measurements was achieved and marked as σ using the following equation. σ is the standard deviation of the blank measurements; n is the tested number of blank measure (n= 11); \bar{x} is the mean of the blank measures; X_i is the values of the blank measures.

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

In this manuscript, we use the following equation for the calculation of detection limit:

Detection limit = $3\sigma/s$

s is the slope of fluorescence intensity versus HOCl concentrations.

1.4 Absorption and fluorescence measurement

Naph-1 or NaPh-2 was dissolved in an appropriate amount of DMSO to obtain 5 mM stock solution. NaOCl and other biological analytes were prepared as 1 mM or 10 mM stock solutions in PBS buffer. All the measurement was taken under room temperature. Firstly, Naph-1 or Naph-2 was diluted in PBS buffer (10 mM, pH 7.4) to afford a final concentration of 5 μ M to a 10-mm quartz cuvette. Then, different concentration of NaOCl or other analytes was added into the quartz cuvette for 1 min incubation. After that, the absorption or fluorescence was measured using Shimadzu 1700 UV/vis Spectrometer and FluoroMax-4 fluorescence photometer respectively. Specially, for the preparation of reactive oxygen species, such as HO•, O2^{•-}, •O^tBu, the protocol from Yang's group was followed.^[2]

[2] Jun Jacob Hu, Nai-Kei Wong, Sen Ye, Xingmiao Chen, Ming-Yang Lu, Angela Qian Zhao, Yuhan Guo, Alvin Chun-Hang Ma, Anskar Yu-Hung Leung, Jiangang Shen, and Dan Yang, *J. Am. Chem. Soc.*, **2015**, 137, 6837–6843.

1.5 MTS assay

HeLa cells were seeded into 96-well plates at a density of 5×10^4 cells per well in

200 μ L of DMEM supplemented with 10% heat-inactivated FBS (fetal bovine serum) and 1% penicillin/streptomycin. Cells were allowed to grow overnight under an atmosphere of 5% CO₂ at 37 °C. The culture media was replaced by the new fresh medium with the various concentration of Naph-1 or Naph-2 (0, 5, 10, 20, 50 μ M). The cells were incubated at the same condition for 24 h. Subsequently, 20 μ L of [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS, 2 mg/mL) was added and the cells were further cultured for another 2 h. Finally, measure the absorbance of treated cells using the plate reader at OD=490nm.

1.6 Cell culture

HeLa cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin and streptomycin). Approximately 10⁵ cells were seeded in a confocal dish (35 mm). 4T1 cells and RAW 264.7 cells maintained in the same manner as HeLa cells.

1.7 Confocal fluorescence imaging

For HeLa cells and 4T1 cells imaging, the cells were treated with Naph-1 or Naph-3 (10 μ M) at 37 °C for 30 min and then incubated with NaOCI (50 μ M) for another 30 min. Cells treated with Naph-1 or Naph-2 (10 μ M) alone were used as a control.

For endogenous HOCl test, RAW 264.7 cells were incubated with lipopolysaccharides (LPS, 5 μ g/mL) and phorbol myristate acetate (PMA, 5 μ g/mL) for 12 h, then Naph-1 or Naph-2 (10 μ M) was added into the medium for 1 h incubation. For inhibition experiment, RAW 264.7 cells were incubated with lipopolysaccharides (LPS, 5 μ g/mL), phorbol myristate acetate (PMA, 5 μ g/mL) and 4-aminobenzoic acid hydrazide (ABH, 400 μ M) for 12 h, then Naph-1 or Naph-2 (10 μ M) was added into the medium for 1 h incubation. Fluorescence images were taken using a Leica TCS SPE Confocal Scanning Microscope. Ex: 405 nm, Em: 505-555 nm for Naph-1, 485-535 nm for Naph-2.

2 Supplemental tables and figures

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2017, 53, 12349	Chem. Commun.	470	540	23	9.7	Within seconds	PBS (PH 7.4)	Endogenous / HeLa cells
	2017, 53, 12349							

Table S1. Properties of representative fluorescent HOCl probes

STP-HOCl Adv. Funct. Mater. 2016, 26, 8128	810	470/ 520	20.1	~	1 minute	PBS (pH 7.4)	Exogenous/HeLa cells and liver tissues; Endogenous /RAW 264.7 macrophage cells
RTP-HOCl 1 Chem. Commun. 2017, 53, 10800	900	598/ 633	~	34.8	within seconds	PBS (pH 7.4)	Endogenous /HeLa cells and mouse brain tissues
NCS-BOD-OCH3 J. Mater. Chem.B 2017, 5, 5854	800	656/ 688	1.5	1150	10 minutes	PBS/THF (1/1, pH 7.4)	Exogenous /mitochondria of A357 cells
SF-1 Chem. Commun. 2018, 54, 1849	550	586	~	4.3	within 15 min	PBS/ACN (9/1, v/v)	Endogenous / RAW 264.7 cells
FDOCl-1 Chem. Sci., 2018, 9, 495	620	686	2068	2.62	within 30 seconds	PBS (pH 7.2) and 0.1% EtOH	Endogenous / RAW 264.7 cells
TPP-TCF Analyst, 2018, DOI:10.1039/C8A N00586A	488	660	582	290	within several seconds	PBS/DMSO (8/2, pH 7.4)	Exogenous / Hela cells and nude mouse
HySOx J. Am. Chem. Soc. 2007, 129, 7313	555	575	~	~	within seconds	PBS (pH 7.4)	Endogenous / phagosomes
Hypo-SiF J. Am. Chem. Soc. 2013, 135, 13365	586	606	2	~	within 80 seconds	PBS (pH 7.4)	No data
Naph-2 This work	364	510	900	8.2	45 seconds	PBS (pH 7.4)	Exogenous / Hela cells Endogenous / RAW 264.7 cells
Naph-1 This work	390	530	4600	2.37	25 seconds	PBS (pH 7.4)	Exogenous / Hela cells Endogenous / RAW 264.7 cells

^a The fluorescence enhanced factor or emission ratio changes before and after interaction with HOCI; ^b The reported detection limit of corresponding probes; ^c The reaction time for responding to HOCI; ^d The solution and pH used for spectrum measurement; ^e Imaging of endogenous or exogenous HOCI in cells or tissues.



Fig. S1 (a) Fluorescence spectra of Naph-1 (5 μ M) in the presence of a series of HOCl (0-5 μ M) in PBS buffer (10 mM, pH 7.4); (b) The linearity between the fluorescent intensity at 530 nm and different concentration of HOCl.



Fig. S2 (a) Fluorescence spectra of Naph-2 (5 μ M) in the presence of a series of HOCl (0-5 μ M) in PBS buffer (10 mM, pH 7.4); (b) The linearity between the fluorescent intensity at 510 nm and different concentration of HOCl.



Fig. S3 ¹H NMR of Naph-Cl-1 from the reaction of Naph-OH-1 and HOCl (d^{6} -DMSO).



Fig. S4 Mass spectrum of Naph-Cl-1 from the reaction of Naph-OH-1 and HOCl.



Fig. S5 TLC images of the reaction between Naph-OH-1 and HOCl under a hand UV lamp. (A): Naph-OH-1; (B): mixture of (A) and (C); (C): reaction solution of Naph-OH-1 and HOCl.



Fig. S6 Mass spectrum of the reaction buffer of Naph-2 and HOCl in methanol.



Fig. S7 ¹H NMR spectrum of Naph-2, Naph-OH-2 and the isolated Naph-Cl-2.



Fig. S8 The differentiation of fluorescence spectrum of Naph-2, Naph-OH-2, reaction of Naph-2 and HOCl, reaction of Naph-OH-2 and HOCl, and Naph-Cl-2.



Fig. S9 Mass spectrum of Naph-Cl-2 from the reaction of Naph-OH-2 and HOCl.



Fig. S10 ¹H NMR of Naph-Cl-2 from the reaction of Naph-OH-2 and HOCl (d^6 -DMSO).



Fig. S11 MTS assay with different concentrations of Naph-1 (a) and Naph-2 (b) in HeLa cells after 24 h incubation.



Fig. S12 Stability test of Naph-1 under continuous irradiation at 405 nm with or without HOCI. (a) three regions of in the different cells; (b) the average fluorescence intensity and standard deviation in (a) with 1 s intervals for the duration of 4 min.



Fig. S13 Confocal images of Naph-1 or Naph-2 (10 μ M) in HeLa cell with pre-incubation of different concentrations of HOCI.



Fig. S14 ¹H NMR spectrum of Naph-1 (CDCl₃).





Fig. S16 Mass spectrum of Naph-1.



Fig. S18 ¹³C NMR spectrum of Naph-2 (CDCl₃).



Fig. S19 Mass spectrum of Naph-2.



Fig. S20 ¹H NMR spectrum of Naph-OH-2 (d^6 -DMSO).