Electronic Supplementary Information for

A Ratiometric Fluorescent Probe for Rapid and Sensitive Visualizing Hypochlorite in Living Cells

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1. Experimental section

1.1 General

Reagents and Instrumentation. All chemicals and solvents were of analytical grade and were used without further purifications. The $^1$H NMR and $^{13}$C NMR spectra were recorded on a Bruker AV-400 spectrometer with tetramethylsilane (TMS) as the internal standard. The chemical shift was recorded in ppm and the following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. Mass spectra were measured on a HP-1100 LC-MS spectrometer. UV-vis spectra were recorded on Hitachi spectrometer. Fluorescence spectra were recorded on a Hitachi FL-4500 fluorometer. Fluorescent images were acquired on a Nikon A1 confocal laser-scanning microscope with a 60 objective lens. The solvents used for UV-vis and fluorescence measurements are of HPLC grade.

1.2 Synthesis of probe

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\begin{align*}
\text{ON} & \quad \text{O} \\
\text{N} & \quad \text{I} \\
\text{O} & \quad \text{N} \\
\text{I} & \quad \text{O} \\
\text{C} & \quad \text{M} \\
\text{C} & \quad \text{Y} \\
\text{H} & \quad \text{A} \\
\text{c} & \quad \text{E} \\
\text{t} & \quad \text{O} \\
\text{H}
\end{align*}
\]

1-Methyl-2,3,3-trimethyl-3$^H$-indolium (133 mg, 0.44 mmol) and diethlyamino coumarin-aldehyde (104 mg, 0.42 mmol) were placed in a round bottom flask with 20 mL anhydrous ethanol. Then, two drops of acetic acid and piperidine were added. The reaction mixture was refluxed for 6 h. After the reaction was completed, the solvent was removed under reduced pressure, and the resulting residue was purified by column chromatography. (CH$_2$Cl$_2$/C$_2$H$_5$OH = 15:1) on silica gel to give the product CMCY as a purple powder (142 mg, yield: 61%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 10.11 (s, 1H), 8.62 (d, $J = 16.0$ Hz, 1H), 8.15 (d, $J = 10.0$ Hz, 1H), 8.04 (d, $J = 16.0$ Hz, 1H), 7.60 – 7.50 (m, 3H), 7.45 (t, $J = 7.6$ Hz, 1H), 6.72 (dd, $^1J = 7.2$ Hz, $^3J = 2.4$ Hz, 1H), 6.48 (d, $^3J = 2.4$ Hz, 1H), 4.33 (s, 3H), 3.54 (q, $J = 7.2$ Hz, 4H), 1.85 (s, 6H), 1.30 (t, $J = 7.2$ Hz, 6H). $^{13}$C NMR (100 MHz, d$_6$-DMSO): $\delta$ 181.3, 169.8, 158.0, 154.4, 150.6, 149.7, 143.5, 142.4, 132.8, 129.4, 129.0, 123.2, 114.9, 112.7, 111.8,
HRMS (ESI) m/z calcd for C_{26}H_{29}N_{2}O_{2} (M^+): 401.2260. Found 401.2236.

1.3 Determination of the detection limit

The fluorescence spectrum of CMCY was measured three times and the standard deviation of a blank measurement was achieved. The fluorescence intensity ratio (I_{480}/I_{631}) was plotted as a concentration of ClO\textsuperscript{−}. The detection limit was calculated by using the equation: detection limit = 3 \sigma/k: where \sigma is the standard deviation of the blank measurement, k is the slope between the fluorescence ratios versus ClO\textsuperscript{−} concentration.

1.4 Cell culture and fluorescence imaging

HeLa cells (Perking Union Medical College, China) were cultured in Dulbecco’s modified Eagle’s medium (DMEM), supplemented with 10% fetal bovine serum (Invitrogen Corp., Carlsbad, CA) and penicillin (100 units/mL)-streptomycin (100 μg/mL) liquid (Invitrogen Corp., Carlsbad, CA) at 37 °C in a humidified incubator containing 5% CO\textsubscript{2} in air. The cells were incubated for 2 days before dye loading on an uncoated 35 mm diameter glass-bottomed dish (D110100, Matsunami, Japan). Then, the cells were rinsed with PBS, incubated with DMEM containing 10% FBS, 5 μM probe CMCY for 10 min at 37 °C, washed with PBS twice, and mounted on the microscope stage. Fluorescence images were captured using a Nikon A1 Application. The cells were furthermore incubated with 50 μM Ca(ClO)\textsubscript{2} for 30 minutes, and then washed with PBS twice for confocal laser-scanning microscopy measurement. Fluorescence images were captured using a Nikon A1 Application.
Fig. S1 (a) Fluorescence responses of CMCY (10 µM) to various species (10 equiv.) in PBS solution (50 mM, pH = 7.40); (b) Fluorescence response of CMCY (10 µM in PBS solution, pH = 7.4) to H₂O₂ with various concentrations. 1, blank; 2, 10 equiv. ClO⁻; 3, 20 equiv. H₂O₂; 4, 40 equiv. H₂O₂; 5, 60 equiv. H₂O₂; λₑₓ = 460 nm. The data were collected after ClO⁻ was added into the CMCY (10 µM) solution for 20 minute.
Fig. S2 The proposed mechanism for sensing of ClO⁻
Fig. S3 $^1$H NMR spectrum of CMCY in CDCl$_3$ (400 M Hz)

Fig. S4 $^{13}$C NMR spectrum of CMCY in d$_6$-DMSO (100 M Hz)
Fig. S5 HR-MS (ESI) spectrum of CMCY