Electronic Supplementary Information for

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

Jiayu Zha,[a] Boqiao Fu, [b] Caiqin Qin,[b] Lintao Zeng*[a,b] and Xichao Hu*[b]

[a] *School of Chemistry & Chemical Engineering, Tianjin University of Technology, Tianjin 300384, PR China. Fax: +86 22 60214252; E-mail: zlt1981@126.com*

[b] *Department of Chemistry and Material Sciences, Hubei Engineering University, Hubei Xiaogan 432000, PR China. Fax: +86 712 2345265.*

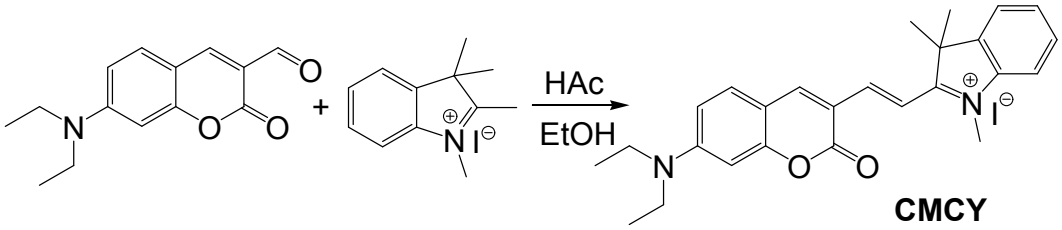
Table of Contents

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



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_2Cl_2/C_2H_5OH = 15:1) on silica gel to give the product **CMCY** as a purple powder (142 mg, yield: 61%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 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, ^{1}J = 7.2 Hz, ^{3}J = 2.4 Hz, 1H), 6.48 (d, ^{3}J = 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): δ 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,

110.6, 109.8, 97.1, 51.9, 45.3, 34.2, 26.3, 13.0. HRMS (ESI) m/z calcd for $C_{26}H_{29}N_2O_2$ (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^-. The detection limit was calculated by using the equation: detection limit = 3 σ/k: where σ is the standard deviation of the blank measurement, k is the slope between the fluorescence ratios versus ClO^- 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 ^oC in a humidified incubator containing 5% CO_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 ^oC, 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 $Ca(ClO)_2$ for 30 minutes, and then washed with PBS twice for confocal laser-scanning microscopy measurement. Fluorescence images were captured using a Nikon A1 Application.

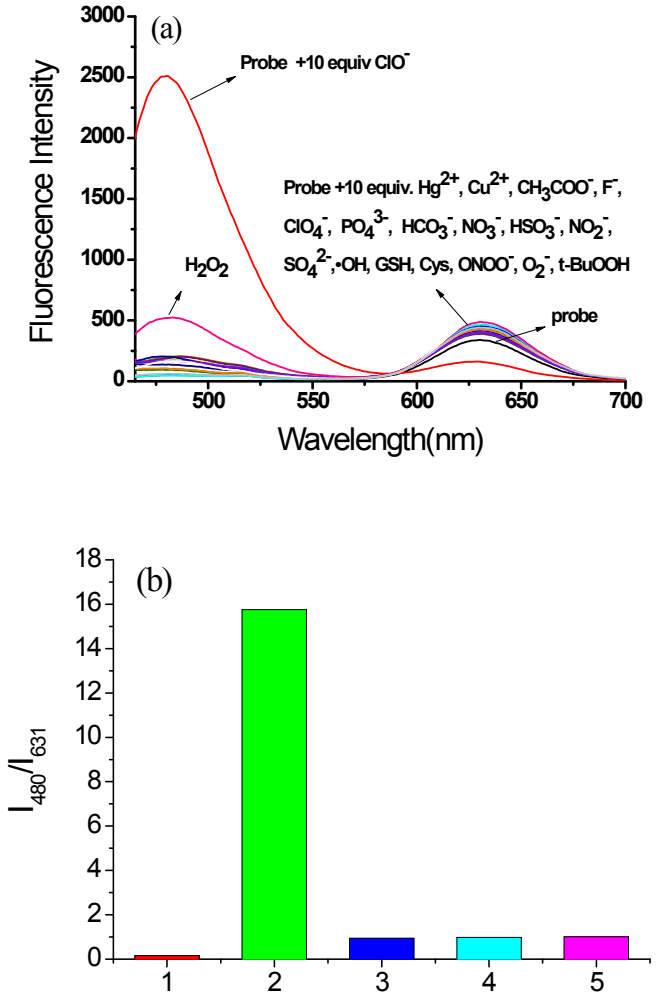
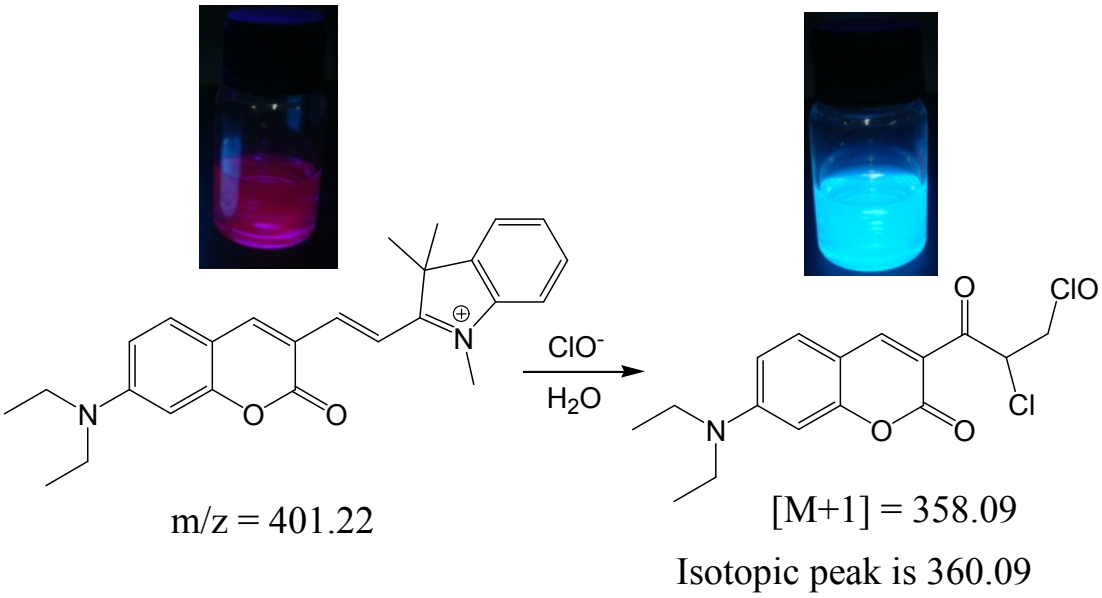


Fig. S1 (a) Fluorescence responses of **CMCY** (10 μM) to various species (10 equiv.) in PBS solution (50 mM, pH = 7.40); (b) Fluorescence response of **CMCY** (10 μM in PBS solution, pH = 7.4) to H_2O_2 with various concentrations.1, blank; 2, 10 equiv. ClO^-; 3, 20 equiv. H_2O_2; 4, 40 equiv. H_2O_2; 5, 60 equiv. H_2O_2; λ_{ex} = 460 nm. The data were collected after ClO^- was added into the **CMCY** (10 μM) solution for 20 minute.



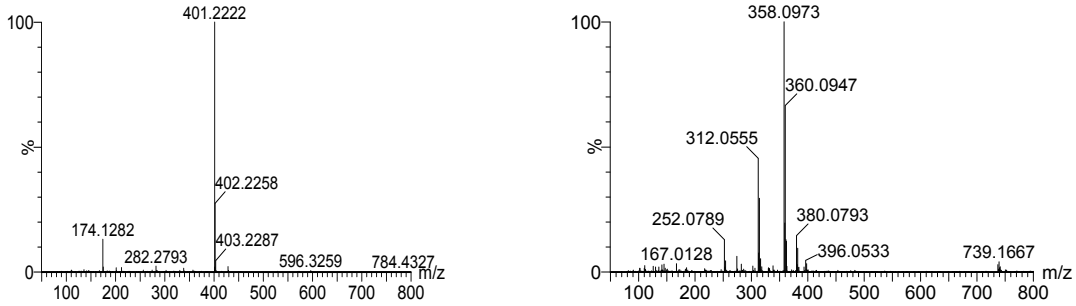


Fig. S2 The proposed mechanism for sensing of ClO^-

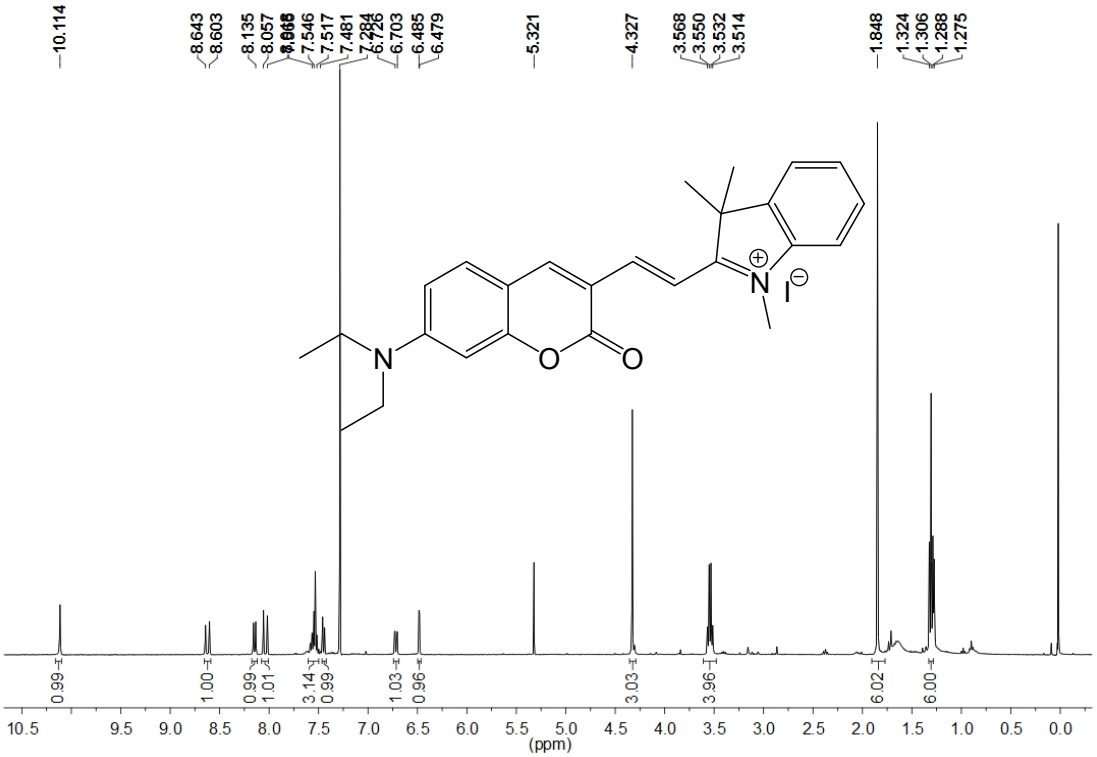


Fig. S3 ^{1}H NMR spectrum of **CMCY** in $CDCl_3$ (400 M Hz)

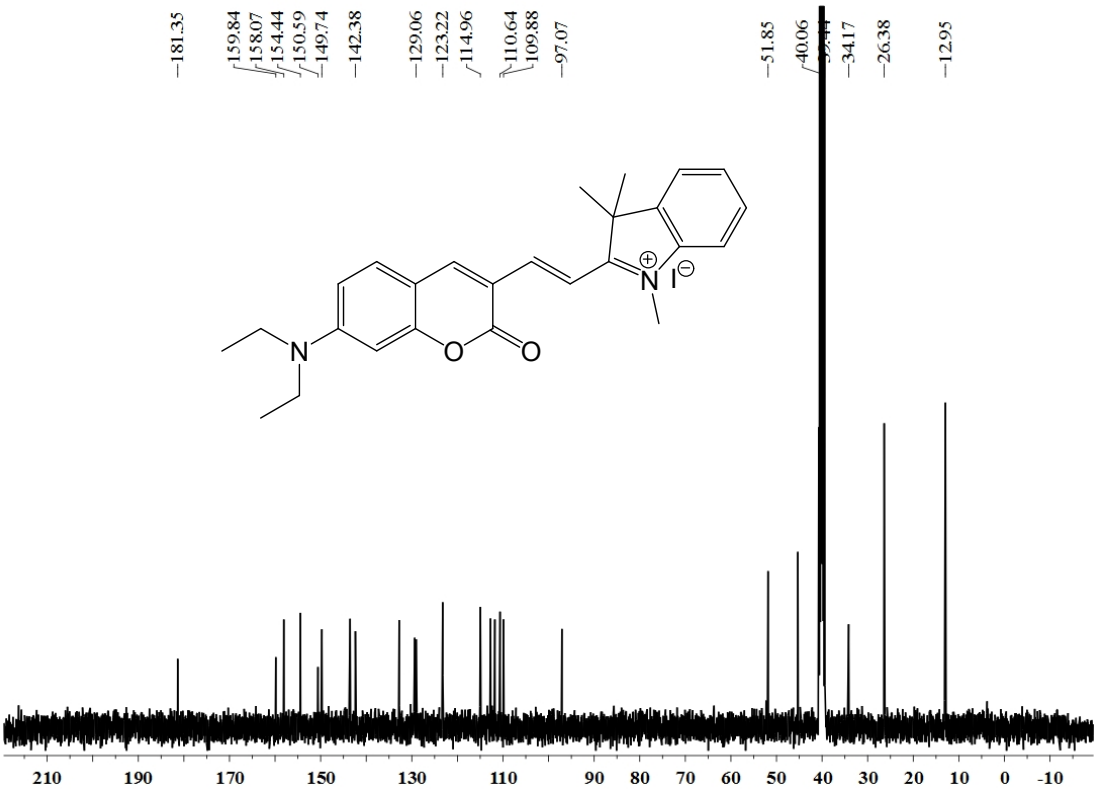


Fig. S4 ^{13}C NMR spectrum of **CMCY** in d^{6}-DMSO (100 M Hz)

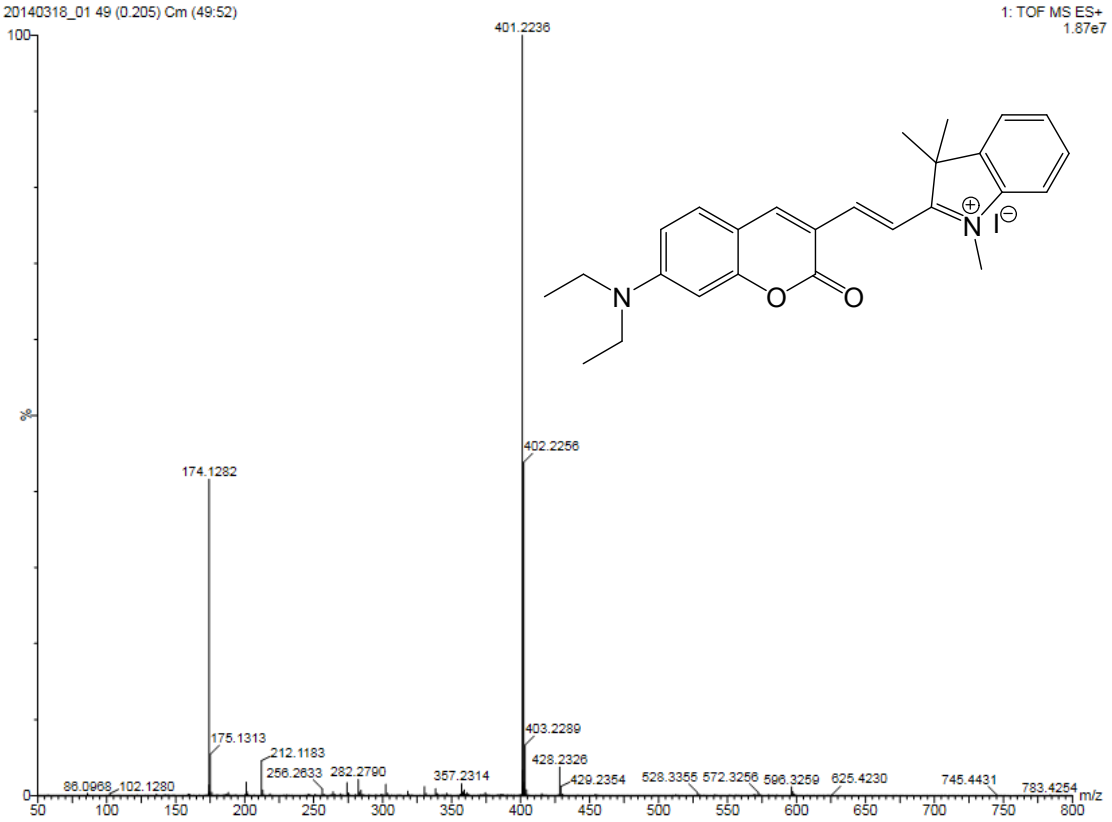


Fig. S5 HR-MS (ESI) spectrum of **CMCY**