Supporting Information for

Recognition of HClO in Live Cells with Separate Signals Using a Ratiometric Fluorescent Sensor with Fast Response

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Content

Materials and methods

All of the solvents used were of analytical grade. **BRClO** was dissolved in dimethyl sulphoxide (DMSO) at a concentration of 5 mM as the stock solution. Slight pH variations in the solutions were achieved by adding the minimum volumes of NaOH or HCl (1 M). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a VARIAN INOVA-400 spectrometer with chemical shifts (δ) reported as ppm (in $CDCl_3$, TMS as the internal standard). Mass spectrometry data were obtained with an HP1100LC/MSD mass spectrometer and an LC/Q-TOF MS spectrometer. Fluorescence measurements were performed on a VARIAN CARY Eclipse fluorescence spectrophotometer (Serial No. FL0812-M018). Excitation and emission slit widths were modified to adjust the fluorescence intensity to a suitable range. Absorption spectra were measured on a Lambda 35 UV/VIS spectrophotometer (Perkin Elmer). All pH measurements were made with a Model PHS-3C meter.

Live cell imaging experiments

MCF-7 cells were cultured in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen). Cells were seeded in 24-well flat-bottomed plates and incubated for 24 h at 37 °C under 5% CO_2. **BRClO** (1 μM) was added (the concentration of DMSO was maintained to be less than 0.2%) and cells were further incubated for 20 min, followed by washing thrice with phosphate-buffered saline (PBS). After incubation with NaOCl (100 μM), the MCF-7 cells were rinsed three times with PBS and the fluorescence imaging was performed with OLYMPUS FV-1000 inverted fluorescence microscope with 100× objective lens. Under the confocal fluorescence microscope, **BRClO** was excited at 488 nm and emission was collected at green channel (490-520 nm) and red channel (560-600 nm).

Computational calculation

All the quantum-chemical calculations were done with the Gaussian 09 suite[1]. The parameter referred to the previous work[2-3]. The geometry optimizations of the dyes were performed using density functional theory (DFT) with Becke's three-parameter hybrid exchange function with Lee-Yang-Parr gradient-corrected correlation functional (B3-LYP functional) and 6-31G** basis set[4]. No constraints to bonds/angles/dihedral angles were applied in the calculations and all atoms were free to optimize.

Determination of the detection limit

The detection limit was calculated based on the fluorescence titration curve of **BRClO** in the presence of NaClO (0-100 μM). The fluorescence intensity of **BRClO** was measured by three times and the standard deviation of blank measurement was achieved. The detection limit was calculated by using detection limit was calculated with the following equation:

Detection limit = $3\sigma/k$

Where σ is the standard deviation of the blank measurement, k is the slope between the fluorescence ratios versus NaClO concentration.

Determination of quantum yields

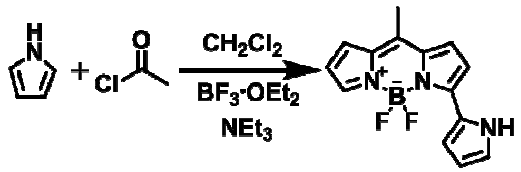
The fluorescence quantum yields of **BRClO** and **BORClO** were determined according to the method below[5].

$$\varphi_u = \frac{(\varphi_s)(FA_u)(A_s)(\lambda_{exs})(\eta_u^2)}{(FA_s)(A_u)(\lambda_{exu})(\eta_s^2)}$$

Where φ is fluorescence quantum yield; FA is integrated area under the corrected emission spectra; A is the absorbance at the excitation wavelength; λ_{ex} is the excitation wavelength; η is the

refractive index of the solution; the subscripts *u* and *s* refer to the unknown and the standard, respectively. We chose Rhodamine B as standard, which has a fluorescence quantum yield of 0.49 in ethanol[6].

Synthesis of BRClO



BRClO

Pyrrole (1.36 g, 20 mmol) was dissolved in 5 mL CH_2Cl_2, and acetyl chloride (0.22 g, 3 mmol) was added. After the reaction mixture was stirred under N_2 atmosphere at rt for 6 h, triethylamine (2 mL) and $BF_3 \cdot OEt_2$ (4 mL) were added dropwise, and the mixture was stirred at 40 □ overnight. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (silica, CH_2Cl_2/petroleum ether = 1/1, v/v) to obtain **BRClO** (0.95 g, 17.3 %). ^{1}H NMR (400 MHz, $CDCl_3$), δ: 10.48 (s, 1H, NH), 7.60 (s, 1H, CH), 7.32 (s, 1H, CH), 7.16 (s, 1H, CH), 7.01 (s, 1H, CH), 6.90 (s, 1H, CH), 6.47 (s, 1H, CH), 6.38 (s, 1H, CH), 2.53 (s, 3H, CH_3). ^{13}C NMR (400 MHz, $CDCl_3$), δ: 151.25, 137.90, 137.03, 136.20, 133.84, 125.80, 123.50, 121.86, 120.45, 118.07, 115.19, 111.44, 29.55, 15.83. TOF MS: m/z calcd for $C_{14}H_{13}BN_3F_2^+$ $[M+H]^+$ 272.1092, found: 272.1174.

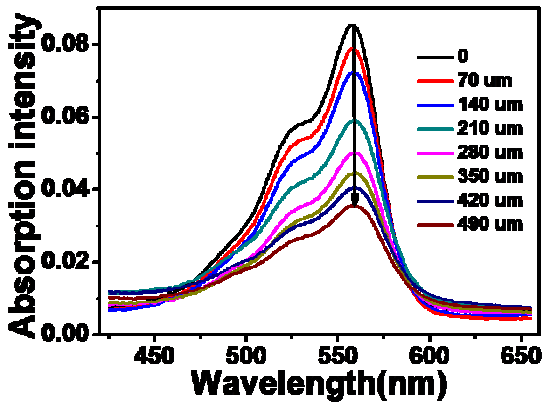


Figure S1 Absorption spectra of **BRClO** (1 μM) upon the titration of NaClO (0-490 μM) in PBS (0.01 M) solution (ethanol/water = 1/9, v/v, pH 7.4).

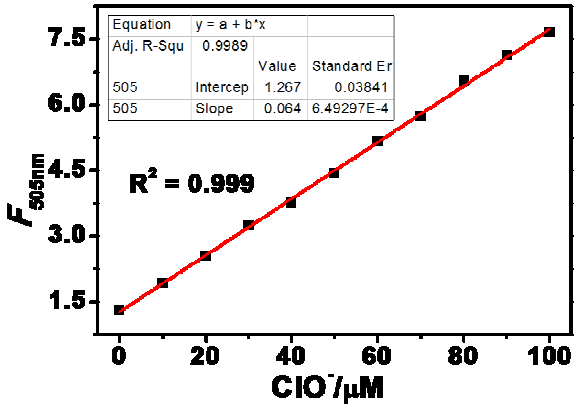


Figure S2 Fluorescence intensity at 505 nm of **BRClO** (1 μM) change as a function of NaClO (0-100 μM) in PBS (0.01 M) solution (ethanol/water = 1/9, v/v, pH 7.4). λ_{ex} = 480 nm.

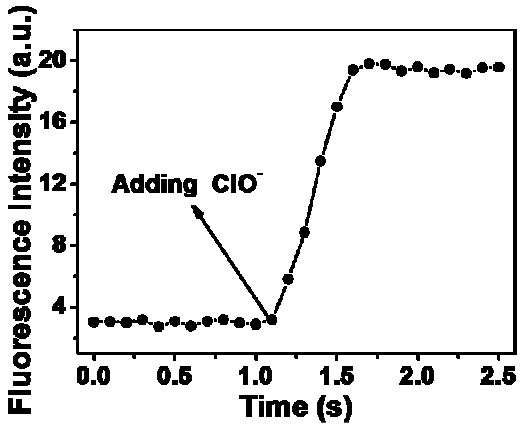


Figure S3 The time courses of Fluorescence intensity at 505 nm of **BRClO** (1 μM) after adding 300 μM NaClO in PBS (0.01 M) solution (ethanol/water = 1/9, v/v, pH 7.4). Time range: 0-2.5 s. λ_{ex} = 480 nm.

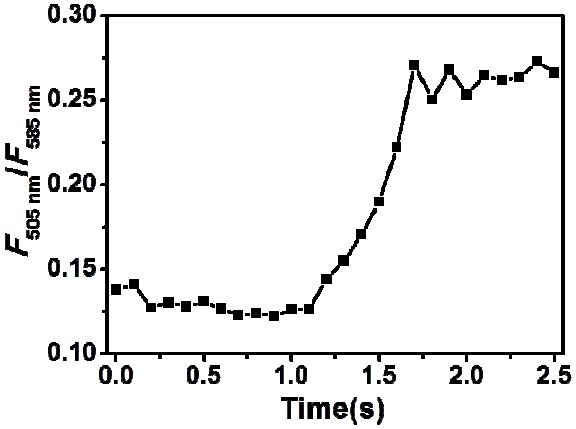


Figure S4 The time courses of Fluorescence ratio ($F_{505\ nm}/F_{585\ nm}$) of **BRClO** (1 μM) after adding 10 μM NaClO in PBS (0.01 M) solution (ethanol/water = 1/9, v/v, pH 7.4). Time range: 0-2.5 s. λ_{ex} = 480 nm.

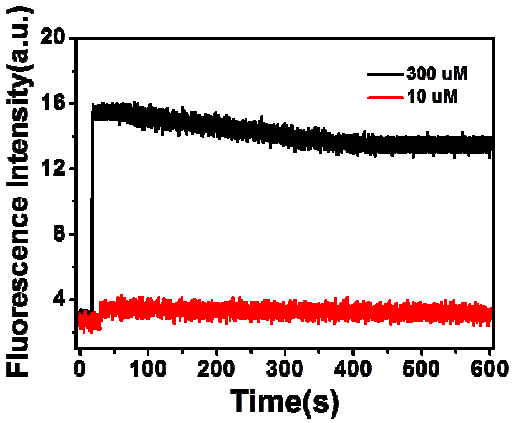


Figure S5 The time courses of Fluorescence intensity at 505 nm of **BRClO** (1 μM) after adding 300 and 10 μM NaClO in PBS (0.01 M) solution (ethanol/water = 1/9, v/v, pH 7.4). Time range: 0-600 s. λ_{ex} = 480 nm.

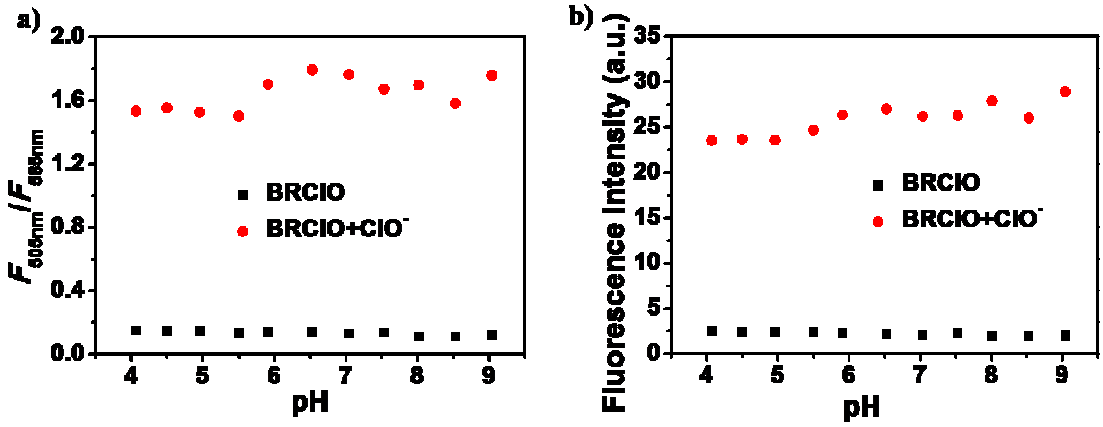


Figure S6 Effect of pH on fluorescence ratios ($F_{505\ nm}/F_{585\ nm}$) (a) and intensities at 505 nm (b) of **BRClO** and **BORClO** in PBS (0.01 M) solution (ethanol/water = 1/9, v/v, pH 7.4). Fluorescence intensity was measured at λ_{ex} = 480 nm.

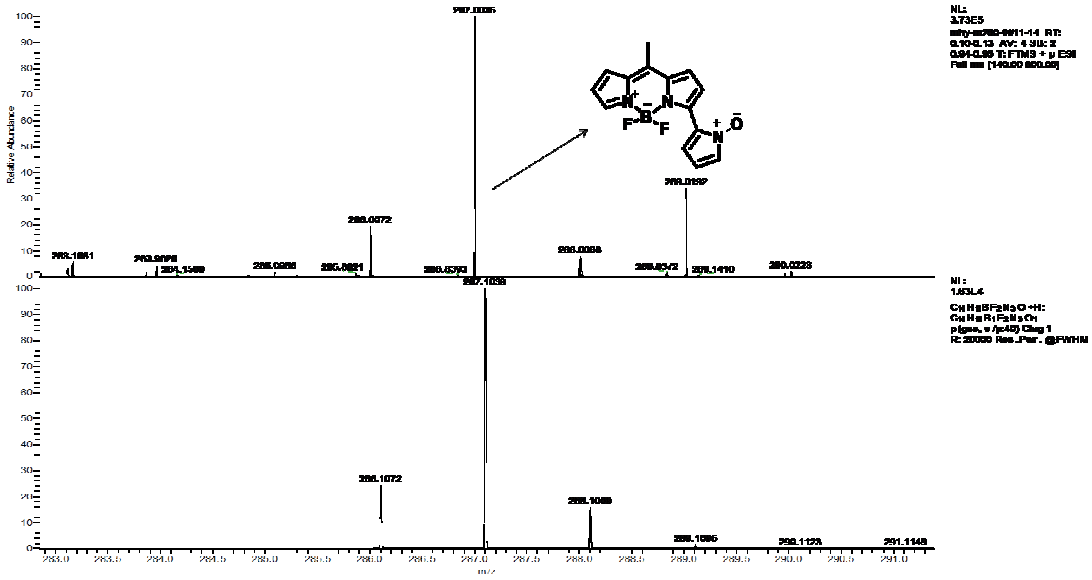


Figure S7 HRMS spectra of **BORClO**.

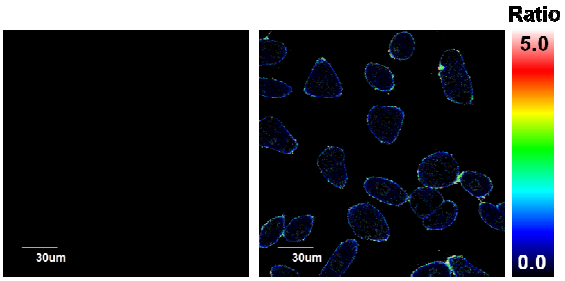


Figure S8 Confocal ratio images ($I_{490\text{-}520nm}/I_{560\text{-}600nm}$) of without (left) and with (right) exogenous NaClO (100 μM) in MCF-7 cells using sensor **BRClO** (1 μM) for 30 min at 37 ^{o}C. λ_{ex} = 488 nm. Scale bar = 30 μm.

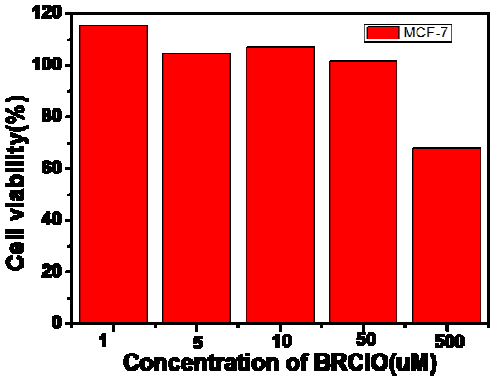


Figure S9 Cytotoxicity assays of **BRClO** at 1、5、10、50 and 500 μM against MCF-7 cells for 24 h.

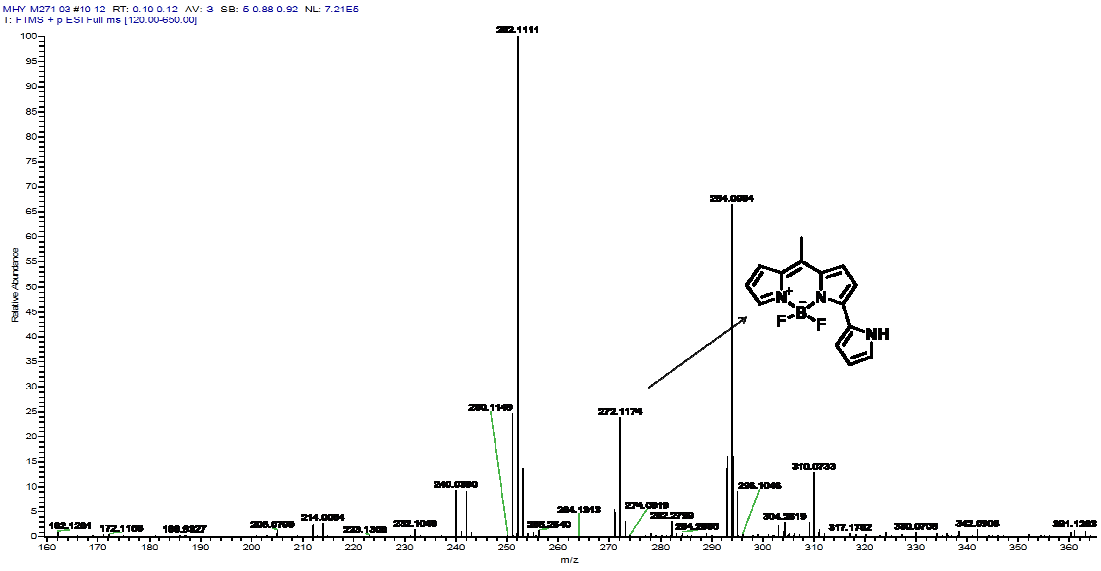


Figure S10 HRMS spectra of **BRClO**.

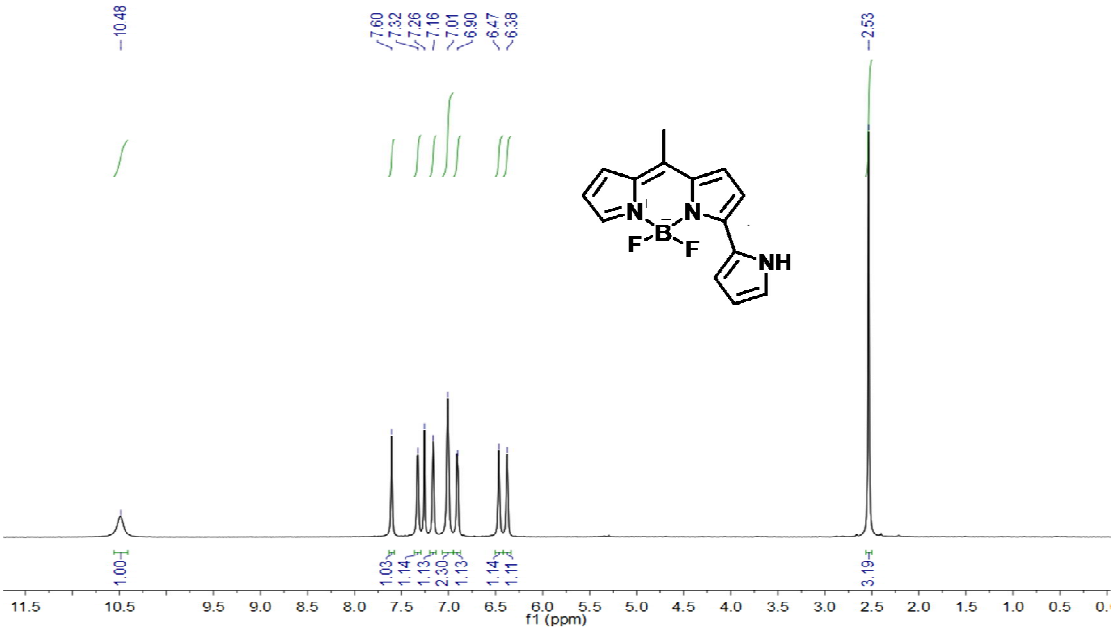


Figure S11 [1]HNMR of **BRClO.**

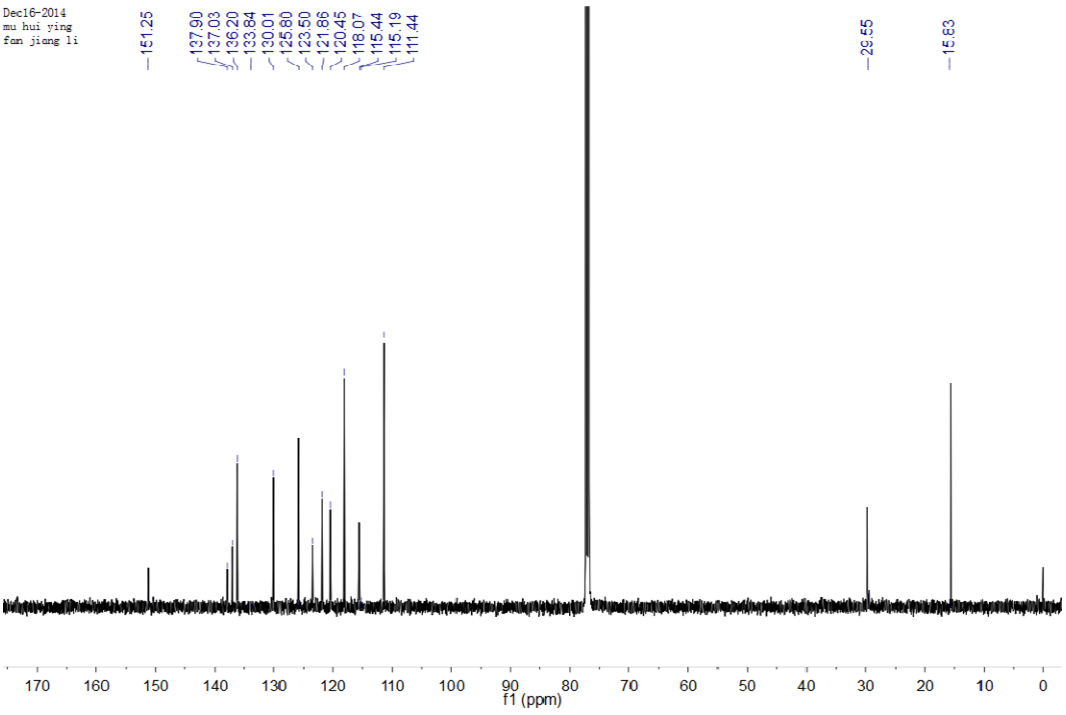


Figure S12 [13]CNMR of **BRClO**.

Reference

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(4) Dreizler, M. R. G., E. K. U. *Density Functional Theory* **1990**.

(5) Karstens, T.; Kobs, K., Rhodamine B and rhodamine 101 as reference substances for fluorescence quantum yield measurements. *J. Phys. Chem.* **1980,** *84*, 1871-1872.

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