Supporting Information

A novel highly specific and ultrasensitive fluorescent probe for monitoring hypochlorous acid and its applications in live cells

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1. Determination of the detection limit

The detection limit was calculated based on the fluorescence titration. The fluorescence spectra of free probe **TCFL** were measured by eight times and its standard deviation was obtained. To gain the slope, the fluorescence intensities at 515 nm were plotted as the increasing concentrations of HOCl. So the detection limit was calculated with the following equation (1):

$$\text{Detection limit} = 3\sigma/k \quad (1)$$

Where σ is the standard deviation of blank measurement, k is the slope between the fluorescence intensities versus the concentrations of HOCl.

2. Cell culture and bioimaging

The live RAW 264.7 macrophage cells were cultured in DMEM containing 10% fetal bovine serum, 1% penicillin, and 1% streptomycin at 37 °C (w/v) in a 5% CO_2 / 95% air incubator MCO-15AC (Sanyo, Tokyo, Japan). The concentrations of counted cells were adjusted to 1×10^6 cells mL^{-1} for confocal imaging in high-glucose DMEM (4.5 g of glucose/L) supplemented with 10% fetal bovine serum (FBS), $NaHCO_3$ (2.0 ng/L), and 1% antibiotics (penicillin/streptomycin, 100.0 U/mL). Cultures were maintained at 37 °C under a humidified atmosphere containing 5% CO_2.

After 24 h, the cells were incubated with 10 μM **TCFL** in culture media for 30 min at 37 °C, and washed with PBS three times. Then the fluorescence imaging of the cells was carried out. After adding HOCl (20 μM) to the above cells for 10 min, and washed with PBS three times. Then the fluorescence imaging of the cells was performed. On the other hand, the cells pretreated with PMA (2.0 μg mL^{-1}) or NAC (500 μM) for 60 min were incubated with 10 μM **TCFL** in culture media for another 30 min at 37 °C, and washed with PBS three times. Then the fluorescence imaging of cells was carried out. The fluorescence imaging of live RAW 264.7 macrophage cells

was observed under Leica TCS SP5.

3. Cytotoxicity assays

The live RAW 264.7 macrophage cells (1×10^6 cells mL^{-1}) were dispersed within replicate 96-well microtiter plates to a total volume of 200 μL $well^{-1}$. Plates were maintained at 37 °C in a 5% CO_2 / 95% air incubator for 24 h. Then the live RAW 264.7 macrophage cells were incubated for 12 h upon different probe concentrations of 5, 10, 20, and 30 μM. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg mL^{-1}, HEPES) was then added to each well. After 4 h, the remaining MTT solution was removed, and 150 μL of DMSO was added to each well to dissolve the formazan crystals. Absorbance was measured at 490 nm in a Triturus microplate reader.

4. Preparation of reactive oxygen species and reactive nitrogen species

Hydrogen peroxide (H_2O_2), sodium hypochlorite (NaOCl), and *tert*-butylhydroperoxide (TBHP) were diluted from the commercially available solution to 0.1 M in ultrapure water. Hydroxyl radical ($^{\cdot}OH$) and *tert*-butoxy radical ($^{\cdot}O^tBu$) were generated by Fenton reactions. Superoxide anion (O_2^-) was prepared from KO_2 in DMSO. Singlet oxygen ($^1O_2$) was generated from HOCl and H_2O_2. Nitric oxide (NO) was generated from potassium nitroprusside dihydrate. Peroxynitrite was prepared according to the reported method (*J. Am. Chem. Soc.*, 1974, 96, 1248). The concentration of H_2O_2 was determined from the absorbance at 240 nm (ε = 43.6 M^{-1} cm^{-1}). The concentration of ^-OCl was determined from the absorbance at 292 nm (ε = 350 M^{-1} cm^{-1}). The concentration of $ONOO^-$ was determined from the absorbance at 302 nm (1670 M^{-1} cm^{-1}).

5. Characterization of probe TCFL

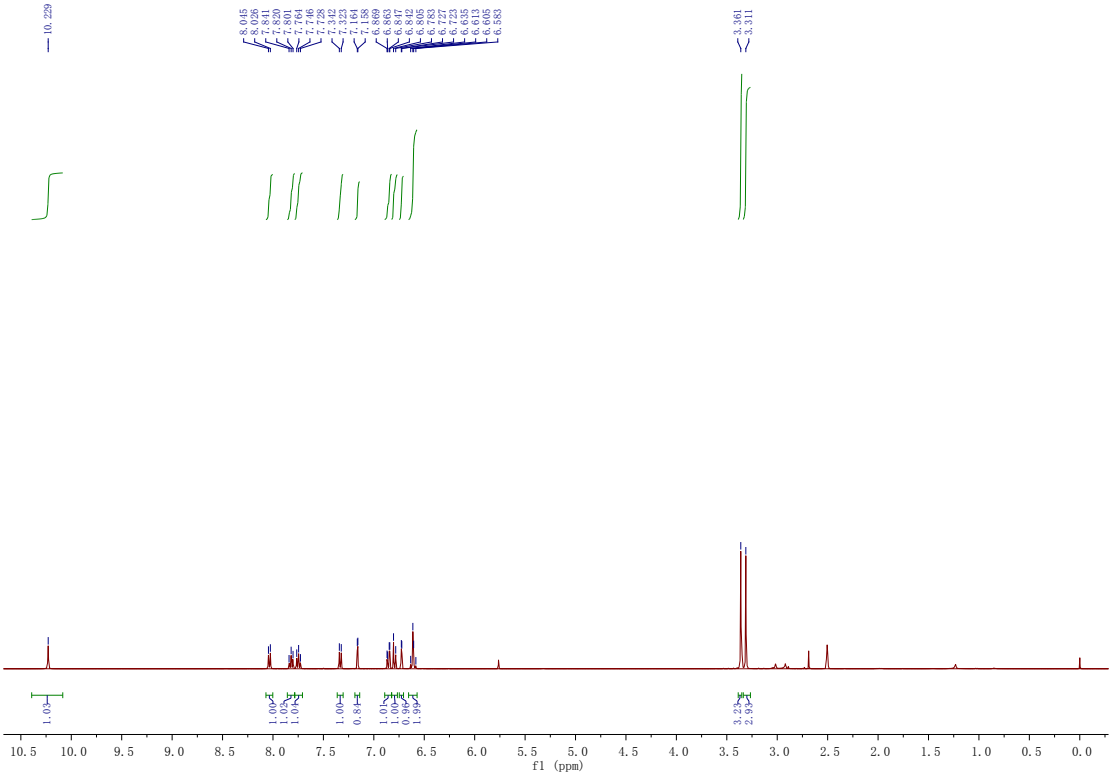

Fig. S1 ^{1}H-NMR of probe **TCFL**

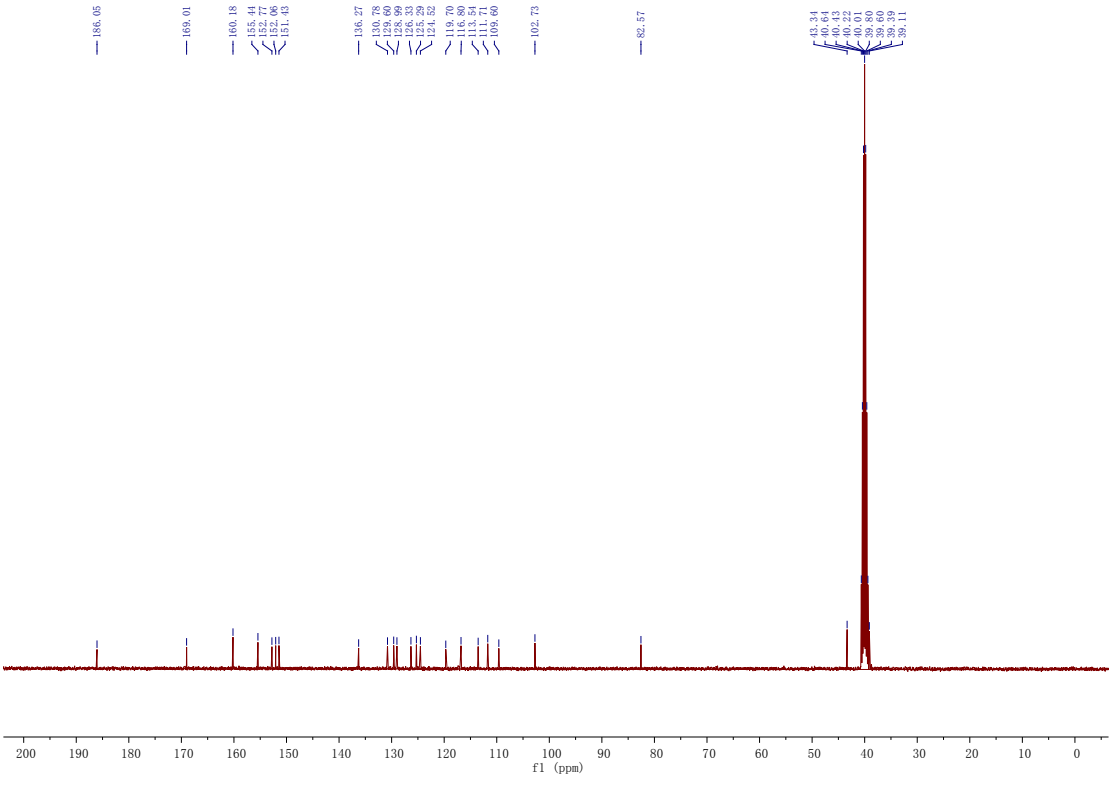

Fig. S2 ^{13}C-NMR of probe **TCFL**

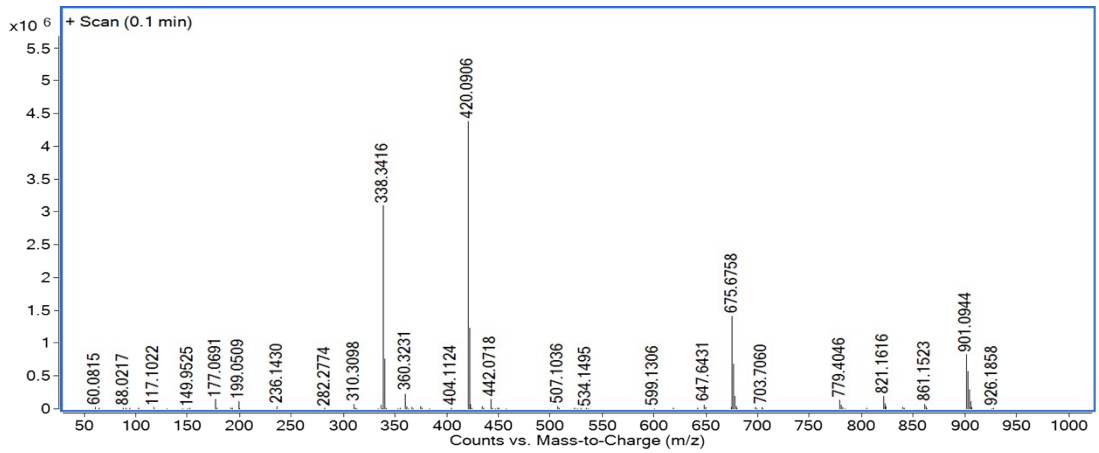

Fig. S3 HRMS of probe **TCFL**

6. HRMS of the reaction products of probe TCFL and HOCl

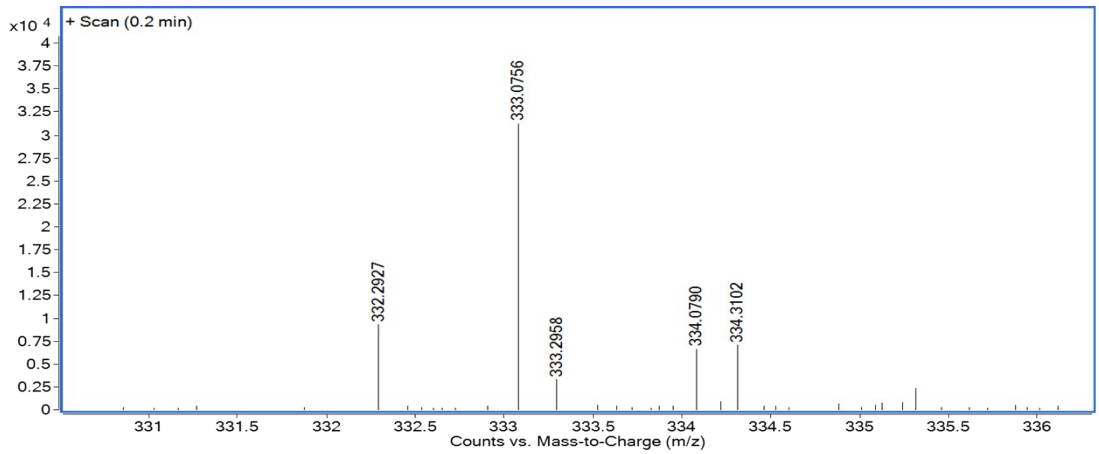

Fig. S4 HRMS of the reaction products of probe **TCFL** and HOCl