**Supporting Information**

**for**

# A ratiometric fluorescent probe based on AIEgen for detecting HClO in living cells†

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## 1. Materials and chemicals.

All of the solvents used were of analytical grade. The water used in the experiments was the double-distilled water which was further treated by ion exchange columns and then by a Milli-Q water purification system. All the chemicals were purchased from J&K Chemicals or Sigma-Aldrich and were used as received without further purification. Stock solutions of AIEgen (TPE-RNS) were prepared and stored as previously reported. Photoluminescence (PL) spectra were recorded on a Perkin-Elmer LS55 spectrofluorometer. Solid state quantum efficiency was measured using a Hamamatsu C11347 Quantaurus-QY integrating sphere at an excitation wavelength of 530 nm. High-resolution mass spectra (HRMS) were obtained on a GCT Premier CAB 048 mass spectrometer operated in MALDI-TOF mode. Fluorescent images were collected on an Olympus BX41 fluorescence microscope. Laser confocal scanning microscope images were collected on Zeiss laser scanning confocal microscope (LSM7 DUO) and analyzed using ZEN 2009 software (Carl Zeiss).

## 2. Determination of detection limit

The detection limit was calculated based on the fluorescence titration curve of TPE-RNS in the presence of NaClO (0-1.2μM). The fluorescence intensity of TPE-RNS 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 = 3σ/slope

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

## 3. Cell culture

HeLa cell line, MCF-7 cell line, COS-7cell line and RAW 264.7 macrophage cell line were provided by American Type Culture Collection. All cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum and antibiotics (100 units/mL penicillin and 100 μg/mL streptomycin), maintaining at 37

˚C in a humidified atmosphere of 95% air and 5% $CO_2$.

**4. Cell viability Test**

Hela cells were seeded in 96-well plates at density of 5000 cells/well and incubated for 24 h. In the 2 day, the medium in each well were replaced by fresh DMEM containing different concentrations of TPE-RNS (0, 5, 10, 20, 50, 75, 100μM, respectively). After 24 hours treatment, into each well, 10 μL MTT solution (5 mg/mL in phosphate buffer solution) was added. After 4 hours incubation at 37 °C, 100 uL SDS-HCl solution (10% SDS and 0.01 M HCl) was added to each well. After 6 hours incubation at 37 °C, the absorbance of each wells at 570 nm was recorded by the plate reader (Perkin-Elmer Victor3TM).

**5. Confocal fluorescence imaging**

5.1 For imaging exogenously generated $ClO^-$ in living cells, HeLa cells was seeded in confocal dish (35 mm) in culture medium with a density of $1\times10^5$ cells per dish. After incubated for 24 h, the cells were treated with TPE-RNS (5 μM) at 37℃ for 30 min and then incubated with NaClO (10 μM) for another 30 min as experimental group 1. TPE-RNS loaded Hela cells were pretreated with NAC (10μM) for 30 min, and then incubated with 1 NaClO (10 μM) for 30 min as experimental group 2. Furthermore, the cells treated with TPE-RNS (5 μM) alone at 37 ºC for 30 min were used as a control. Before imaging, the culture medium was removed and washed three times with PBS (pH 7.4), followed by fluorescence imaging with excitation at 405 nm and emission ranges:420-530nm and 570-680 nm.

5.2 For imaging endogenously generated $ClO^-$ in living cells, Raw 264.7 macrophage cells were pretreated with LPS ($1\mu g\cdot mL^{-1}$) for 12 h and then with PMA ($1\mu g\cdot mL^{-1}$) and TPE-RNS (5 μM) for 30 min were added for another 2 h incubation. The cells treated with TPE-RNS without LPS and PMA as control. Before imaging, the culture medium was removed and washed three times with PBS (pH 7.4), followed by fluorescence imaging with excitation at 405 nm and emission ranges:

420-530nm and 570-680 nm.

5.3 For imaging endogenously generated $ClO^-$ in living cells infected by Bacteria, Raw 264.7 macrophage cells were infected by *E. coli* with a concentration of about 5×106 CFU/mL, followed by culturing for7 h at 37 °C. Then, the cells were washed three times with PBS (pH7.4), and incubated with TPE-RNS (5μM) for 30 min at 37 °C. The cells which not infected by *E. coil* as control. Before imaging, the culture medium was removed and washed three times with PBS (pH 7.4), followed by fluorescence imaging with excitation at 405 nm and emission ranges: 420-530nm and 570-680 nm.

**6. Fluorescence imaging**

Fluorescent inverted microscope was used to investigate the detection ability of TPE-RNS in the same condition as confocal fluorescence imaging. For imaging co-culture cells, Hela cells or MCF-7 cells co-cultured with COS-7 cells for 24 h at 37 °C. Then, the cells were washed three times with PBS (pH 7.4), and incubated with TPE-RNS (10 μM) for 30 min at 37 °C, followed by fluorescence imaging with excitation ranges: 330-385nm and 510-550 nm.

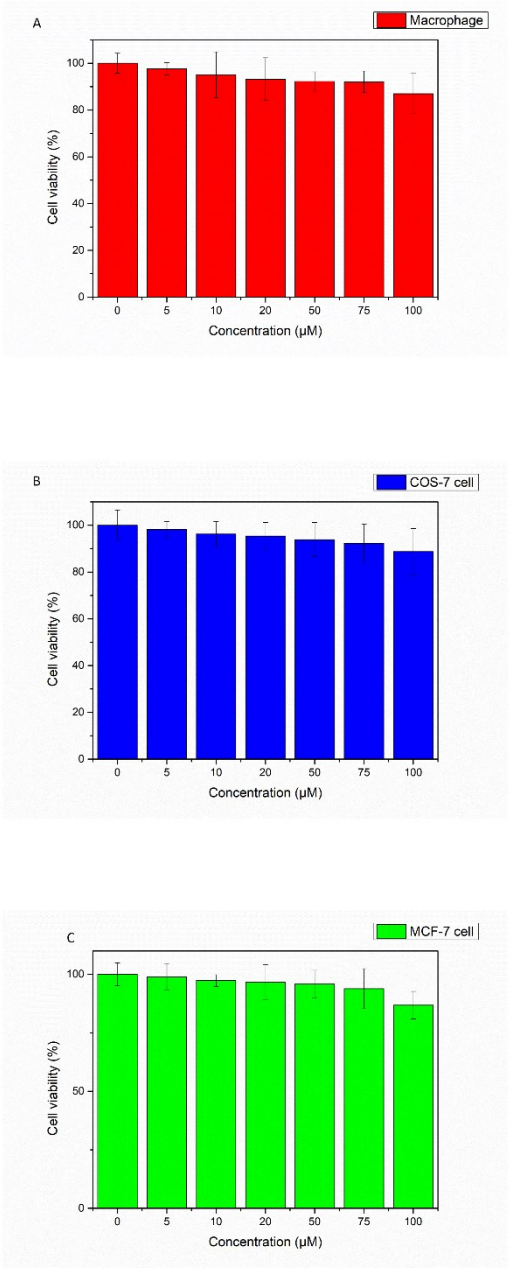


**Figure S1**. Cytotoxicity experiments of TPE-RNS were evaluated on Macrophages (A), COS-7 cells (B) and MCF-7 cells (C) by MTT assay, respectively.

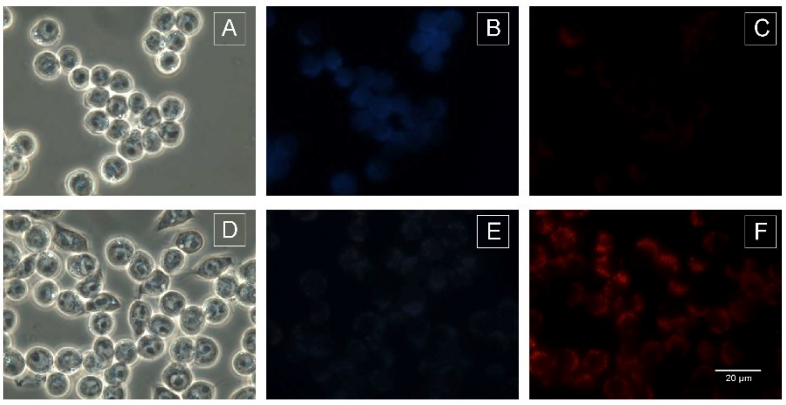


**Figure S2**. Images of endogenously produced HOCl in living RAW 264.7 macrophages treated with probe TPE-RNS. (A-C) Cells were only incubated with TPE-RNS (5 μM) for 30 min (control); (D-F) Cells were first incubated with LPS (1 μg·mL$^{-1}$) for 12h and then incubated with PMA (1 μg·mL$^{-1}$) and TPE-RNS (5 μM) for 30 min.

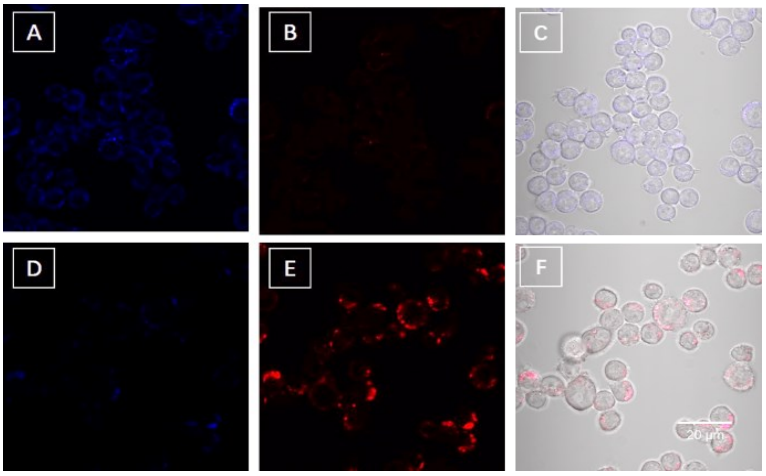


**Figure S3.** Confocal fluorescence imaging of RAW264.7 cells during E. coli infection. (A-C) Images of RAW264.7 Cells were only incubated with TPE-RNS (5 mM) for 30 min (control); (D-F) Images of RAW264.7 Cells were pre-treated with E. coli at a concentration of 5 × 106 CFU mL-1 for 7 h, and then incubated with TPE-RNS (5 mM) for 30 min. Excitation wavelength = 405 nm; Emission ranges: (A, D) and G) 420−530 nm and (B, E and H) 570−680 nm; (C, F, I) Bright.

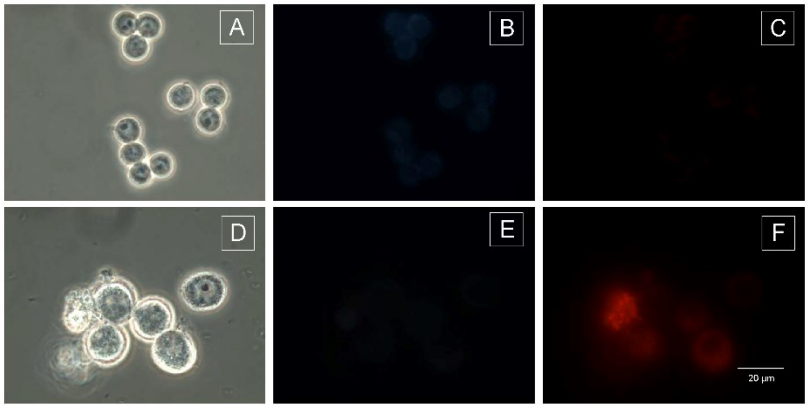


**Figure S4.** Images of endogenously produced HOCl in living RAW 264.7 macrophages during *E. coli* infection treated with probe TPE-RNS. (A-C) Cells were only incubated with TPE-RNS (5 mM) for 30 min (control); (D-F) Cells were pre-treated with E. coli at a concentration of 5 × 106 CFU.

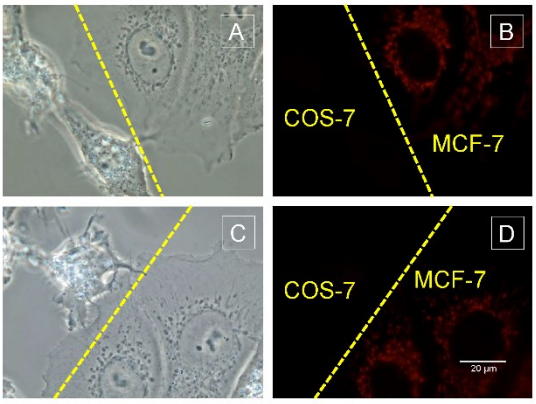


**Figure S5.** Normal and cancer cells co-cultured and stained with TPE-RNS (10 μM) for 30 min. (A and C) Bright-field of co-cultured cancer (MCF-7) and normal cells (COS-7); (B and D) fluorescence imaging of co-cultured cancer (MCF-7) and normal cells (COS-7). Excitation ranges: (B and E) 510-550 nm and (C and F) 330-385 nm.