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Electronic Supplementary Information

Ratiometric detection and imaging of endogenous hypochlorite in live cells and in vivo achieved by using an aggregation induced emission (AIE)-based nanoprobe

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Experimental section

Reagents and Materials: 4-Bromobenzophenone and 4,4'-dimethoxybenzophenone were purchased from Sigma Aldrich and used as received. 4-Formylphenylboronic acid, bis(benzonitrile)palladiuM (II) dichloride ($Pd(PhCN)_2Cl_2$), malononitrile (99%) and titanium(Ⅳ) chloride (99%, $TiCl_4$) were purchased from J&K Chemical Ltd. N,N-Dimethyl-formamide (DMF) was dried with CaH_2 and vacuum distilled. 1,2-Dichloroehane and tetrahydrofuran were analytically pure solvents and distilled before use. 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. Tert-butylhydroperoxide (t-BuOOH, 70%), KO_2, $NaNO_2$, $NaNO_3$, $NaHCO_3$, Na_2SO_4, chloride salts of metal ions (Cu^{2+}, Fe^{2+}, Fe^{3+}, Mg^{2+}, Zn^{2+}, Ca^{2+}), sodium hypochlorite (NaClO, 14.5% available chlorine), CTAB and hydrogen peroxide solution (30%) were purchased from Aladdin. Triton X-100 was purchased from Alfa Aesar. Fe^{II}(EDTA) was obtained from Jinan Great Chemical Co. Ltd. Lipopolysaccharide (LPS) and phorbol myristate acetate (PMA) were purchased from Sigma Aldrich. RAW254.7 (murine macrophage cells) was purchased from KeyGen Biology Co. Ltd.

Synthesis of 1: To the round-bottom flask were added zinc dust (25.75 mmol, 1.68 g), 4-bromobenzophenone (6.18 mmol, 1.613 g) and 4,4'-dimethoxybenzophenone (5.15 mmol, 1.246 g), and then the flask was evacuated under vacuum and flushed with dry nitrogen three times. After addition of 70 mL of anhydrous THF, the mixture was cooled to -20 °C and $TiCl_4$ (2.26 mL, 20.6 mmol) was slowly injected. The mixture was stirred for 0.5 h at room temperature, and then refluxed for 24 h. The reaction was quenched by 10% aqueous K_2CO_3 solution and extracted with ethyl acetate three times and the combined organic layer was washed with brine twice. The mixture was dried over anhydrous sodium sulfate. The crude product was purified on a silica-gel column using DCM/petroleum ether (v/v 4:1, R_f = 0.50) as eluent. A white solid of 1 was obtained in 45% yield. ^{1}H NMR ($CDCl_3$, 600 MHz, ppm): 7.22-7.20 (d, 2H), 7.12-7.08 (m, 3H), 7.01-6.98 (m, 2H), 6.95-6.87 (m, 6H), 6.67-6.64 (d, 2H), 6.64-6.61 (d, 2H), 3.77-3.74 (s, 3H), 3.74-3.70 (s, 3H). MS(ESI): m/z 471.5 $[M+H]^+$.

Synthesis of 2: $Pd(PhCN)_2Cl_2$ (1.25 mg) was added into a mixture of compound 1 (118 mg, 0.250 mmol), 4-formylphenylboronic acid (56 mg, 0.375 mmol) and K_2CO_3 (86 mg, 0.625 mmol) in DMF (4 mL) under nitrogen. The reaction mixture was stirred and heated to 80 °C for 48 h. After cooling to room temperature, the solution was poured into the distilled water and extracted with ethyl acetate three times and the combined organic layer was washed with brine twice. The mixture was dried over anhydrous sodium sulfate. The crude product was purified on a silica-gel column using DCM/petroleum ether (v/v 50:1-10:1) as eluent. A yellow solid of 2 was obtained in 80% yield. ^{1}H NMR ($CDCl_3$, 600 MHz, ppm): 10.03 (s, 1H), 7.91 (d, J = 7.8 Hz, 2H), 7.72 (d, J = 7.9 Hz, 2H), 7.40 (d, J = 7.9 Hz, 2H), 7.14-7.09 (m, 5H), 7.06 (d, J = 7.4 Hz, 2H), 6.98 (d, J = 8.4 Hz, 2H), 6.95 (d, J = 8.4 Hz, 2H), 6.65 (dd, J = 12.6, 8.5 Hz, 4H), 3.74 (s, 6H). MS(ESI): m/z 529.1 $[M+CH_3OH]^-$.

Synthesis of MTPE-M: Triethylamine (130 μL) was added to a solution of compound 2 (50 mg, 0.1 mmol) and (13 mg, 0.2 mmol) in CH_2Cl_2 (5 mL) and the mixture was stirred at room temperature for 4 h. Then the reaction mixture was diluted with dichloromethane and washed with water and brine (10 mL), and then dried over sodium sulfate. The crude product was purified on a silica-gel column using DCM/petroleum ether (v/v 1:1 R_f = 0.45) as eluent. An orange solid of MTPE-M was obtained in 85% yield. ^{1}H NMR ($CDCl_3$, 600 MHz, ppm): 7.97-7.92 (d, 2H), 7.75 (s, 1H), 7.74-7.70 (d, 2H), 7.43-7.38 (d, 2H), 7.15-7.10 (m, 5H), 7.07-7.03 (m, 2H), 7.00-6.97 (m, 2H), 6.69-6.61 (m, 4H), 3.74 (s, 6H). MS(ESI): m/z 567.4 $[M+Na]^+$.

Preparation of Micellar Nanoprobe: The probe molecules were loaded into surfactant micelles by adopting a simple procedure. The probe molecules were first dissolved in DMSO at ambient temperature to make a DMSO solution with the concentration of 1 mM; and 20 μL of the solution was then added into 1.980 mL of PBS buffer solution containing 1 mM of CTAB or Triton X-100 under agitation at ambient temperature, so as to form the micellar nanoprobe.

Generation of Various ROS and RNS: Various ROS and RNS including ClO^-, $^{\cdot}OH$, H_2O_2, TBHP, TBO ; NO_2^-, NO_3^-, NO, $ONOO^-$ and O_2^- were prepared according to

the reported methods.[1-6] Hypochlorite (ClO^-) was prepared from sodium hypochlorite; the concentration of hypochlorite (ClO^-) was determined by using an extinction coefficient of 350 $M^{-1}cm^{-1}$ (292 nm) at pH 12.[1] The hydroxyl radical (·OH) was generated by Fenton reaction between Fe^{II}(EDTA) and H_2O_2 quantitatively, and Fe^{II}(EDTA) concentrations represented ·OH concentrations.[2] The concentration of the commercially available stock H_2O_2 solution was estimated by optical absorbance at 240 nm (43.6 $M^{-1}cm^{-1}$).[3] T-BuOOH (TBHP) was obtained from Aladdin and was diluted to the required concentration. Tert-butoxy radical (TBO ·) were generated by Fenton reaction of TBHP with Fe^{II}(EDTA) quantitatively.[4] The source of NO_2^- and NO_3^- was from $NaNO_2$ and $NaNO_3$. Nitric oxide (NO) was generated from diethylamine NONOate.[5] Peroxynitrite ($ONOO^-$) was prepared as in the reported method;[6] the concentration of peroxynitrite was estimated by using an extinction coefficient of 1670 $M^{-1}cm^{-1}$ (302 nm). Superoxide (O_2^-) was prepared from KO_2.

Cell viability assay: To examine the toxicity of the probe in living cells, RAW264.7 cells were incubated in DMEM medium supplemented with 10% fetal bovine serum (FBS) and allowed to grow for 24 h at 37 °C with 5% CO_2. After removal of the medium, cells were treated with various concentrations of probe and incubated for an additional 24 h. The cytotoxicity of the probe against RAW264.7 cells was assessed by MTT assay according to ISO 10993-5. For each independent experiment, the assays were performed in eight replicates. And the statistic mean and standard derivation were utilized to estimate the cell viability.

Cell imaging: For imaging endogenously generated ClO^- in Raw 264.7 macrophage cells (endogenous generation of ClO^- was induced by the stimulants, lipopolysaccharide (LPS) and phorbol myristate acetate (PMA)). Raw 264.7 cells were incubated in DMEM medium supplemented with 10% Fetal Bovine Serum (FBS, Invitrogen) at 37 ℃ in a humidified incubator containing 5% CO_2 gas. After one day, Raw 264.7 cells were pretreated without or with LPS for 12 h, then further incubated with PMA for 30 min, and subsequently treated with MTPE-M (10 μM) for 30 min at 37 °C. After that, the culture dishes were washed with PBS some times to remove the

culture medium, then imaged on an Olympus IX71 inverted fluorescence microscope equipped with a DP72 color CCD.

Fluorescence imaging in zebrafish:

In this study, wild-type zebrafishes were provided by Key Laboratory of Zebrafish Modeling and Drug Screening for Human Diseases of Guangdong Higher Education Institutes, Department of Cell Biology, Southern Medical University. All animal experiments were performed in full compliance with international ethics guidelines. The zebrafishes were maintained at 28 °C with optimal breeding conditions. Both the female and male zebrafish were kept in the same tank at 28 °C on a 12 h light and 12 h dark cycle, and then gave light stimulation in the morning to trigger the egg spawning. Then, zebrafish eggs were collected in a sedimentation tank. After fertilization, the embryos were put into a 50 mm Petri dish filled with E3 media (15 mM NaCl, 0.5 mM KCl, 1mM $MgSO_4$, 1 mM $CaCl_2$, 0.15 mM KH_2PO_4, 0.05 mM Na_2HPO_4 and 0.7 mM $NaHCO_3$; pH 7.5) by using a disposable transfer pipette. Afterwards these embryos were kept in an incubator at 28 °C for 5 days with the E3 media being replenished every day.

For the imaging endogenous ClO^-, 5-day-old larvae were transferred into a 96-well microplate by using a disposable transfer pipette, then the larvae were incubated in 100 μL of E3 media containing 2 μg mL^{-1} LPS for 6 h and 24 h (One the contrast, the control group larvae were only incubated in 100 μL of E3 media). Then, the media solution was removed and the fishes were washed for three times with 100 μL E3 media to remove the remaining LPS. The fishes were incubated with E3 media containing 10 μM of the probe for another 60 min at 28 °C before the removal of the media solution. After that, the fishes were washed for three times with 100 μL E3 media to remove the remaining probe for subsequent fluorescence image.

The fluorescence image for zebrafish larvae were observed on an Olympus IX71 inverted fluorescence microscope equipped with a DP72 color CCD. All the photographs were taken under identical exposure condition.

Measurements. ^1H NMR spectra were recorded on a Bruker Avance 600 MHz NMR spectrometer. Mass spectra were obtained through a Bruker Esquire HCT Plus mass

spectrometer. UV-vis spectra were recorded on a Hitachi U-3010 UV-vis spectrophotometer. Fluorescence spectra were recorded on a Hitachi F-4600 fluorescence spectrophotometer. The particle size and distribution was determined through dynamic light scattering (DLS) on a Malvern Nano-ZS90 particle size analyzer at a fixed angle of 90 ºat 25 °C. Transmission electronic microscopy (TEM) experiments were carried out by mounting a drop of the solution onto a carbon-coated copper grid and observation was carried out on a JEM-2010HR transmission electron microscopy (Japan). Fluorescence images were obtained using an Olympus IX 71 fluorescence microscope with a DP72 color CCD.

Reference:

1. J. C. Morris, J. Phys. Chem., 1966, 70, 3798-3805.

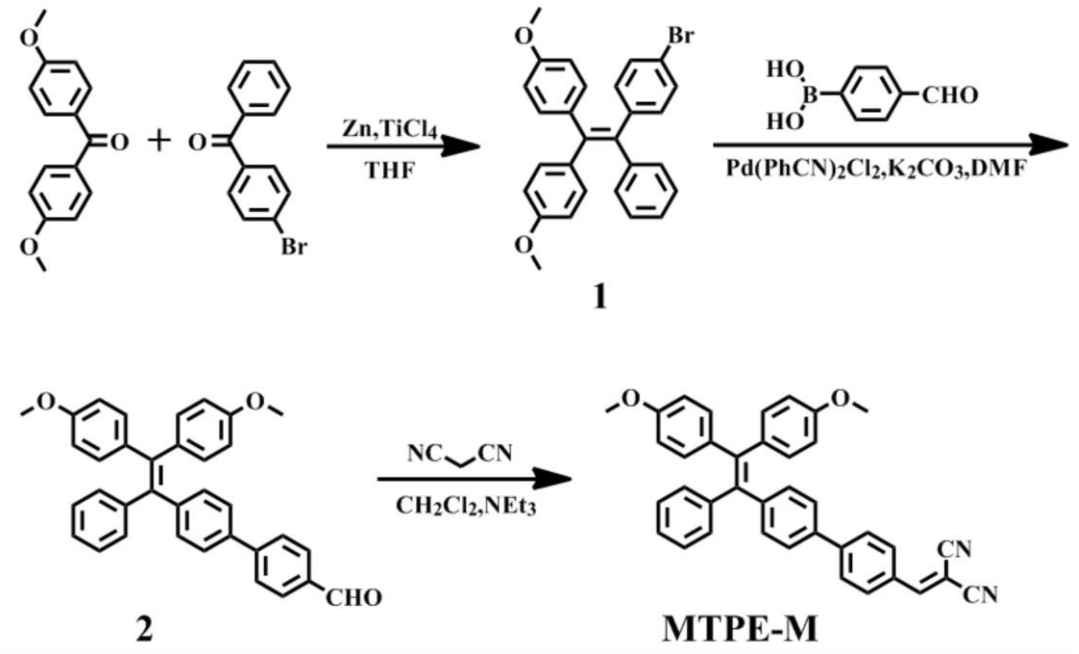
2. 2. K. Setsukinai, Y. Urano, K. Kakinuma, H. J. Majima and T. Nagano, J. Biol. Chem., 2003, 278, 3170-3175.

3. F. Auchère, G. Bertho, I. Artaud, J. P. Girault and C. Capeillère-Blandin, Eur. J. Biochem. 2001, 268, 2889-2895.

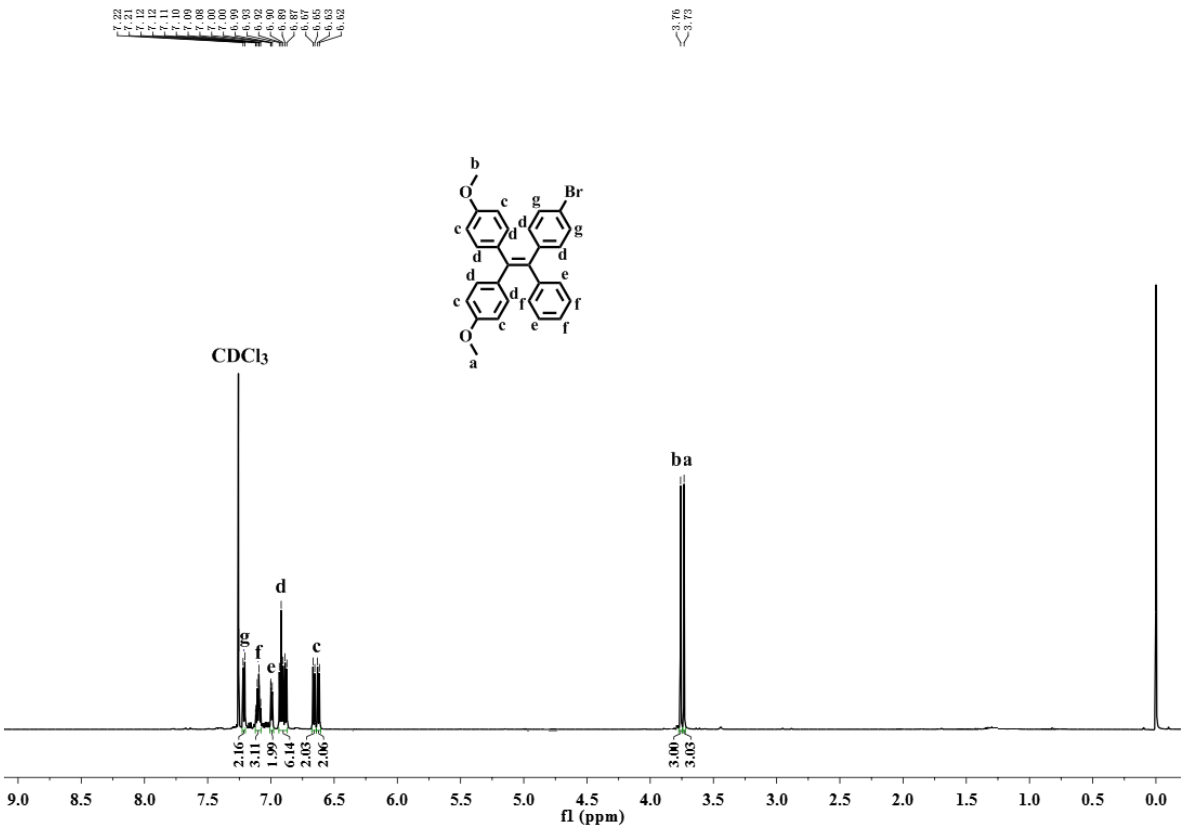
4. G. W. Chen, F. L. Song, J. Y. Wang, Z. G. Yang, S. G. Sun, J. L. Fan, X. X. Qiang, X. Wang, B. R. Dou, X. J. Peng, Chem. Commun., 2012, 48, 2949-2951.

5. P. S. Zhang, J. Li, B. W. Li, J. S. Xu, F. Zeng, J. Lv and S. Z. Wu, Chem. Commun., 2015, 51, 4414-4416.

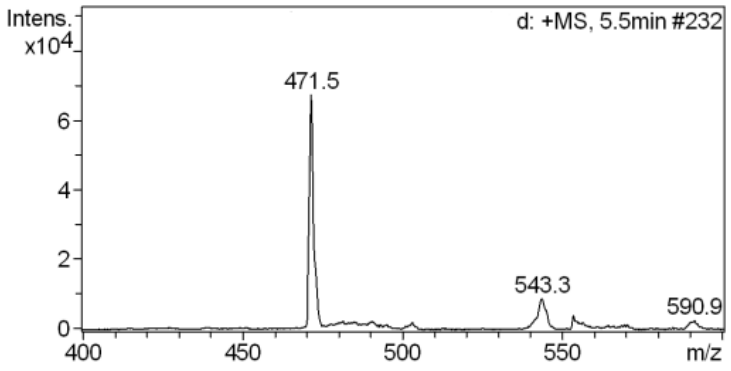
6. R. M. Uppu, W. A. Pryor, Anal. Biochem., 1996, 236, 242-249.



Scheme S1. Synthetic route of the probe (**MTPE-M**).



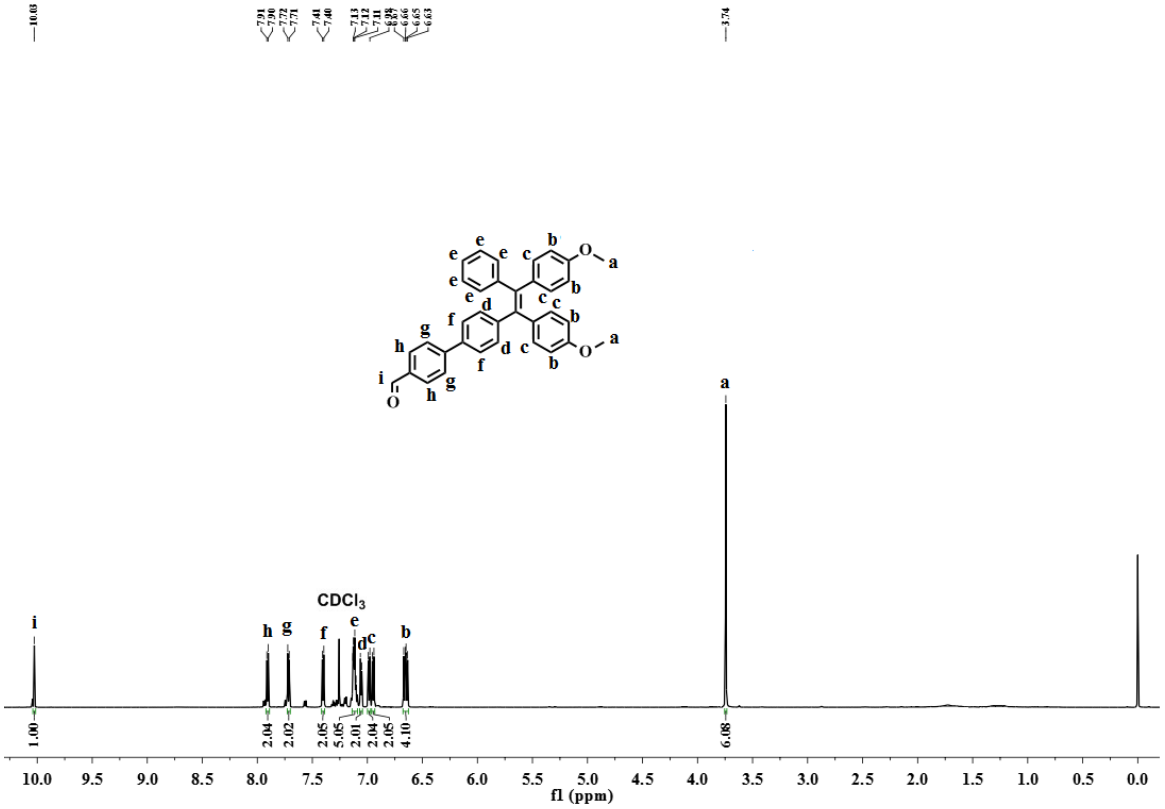
(A)



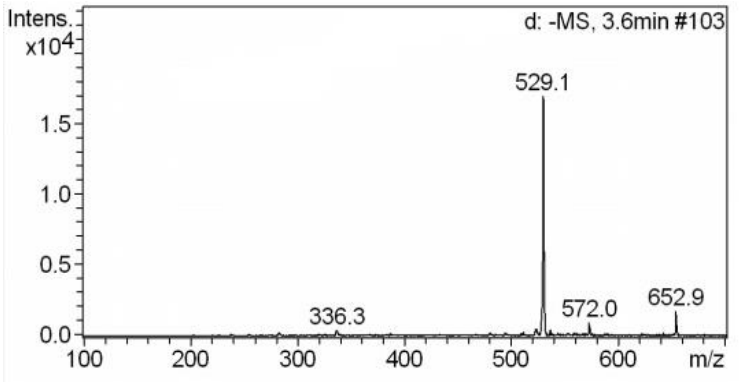
#	m/z	I
1	471.5	67347
2	481.3	2261
3	484.1	2019
4	484.7	2061
5	490.5	2249
6	543.3	8652
7	553.2	3837
8	554.0	2415
9	590.2	2120
10	590.9	2441

(B)

Figure S1. (A) ^{1}H-NMR spectrum ($CDCl_3$) and (B) mass spectrum of **1** (m/z 471.5 $[M+H]^+$).



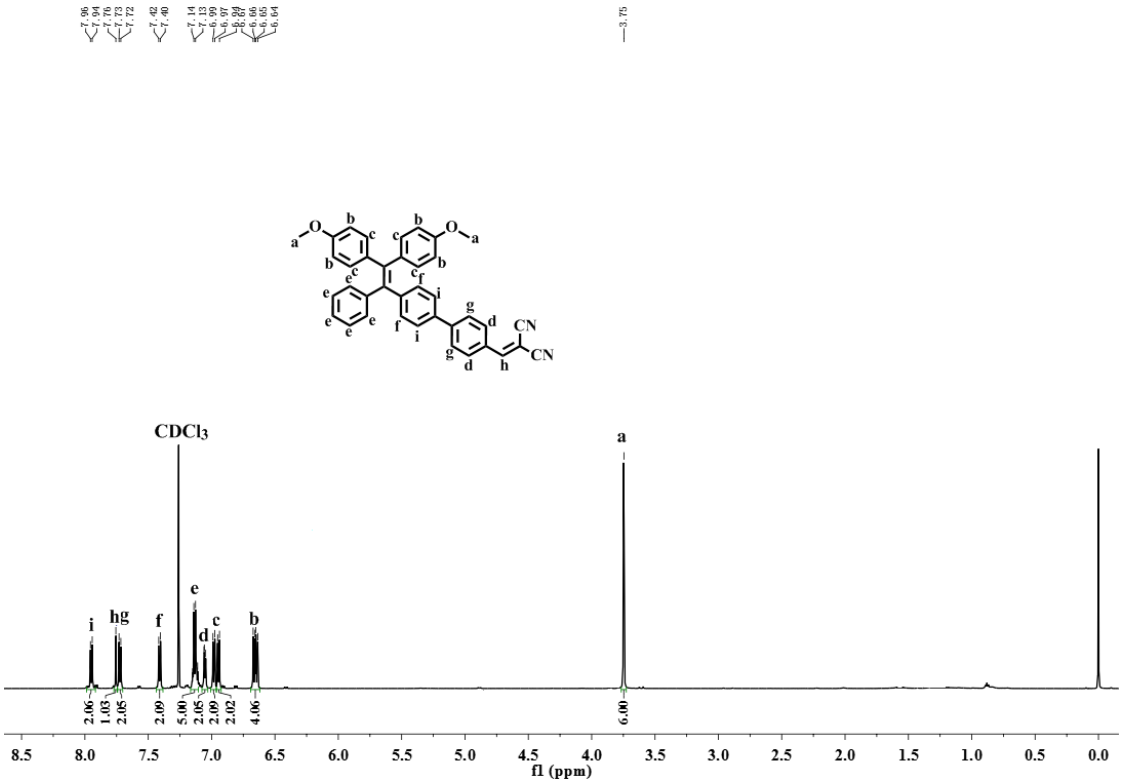
(A)



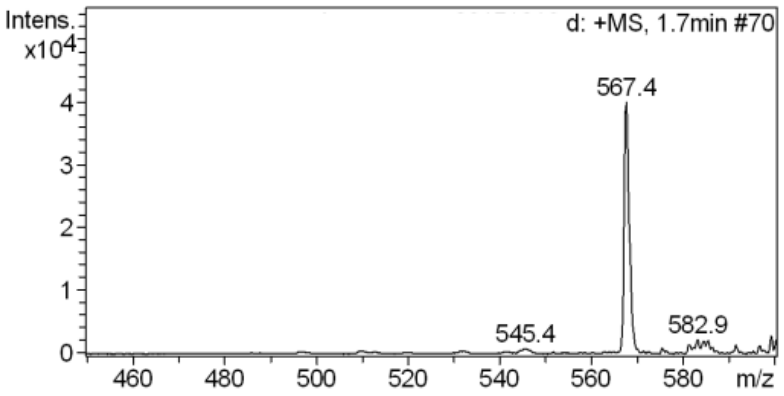
#	m/z	I
1	336.3	355
2	336.8	293
3	521.8	438
4	522.8	424
5	529.1	16917
6	535.7	371
7	572.0	902
8	572.9	476
9	652.9	1707
10	653.8	661

(B)

Figure S2. (A) ^{1}H-NMR spectrum (in $CDCl_3$) and (B) mass spectrum of **2** (m/z 529.1 $[M+CH_3OH]^-$).



(A)



#	m/z	I
1	567.4	39973
2	581.1	1387
3	582.1	1035
4	582.9	2246
5	584.3	2001
6	585.1	2107
7	586.1	996
8	591.2	1392
9	596.3	1226
10	598.9	2849

(B)

Figure S3. (A) ^{1}H-NMR spectrum ($CDCl_3$) and (B) mass spectrum of **MTPE-M** (m/z 567.4 $[M+Na]^+$).

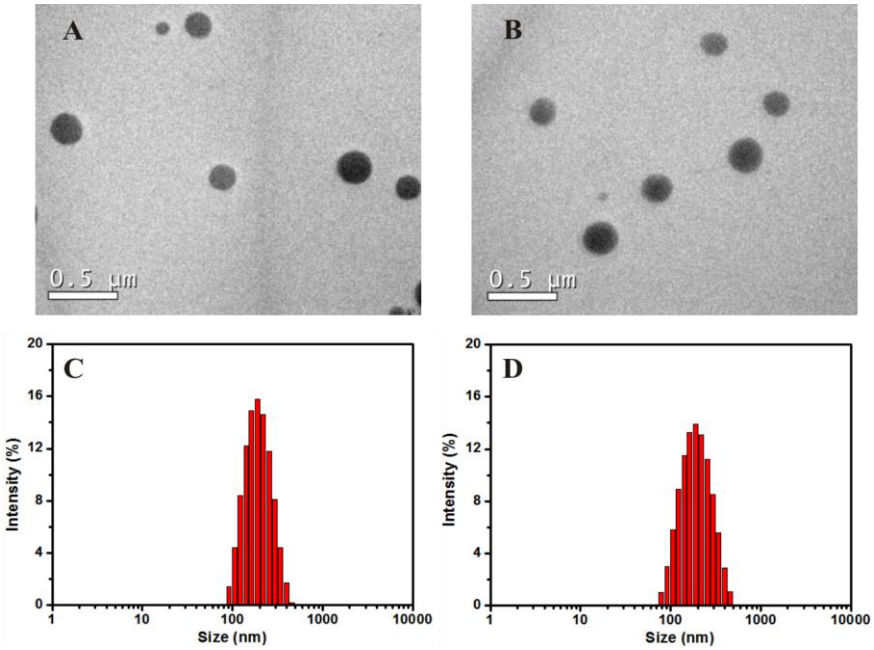


Figure S4. TEM images of the nanoprobe systems before (A) and after (B) addition of 40 μM ClO^-. Scale bar: 0.5μm. Size distribution of the assay system determined by dynamic light scattering (DLS) before (C) and after (D) addition of 40 μM ClO^-.

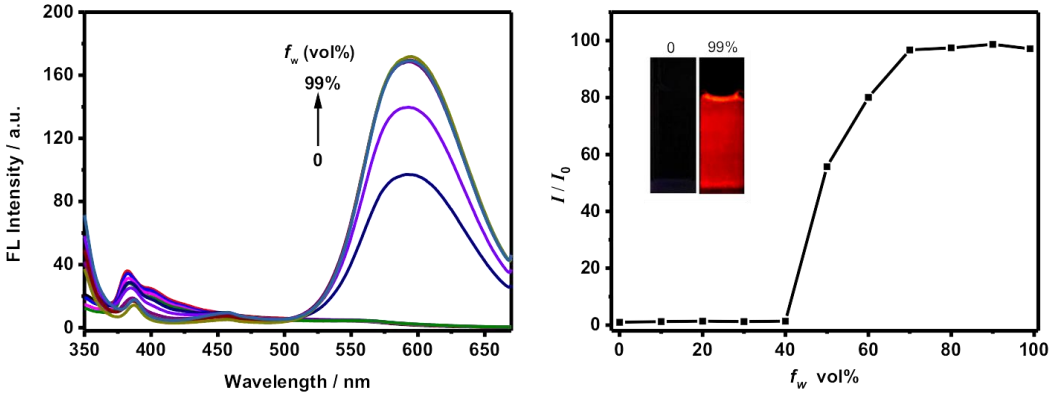


Figure S5. (A) Fluorescence emission spectra of MTPE-M in water/DMSO mixtures with different concentrations of water. (B) Plot of I/I_0 at 595 nm versus f_w, where I_0 is the fluorescence intensity in pure DMSO solution. λ_{ex} = 340 nm

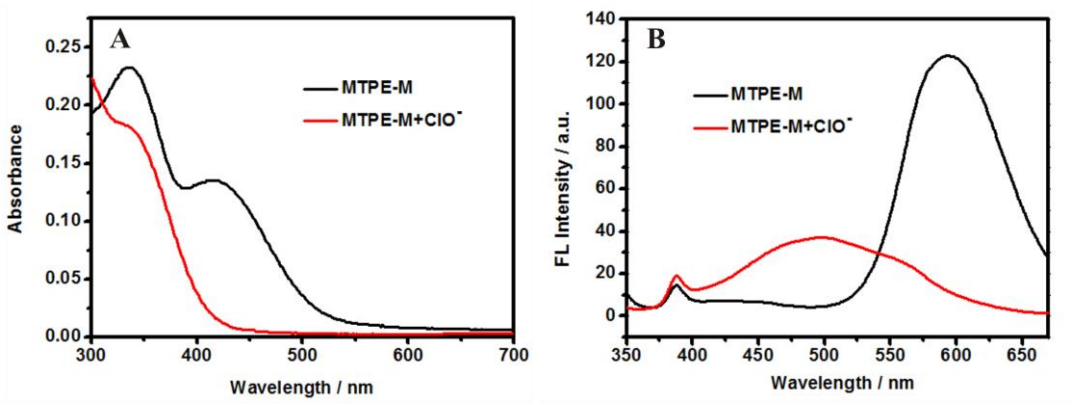


Figure S6. The absorption (A) and emission spectra (B) of MTPE-M (10 μM) before (black line) and after (red line) reaction with ClO^-.

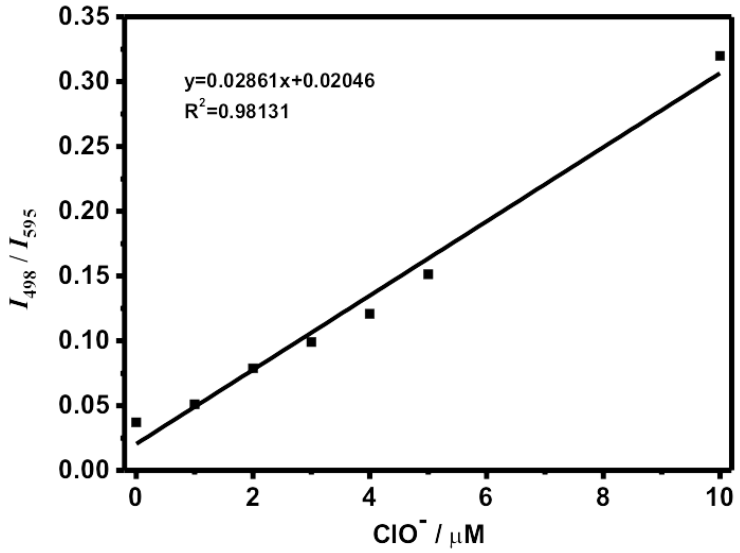


Figure S7. Fluorescence intensity ratio I_{498}/I_{595} versus ClO^- concentrations.

Determination of the detection limit:

First the calibration curve was obtained from the plot of fluorescence intensity ratio (I_{498}/I_{595}) versus ClO^- concentrations. The regression curve equation was then obtained for the lower concentration part.

The detection limit = 3 × S.D. / k

where k is the slope of the curve equation, and S.D. represents the standard deviation for the fluorescence intensity ratio of MTPE-M in the absence of ClO^-.

$I_{498}/I_{595} = 0.02046 + 0.02861 \times [ClO^-]$ (R^2=0.98)

LOD = 3 × 0.0045 / 0.02861 = 0.47 μM

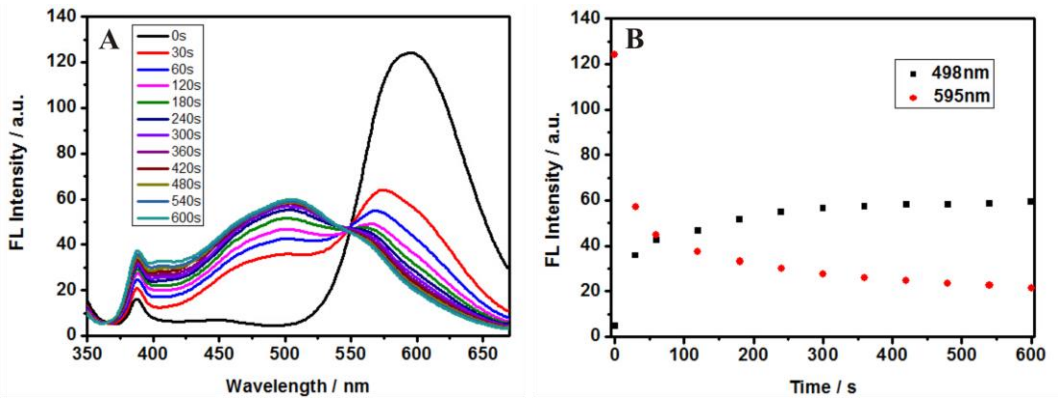
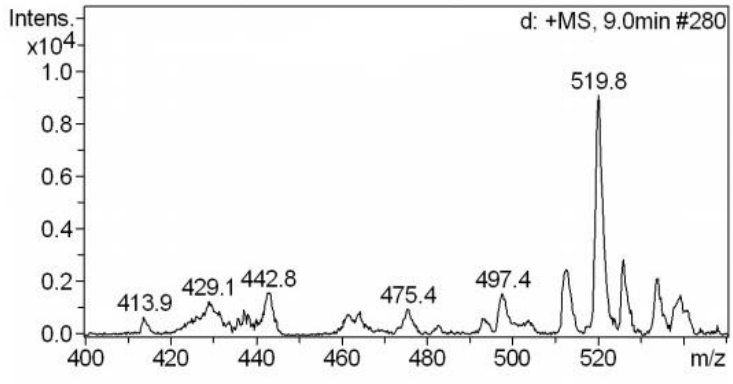


Figure S8. (A) Time-dependent fluorescence spectra for MTPE-M (10 μM) solution upon addition of ClO^- (40 μM). Fluorescence spectra were measured in PBS (pH 7.4, 10 mM, containing 1% DMSO and 1 mM CTAB). λ_{ex}=340 nm; (B) Plot of fluorescence intensity versus time for MTPE-M (10 μM) solution upon addition of ClO^- (40 μM) at 498 and 595 nm.



#	m/z	I
1	429.1	1258
2	442.8	1586
3	475.4	944
4	497.4	1550
5	512.4	2469
6	519.8	9057
7	525.7	2827
8	533.6	2117
9	538.9	1482
10	540.5	937

Figure S9. ESI-MS spectrum of the product of MTPE-M reacted with ClO^-.

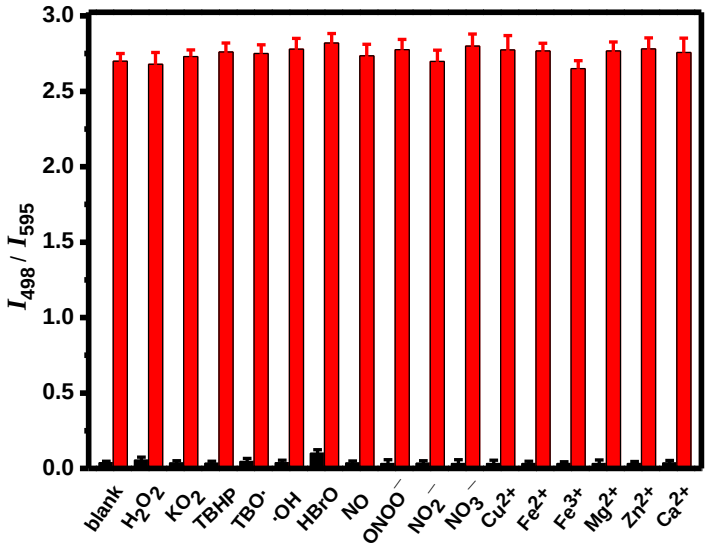


Figure S10. Fluorescence intensity ratio of MTPE-M (10 μM) in the absence(black) and presence(red) of 40 μM ClO^- and simultaneously with the addition of 100 μM other ROS/RNS and ions respectively. Fluorescence intensities were measured in PBS (pH 7.4, 10 mM, containing 1% DMSO and 1 mM CTAB). λ_{ex}=340 nm.

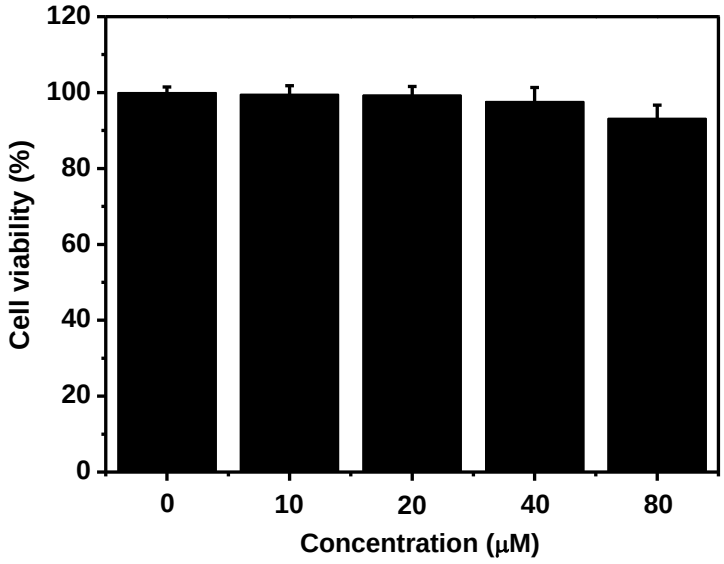


Figure S11. Viability for Raw264.7 macrophage cells in the presence of the nanoprobe (prepared with Triton X-100) with varied concentrations for 24 h.

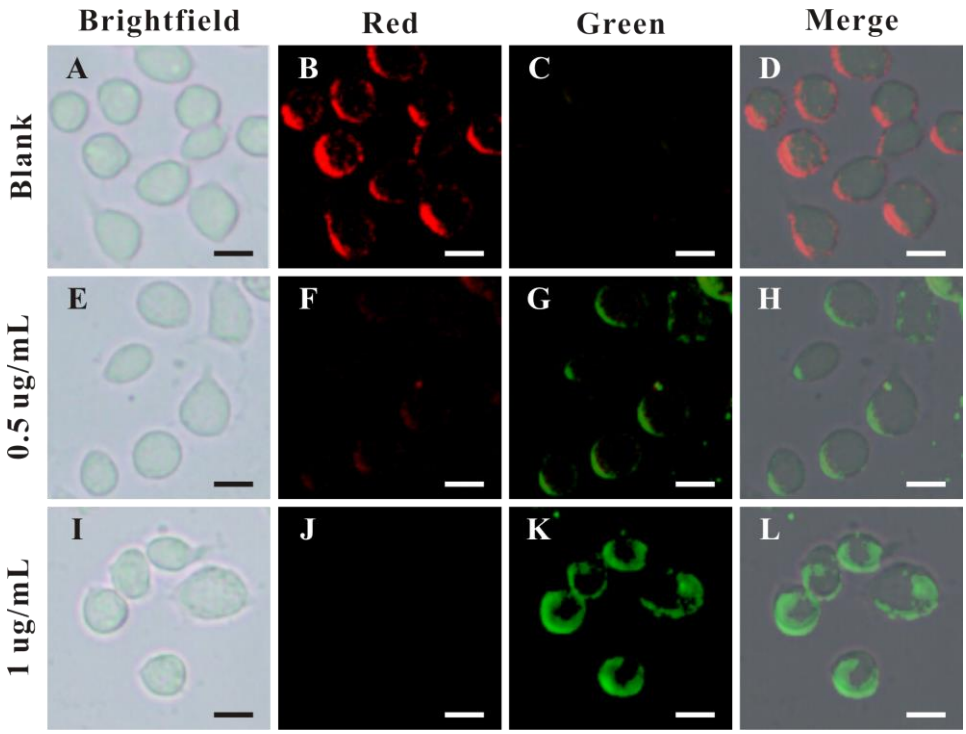


Figure S12. Fluorescence microscopy images for Raw264.7 cells upon stimulation by LPS and PMA with varied concentrations.(A-D): The blank, only incubated with probe; (E-H): Cells treated with LPS (0.5 μg mL^{-1}) and PMA (0.5 μg mL^{-1}) and then incubated with probe; (I-L):Cells treated with LPS (1 μg mL^{-1}) and PMA (1 μg mL^{-1}) and then incubated with probe. Scale bar: 15 μm.