Supplementary material

A 1,8-naphthalimide-based fluorescent probe for selective and sensitive detection of peroxynitrite and its applications in living cell imaging

XiulanLi,[‡a]JingliHou,[‡a]ChaoPeng,[a] Li Chen,[a]WenboLiu[a]and Yangping Liu*[a]

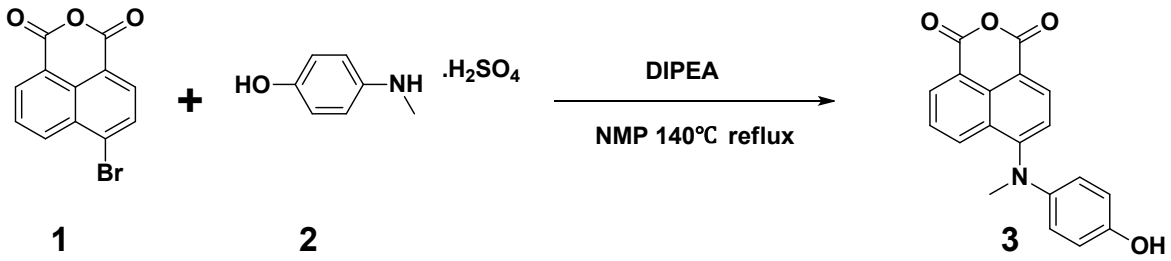
[a]*Tianjin Key Laboratory on Technologies Enabling Development of Clinical Therapeutics and Diagnostics, School of Pharmacy, Tianjin Medical University, Tianjin 300070, P. R. China.* E-mail: liuyangping@tmu.edu.cn
[#]These authors contribute equally to this work.

1. General methods

All reagents and solvents were purchased from commercial sources and were of the analytic grade. All reactions were performed in oven-dried apparatus under argon atmosphere.

Ultraviolet absorption spectra were taken on Hitachi U-3900 spectrophotometer. Fluorescence spectra were taken on Hitachi F-4600 fluorescence spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance-400 FT nuclear magnetic resonance spectrometer. The following abbreviations were used to explain the multiplicities: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; br = broad. HPLC and LC-MS analysis was performed with an Agilent 1260 HPLC system and an Agilent 1200-Bruker HCT LC-MS system, respectively. High resolution mass spectra were obtained on a Varian QFT-ESI mass spectrometer. Confocal images were scanned by Laser Confocal Scanning Biological Microscope OlympusFV1200.

2. Synthesis of Probes

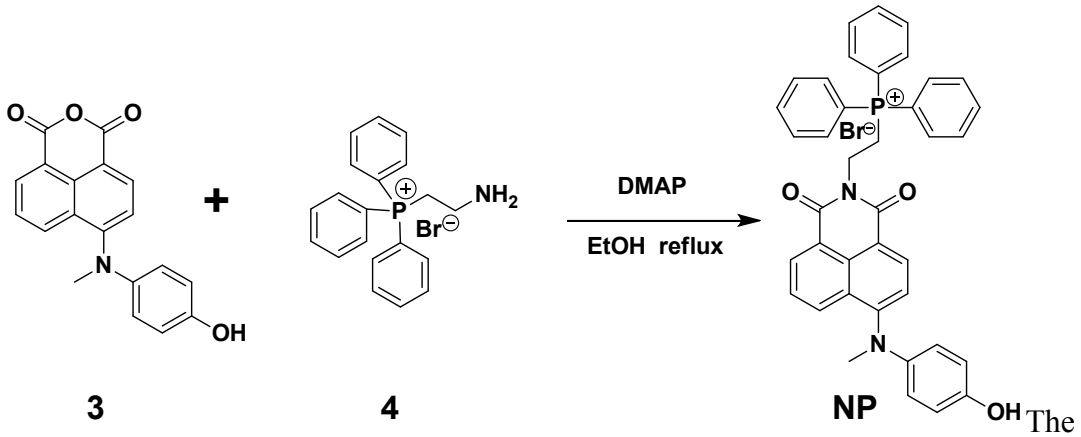


1 2 3

The mixture of 4-Bromo-1,8-naphthalimide (0.1 g, 1 mmol, 1.0 eq.) and 4-methylaminophenol sulfate (1.244 g, 5 mmol, 5.0 eq.) in NMP (5 mL) was stirred for 10min at room temperature, then DIPEA (0.6 mL)was added. The resulting solution was stirred and refluxed under argon for 5hat 140 °C. After the reaction was completed, the solvent was removed under vacuum. The crude product was dissolved in acetone, filtered insoluble residues and the filtrate was concentrated in vacuum [1]. The concentrated solution was crystallized in a low temperature and furnished the desired product as reddish brown powder. Yield: 0.121 g, 52%.

^{1}H-NMR (400 MHz, DMSO) δ 9.44 (s, 1H), 8.46 (dd, *J* = 8.3, 1.6 Hz, 1H), 8.36 (d, *J*

= 7.3 Hz, 1H), 8.03 (d, *J* = 1.7 Hz, 1H), 7.56 – 7.50 (m, 1H), 7.46 (dd, *J* = 8.4, 1.3 Hz, 1H), 6.94 – 6.87 (m, 1H), 6.73 – 6.69 (m, 1H), 3.47 (s, 1H). ^{13}C-NMR (100 MHz, DMSO) δ 161.2, 160.2, 154.4, 153.5, 142.6, 134.5, 132.8, 132.2, 132.0, 125.1, 124.7, 124.5, 119.1, 116.4, 116.1, 110.0, 43.9



The obtained compound 3 (0.103 g, 1 mmol, 1.0 eq.) and DMPA (0.042 g, 1.10 mmol, 1.1 eq.) were added to stirred solution of compound 4 (0.133 g, 1.10 mmol, 1.1 eq.) in ethanol. The mixture was then heated at reflux for 7 h under argon atmosphere, and monitored by TLC. After the reaction was completed, the solvent was evaporated.[2,3] The crude product was then purified by column chromatography with CH_2Cl_2/MeOH (20:1, v/v), to give NP as a brown solid, Yield: 0.101 g, 47%.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.52 (s, 1H), 8.30 (d, *J* = 8.3 Hz, 1H), 8.14 (d, *J* = 7.0 Hz, 1H), 7.97 (d, *J* = 8.4 Hz, 1H), 7.86 – 7.58 (m, 5H), 7.14 (dd, *J* = 15.6, 7.8 Hz, 1H), 7.02 (d, *J* = 8.7 Hz, 1H), 6.76 (d, *J* = 8.7 Hz, 1H), 4.68 – 4.49 (m, 1H), 3.93 (s, br, 1H),3.43 (s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.8, 164.3, 163.5, 155.6, 154.2, 142.6, 135.4, 133.6, 133.5, 130.7, 130.6, 124.7, 124.6, 124.5, 121.4, 118.1, 117.5, 117.2, 114.6, 113.0, 44.3, 34.1, 21.7. HRMS (ESI) calcd for C39H32N2O3P [M-Br]$^+$: 607.2145, found: 607.2150.

3. Reaction of NP with $ONOO^-$ analyzed by HPLC and LC-MS

To a solution of NP in DMSO (4 μM, 175 μL) was added PBS (100 mM, 455 μL) and an alkaline solution of peroxynitrite (100 μM, 70 μL). After stirred for 10 min, the reaction mixture was analyzed by HPLC and LC-MS. The samples were eluted from a XBridge C18 column (4.6 X 250 mm, 5 μM) with CH_3COONH_4 (pH = 6.8, 20 mM)

and CH_3CN as eluent (20% - 40% CH_3CN in 5 min, then 40% - 60% CH_3CN from 5 min to 25 min) at a flow rate of 1 mL/min.

4. Determination of the fluorescence quantum yield.

Fluorescence quantum yields for NP and NP-P were determined by using Rhodamine-B (Φ_F = 0.89 in ethanol) as a fluorescence standard. The quantum yield was calculated using the following equation:

$$\Phi_{F(sample)} = \Phi_{F\ (reference)} (A_{reference}/A_{sample}) (F_{sample}/F_{reference}) (n_{sample}/n_{reference})^2$$

where Φ_F is the fluorescence quantum yield, A is the absorbance at the excitation wavelength, F is the area under the corrected emission curve, and n is the refractive index of the solvents used.

5. Cytotoxicity assays

Raw 264.7 macrophage cells were grown in RPMI 1640 medium supplemented with10 % FBS (Fetal Bovine Serum) and 1% antibiotics at 37 ºC in humidified environment of 5% CO_2. Immediately before the experiment, the cells well placed in a 96-well plate, followed by addition of increasing concentrations of NP (0.625 μM，1.25 μM，2.5 μM，5.0 μM，10.0 μM). The cells were then incubated at 37 ºC in an atmosphere of 5% CO_2 and 95% air for 9 h, followed by MTT assays (n = 6).Untreated assay with RPMI 1640 medium (n = 6) was also conducted under the same conditions.（Fig S8）.

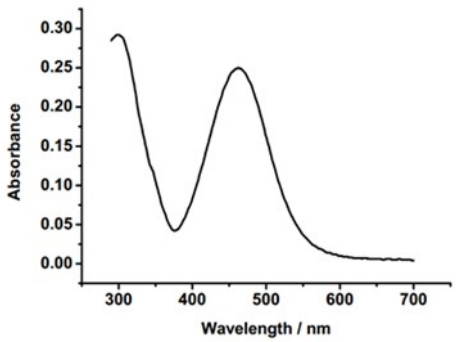
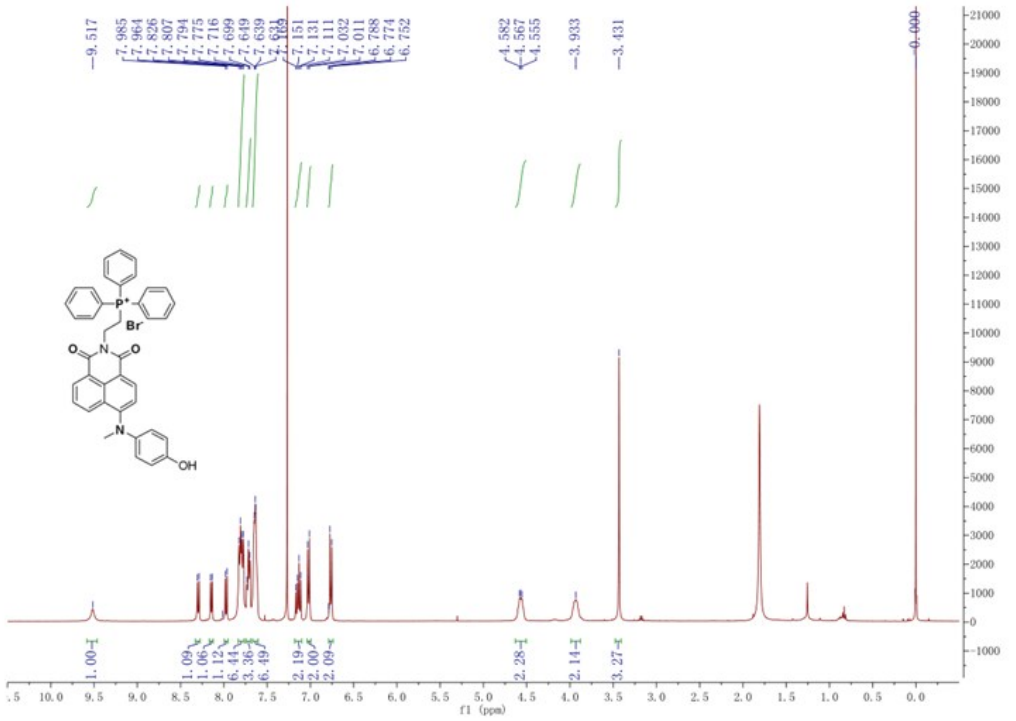


Fig S1.The UV-Absorption of NP (5 μM)



FigS2. ^{1}H-NMRspectrumof NP in $CDCl_3$

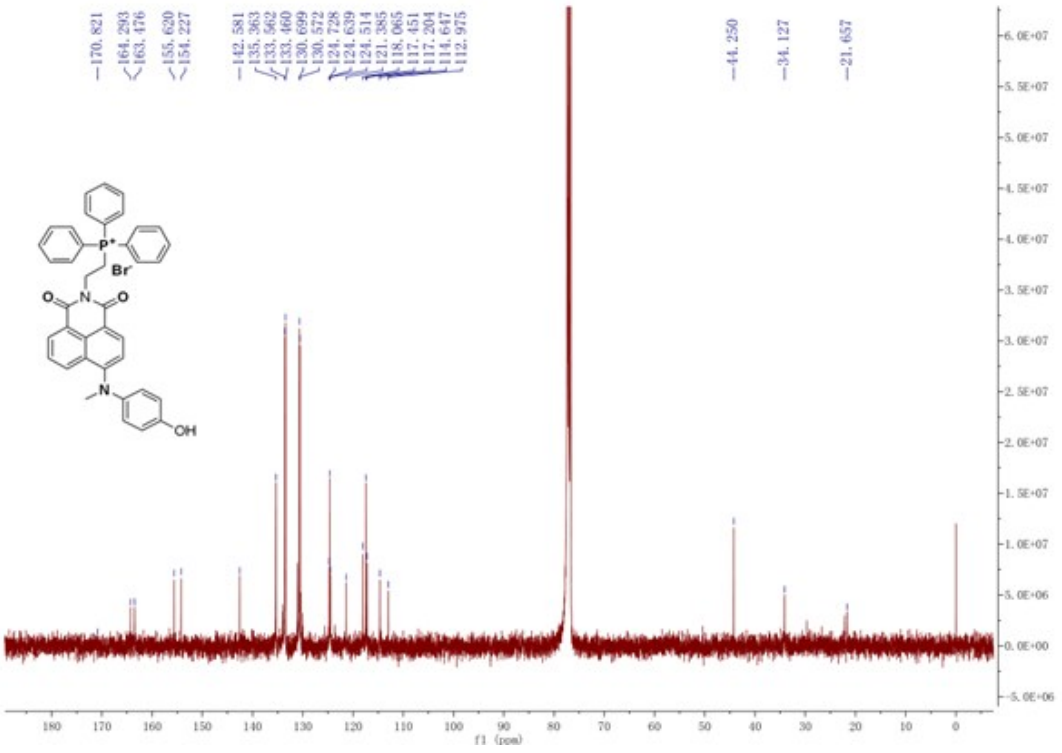


Fig S3. ^{13}C-NMR spectrum of NP in $CDCl_3$

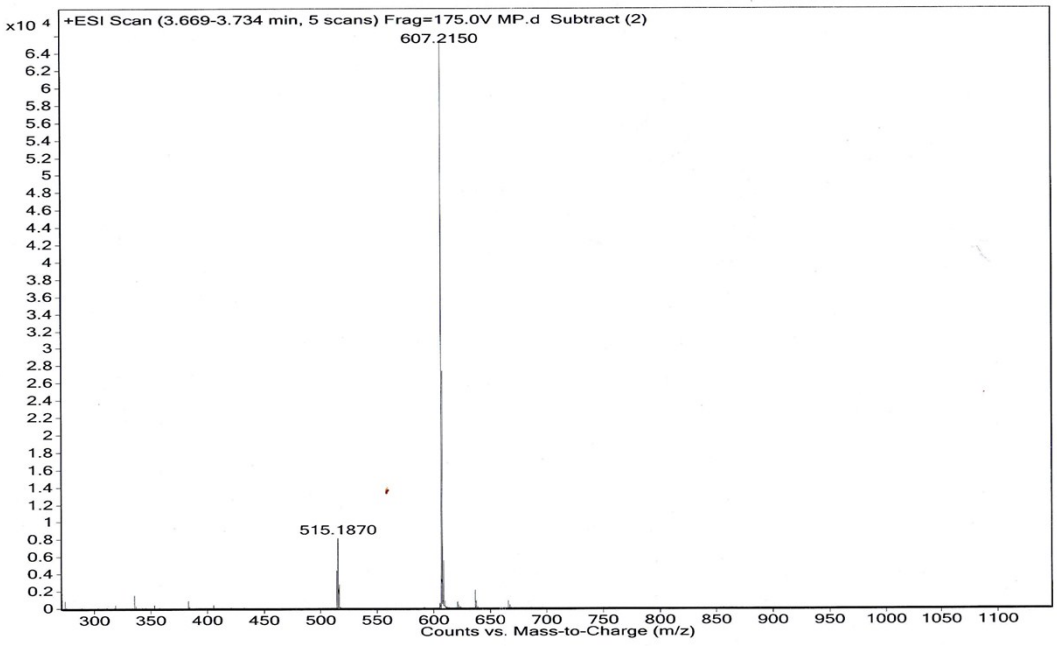


Fig S4. HRMS spectrum of NP in $CDCl_3$

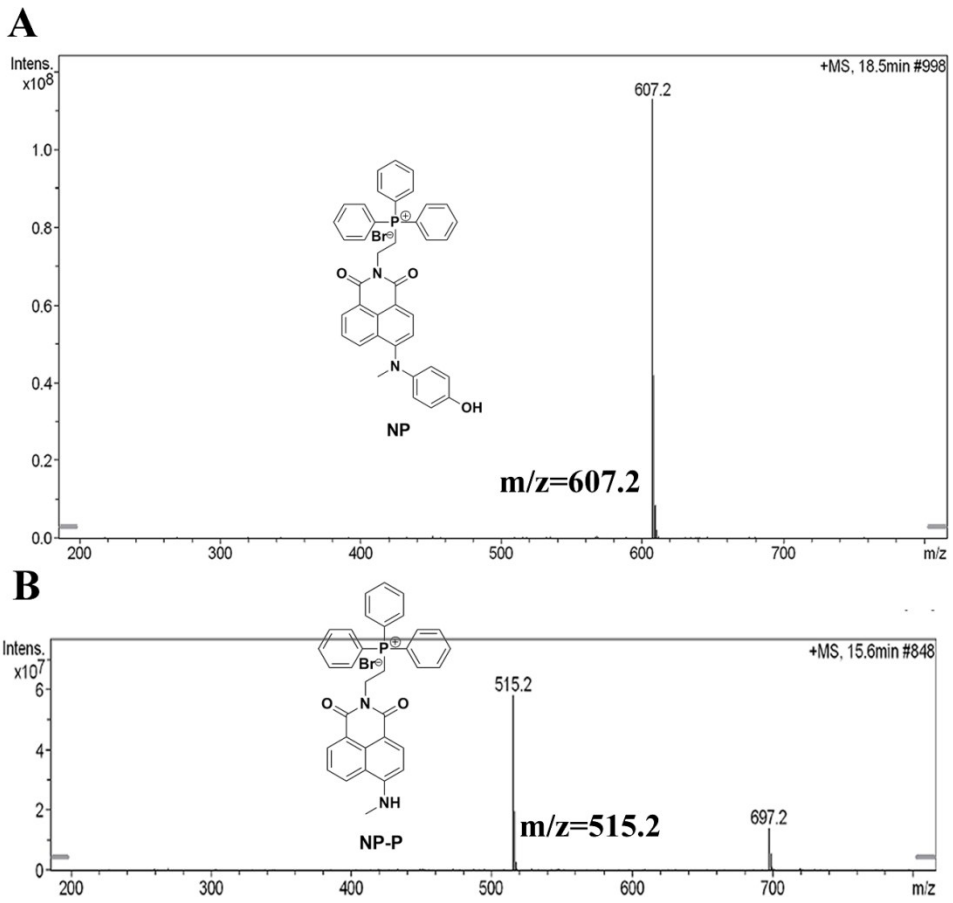


Fig S5. Mass spectra of NP and NP-P

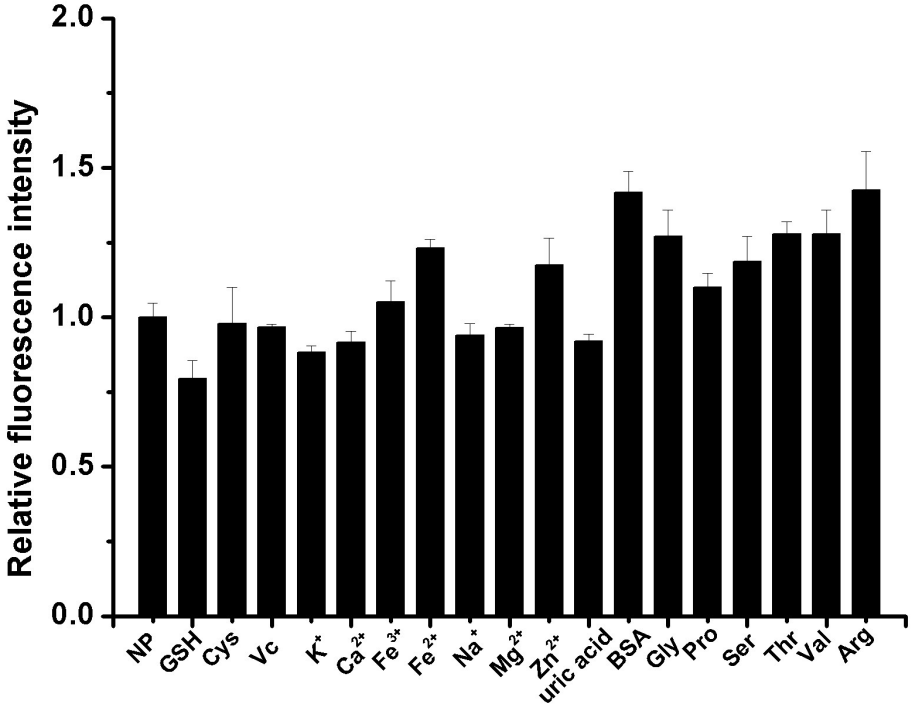


Fig S6: Fluorescence intensity changes of NP (1 μM) towards reducing reagents, metal ion and amino acids.

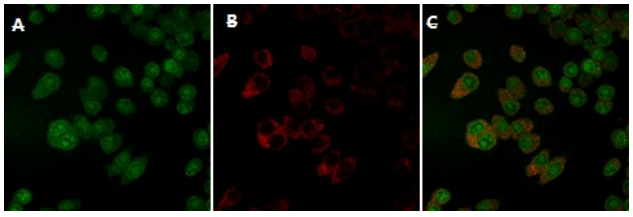


Fig S7 (A-C) Fluorescence images of Raw 264.7 macrophage cells costained by NP (5 μM, 30min), Mito Tracker Red FM (1.0 μM, 30min) and then SIN-1 (50 μM, 30 min): (A) image from brand path of 500-600 nm upon excitation of NP at 488 nm; (B) image from band path of 640-730 nm upon excitation of MitoTracker Red FM at 633 nm. (C) the overlay imaging of A and B.

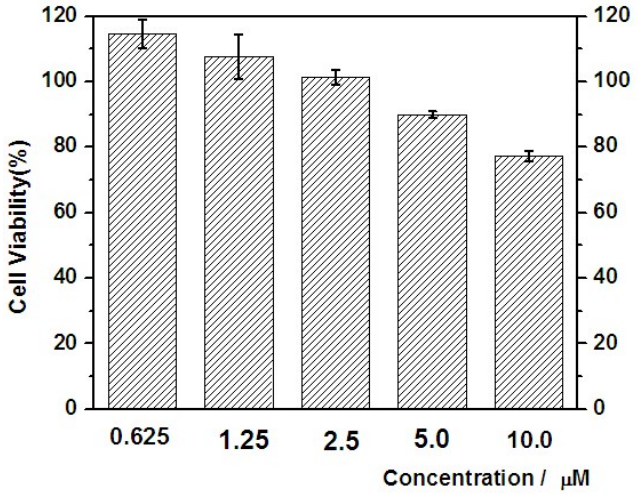


Fig S8. Percentage of viable Raw 264.7 macrophage cells after treatment with indicated concentrations of NP after 9 hours.

Reference

(1) Naumenko, A. In *AACE, WebNet 98 - World Conference of the WWW, Internet and Intranet* 1998, p 682.

(2) Sunduru, N.; Srivastava, K.; Rajakumar, S.; Puri, S. K.; Saxena, J. K.; Chauhan, P. M. S. *Bioorganic & Medicinal Chemistry Letters* 2009, *19*, 2570.

(3) Tian, H.; Liu, P. H.; Zhu, W.; Gao, E.; Wu, D. J.; Cai, S. *Journal of Materials Chemistry* 2000, *10*, 2708.