Electronic Supplementary Material (ESI) for Analyst.

Table of Contents

1. Materials and instruments

Unless otherwise stated, chemical reagents were purchased from commercial vendor and were used as received. Absorption spectra were carried out using a UV-3101PC spectrophotometer. Fluorescence emission spectra were performed using a Horiba FluoroMax-4 spectrophotometer. The slit width was 5.0 nm for both excitation and emission. High resolution mass spectra (HRMS) were carried out using a LC-MS2010A instrument. Fluorescence imaging of $ONOO^-$ in live RAW 264.7 cells and zebrafish were carried out on an Olympus FV1000-IX81 confocal fluorescence microscope.

2. Determination of the detection limit

The detection limit was calculated based on the fluorescence titration. The detection limit (LOD) was calculated as follows:

$$LOD = 3\sigma/k,$$

$$\sigma = \sqrt{\frac{\sum(\bar{x} - x_i)^2}{n - 1}}$$

where σ is the standard deviation of the blank solution, $\bar{x}$ is the mean of the blank measures, x_i is the value of blank measures, n is the number of tested blank measures (n = 8), and k is the slope between the fluorescence intensities versus the concentrations of $ONOO^-$.

3. Cytotoxicity assays

The RAW 264.7 macrophage cells were purchased fromthe Shanghai Institute of Biochemistry and Cell Biology, China Academy of Sciences (Shanghai, China). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with

10% fetal bovine serum and 1% penicillin-streptomycin and incubated under a humidified atmosphere containing 5% CO_2 at 37 °C. The cell viability of RAW 264.7 cells, treated with probe **PDPE-PN**, was assessed by a cell counting kit-8 (CCK-8; Dojindo Molecular Technologies, Tokyo, Japan). Briefly, RAW 264.7 cells, seeded at a density of 1×10^6 cells·mL^{-1} on a 96-well plate, were maintained at 37 °C in a 5% CO_2 / 95% air incubator for 12 h. Then the live RAW 264.7 cells were incubated with various concentrations (0, 5, 10, 20 and 30 μM) of probe **PDPE-PN** suspended in culture medium for 10 h. Subsequently, CCK-8 solution was added into each well for 2 h, and absorbance at 450 nm was measured.

4. Behavioral analysis of zebrafish

Healthy male and female zebrafish (AB stain) were maintained in different tanks with a 14 h light / 10 h dark cycle at 28 ± 1 °C. Then, sexually mature zebrafish were selected to induce spawning in tanks and the zebrafish eggs were obtained by giving light stimulation in the morning. After sterilizing and cleaning, the fertilized eggs were added to zebrafish embryo culture water (5 mM NaCl, 0.17 mM KCl, 0.4 mM $CaCl_2$, 0.16 mM $MgSO_4$) and cultured in illumination incubator in a 14 h light (~1000 lux): 10 h dark (LD) cycle at 28 ± 1 °C.

Behavioral test was conducted to evaluate the safety of probe **PDPE-PN**. The normal zebrafish larvae at 72 (hpf, hours post fertilization) were randomly divided into 2 well plates and exposed to different concentrations of probe **PDPE-PN** (0, 10 μM) dissolved in the bathing medium. Then the zebrafish larvae were cleaned in bathing medium and placed in 48-well plates (one larva per well) at 96 hpf. After a 10 min acclimation period, the locomotor activity of each larva was monitored for 20 min in a silent room using an automated computerized video-tracking system

(Viewpoint, Lyon, France), and the detailed track was recorded with Zebralab software (Viewpoint). The swimming duration, movement distance and speed were analyzed. Additionally, to reduce possible diurnal factors on level of locomotor activity, all behavioral tests were performed at zeitgeber time 8-12 (ZT8-12).

5. Preparation of reactive oxygen species (ROS) and reactive nitrogen species (RNS)

TBHP

tert-butylhydroperoxide (TBHP) were diluted from the commercially available solution to 0.1 M in ultrapure water.

•OH

Hydroxyl radical (•OH) was generated by Fenton reactions. To prepare •OH solution, hydrogen peroxide (H_2O_2, 2 eq) was added to $FeSO_4$ in deionised water.

•O^{t}Bu

tert-butoxy radical (•O^{t}Bu) was generated by Fenton reactions.

O_2^-

Superoxide (O_2^-) was generated from KO_2 in DMSO.

$^1O_2$

Singlet oxygen ($^1O_2$) was generated from HOCl and H_2O_2.

NO

Nitric oxide (NO) was generated from potassium nitroprusside dihydrate.

H_2O_2

The concentration of hydrogen peroxide (H_2O_2) was determined from the absorption at 240 nm (ε = 43.6 $M^{-1} \cdot cm^{-1}$).

NaOCl

The concentration of sodium hypochlorite (NaOCl) was determined from the absorbance at 292 nm (ε = 350 M^{-1} cm^{-1}).

$ONOO^-$

Simultaneously, 0.6 M KNO_2, 0.6 M HC1 and 0.7 M H_2O_2 was added to a 3 M NaOH solution at 0 ºC. The concentration of peroxynitrite was estimated by using the extinction co-efficient of 1670 cm^{-1} • M^{-1} at 302 nm in 0.1 M sodium hydroxide aqueous solutions.

6. Characterization data of probe PDPE-PN

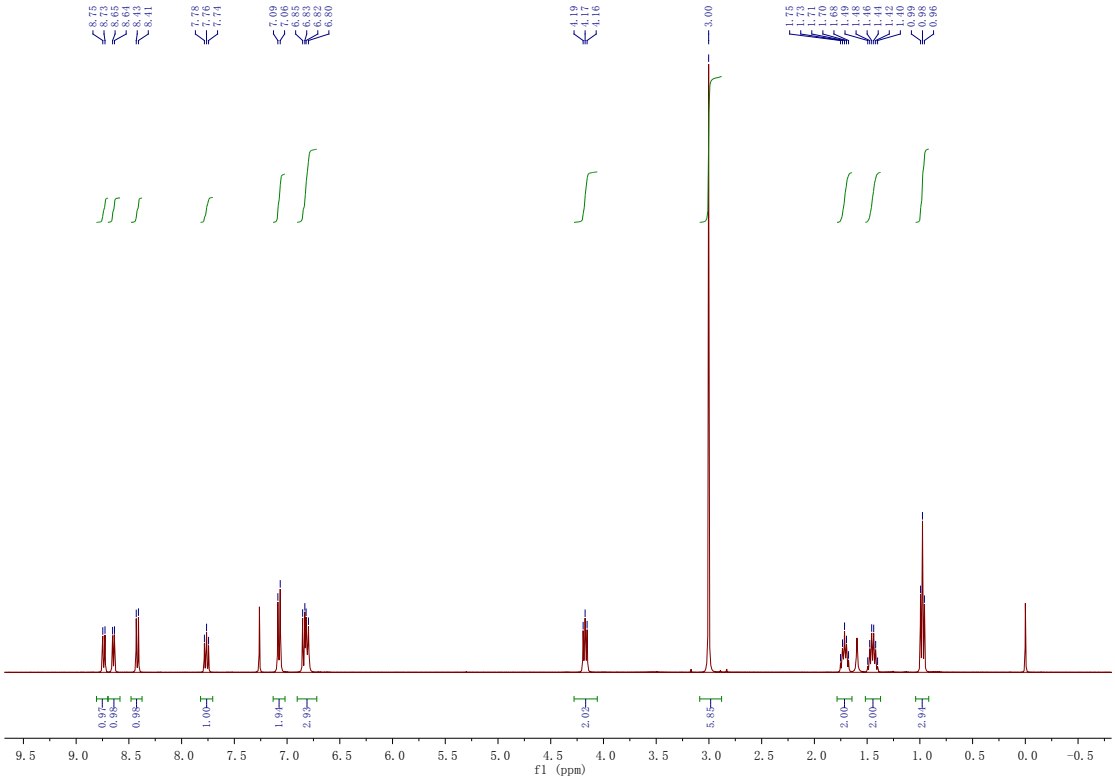


Figure S1. ^{1}H-NMR data of probe **PDPE-PN**.

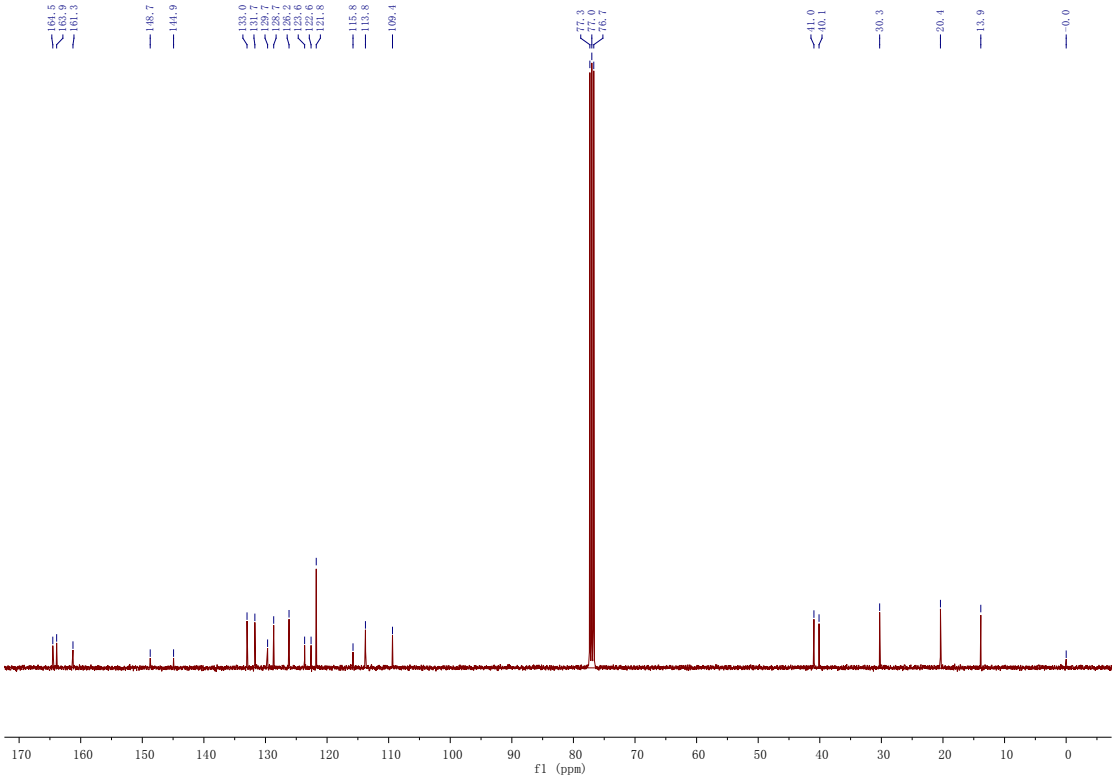
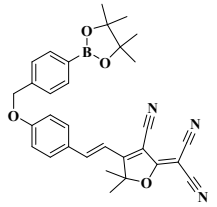
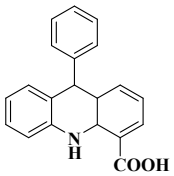
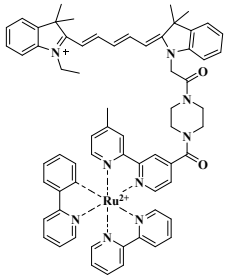
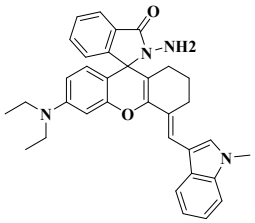
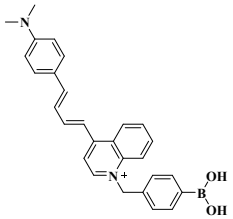
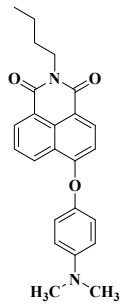


Figure S2. ^{13}C-NMR data of probe **PDPE-PN**.

9. Table of comparison between reported $ONOO^-$ probes and PDPE-PN

Table S1. Comparison of fluorescent probes for $ONOO^-$.

Probes	Solution	Detection limit	Response time	Imaging	References
	PBS/DMSO = 8/2	-	< 20 s	living cells	Chem. Comm. 2017, 53, 12822-12825.
	PBS solution with 0.1% DMSO	16 nM	within 5 s	living cells	ACS Sens. 2017, 2, 501-505.
	PBS solution	280 nM	~ 20 min	living cells	Chem. Comm. 2018, 54, 13698-13701.
	PBS/ACN = 8/1	25 nM	< 2 s	living cells	Sens. Actuators B 2018, 269, 15-21.
	PBS/DMSO = 6/4	1.69 nM	5 min	living cells, zebrafish and mice	Anal. Chem. 2020, 92, 14667-14675.
	PBS/DMF = 9/1	69 nM	< 3 s	living cells and zebrafish	This work.