Supporting Information

A highly selective and sensitive red-emitting fluorescent probe for visualization of endogenous peroxynitrite in living cells and zebrafish

Zilu Li,[a] Caiyun Liu,[a] Chen Yu,[a] Yanan Chen,[a] Pan Jia,[a] Hanchuang Zhu,[a] Xue Zhang,[a] Yamin Yu,[a] Baocun Zhu,[a,*] and Wenlong Sheng [b,*]

[a] School of Water Conservancy and Environment, University of Jinan, Shandong Provincial Engineering Technology Research Center for Ecological Carbon Sink and Capture Utilization, Jinan 250022, China.

[b] Biology Institute, Qilu University of Technology (Shandong Academy of Sciences), Jinan 250103, China.

*Corresponding author. Fax: +86-531-82767617; Tel.: +86-531-82767617

E-mail address: lcyzbc@163.com (B. Zhu) and 15618694162@163.com (W. Sheng)

Table of Contents

1. Materials and instruments

Except for special labels, chemical reagents were obtained from commercial vendor and employed without further purification. High resolution mass spectra (HRMS) were obtained by LC-MS2010A instrument. ^{1}H and ^{13}C NMR data were obtained by Bruker AV-400 NMR spectrometer. Absorption spectra were obtained by UV-3101PC spectrophotometer. Fluorescence spectra were obtained by Horiba FluoroMax-4 spectrophotometer. Fluorescence imaging of $ONOO^-$ in live RAW 264.7 macrophage 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 fluorescence spectra of free probe **Red-PN** were measured by five times and its standard deviation was obtained. To gain the slope, the fluorescence intensities at 585 nm were plotted as the increasing concentrations of $ONOO^-$, so the detection limit was calculated with the following equation (1):

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

Where σ is the standard deviation of blank measurement, k is the slope between the fluorescence intensities versus the concentrations of $ONOO^-$.

3. Cytotoxicity assays

The cell viability of RAW 264.7 macrophage cells, treated with probe **Red-PN**, was assessed by a cell counting kit-8 (CCK-8; Dojindo Molecular Technologies, Tokyo, Japan). Briefly, RAW 264.7 macrophage 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 macrophage cells were incubated with various concentrations (0, 5, 10, 20, and 30 μM) of probe **Red-PN** suspended in culture medium for 6 h. Subsequently, CCK-8 solution was added into each well for 2 h, and absorbance at 450 nm was measured.

4. Imaging studies of live cells

The RAW 264.7 macrophage cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum and 1% penicillin-streptomycin and incubated under an atmosphere containing 5% CO_2 at 37 °C humidified air for 24 h. Before imaging by confocal fluorescence microscope, probe **Red-PN** (10 μM) was used as a bioimaging reagent to incubate RAW 264.7 macrophage cells for 30 min, then removed culture medium and washed with phosphate-buffered saline for three times. After that, these cells were further incubated upon addition of $ONOO^-$ (20 μM, 40 μM) for 30 min. On the other hand, the cells pretreated with PMA (1.0 μg mL^{-1}) or LPS (1.0 μg mL^{-1}) for 1 h were incubated with probe **Red-PN** (10 μM) in culture media for another 30 min, and washed with culture water. Then the fluorescence imaging of cells was carried out by confocal fluorescence microscope.

5. Imaging studies 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 °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 at 28 °C. The 4-day-old zebrafish were incubated with probe **Red-PN** (10 μM) for 30 min, and then washed with culture water to remove the remaining probe. After that, the zebrafish were further incubated with $ONOO^-$ (20 μM) for 30 min. On the other hand, zebrafish pretreated with PMA (1.0 μg mL^{-1}) or LPS (1.0 μg mL^{-1}) for 30 min were incubated with probe **Red-PN** (10 μM) in for another 30 min, and washed with culture water. Then the fluorescence imaging of zebrafish was carried out by confocal fluorescence microscope.

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

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 ($O_2^{\cdot-}$) 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. The concentration of H_2O_2 was determined from the absorption at 240 nm (ε = 43.6 M^{-1} cm^{-1}). The concentration of $^{\cdot}OCl$ was determined from the absorbance at 292 nm (ε = 350 M^{-1} cm^{-1}). $ONOO^-$ was prepared according to the reported method and the concentration was determined based on the absorbance at 302 nm (1670 M^{-1} cm^{-1}).