Supporting Information

From BODIPY-Rhodamine Scaffold to Ratiometric Fluorescent Probe for Nitric Oxide

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General information

All regents such as $ClCH_2CH_2Cl$, $POCl_3$, acetonitrile and triethylamine were purchased from commercial suppliers and used without further purification. Column chromatography was performed with silica gel (200-300 mesh). RPMI 1640 culture medium with L-glutamine was purchased from GIBCO (Invitrogen, USA), FBS (fetal calf serum) was purchased from GIBCO (Invitrogen, USA).
Melting points were determined using X-6 melting point apparatus and uncorrected. ^{1}H-NMR and ^{13}C-NMR were measured on Varian MERCURY 400 spectrometer in $CDCl_3$ with TMS as internal reference. Multiplicities of signals are described as follows: s---singlet, br. s---broad singlet, d---doublet, t---triplet, m---multiplet. Coupling constants (*J*) are given in Hz. Mass spectra were measured on a HP 1100 LC-MSD, Gas chromatography/TOF Mass spectrometers and the UPLC/Q-TOF Mass spectrometers. Fluorescence spectra were measured on Recording Spectroflurophotometer FP-6500. Absorbance spectra were recorded on a UV-vis Spectrophotometer HP-8453. An inverted confocal fluorescent microscopy (IX81, Olympus, Japan) equipped with an objective lens (×100 oil, 1.4 Numerical Aperture (NA), Scan mode XY) was used in the imaging of living cells.

Experimental

Preparation and calibration of Reactive Oxygen/Nitrogen Species

Hydrogen peroxide (H_2O_2) was diluted immediately from a stabilized 30% solution and was assayed by using its molar absorption coefficient of 43.6 $M^{-1}cm^{-1}$ at 240 nm.[1] Freshly prepared aqueous solutions of $NaNO_2$ and $NaNO_3$ were used a nitrite (NO_2^-) and nitrate (NO_3^-) sources, respectively, and their concentrations were determined by Griess reagent. Singlet oxygen was chemically generated from the $^-OCl/H_2O_2$ system in buffer.[2] Hydroxyl radicals (·OH) were generated in the Fenton system from ferrous ammonium sulfate and hydrogen peroxide.[3] Peroxynitrite was synthesized from sodium nitrite (0.6 M) and H_2O_2 (0.65 M) in a quenched-flow reactor (excess H_2O_2 was used to minimize nitrite contamination). After the reaction, the solution was treated with MnO_2 to eliminate the excess H_2O_2. The concentration

of the $ONOO^-$ stock solution was determined by measuring the absorbance at 302 nm with a molar extinction coefficient of $1670 m^{-1}cm^{-1}$.[4] NO solution was prepared by bubbling NO through deoxygenated water for about 20 min. The concentration of NO was determined by Griess reagent.

Culture of MCF-7 cells and fluorescent imaging

MCF-7 (human breast carcinoma) were obtained from Institute of Basic Medical Sciences (IBMS) of Chinese Academy of Medical Sciences (CAMS) and cultured in RPMI 1640 supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO_2 and 95% air at 37 °C. Grow MCF-7 Cells in the exponential phase of growth on 35-mm glass-bottom culture dishes (Φ 20 mm) for 1-2 days to reach 70-90% confluency. The cells was washed three times with RPMI 1640, and then incubated for 10 min in an atmosphere of 5% CO_2 and 95% air at 37 °C with 2 mL RPMI 1640 containing a certain concentration of fluorescent probe. Wash cells twice with 1 mL PBS at room temperature, and then add 1 mL RPMI 1640 culture medium and observe under a confocal microscopy (Olympus FV1000).

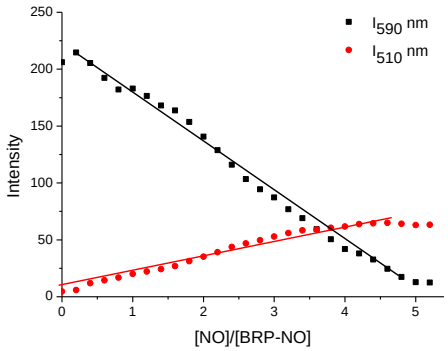

Figure S1 Emission change at 590 nm and 510 nm in the presence of various amounts of NO solution (0-1.24 μM), respectively

The limit of detection of BRP-NO

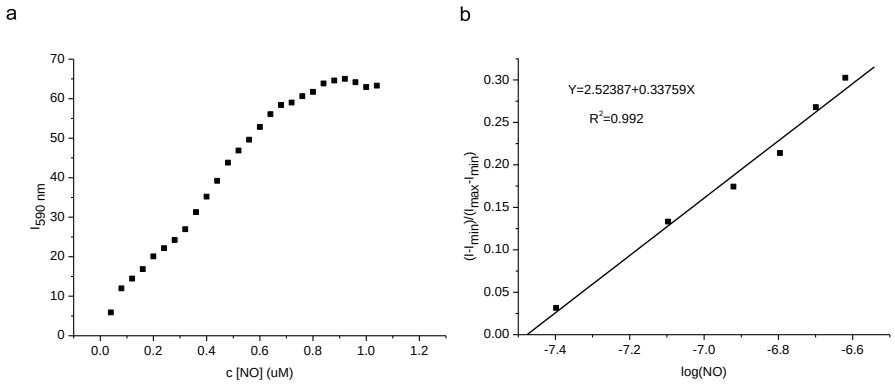

Figure S2 (a) Response of the fluorescence signal at 590 nm to changing NO concentrations. (b) A linear regression curve was then fitted to these fluorescence intensity data, and the point at which this line crossed the horizontal axis was considered as the detection limit 2.74×10^{-8} M for NO

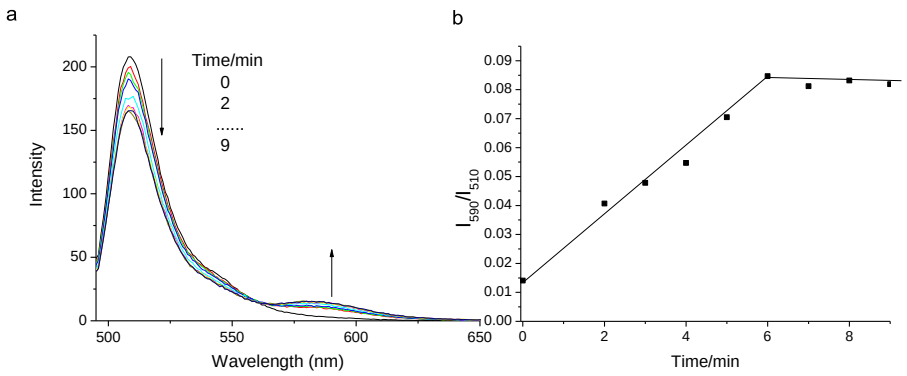

Figure S3 (a) Fluorescence changes of BRP-NO (0.2 μM) in the presence of NO (0.6 equiv) versus time (b) time course of ratio intensity at 590 nm and 510 nm depending on 0.6 equiv NO.

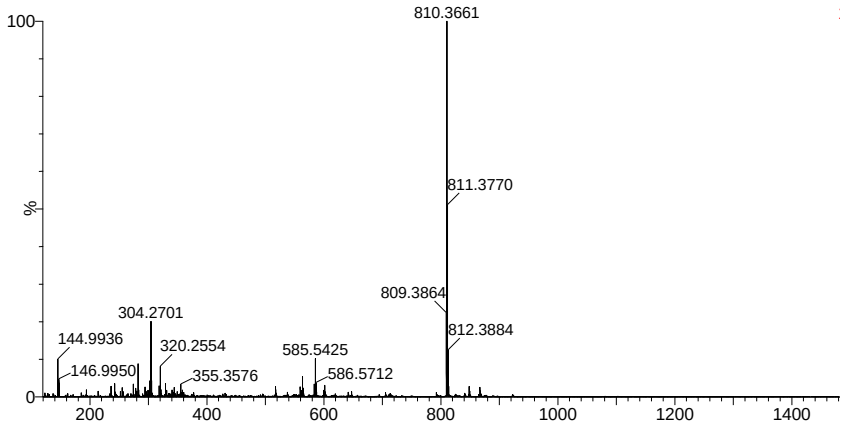

Figure S4 ESI-MS (positive) spectrum of BRP-NO (2 μM) in the presence of NO (5 equiv.) in H_2O/CH_3CN.

Reference:

[1] Lei, B.; Adachi, N.; Arai, T. *Brain Res. Protoc.* 1998, **3**, 33.
[2] Aubry, J. M. *J. Am. Chem. Soc.* 1985, **107**, 5844
[3] Setsukinai, K.; Urano, Y.; Kakinuma, K.; Majima, H. J.; Nagano, T. *J. Biol. Chem.* 2003, **278**, 3170
[4] Miyamoto, S.; Martinez, G. R.; Martins, A. P. B.; Medeiros, M. H. G.; Mascio, P. D. *J. Am. Chem. Soc.* 2003, **125**, 4510

NMR spectra of BRP-NO