Electronic Supplementary Information

A near-infrared fluorescent probe for the selective detection of HNO in living cells and in vivo

Ping Liu,[‡a] Xiaotong Jing,[‡a] Fabiao Yu,[ab*] Changjun Lv,[b] and Lingxin Chen[ab*]

[a] Key Laboratory of Coastal Environmental Processes and Ecological Remediation; The Research Center for Coastal Environmental Engineering and Technology, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, China
[b] Medicine Research Center, Binzhou Medical University, Yantai 264003, P.R. China

E-mail addresses: fbyu@yic.ac.cn, lxchen@yic.ac.cn
‡These authors contributed equally.

Contents:

1. General experimental section

Instruments: The purity of Cyto-JN was separated on a HPLC system (LC-20AT, Shimadzu) equipped with fluorescence and UV-vis absorption detectors (>99.92%). The absorption spectra were recorded on TU-1810DSPC UV-visible spectrophotometer (Beijing Persee). The fluorescence spectra and relative fluorescence intensity were measured on Shimadzu RF-5301PC Spectrofluorometer with a Xenon lamp and 1.0-cm quartz cells. ^{1}H and ^{13}C NMR were recorded on a 500 MHz Bruker Super Conducting NMR Spectrometer (AVANCE IIITM 500, Bruker). Cell images of fluorescence were acquired with an Olympus Laser Scanning Confocal Microscope (FV1000, Olympus) at × 40 magnifications. Intracellular fluorescence detection was carried out on flow cytometry (Aria, BD) with excitation at 633 nm and emission at 750-810 nm. Fluorescent images in vivo were detected on whole-body fluorescent imaging system (In-vivo Imaging System, Bruker).

Materials: All chemicals were purchased from Sigma-Aldrich or Aladdin and of analytical reagent grade. Double-distilled water purified from a Millipore Milli-Q system (Bedford, MA, USA) was used throughout all experiments. RAW264.7 cells were purchased from the Committee on Type Culture Collection of the Chinese Academy of Sciences. Dulbecco's modified Eagle's medium (DMEM), Fetal Bovine Serum (FBS) and trypsin were purchased from Gibco (Grand Island, USA). The BALB/c mice were supplied by Luye Pharma Group (Yantai, China).

Preparation of analytes: Cyto-JN (1 mM) was prepared in DMSO and stored at 4 °C in darkness. Angeli's salt was prepared as reported[1] and stored anhydrous at -20 °C. S-nitrosoglutathione (GSNO) was synthesized from GSH according to the published procedure.[2] Peroxynitrite ($ONOO^-$) solution was synthesized as reported.[3] NO was generated in form of 3-(Aminopropyl)-1-hydroxy-3-isopropyl-2-oxo-1-triaz-ene (NOC-5, 100 μM/ml).[4] NO_2^- was generated from $NaNO_2$. O_2^- was created by the enzymatic reaction of xanthine/xanthineoxidase (XA/XO, 6.0 μM/3mU) at 25 °C for 5 min.[5] Methyl linoleate (MeLH) and 2,2'-azobis-(2,4-dimethyl) valeronitrile (AMVN) were used to produce MeLOOH.[6] ClO^- was generated from NaClO.

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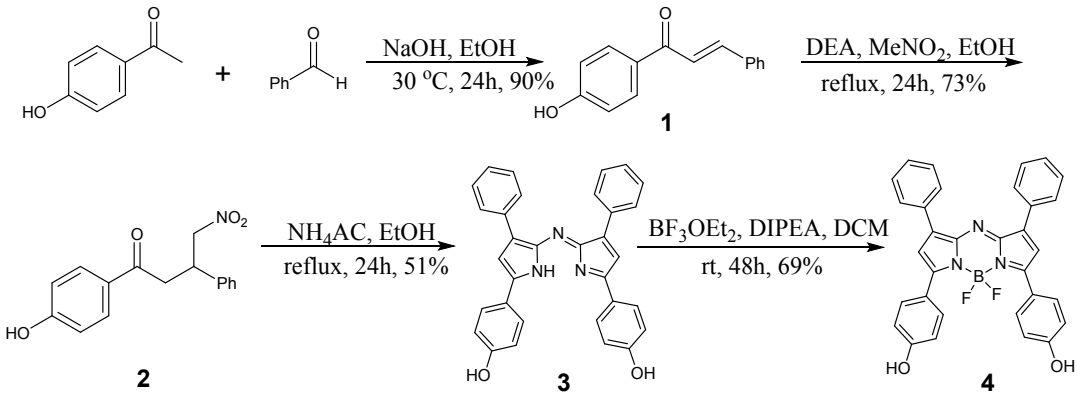
2. Synthesis of fluorophore

Scheme S1. Synthesis of Aza-BODIPY fluorophore



Synthesis of 1: 4'-Hydroxyacetophenone (13.6 g,100 mmol) in ethanol (140 mL) was added to the solution of sodium hydroxide (8.4 g, 210 mmol) in 60 mL water under ice bath，then benzaldehyde（10.13 mL, 100 mmol）diluted with ethanol (140mL) was dropwised to the cold solution. The resulting solution was stirred at room temperature for 15 h, and then the HCl was added to the solution. Finally, the solution was cold in ice water, filtered, and crystallized with ethanol to yield light yellow crystal chalcone **1** (20.16 g, 90%), m.p.180-182 ºC. ^{1}H NMR (500 MHz, $CDCl_3$-D_1) δ (ppm): 10.41 (s, 1H), 8.08-8.05 (m, 2H), 7.92-7.85 (m, 3H), 7.69-7.66 (m, 1H), 7.47-7.43 (m, 3H), 6.91-6.88 (m, 2H). LC-MS (ESI^+): m/z $C_{15}H_{12}O_2$ calcd. 224.0837, found $[M-H]^-$ 223.0758.

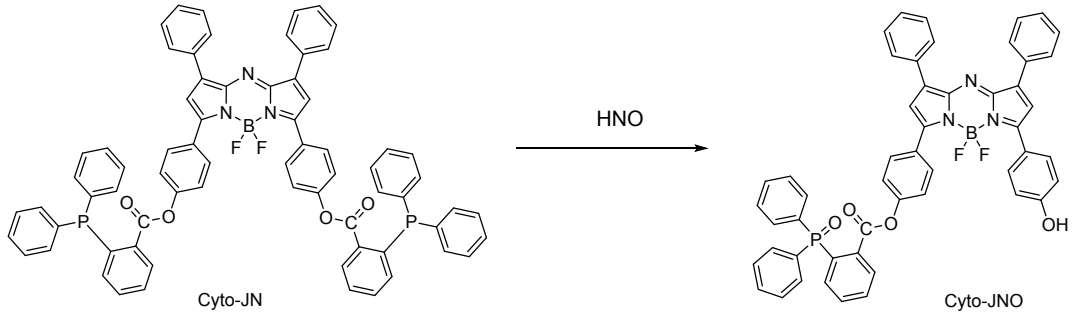
Synthesis of 2: A solution of chalcone **1** (2.02 g, 9 mmol) in EtOH (15 mL) was treated with diethylamine (4.6 mL, 45 mmol) and nitromethane (4.8 mL, 90 mmol), then heated under reflux for 24 h. The solution was cooled and then neutralized with HCl, partitioned between EtOAc and H_2O (1:1). The organic layer was separated, dried over sodium sulfate and evaporated. The residue was stirred in cold Et_2O for 10 min and filtered to give the product as yellowish crystalline compound 2 (1.87 g, 73%), m.p. 112-113 ºC. ^{1}H NMR (500 MHz, DMSO-D_6) δ (ppm): 10.20 (s, 1H), 7.77 (d, 2H), 7.28-

7.20 (m, 5H), 6.78 (m, 2H), 4.86 (m, 2H), 3.93 (m, 1H), 3.81 (m, 2H). LC-MS (ESI^+): m/z $C_{16}H_{15}NO_4$ calcd. 285.1001, found $[M-H]^-$ 284.0928.

Synthesis of 3: Compound 2 (2.0 g, 7.0 mmol) and ammonium acetate (18.9 g, 245 mmol) in EtOH (50 mL) were heated under reflux for 24 h. The reaction was cooled to room temperature. Then, the product was spin steamed and extracted in EtOAc and H_2O (1:1).The organic layer was evaporated to dry and yielded **3** as blue-black solid (1.72 g, 51%), mp 245-246 °C. ^1H NMR (500 MHz, DMSO-D_6) δ (ppm): 10.26-10.21 (s, 2H), 7.53-7.24 (m, 13H), 7.10-7.07 (m, 3H), 6.49-6.47 (d, 4H), 5.26 (s, 1H). LC-MS (ESI^+): m/z $C_{32}H_{23}N_3O_2$ calcd. 481.1790, found $[M-H]^-$ 480.1717.

Synthesis of 4: Compound 3 (0.15 g, 0.31 mmol) was dissolved in anhydrous CH_2Cl_2, treated with diisopropylethylamine (0.54 mL, 3.11 mmol) and BF_3diethyletherate (0.55 mL, 4.35 mmol), and stirred under argon for 48 h. The colour of solution changed from blue to green. The course of the reaction is confirmed by thin-layer chromatography (TLC).The product was extracted in CH_2Cl_2/H_2O (1:1), and the organic layer evaporated to dryness, and then purified by column chromatography on silica eluting with CH_2Cl_2/EtOAc (4:1) gave the Aza-BODIPY as atropurpureus solid (0.11 g, 69%), m.p 253-254 °C. ^1H NMR (500 MHz, DMSO-D_6) δ (ppm): 10.46 (s, 2H), 8.16-7.80 (m, 8H), 7.56-7.48 (m, 6H), 7.48-7.45 (m, 2H), 6.96-6.94 (4H). LC-MS (ESI^+): m/z $C_{32}H_{22}BF_2N_3O_2$ calcd. 529.1773, found $[M-H]^-$ 528.1702.

Scheme S2. Synthesis of Cyto-JNO in vitro



Characterization of Cyto-JNO： A solution of Cyto-JN (110.5 mg, 0.1 mmol) in acetonitrile was treated with Angeli's salt (5 mmol) in HEPES buffer (10 mM, pH = 7.4). The mixture was stirred for 1 hour at room temperature. Then solvent was evaporated and partitioned between CH_2Cl_2 and H_2O, the organic layer evaporated to dryness. The resulted residue was purified by column chromatography. Compound Cyto-JNO was obtained as a green solid, 72.5 mg, 87%. ^1H NMR (500 MHz, DMSO-D_6) δ (ppm): 8.39-8.29 (m, 1H), 8.03-7.92 (m, 1H), 7.80-7.65 (m, 8H), 7.53-7.40 (m,

13H), 7.27-7.16 (m, 8H), 7.00-6.94 (m, 2H). ^{13}C NMR (125 MHz, DMSO-D_6) δ (ppm): 169.88, 166.90, 157.21, 154.06, 153.46, 153.45, 152.08, 151.82, 136.45, 136.15, 135.16, 135.15, 134.66, 134.34, 132.86, 132.12, 131.50, 131.20, 131.11, 118.18, 106.10. ^{31}P NMR (200 MHz, $CDCl_3$-D) δ (ppm): 28.91. LC-MS (ESI^+): $C_{51}H_{35}BF_2N_3O_4P$ calcd. 833.2426 found $[M-H]^-$ 832.2354.

3. Effects of pH values on probe

The effects of pH on the fluorescence intensity and reactivity of Cyto-JN (5 μM) was examined in the absence and presence of HNO in HEPES buffer solution (10 mM, 0.5% TW 80, pH 7.4). The fluorescence intensity of Cyto-JN was stable in the pH range of 4.0-9.0. Cyto-JNO remained the strong fluorescence intensity during the pH range of 4.0-7.6. However, the fluorescence intensity could be severely affected when exposed Cyto-JNO at pH > 7.8. The results indicate that our probe can work well under physiological conditions (10 mM HEPES buffer, pH 7.4).

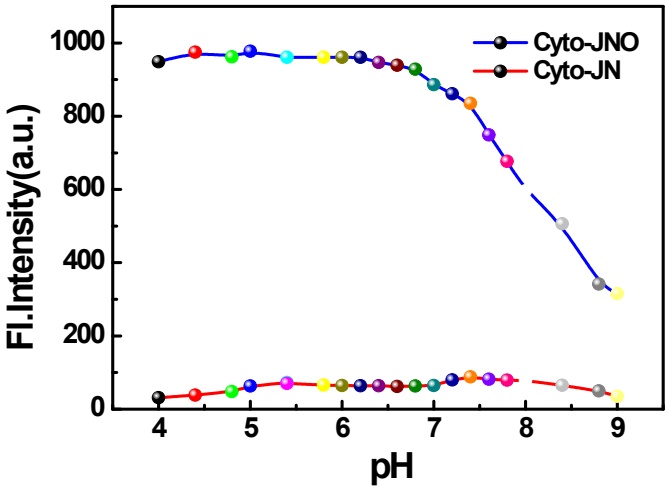


Fig. S1 The effects of pH values on the fluorescence intensity (λ_{ex}= 682 nm, λ_{em}= 700 nm) of probe Cyto-JN (5 μM) and Cyto-JNO (5 μM) in HEPES (10 mM, 0.5% DMSO, 0.5% TW 80). pH values: 4.0, 4.4, 4.8, 5.0, 5.4, 5.8, 6.0, 6.2, 6.4, 6.8, 7.0, 7.2, 7.4, 7.6, 7.8, 8.0, 8.4, 8.8, 9.0.

4. Effect of Tween 80 to the fluorescence intensity

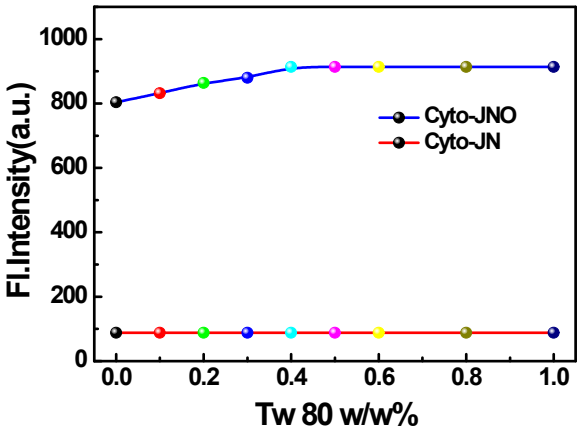


Fig.S2 The effect of Tween 80 on the fluorescence intensities (λ_{ex}= 682 nm, λ_{em}= 700 nm) of Cyto-JN (5 μM) and Cyto-JNO (5 μM) in HEPES (10 mM, pH 7.4).

5. Determination of quantum yields

The fluorescence quantum yields of Cyto-JN and Cyto-JNO were determined according to the following expression:[7]

$$\varphi_u = \frac{(\varphi_s)(FA_u)(A_s)(\lambda_{exs})(\eta_u^2)}{(FA_s)(A_u)(\lambda_{exu})(\eta_s^2)}$$

Where φ is fluorescence quantum yield; the subscripts u and s refer to the unknown and the standard, respectively; F is integrated fluorescence intensity under the corrected emission spectra; A is the absorbance at the excitation wavelength; η is the refractive index of the solution. We chose Mg-tetra-tert-butylphthalocyanine as standard, which has a fluorescence quantum yield of 0.84 according to the literature.[8]

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6. Effects of common physiological metal ions and anions

The fluorescence responses of Cyto-JN to common metal ions and anions were also investigated in HEPES buffer (10 mM, pH 7.4, 0.5% Tween 80). As shown in Fig. S3, the K^+, Na^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, Cu^{2+}, Cl^-, Br^-, SO_4^{2+}, CO_3^{2-}, $H_2PO_4^{2-}$, and critrate did not trigger any fluorescence enhancement.

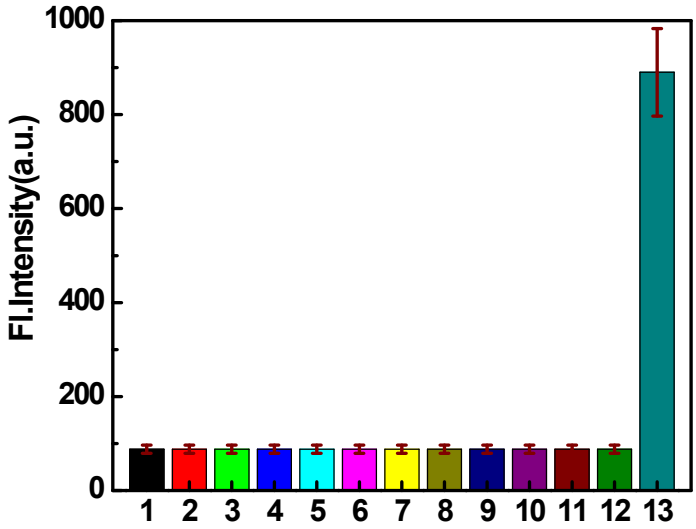

Fig. S3 Fluorescence responses of Cyto-JN to various metal ions and anions. (1) K^+ (1 mM), (2) Na^+ (1 mM), (3) Ca^{2+} (1 mM), (4) Mg^{2+} (1 mM), (5) Zn^{2+} (1 mM), (6) Cu^{2+} (1 mM), (7) Cl^- (1 mM), (8) Br^- (1 mM), (9) SO_4^{2-} (1 mM), (10) CO_3^{2-} (1 mM), (11) $H_2PO_4^-$ (1 mM), (12) Critrate (1 mM), (13) AS (10 μM). Data were recorded in 10 mM HEPES buffer (pH 7.4, 0.5% Tween 80) for 20 min. λ_{ex} = 682 nm, λ_{em} = 734 nm.

7. Bright field images of cells

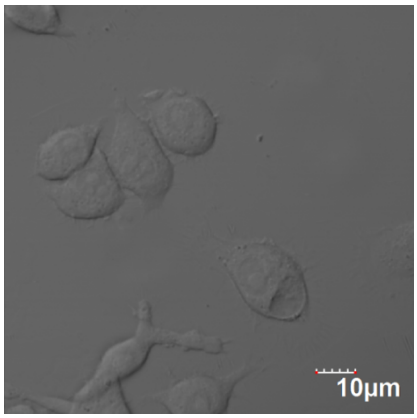

Fig. S4 DIC images of RAW264.7 cells incubated with Hoechst 33342, Calcein-AM and Cyto-JN before cultured with 100 μM AS.

8. X-ray images of mice

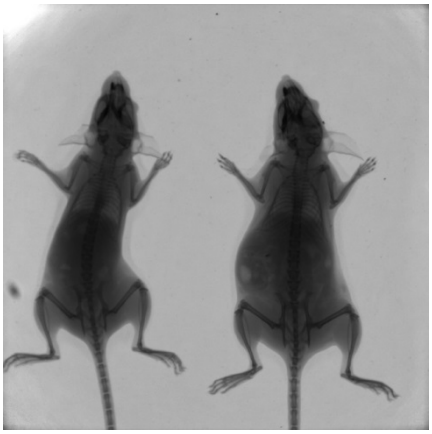

Fig. S5 X-ray imaging in peritoneal cavity of the BALB/c mice.