

# Supporting Information

## **A 6-acetyl-2-naphthol based two-photon fluorescent probe for the selective detection of nitroxyl and imaging in living cells**

**Pingzhu Zhang <sup>a,b</sup> Pingping Lian <sup>a</sup>, Xiaofen Wang <sup>a</sup>, Xueyan Li <sup>a</sup>, Chao Wei <sup>\*a,b</sup> and Xiaoliu Li <sup>\*a,b</sup>**

<sup>a</sup> Key Laboratory of Chemical Biology of Hebei Province, College of Chemistry and Environmental Science, Hebei University, Baoding 071002, P. R. China.

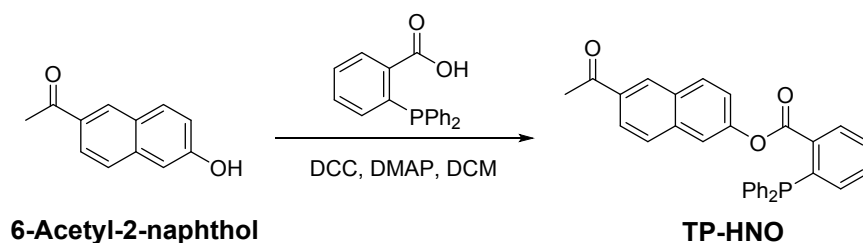
<sup>b</sup> Key Laboratory of Medicinal Chemistry and Molecular Diagnosis of Ministry of Education, Hebei University, Baoding 071002, P. R. China.

\*corresponding author: weichao@hbu.edu.cn; lixl@hbu.cn.

## 1. Reagents and instruments

All solvents were of analytic grade. All chemicals and solvents used for synthesis were purchased from commercial suppliers and applied directly in the experiment without further purification. The progress of the reaction was monitored by TLC, and spots were visualized by UV light. Silica gel 60 (100-200 mesh) was used for general column chromatography purification.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were recorded on a Bruker 600 (600 MHz  $^1\text{H}$ ; 150 MHz  $^{13}\text{C}$ ) spectrometer at room temperature. Chemical shifts are reported in parts per million relative to internal standard tetramethylsilane ( $\text{Si}(\text{CH}_3)_4 = 0.00$  ppm) or residual solvent peaks ( $\text{CDCl}_3 = 7.26$  ppm).  $^1\text{H}$  NMR coupling constants ( $J$ ) are reported in Hertz (Hz), and multiplicity is indicated as the following: s (singlet), d (doublet), bs (board singlet), m (multiple). High-resolution mass spectra (HRMS) were obtained on Varian 7.0 T FTICR-MS. The UV-visible spectra were recorded on a Cary 5000 Bio UV-VIS spectrophotometer (Agilent, USA). Fluorescence study was carried out using F-7000 spectrophotometer (Hitachi, Japan). Bioimaging of cells was performed on a confocal microscope (Zeiss LSM 880).

## 2. Synthesis of TP-HNO



Scheme S1. Synthesis of probe TP-HNO

DCC (248 mg, 1.20 mmol) was firstly added to a DCM solution (5 mL) of 6-acetyl-2-naphthol (186 mg, 1.00 mmol). After stirring 30 min, 2-(diphenylphosphanyl)benzoic acid (322 mg, 1.05 mmol) and DMAP (183 mg, 1.50 mmol) were successively added at room temperature. The solution was stirred at room temperature overnight. TLC showed no starting material, and then the solution was poured into 100 mL of ice-water. The precipitate was filtered off to give the desired produce as a yellow solid. The product **TP-HNO** was purified by column chromatography on silica gel to give a light yellow solid (403 mg, yield 85 %). TLC (etroleumether:EtOAc = 3:1):  $R_f = 0.5$ ; m.p. 118-120 °C;  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 8.43 (s, 1H), 8.31 (bs, 1H), 8.02 (d,  $J = 8.4$  Hz, 1H), 7.91 (d,  $J = 9.0$  Hz, 1H), 7.78 (d,  $J = 8.4$  Hz, 1H), 7.51-7.46 (m, 2H), 7.41 (s, 1H), 7.37-7.31 (m, 10H), 7.16 (d,  $J = 8.4$  Hz, 1H), 7.03 (bs, 1H), 2.71 (s, 3H);  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 197.7, 165.1, 150.2, 141.5, 141.3, 137.5, 137.4, 136.1, 134.4, 134.3, 133.9, 133.3, 132.6, 131.3, 130.9, 130.4, 129.8, 128.8, 128.6, 128.5, 128.1, 124.5, 122.1, 118.6, 26.6; HRMS  $m/z$   $[\text{M}+\text{H}]^+$  Calcd. for  $\text{C}_{31}\text{H}_{24}\text{O}_3\text{P}$ : 475.1458; found: 475.1460.

### 3. Spectroscopic analysis of TP-HNO

Spectroscopic measurements were performed in PBS (20 mM, pH 7.4, containing 50%  $\text{CH}_3\text{CN}$ ) buffer at room temperature. Fresh Angeli's salt (AS) dissolved in degassed PBS was used as HNO source. **TP-HNO** was diluted in PBS to afford the final concentration of 5  $\mu\text{M}$ . For the selectivity experiments, different biologically relevant molecules (100 mM stock solution) were prepared. Appropriate amount of biologically relevant species were added to separate portions of the probe solution and mixed thoroughly in PBS. The reaction mixture was shaken uniformly before emission spectra were measured. For the time-course experiments, 5  $\mu\text{M}$  probe **TP-HNO** in PBS were added with 15  $\mu\text{M}$  HNO at room

temperature, and the fluorescence intensity was measured at different time points. For the pH-dependent experiment, **TP-HNO** (5  $\mu$ M) and HNO (15  $\mu$ M) were incubated with PBS at different pH values.

#### **4. Cell culture and confocal microscope imaging**

HeLa cells were cultured at 37 °C, 5% CO<sub>2</sub> in DMEM/HIGH GLUCOSE (GIBICO) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, 100  $\mu$ g/ml streptomycin, and 4 mM L-glutamine. The cells were maintained in exponential growth, and then seeded in glass-bottom 35 mm plate at the density about  $2 \times 10^4$ /well. Cells were passaged every 2-3 days and used between passages 3 and 10. HeLa cells were imaged on a confocal microscope (Zeiss LSM 880) with a 40  $\times$  objective lens. Emission was collected at cyan channel (430-530 nm) with 405 nm excitation. Emission was collected at cyan channel (430-550 nm) with 750 nm two-photon excitation.

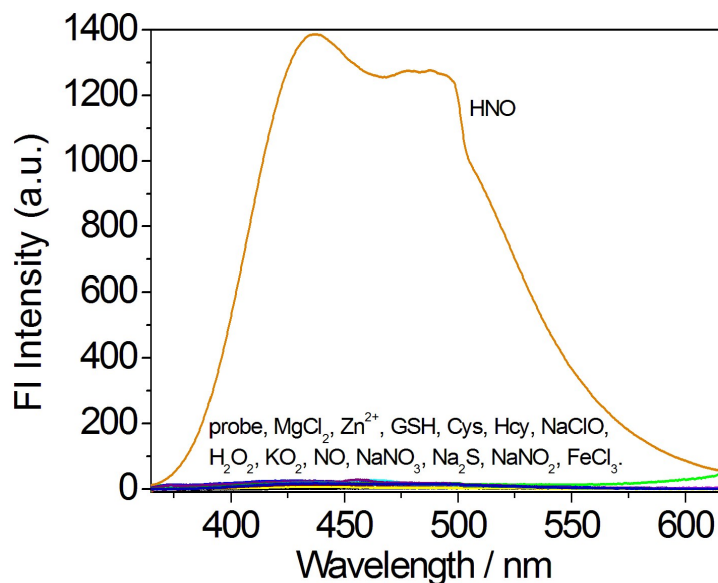
For living cell imaging, HeLa cells were treated with **TP-HNO** (5  $\mu$ M) at 37 °C, 5% CO<sub>2</sub> for 30 min, washed with PBS, and then treated with HNO (20  $\mu$ M and 50  $\mu$ M) for another 30 min. Control cells were only treated with **TP-HNO** at 37 °C, 5% CO<sub>2</sub>.

For imaging of NaAsc and SNP-induced endogenous HNO, cells were first treated with NaAsc (2 mM) and SNP (2 mM) at 37 °C, 5% CO<sub>2</sub> for 2 h, washed with PBS, and then treated with **TP-HNO** (5  $\mu$ M) for another 1 h.

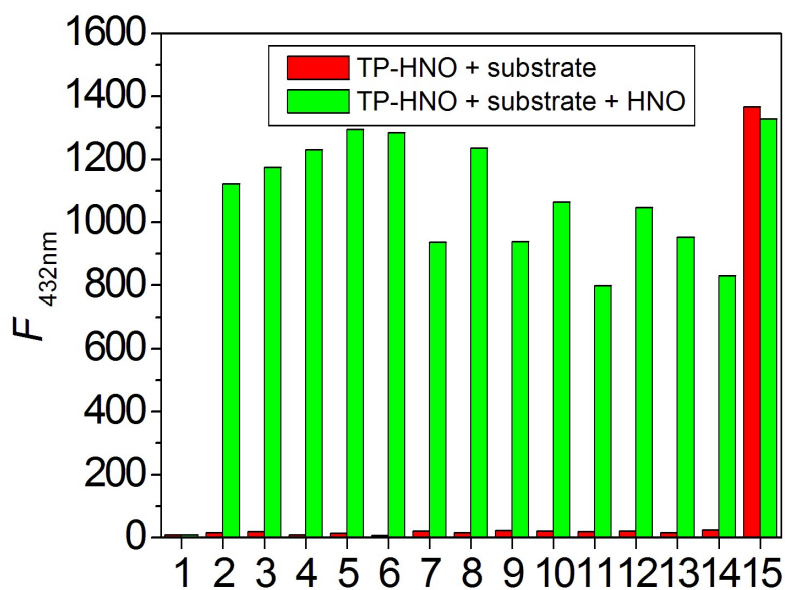
#### **5. Cell culture and confocal microscope imaging**

Cell culture and confocal microscope imaging

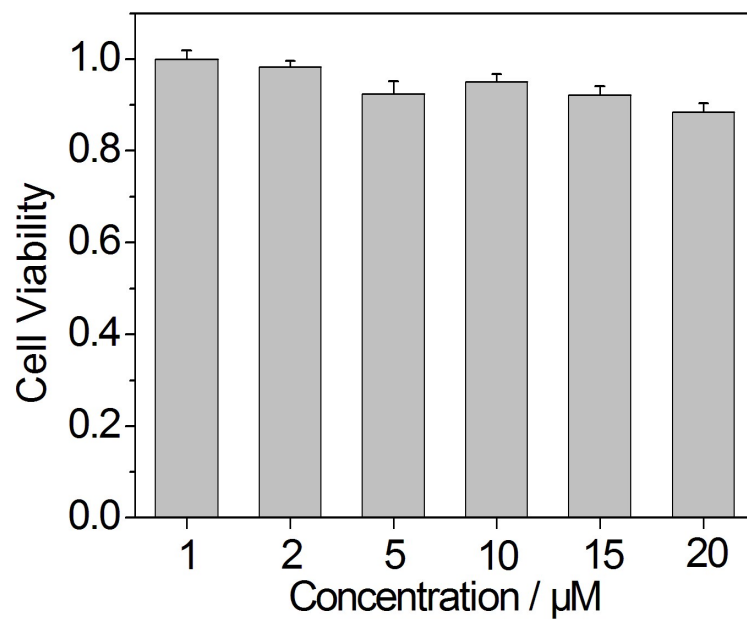
## 6. Supporting figures.



**Fig. S1.** Fluorescence intensity of TP-HNO (5  $\mu M$ ) incubated with various analytes (Probe only,  $Mg^{2+}$ ,  $Zn^{2+}$ ,  $Fe^{3+}$ ,  $NO_3^-$ ,  $OCl^-$ ,  $H_2O_2$ ,  $KO_2$ , NO,  $NO_2^-$ ,  $H_2S$ , Cys or Hcy was 1 mM; GSH was 5 mM, HNO was 15  $\mu M$ ) for 30 min.

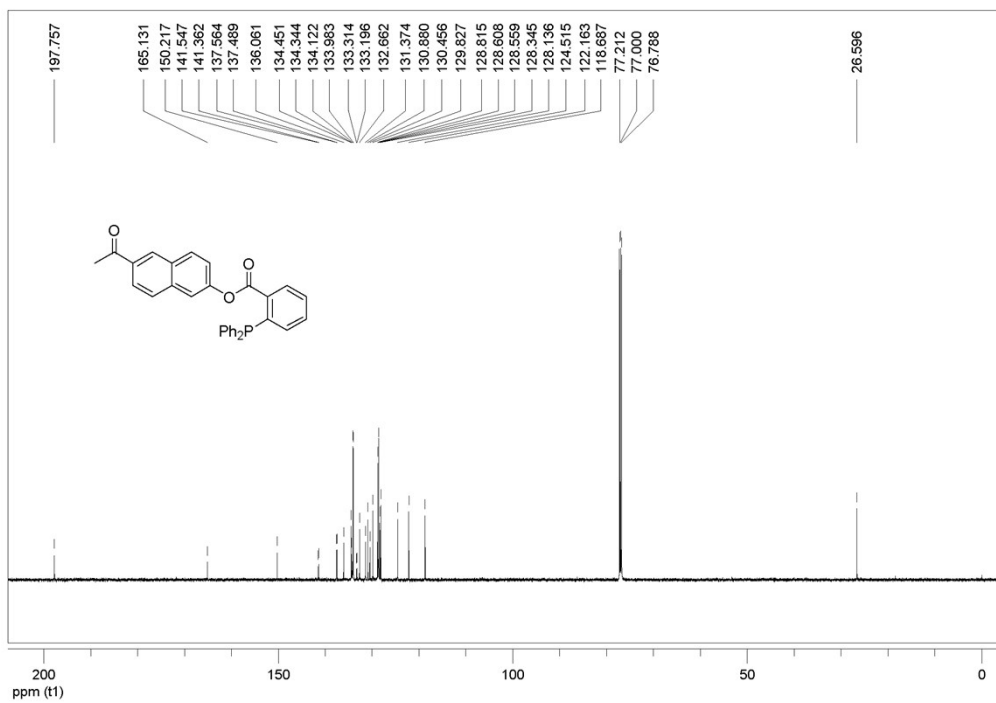
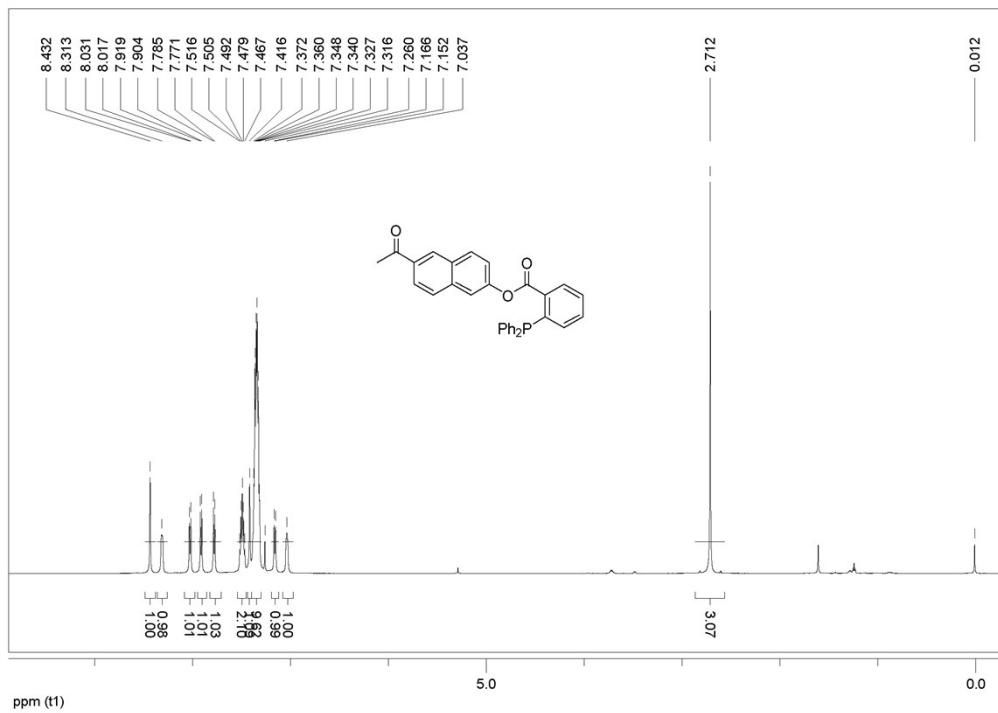


**Fig. S2.** The competitive experiments of TP-HNO (5  $\mu M$ ) towards various analytes. (1. Probe only; 2.  $Mg^{2+}$ ; 3.  $Zn^{2+}$ ; 4.  $Fe^{3+}$ ; 5.  $NO_3^-$ ; 6.  $OCl^-$ ; 7.  $H_2O_2$ ; 8.  $KO_2$ ; 9. NO; 10.  $NO_2^-$ ; 11.  $H_2S$ ; 12. Cys; 13. Hcy; 14. GSH; 15. HNO) ( $OCl^-$ ,  $H_2S$ , Cys, Hcy, and GSH were 250  $\mu M$ ; HNO was 15  $\mu M$ ; others was 1 mM) for 30 min.



**Fig. S3.** The cytotoxicity of the probes **TP-HNO** evaluated by MTT assay. Error bars are  $\pm$  SEM.

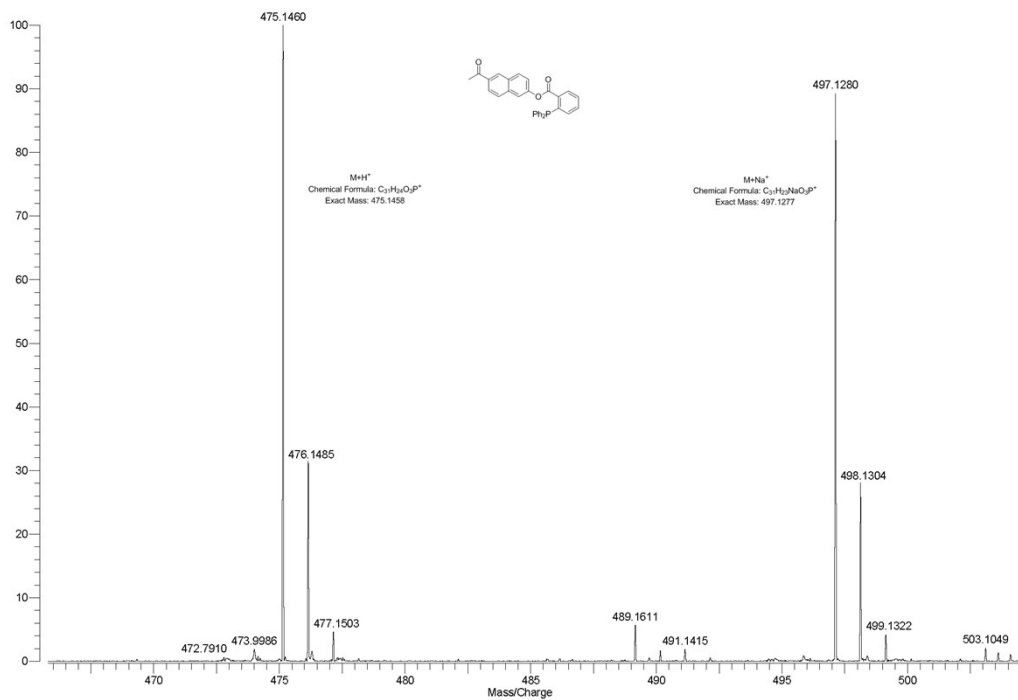
## 7. Supplementary spectra of NMR and HRMS



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