# Supplementary Information

## A Photo-controlled and Photo-calibrated Nanoparticle Enabled Nitric Oxide-releasing for Anti-bacteria and Anti-biofilm

Li Li[a, ‡], Zhenmei Lin[a, ‡], Xicun Lu[b], Chen chen, Anqi Xie, Yaoping Tang, and Ziqian Zhang [a,*]

*Guangxi Scientific Research Center of Traditional Chinese Medicine, Guangxi University of Chinese Medicine, Wuhe avenue 13, Nanning 530200, China. E-Mail: zhangzq@gxtcmu.edu.cn*

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## S1. General methods

Chemical reagents were provided from TCI, Energy, and Aladdin. All the Solvents of analytical grade were purchased from Sinopharm Chemical Reagent Co., Ltd. The water was acquired from Milli-Q.

The $^{1}$H-NMR and $^{13}$C-NMR spectra were acquired on a Bruker AV-400 spectrometer. Chemicals shifts were referenced to the residue solvent peaks and given in ppm. The results of the HR-MS were received by a Micro mass GCT spectrometer. UV-Vis absorption spectra were recorded on a SHIMADZU UV-2600 plus UV-vis spectrophotometer. Fluorescence emission spectra were acquired on a SHIMADZU RF-3000 fluorescence spectrophotometer. The excitation and emission slits of spectrum experiment were set to 5 nm. The images were captured by a Leica SP5 confocal laser scanning microscope.

## S2. Synthesis

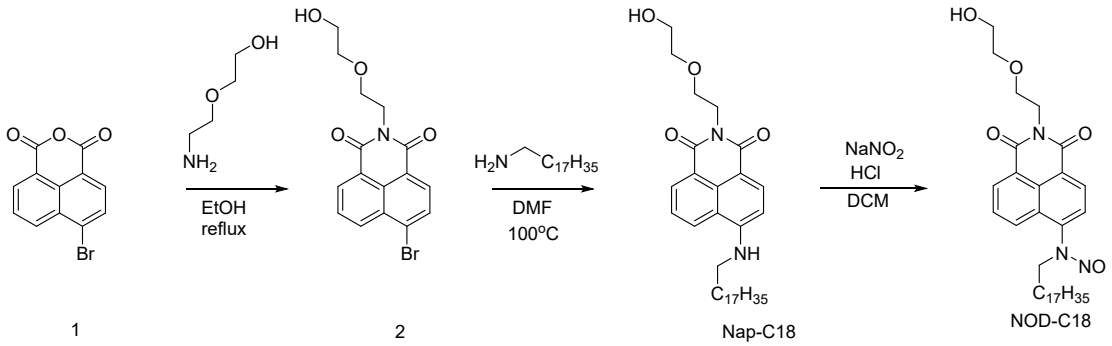



Fig.S1 Synthesis of **NOD-C18**

**Synthetic procedures and characterizations:**

The synthesized method of **compound 2** was based on the report: *Anal. Chem.*, 2016, 88, 7274-7280.

**6-bromo-2-(2-(2-hydroxyethoxy)ethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (2):**

To a EtOH solution (100 mL) of Compound **1** (5.0 g, 18.05 mmol) in a 250 mL round-bottom flask, 1.9 g diglycolamine was added slowly. Then the reaction mixture was stirred in reflux condition for 8 hours. The solvent was cold to room temperature and filtrated to acquire the crude product. The residue was purified via recrystallization to give compound **2**. Yield: 4.8 g, 76%. $^{1}$H-NMR (400 MHz, $CDCl_3$) δ 8.67 (d, $J$ =7.2 Hz, 1H), 8.59 (d, $J$ = 8.4 Hz, 1H), 8.43 (d, $J$ = 8.0 Hz, 1H), 8.05 (d, $J$ = 7.6 Hz, 1H), 7.86 (t, $J$ = 8.0 Hz, 1H), 4.45 (t, $J$ = 5.6 Hz, 2H), 3.86 (t, $J$ = 5.6 Hz, 2H), 3.68 (m, 4H).

**2-(2-(2-hydroxyethoxy)ethyl)-6-(octadecylamino)-1H-benzo[de]isoquinoline-1,3(2H)-dione (Nap-C18):**

Compound **2** (500.0 mg, 1.37 mmol), Octadecylamine (550.0 mg 2.06 mmol) and 25 mL DMF were mixed in a 50 mL round-bottom flask. The mixture was stirred at 100 °C for overnight. Then the reaction solvent was removed via reduced pressure distillation. The residue was dissolved into

50 mL DCM and washed by brine (50 mL×3). The organic layer was dried with $Na_2SO_4$, filtered, and evaporated in sequence. The crude product was purified via a flash chromatography with silica gel to give **Nap-C18**. Yield: 450.1 mg, 59%. $^1$H-NMR (400 MHz, $CDCl_3$) δ：8.46 (d, *J* = 8.0Hz, 1H), 8.38 (d, *J* = 12.0 Hz, 1H), 8.04 (d, *J* = 8.0 Hz, 1H), 7.51 (t, *J* = 8.0 Hz, 1H), 6.60 (d, *J* = 12.0 Hz, 1H), 5.53 (br, 1H), 4.41 (t, *J* = 8.0 Hz, 2H), 3.88 (t, *J* = 4.0 Hz, 2H), 3.72 (s，4H)，3.36 (q，*J*=8.0Hz，2H)，2.91(br，1H)1.80 (t，*J*= 8.0Hz，2H), 1.71 (, 2H), 0.87 (t, *J* = 8.0 Hz, 3H).

$^{13}$C-NMR (400MHz,$CDCl_3$) δ:164.99，164.66，149.80，134.70，131.31，129.76，126.18，124.52，122.62，119.98，109.49，104.20，72.06，68.64，61.90，43.71，39.12，31.93，29.70，29.66，29.61，29.58，29.39，29.37，22.70，14.13

HRMS (ESI)m/z: $[M+H]^+$ caculated for $C_{32}H_{52}N_2O_4$, 552.39,found, 552.3925.

**N-(2-(2-(2-hydroxyethoxy)ethyl)-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl)-N-octadecylnitrous amide (NOD-C18):**

**NOD-C18** (250.0 mg, 0.45 mmol) was added into a mixed 10 mL solution contained HCl、acetic acid and dichlormethane (1:1:8, v/v). The mixture was stirring in ice-bath, and slowly added by sodium nitrite (300 mg, 4.35 mmol) solid. The reaction was monitored by TLC, until t recovered to room temperature. Then the reaction solvent was added to a 30 mL sodium bicarbonate saturated solution, then extracted by DCM (15 mL×3). The organic layer was dried with $MgSO_4$, filtered, and evaporated in sequence. The crude product was purified via a flash chromatography with silica gel to give Nap-C18. Yield: 198.5 mg, 75.8%.

$^1$H-NMR (400 MHz,$CDCl_3$) δ 8.67 (m, 1H+1H*0.4) 8.63 (m, 1H+1H*0.4), 8.04 (d, *J* = 8.0 Hz, 1H), 7.79 (t, *J*=8.0 Hz, 1H+H*0.4) 7.68(d, *J*=8.0, 1H+H*0.4H), 7.42，（d，*J*=8.0Hz，1H*0.4H）,4.63 (t,，*J*=4.0Hz，2H*0.4), 4.43 (m，2 H+2H*0.4), 4.11 (t，*J*=4.0Hz，2H), 3.84 (m，2 H+2H*0.4), 3.64 (m, 4H+4H*0.4),2.66（br,1H+1H*0.4），1.42（m，2H+2H*0.4），1.22（m，31H+31H*0.4），0.84（t，*J*=8.0Hz，3H+3H*0.4）

$^{13}$C-NMR (400 MHz, $CDCl_3$) δ 163.2, 162.7, 143.4, 131.4, 130.8, 129.9, 128.5, 128.4, 126.5, 123.5, 122.4, 72.1, 66.8, 60.2, 36.2.

HRMS (ESI) $[M+Na]^+$ calculated for $C_{34}H_{51}N_3O_5$, 581.38, found, 604.3275.

## S3. Spectroscopic Analysis

**1.Preparation and Characterization of NOD-NP polymer**

In a typical oil-in-water microemulsion synthesis procedure, 8 mg of DSPE-PEG2000 were dissolved in 9 mL of deionized water to form solution A. A concentrated solution of **NOD-C18** (1 mg) in tetrahydrofuran (THF,1 mL) formed solution B. Solutions A and B were mixed together followed by sonication for 10 min. The residue THF evaporation during a heat course (45 °C, 1 hour) by violent stirring the suspension to finish the encapsulation, forming **NOD-NP polymer** colloidal solution and used directly. The **NOD-NP polymer** solution of various NOD-C18 or DSPE-PEG2000 concentrations was obtained by the same synthesis process.

**2. The measurement of encapsulation efficiency**

After NOD-C18 was encapsulated into a hydrophilic nanoparticle (NOD-NP ), then NOD-NP centrifuged at 15000 rpm for 15 min and washed with deionized water three time to remove any

precipitates. After purifying, the obtained NOD-NP suspension was dissolved in deionized water. The encapsulation efficiency of the NOD-C18 was calculated by UV−vis absorption of NOD-NP suspension solution. (n=3)

**3. Determination of quantum yield**

The quantum yield was obtained by referencing dye Rhodamine 6G (QY = 95% in ethanol). The quantum yield was calculated using the following equation (G. A. Crosby and J. N. Demas, The Journal of Physical Chemistry, 1971, 75, 991.):

$$\Phi = \Phi_{ST}\left(\frac{\mathrm{Grad}_x}{\mathrm{Grad}_{ST}}\right)\left(\frac{\eta_X^2}{\eta_{ST}^2}\right)$$

Where the subscripts ST and X denote standard (Here, Rhodamine 6G as standard) and test respectively, Φ is the fluorescence quantum yield, Grad the gradient from the plot of integrated fluorescence intensity vs absorbance, and η the refractive index of the solvent.

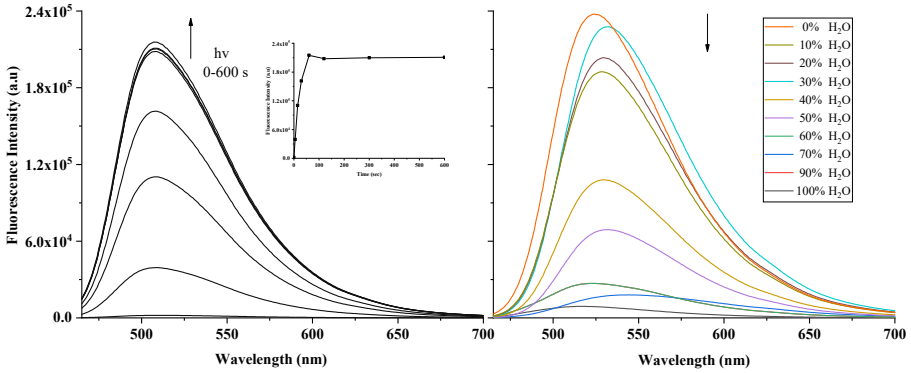



Fig. S2 (a)Fluorescence emission changes of NOD-C18 (10 μM) irradiated with UV light in THF, inset: time-dependent fluorescence intensity recorded after irradiation. (b) Fluorescence spectra of NOD-C18 (10 μM) in THF/water mixture with various water fractions

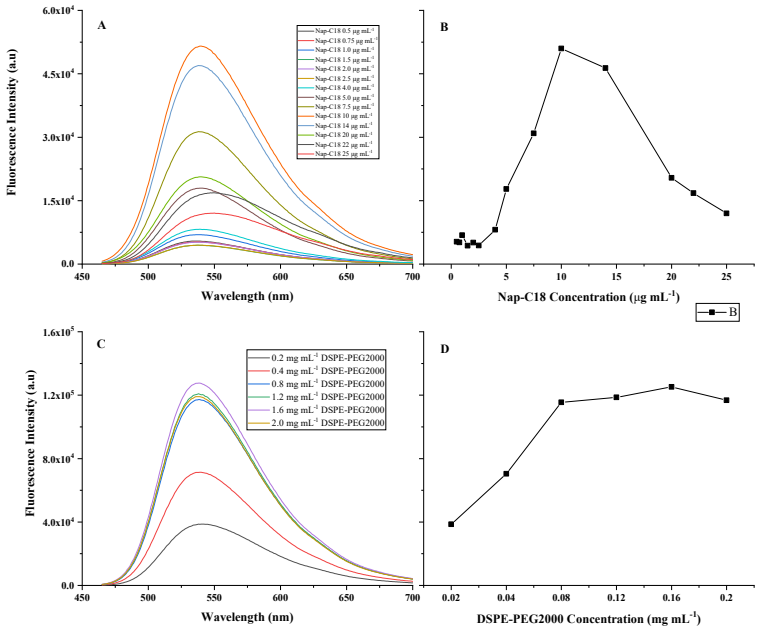



Fig. S3 (A, B) The fluorescence intensity changes of encapsulation with different concentrations of NOD-C18 by 2 mg $mL^{-1}$ DSPE-PEG2000. (C, D) The fluorescence intensity changes of 10 μg $mL^{-1}$ NOD-C18 encapsulated with different concentration of DSPE-PEG2000

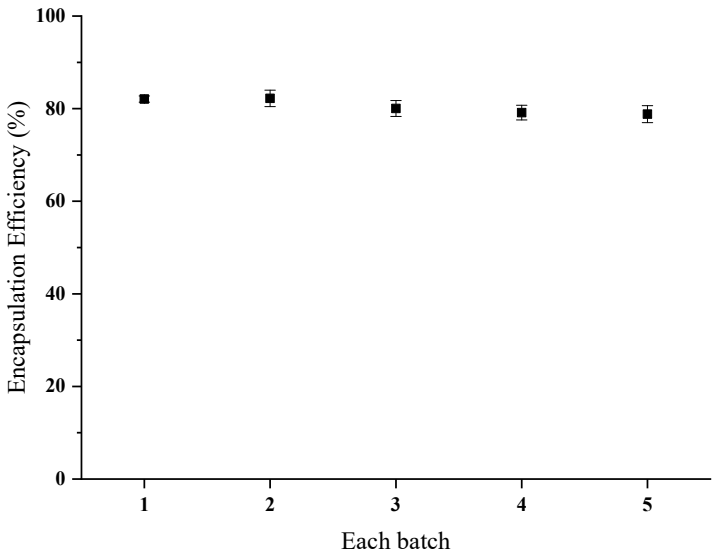



Fig. S4 The encapsulation efficiency of each batch of NOD-C18 with DSPE-PEG

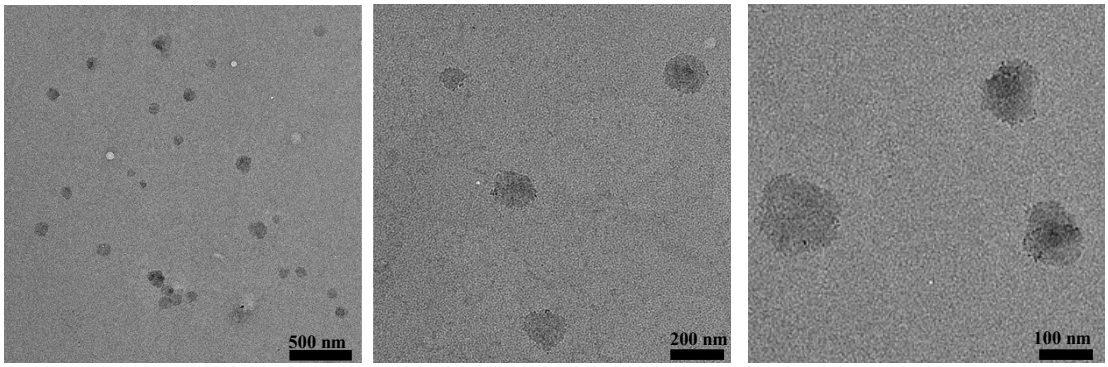



Fig. S5 The TEM images and f NOD-NP at different resolutions

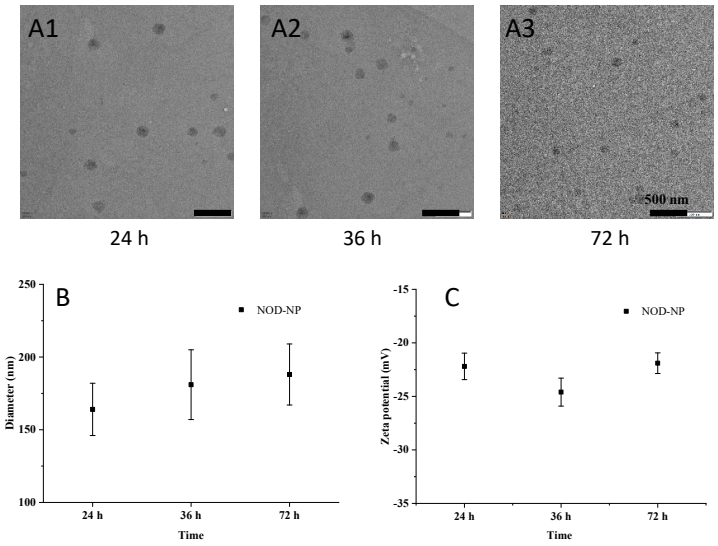



Fig. S6 The TEM images and DLS measurements of NOD-NP at different times. (A1-3) TEM images of NOD-NP (24-72 h)；(B) DLS diameter measurement of TEM images and DLS measurements of NOD-NP (24-72 h). (C) Zeta potential value of NOD-NP (24-72 h).

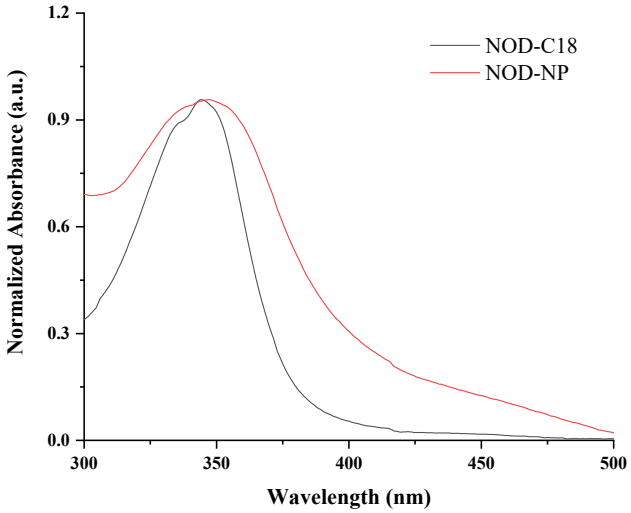



Fig. S7 the UV-vis absorption spectrums of NOD-C18 and NOD-NP

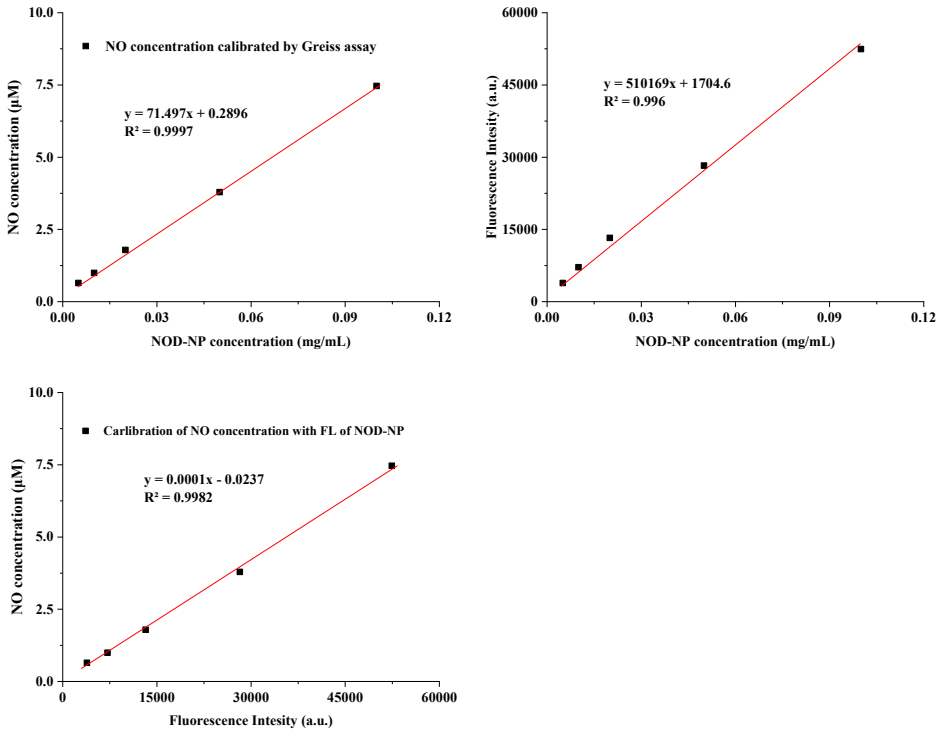
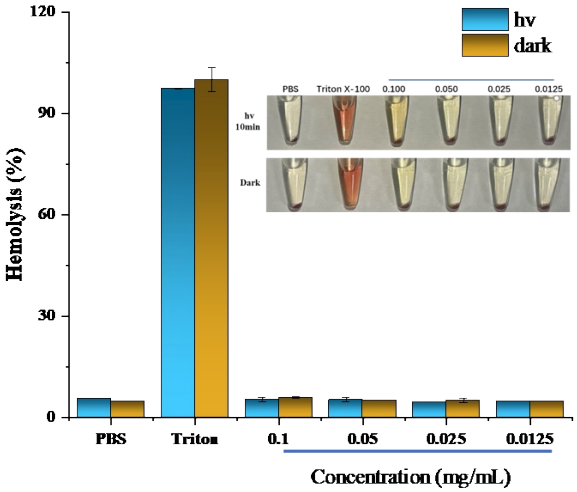



Fig. S8 (a)The NO concentration released from NOD-NP calibrated by Greiss assay; (b) the linear relation between fluorescence intensity and concentration of NOD-NP; (c) the self-calibrated relation with NO Concentration and Fluorescence intensity of NOD-NP.




x`

Fig. S9 The Hemolysis evaluation of different concentration of NOD-NP, and macroscopical imagines of rat hemocytes treated with PBS, Triton and NOD-NP in the dark or under irradiation.

## 4.Cells Assay

### 4.1 Cytotoxicity study

The cytotoxicity of **NOD-NP** and NOD-C18 was evaluated by an CCK-8 assay. Each well of a 96-well plate was inoculated with 200μL suspension of **NOD-NP** with various concentrations **(0.013, 0.25, 0.5 and 1 mg mL$^{-1}$.)** and RAW 264.7 cells ($5\times10^4$). After incubated for 48 h at 37°C and 5% $CO_2$, we processed the cells viability using the CCK-8 kit as per the manufacturer's instructions. Subsequently, the absorbance of each well at 450nm was measured by a multidetection microplate reader. The cytotoxicity of NOD-C18 with various concentrations **(1.6, 3.25, 6.5 and 13 mg mL$^{-1}$.)** was assessed using the parallel method.

### 4.2 Cell imaging of photo-controlled NO release from NOD-NP

RAW 264.7 cells were seeded in the 20 mm plastic-bottomed dishes containing 2 mL of DMEM and incubated at 37°C under 5% $CO_2$. After incubating overnight, the cells were incubated with DAPI (10 μM) and NOD-NP (0.08 mg/mL) for 30 min. Subsequently, the cells were washed with PBS and irradiated with UV (40 mW cm$^{-2}$, 365 nm) or blue light (40mW cm$^{-2}$, 450 nm) for different irradiation time. After that, the cellular images were taken with the confocal microscope (Leica SP5), at an $\lambda_{Ex}$ of 488nm and and $\lambda_{Em}$ of 510nm to 560 nm.

### 4.3 Cell Hemolysis Assay.

Blood collection were by abdominal aortic method in male SD rats. And anaesthesia was given using 10% chloral hydrate (0.2−0.3 mL/100 g IP). 20 mL of rat blood was centrifugated under 2000 r/min for 5 min to collect the RBCs. And the hemoglobin of the ruptured RBCs was washed with PBS three time. Then obtained RBCs were suspended in the 20 mL of PBS solution for used. Subsequently, 300 μL of PBS solution (pH 7.4) which containing different concentration of NOD-NP and 300 μL of RBCs suspension were mixed together followed by shaking for 12 h at 37 °C. 300 μL of Triton X -100 (1%) and 300 μL of PBS solution (pH 7.4) were respectively added into 300 μL of RBCs suspension to form the positive and negative group. After that, all the samples were

centrifugated (2000 r/min, 10 min) to collect supernatant and measured by the UV spectrophotometer.

**4.4 Imaging of NO release from NOD-NP in Bacterial**

The NO release from NOD-NP in *E. coli* and *S.aureus* was visualized by monitoring the fluorescence of Nap-C18. Briefly, *E. coli* suspension and *S.aureus* at a density of $10^7$ CFU/dish were respectively incubated with 200 μL of **NOD-NP** (0.08 mg/mL) for 2 h. Then, the bacterial were irradiated with UV (365 nm, 40 mW $cm^{-2}$) or blue light (450 nm, 40mW $cm^{-2}$) for different irradiation time following washed with PBS for several times, then fluorescence imaging was conducted by CLSM (Leica SP5), Excitation was at 488 nm, emission collection was from 510 - 560 nm.

**4.5 Antibacterial Activity**

The flat colony counting method was used to evaluate the antibacterial activity of **NOD-NP**. Briefly, *E. coli* and *S.aureus* were first cultured in LB medium at 37 °C overnight, then the bacteria suspension was diluted to $10^6$ CFU/mL after washed with PBS. 200 μL of **NOD-NP** (0.4, 0.8 and 1.6 mg/mL) were added into 200 μL of the diluted bacteria solution. After incubated for 1 h at 37 °C, all the bacteria mixture was irradiated with blue light (450 nm, 40mW $cm^{-2}$) for 1 h. Subsequently, 100 μL of the bacteria mixture were plated on LB and incubated for overnight at 37 °C. Finally, counting the bacterial colonies for the assessment of antimicrobial activity. PBS, **Nap-C18** (1.6 mg/mL), Kanamycin (50 mg/mL) and **NOD-NP** (1.6 mg/mL) served as control.

**4.6 Ablation Effect of Bacterial Biofilm In Vitro**

As for the biofilm eradication testing, we used Crystal violet (CV) staining assay to perform the quantitative changes after photo-controlled NO release from **NOD-NP**. To prepare the bacterial biofilm, the bacteria solution (*E. coli and S. aureus*) were first cultured in LB medium overnight, then 400 μL of diluted bacteria solution ($10^7$ CFU/mL) were added to a 24-well plate and cultured at 37 °C for 48 h under static conditions. 400 μL of fresh LB medium containing of NOD-NP (0.25, 0.05 and 0.1 mg/mL) were added into the 24-well plates and incubated at 37 °C for another 2 h, followed by 450 nm laser (40mW $cm^{-2}$) irradiation for 1h. NOD-NP solutions were removed and the residual biofilms were washed gently with the PBS after another 12 h incubation. Finally, 100 μL of 1% CV was stained with biofilms at 37 °C for 30min. The effect of biofilm eradication was photographed by a digital camera after cleaning with PBS three times. To detect quantitatively the residual biofilm, 150 μL of acetic acid (33%) were added into the samples and incubated at 37 °C for 15 min, and absorbance was measured at 595 nm using a multi-detection microplate reader.

## S5. Original NMR Spectra and HRMS Spectra.

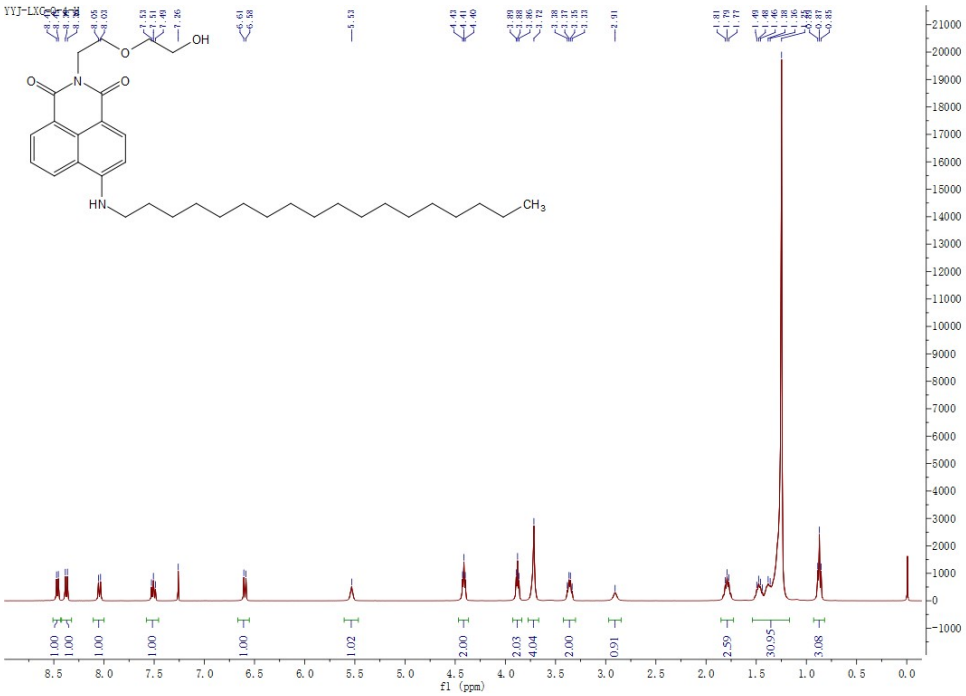



Fig. S10 The $^{1}$H NMR spectrum of Nap-C18.

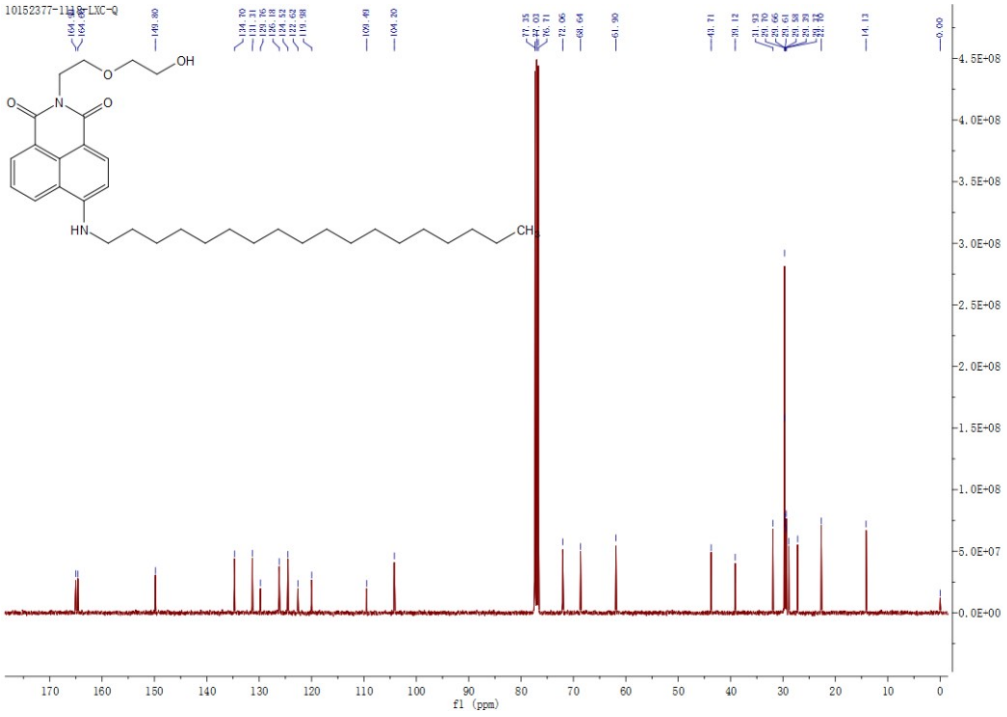



Fig. S11 F The $^{13}$C NMR spectrum of Nap-C18

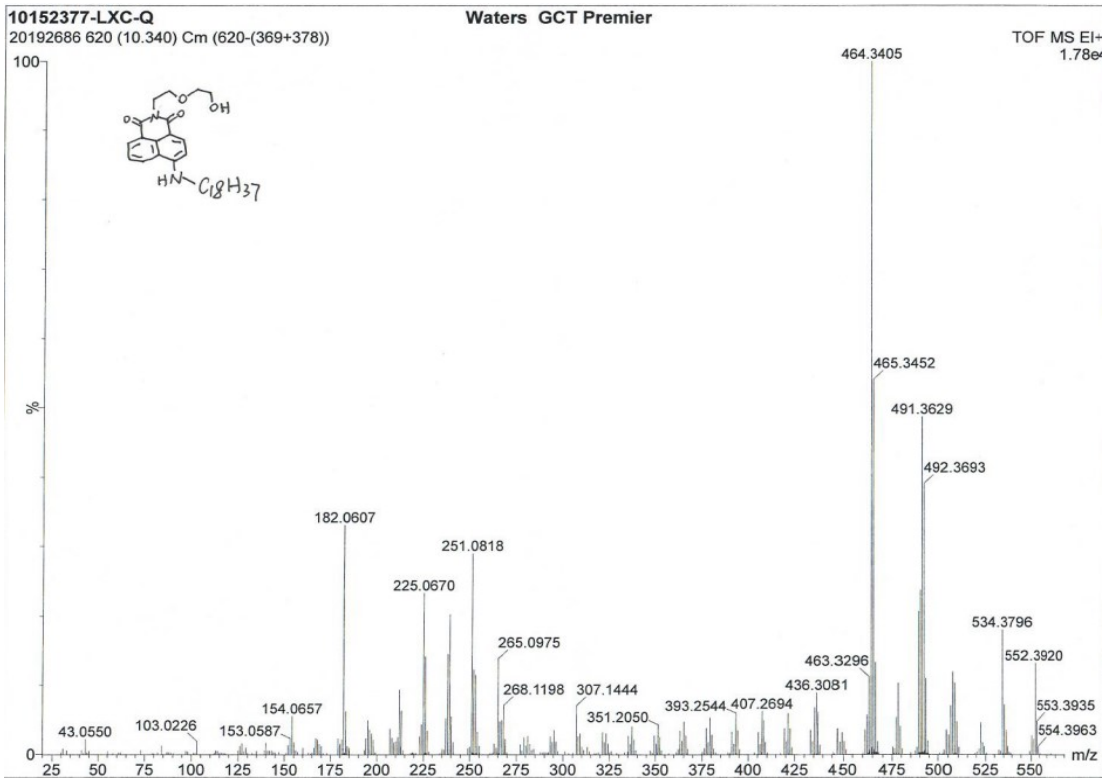



Fig. S12. The $^{1}$H NMR spectrum of compound Nap-C18

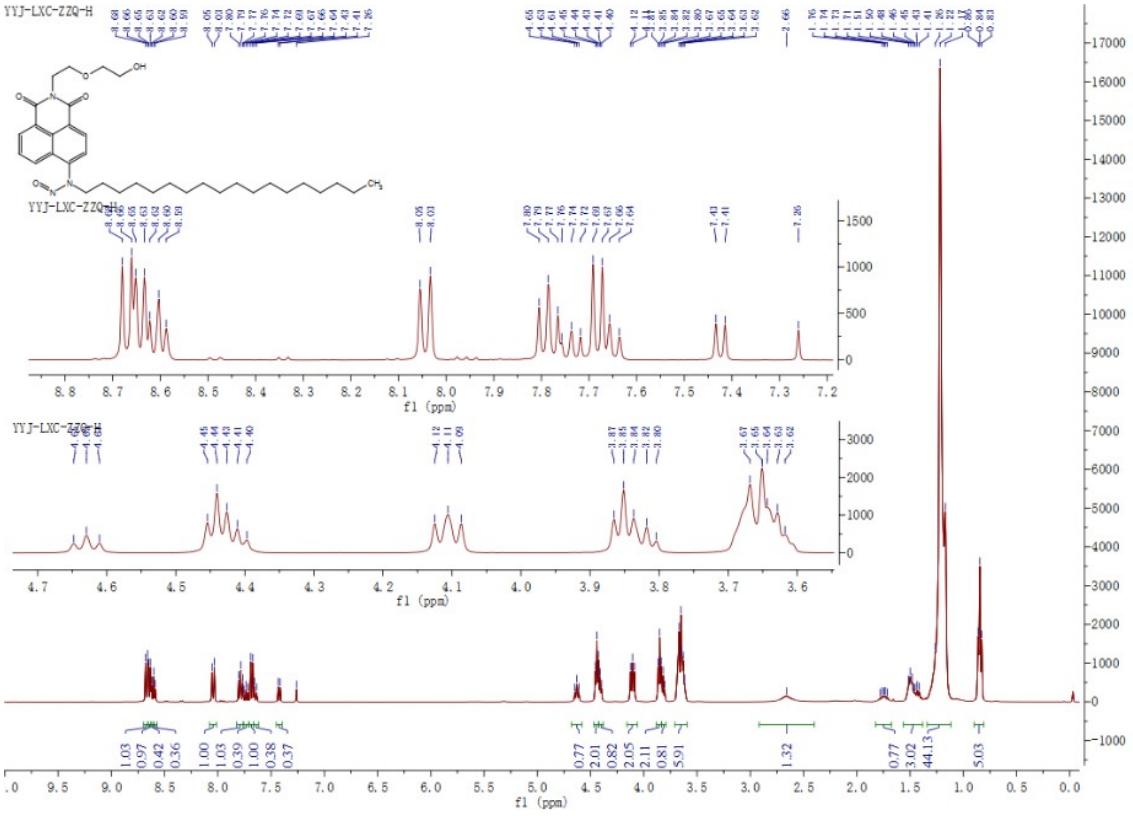



Fig. S13. The $^{1}$H NMR spectrum of compound NOD-C18.

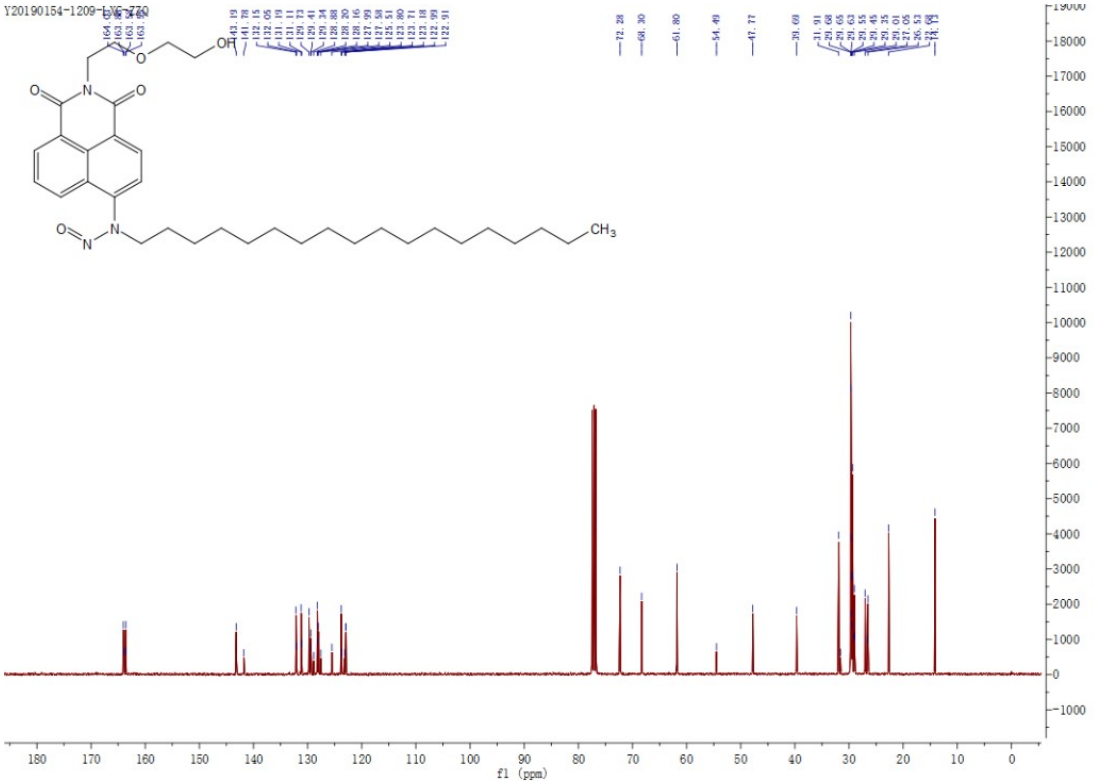



Fig. S14. The $^{13}C$ NMR spectrum of compound NOD-C18.

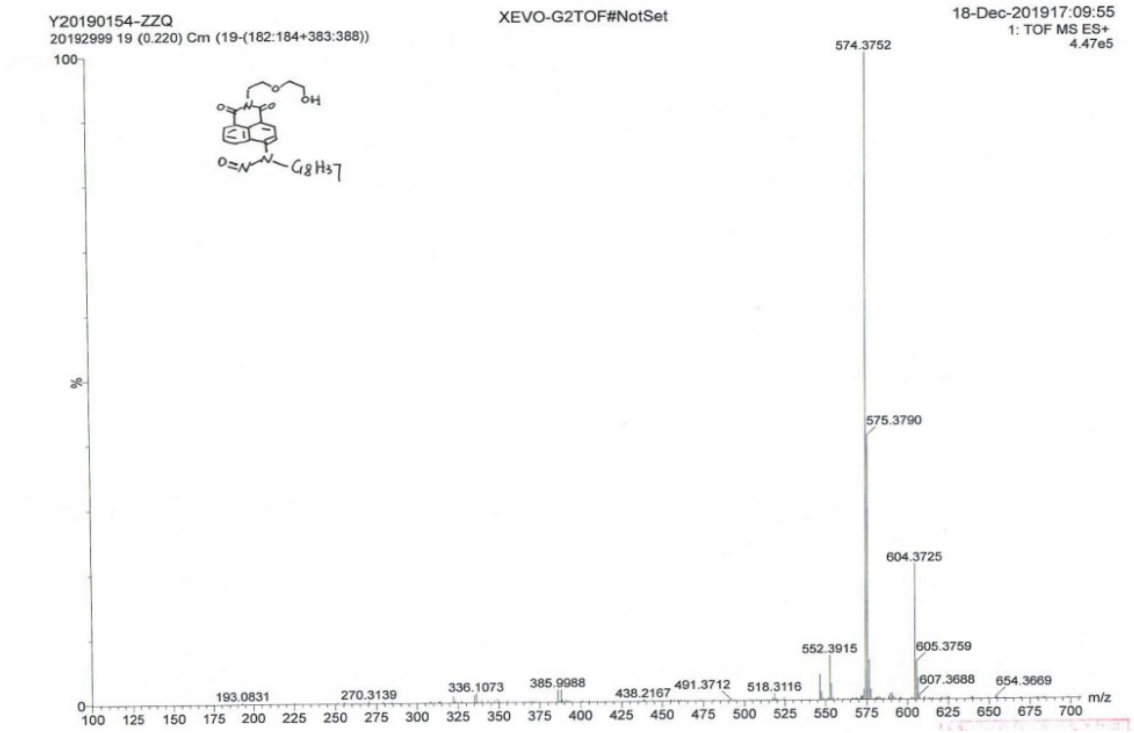



Fig. S15. HRMS spectrum of compound NOD-C18.