

Supporting Information

Optically-Controlled Supramolecular Self-assembly of Antibiotic for Antibacterial Regulation

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Experimental

Materials: All organic solvents were obtained from Beijing Chemical Works and used as received. 4-Phenylazobenzoyl Chloride was purchased from TCI. Norfloxacin was purchased from across. α cyclodextrin was purchased from J&K Scientific Ltd. All chemicals were used without further purification.

Measurements: The ¹H-NMR and ¹³C-NMR were taken from the Bruker Avance III 400MHz HD spectrometer. MS experiments were performed on an Impact HD Q-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany), operated in the positive ion mode. SEM images were collected by JSM-6700F. UV-Vis absorption spectra were determined on a Thermo Scientific Evolution 201 spectrophotometer.

The diameter was collected by Dynamic Light Scattering (Malvern, Nano ZS 90). The calorimetric measurement was taken on MicroCal VP-ITC apparatus at 298.15 K.

Synthetic route of azobenzene-norfloxacin (Azo-Nor): Norfloxacin (0.5 g, 1.5 mmol), triethylamine (0.167 g, 1.65 mmol) were completely dissolved in super dry dimethyl chloride (20 mL), and the reaction system was put into ice bath for 30 min. and the solvent of 4-phenylazobenzoyl chloride (0.919 g, 3.75 mmol) in CH₂Cl₂ (8 mL) was added to the above system dropwisely. The reaction was conducted overnight at room temperature. Water (20 mL) was added to stop the reaction and the mixture was washed with water for three times. Azobenzene-norfloxacin (0.394 g, 0.75 mmol) was purified by column chromatography (CH₂Cl₂/ ethyl acetate/methanol = 1/1/0.1, v/v/v) in yield of 49.8%. ¹H-NMR (400 MHz, CDCl₃, ppm): 8.70 (s, 1H), 8.02 (s, 1H), 7.91 (m, 3H), 7.62(d, 2H), 7.59 (m, 3H) 6.88 (d, 1H) 4.43 (m, 2H) 3.91 (d, 4H) 3.35 (m, 4H) 1.66 (m, 3H). ¹³C-NMR (100 MHz, CDCl₃, ppm): δ177.02, 169.85, 167.02, 154.50, 153.39, 152.48, 147.33, 145.62, 145.54, 137.03, 131.67, 129.22, 128.20, 123.09, 113.25, 113.07, 108.62, 104.34, 49.78, 14.52, 13.74.

Isothermal titration microcalorimetry (ITC) analysis:

In the following equations,

K = binding constant;

N = number of binding ratio

Θ = fraction of sites occupied by compound;

M_t and [M] are bulk and free concentration of bacterium;

X_t and [X] are bulk and free concentration of the repetitive unit of compound in V₀;

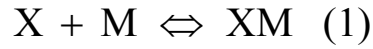
V₀ = the volume of active sample cell;

ΔV_i = injection volume;

Q = heat content;

Q_i = heat content from the completion of the i^{th} injection.

The binding process is described in a simplified fashion by equation (1).



In the process, the binding constant and the bulk concentration of the compound can

be expressed as follows:

$$K = \frac{\Theta}{(1 - \Theta)[X]} \quad (2)$$

$$X_t = [X] + N\Theta M_t \quad (3)$$

Combining equations (2) and (3) above gives:

$$\Theta^2 - \Theta \left[1 + \frac{X_t}{NM_t} + \frac{1}{NKM_t} \right] + \frac{X_t}{NM_t} = 0 \quad (4)$$

The total heat content Q contained in V₀ at fractional saturation Θ is:

$$Q = N\Theta M_t \Delta H V_0 \quad (5)$$

Solving the quadratic equation (4) for Θ and then substituting this into equation (5)

gives:

$$Q = \frac{NM_t \Delta H V_0}{2} \left[1 + \frac{X_t}{NM_t} + \frac{1}{NKM_t} - \sqrt{\left(1 + \frac{X_t}{NM_t} + \frac{1}{NKM_t} \right)^2 - \frac{4X_t}{NM_t}} \right] \quad (6)$$

The calculated heat released from the i^{th} injection, $\Delta Q_{(i)}$ can be expressed as:

$$\Delta Q(i) = Q(i) + \frac{dV_t}{V_0} \left[\frac{Q(i) + Q(i-1)}{2} \right] - Q(i-1) \quad (7)$$

The practical heat released is determined in the i^{th} injection. The fitting process ITC

data involves 1) initial guesses of N, K, and ΔH ; 2) calculation of $\Delta Q_{(i)}$ for each

injection and comparison of these values with the measured heat for the corresponding experimental injection; 3) improvement in the initial values of N , K , and ΔH by standard Marquardt methods; 4) iteration of the above procedure until no further significant improvement in fit occurs with continued iteration.

The characterization of photoisomerization behavior of Azo-Nor in DMSO: All irradiation experiments were performed with M-Ultra violet Light Source (365 nm, MUA-165) and M-Visual light source (white light, MVL-210). All the concentration of Azo-Nor was set as 25 μM when testing the absorption spectra. To measure the time of reaching the balance of *cis* state, the absorption spectra of Azo-Nor was tested each 10s upon exposed to 365 nm light irradiation at the intensity of 5mM/cm². By alternating the irradiation between 365 nm (1min, 5mM/cm²) and white light (1min, 10mM/cm²), the photoswitching cycles was obtained. The absorbance was measured at 325 nm. After exposed to 365 nm light (1min, 5mM/cm²), the half-life of thermal *cis-trans* isomerization was obtained by monitoring the absorbance at 325 nm at intervals of 30mins under dark condition. ¹H NMR was employed to study the ratio of *trans* and *cis* isomers changing in different situations. The concentration of Azo-Nor was set as 500 μM . Firstly, the ratio of *trans* and *cis* isomers in thermal state was tested by ¹H NMR. Under the irradiation of 365 nm (1min, 5mM/cm²), the Azo-Nor transferred to 365 nm thermodynamically photostationary state, then measured the ¹H NMR to obtained the ratio of *trans* and *cis* isomers. Upon the irradiation of white light (1min, 10mM/cm²), the ratio of *trans* and *cis* isomers in white light

thermodynamically photostationary state was also obtained through ^1H NMR.

Bacterial strains and growth solutions: The bacterial strains were *S. aureus* (ATCC6538) and *E. coli* (Top 10). A single colony of *S. aureus* were grown in Luria-Bertani (LB) at 37 °C. *E. coli* were grown in Nutrient Broth (NB) medium supplemented with required antibiotic, 10 µg/ml penicillin at 37 °C.

Antibacterial experiments of Azo-Nor/ α CD and Azo-Nor/ α CD (1/1): After the overnight growth of bacterial, *S. aureus* and *E. coli* were diluted to an OD₆₀₀ of 0.1 and 100 µL of solution was added to 96-well plate.^[1] To test the antibacterial activity after photo-activation, the solutions containing antibiotics were irradiated at 365 nm firstly for 10 min before adding to the cell suspension. The mixture solution of antibiotic and bacterial were grown in a microtiter plate at 37°C under dark condition. The cell density (650 nm) was measured after 12 h with a 10 seconds shaking step before each measurement in a microplate reader. The background graphs were corrected by subtracting the OD₆₅₀ at time 0.

Reference

- [1] W. A. Velema, M. J. Hansen, M. M. Lerch, A. J. M. Driessen, W. Szymanski, B. L. Feringa, *Bioconjugate Chem.* **2015**, 26, 2592-2597.

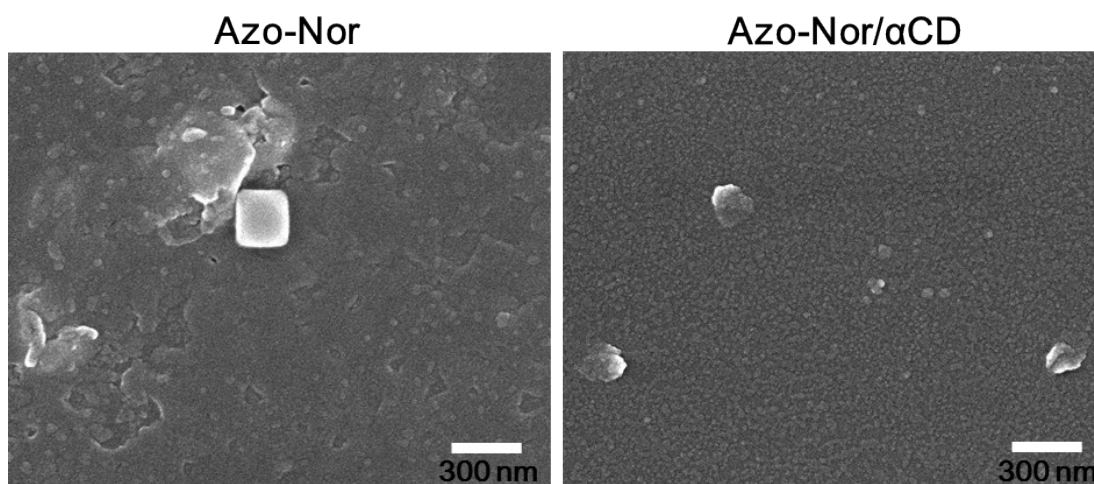


Figure S1. SEM images of Azo-Nor and Azo-Nor/ α CD.

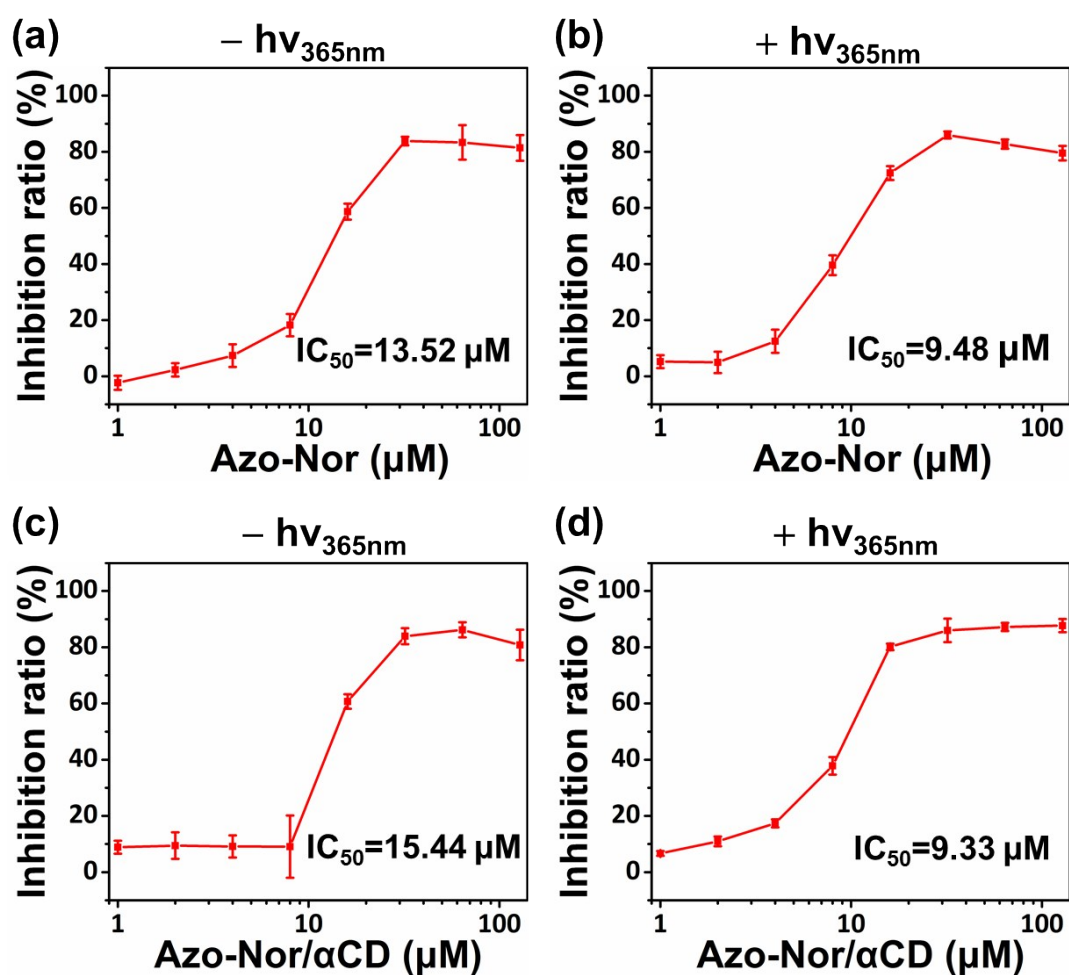


Figure S2. (a-d) Growth rates of *E. coli* at increasing concentrations of Azo-Nor and Azo-Nor/ α CD before and after irradiation.

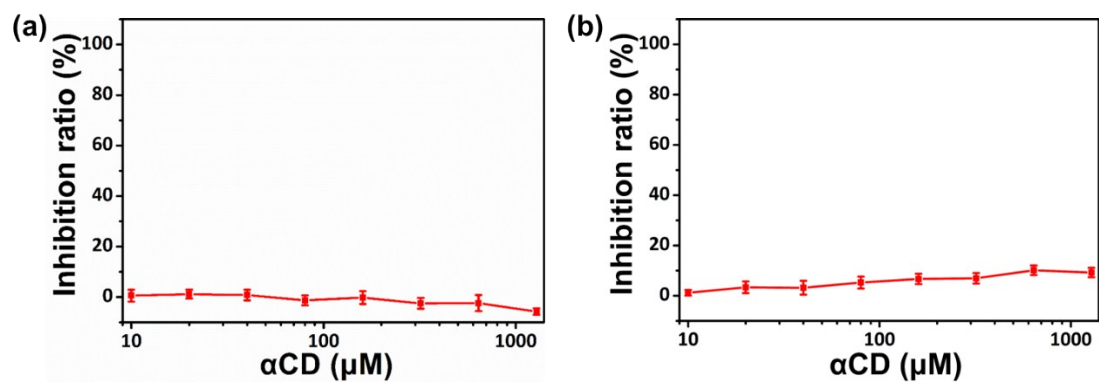


Figure S3. (a) Growth rates of *S. aureus* at increasing concentrations of αCD and IC₅₀ value. (b) Growth rates of *E. coli* at increasing concentrations of αCD and IC₅₀ value.

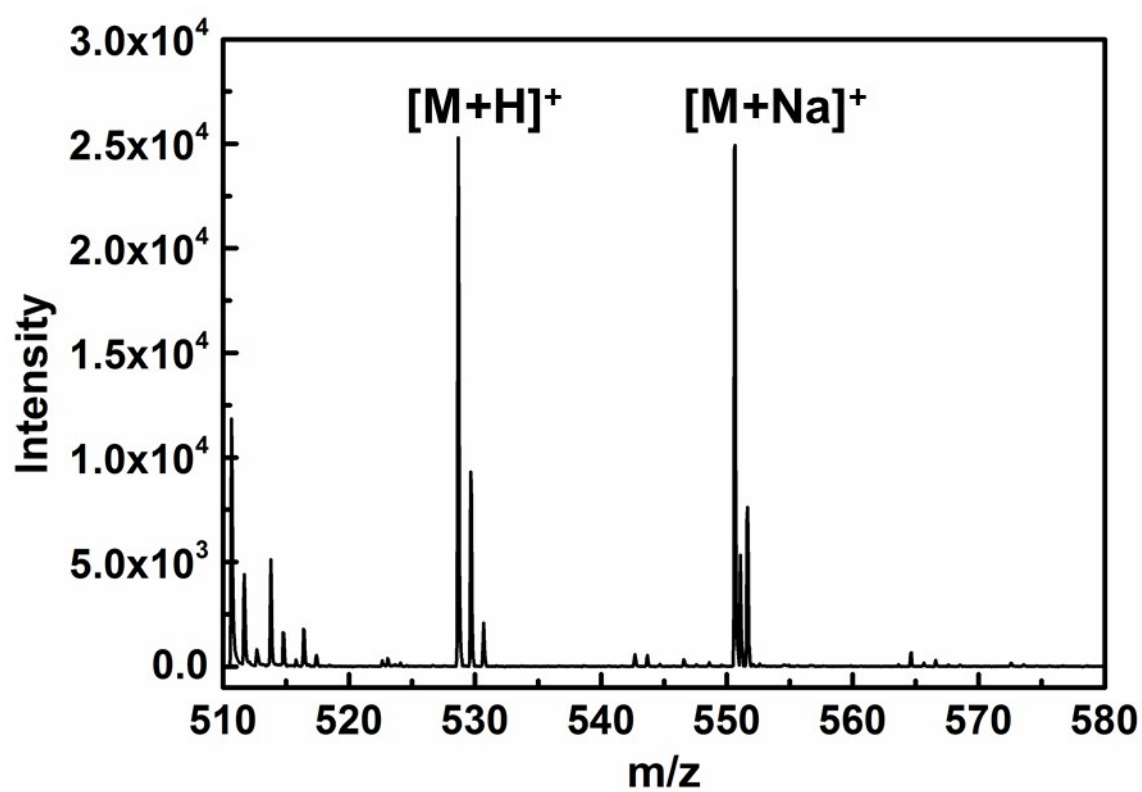


Figure S4. Mass spectrum of azobenzene-norfloxacin.