Electronic Supplementary Information

Near Infrared-Caged D-Amino Acids Multifunctional Assembly for Simultaneously Eradicating Biofilm and Bacteria

1. Preparation of UCNPs (NaYF4:Yb,Tm)

UCNPs were prepared by a solvothermal process using polyvinylpyrrolidone (PVP, K-30) as the chelating agent. All the chemical reagents were used as received without further purification. To obtain rare earth (RE) chlorides, 1 mmol of rare earth oxides Y_2O_3, Yb_2O_3, and Tm_2O_3 with a stoichiometric ratio of 79.5:20:0.5 were dissolved in hydrochloric acid, and then the solution was heated to evaporate the water completely. In a typical synthesis procedure, 1 mmol of RE chlorides with a stoichiometric Y/Yb/Tm ratio of 79.5:20:0.5 was dissolved in 10 mL of ethylene glycol (EG) under vigorous stirring. PVP and NaCl were subsequently added, and the solution was heated to 80 °C to form a homogeneous solution. Another 10 mL of EG containing 4 mmol of NH4F was added dropwise to the above solution under constant stirring until a transparent solution was formed. The above precursor solution was transferred to a 40 mL autoclave, sealed, and maintained at 180 °C for 12 h. After the autoclave was cooled to room temperature naturally, the product was collected by centrifugation and washed with distilled water and ethanol several times, then dried at 80 °C in air.

2. Preparation of UCNP@TiO_2

Titania-coated $NaYF_4$:Yb,Tm particles were prepared through controlling hydrolysis and condensation of a titanium alkoxide in ethanol. A typical procedure for coating titania onto UCNPs, has been described as follows:UCNPs were dispersed in ethanol containing a small trace of Lutensol ON50 aqueous solution. After that, titanium alkoxide dispersed in alcohol was added to the seed particle solution with a controlling speed upon rigorous stirring. The reaction was conducted at room temperature for 20 h. To produce crystalline titania shells, the above precursor solution was transferred to a 40 mL autoclave, sealed, and maintained at 160 °C for 6 h. The resulting product was collected by centrifugation and washed with distilled water and ethanol several times, then dried at 80 °C in air.

3. Strains and media.

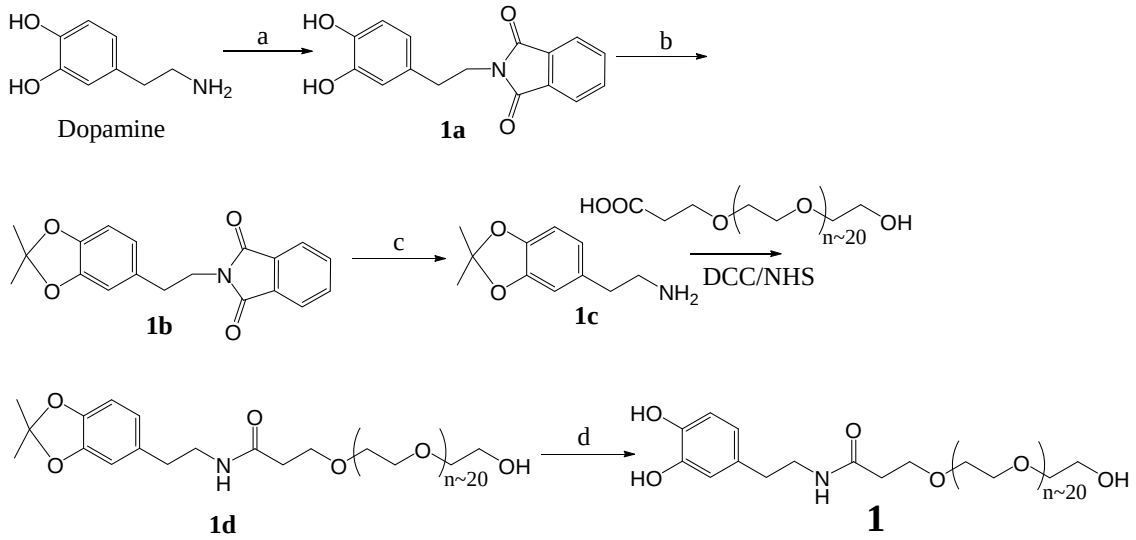
Bacillus subtilis NCIB3610 was grown in Luria-Bertani (LB) medium at 37°C or MSgg medium at 23°C. Solid media contained 1.5% Bacto agar. When appropriate, antibiotics were added at the following concentrations for growth of *B. subtilis*: 10 μg per ml of tetracycline, and 5 μg per ml of erythromycin, 500 μg per ml of spectinomycin.

4. Preparing conditioned medium.

Cells were grown in LB medium to exponential phase. 0.1 ml of culture was added to 100 ml of MSgg medium and grown without shaking in a 500 ml beaker at 23°.

5. Synthesis of compounds 1 and 2

Compounds **1** and **2** were synthesized according to the following routes. (Ref: Tetrahedron Letters 51 (2010) 2403.)



Reagents and conditions: a, *N*-carbethoxyphthalimide, Et_3N; b, DMP, TsOH (4.5%), benzene, reflux; c, H_2NNH_2, DCM; d, 25% TFA in $CHCl_3/H_2O$.

Synthesis of **1a**: To a 500 mL flask were added N-carbethoxyphthalimide (11.0 g, 50 mmol), methanol (300 mL), dopamine hydrochloride (9.0 g, 50 mmol), and a magnetic stirring bar. The mixture was degassed with argon for 30 min, followed by addition of triethylamine (28 mL, 200 mmol). The mixture was stirred at room temperature overnight. After the volatile solvents were reduced by rotary evaporation, the residue was treated with 1M HCl and the mixture was extracted with ethyl acetate. The organic layer was washed with 1N HCl and water, dried over $MgSO_4$, and evaporated to give a yellow solid, which was recrystallized in EtOAc/hexane to give pale yellow crystals.

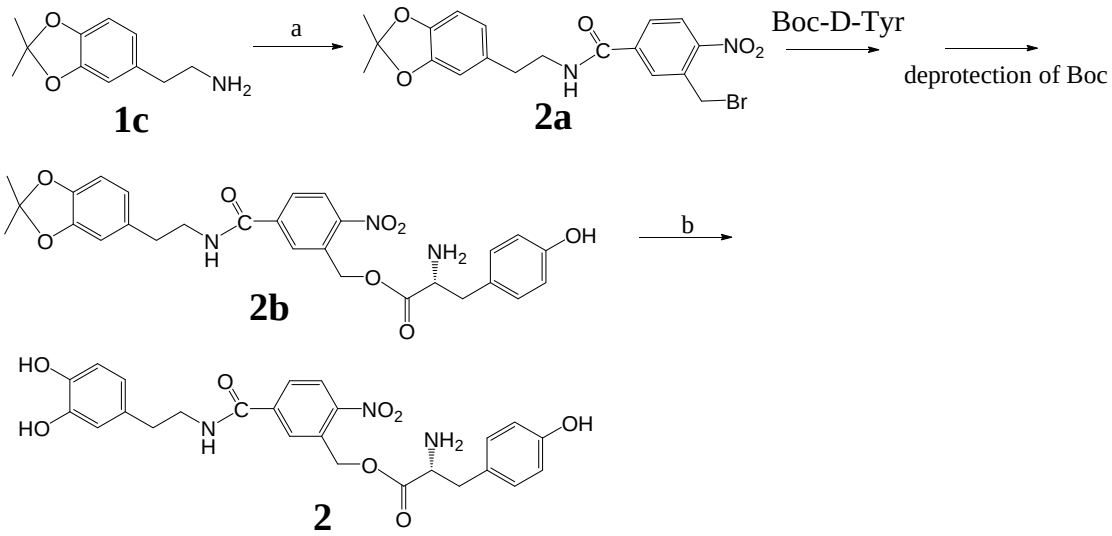
Synthesis of **1b**: To a two-neck 250 mL flask were added **1a** (5 g, 20 mmol), DMP (8.9 mL, 4 equiv), and anhydrous benzene (200 mL). One neck of the flask was fitted with a Soxhlet extractor, the thimble of which was filled with 28 g of granular anhydrous $CaCl_2$; the other neck

of the flask was sealed with a septum for sampling purpose. After the system was degassed with argon for 5 min and then heated to reflux for another 5 min, *p*-toluenesulfonic acid monohydrate (134 mg, 4.0 mol%) was added. The reaction progress was monitored by $FeCl_3$ test. Once a negative test result was achieved, usually in 1-2 h, the reflux was stopped. After cooled down, the mixture was filtered through a short silica-gel column, which was washed with DCM. The combined filtrate and washings were evaporated to produce a light yellow solid, which was recrystallized in DCM/hexane to give white crystals.

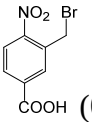
Synthesis of **1c**: To a 250 mL flask were added **1b** (1.62 g), methanol (50mL), DCM (50 mL), and hydrazine hydrate (2.5 mL, 10 equiv). The mixture was stirred at room temperature for 3 days. The white precipitate was removed by filtration and washed with DCM. The combined filtrate was concentrated under reduced pressure. After addition of another portion of DCM (50 mL), the mixture was stirred for another day. White precipitates were filtered off and the combined precipitate was dried and measured (ca. 0.85g). The filtrate was concentrated and dried under vacuum to produce a light yellow oil.

Synthesis of **1d**: To a 100 mL flask were added **1c** (0.48 g, 2.5 mmol), ethanol 100 ml, and DCC (5 mmol), NHS (2.6 mmol), and HOOC-PEG_{20}-OH (2.2 g, ca. 2.5 mmol). The mixture was stirred at room temperature for 24 h. The white precipitate was removed by filtration. The filtrate was concentrated and dried under vacuum.

Synthesis of **1**: To a 50 mL flask sealed with a septum, were added 20 mL degassed ethanol, 2.5 mL TFA, and 0.5 mL water. After the flask was flushed with argon for 2 min, **1d** (1 g) was added with a syringe. The mixture was stirred for 3 h at room temperature. The volatile solvents were removed under vacuum and a light brown residue was obtained, which was re-dissolved in ethanol and stored in freezer.

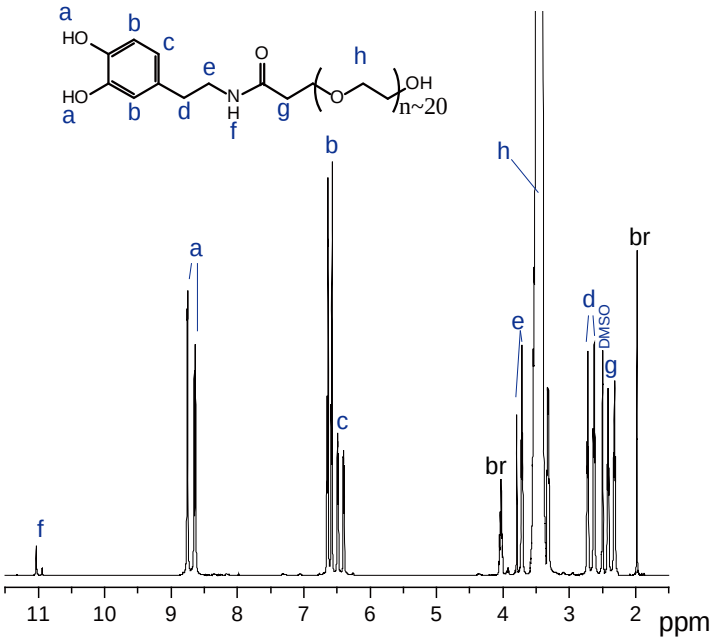


Reagents and conditions: a,  , EDC/NHS, Et_3N, acetonitrile; b, 25% TFA in $CHCl_3/H_2O$.

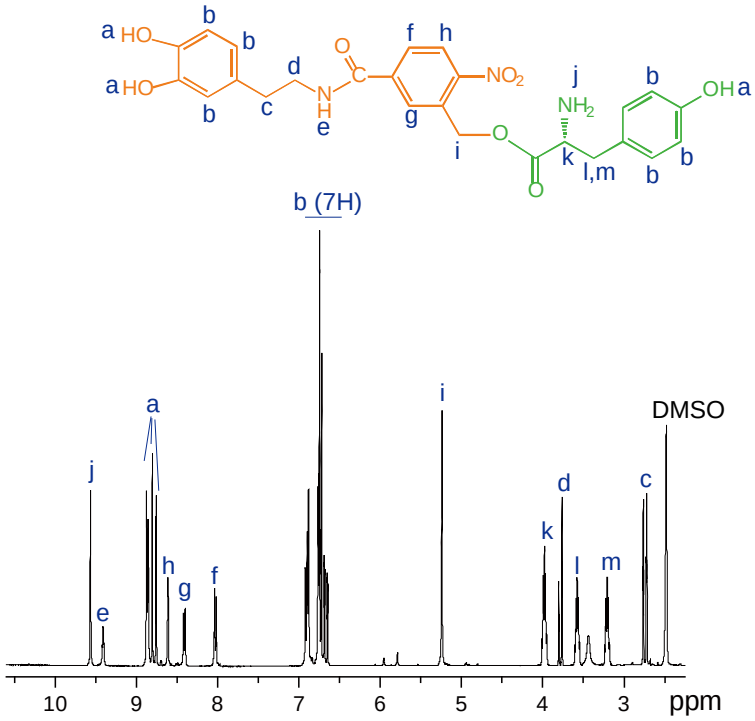
Synthesis of **2a**: To a 100 mL flask were added **1c** (0.48 g, 2.5 mmol), ethanol 100 ml, and EDC (5 mmol), NHS (2.6 mmol), and  (0.65 g, 2.5 mmol). The mixture was stirred at room temperature for 24 h. The white precipitate was removed by filtration. The filtrate was concentrated and dried under vacuum.

Synthesis of **2**: The **2a** was firstly coupled to Boc-D-Tyr under general basic conditions (Et_3N). After deprotection of Boc, the product was further deprotected via the procedure of the synthesis of **1**.

^{1}H NMR (DMSO-d_6) spectra of **1**:



^{1}H NMR (DMSO-d_6) spectra of **2**:



Supporting Figures

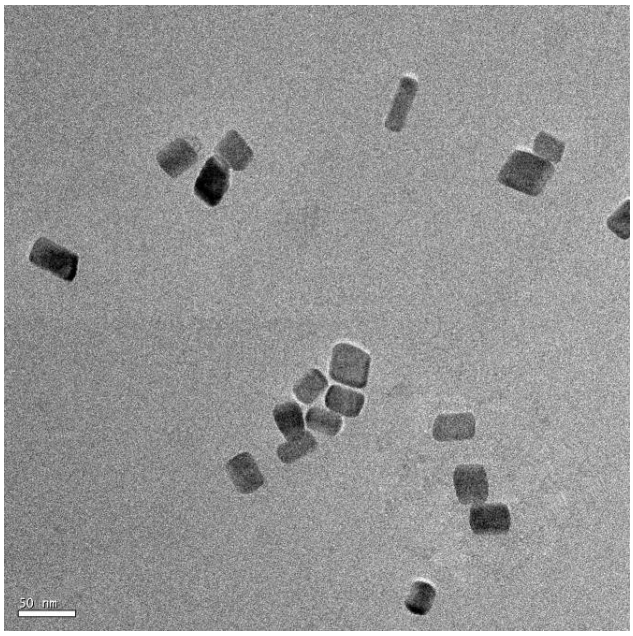


Fig. S1 Transmission Electron Microscopy (TEM) image of the UCNPs (β-$NaYF_4$:Tm^{3+} 0.5 mol%, Yb^{3+} 30 mol%).

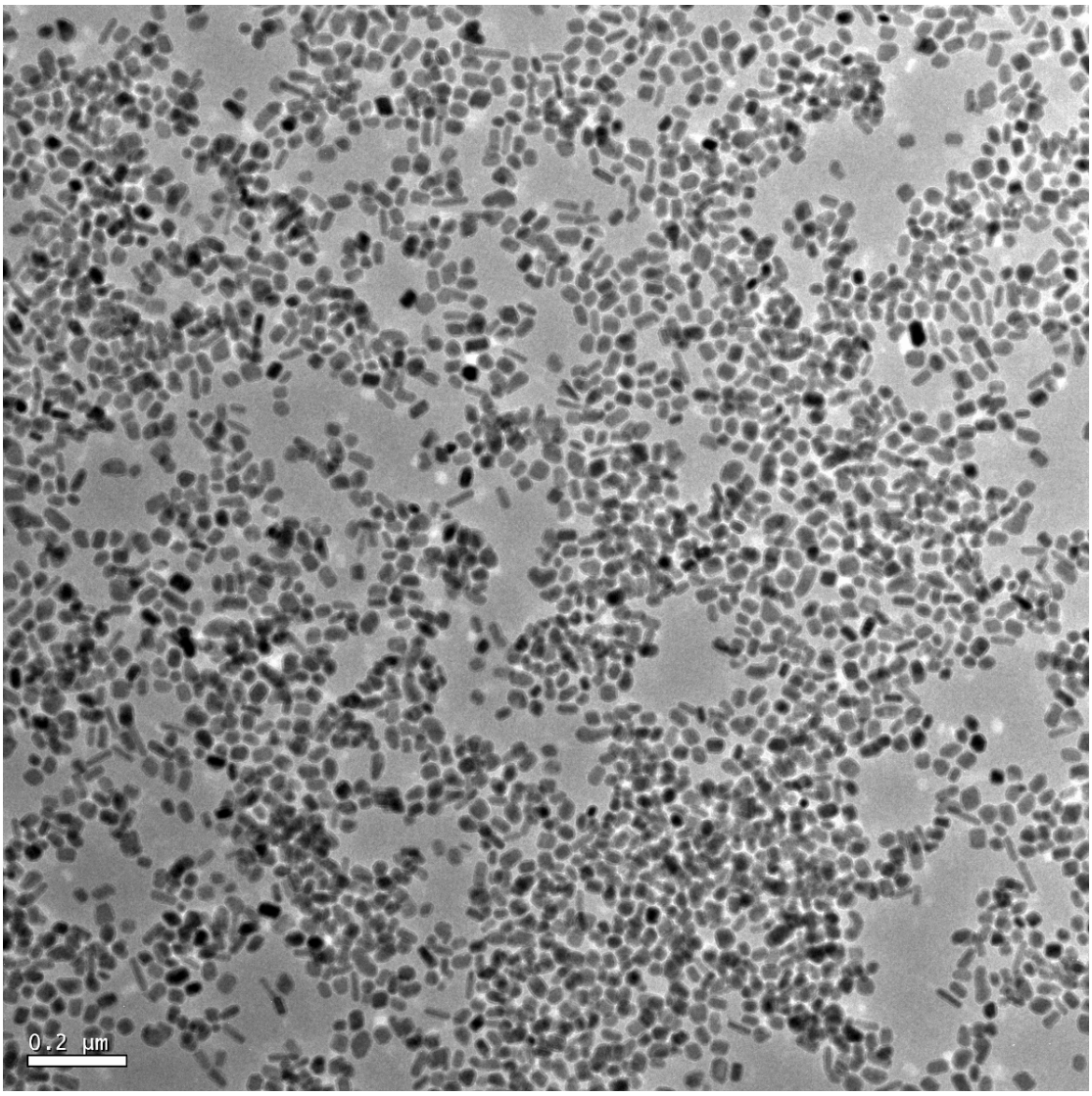


Fig. S2 large scale TEM image of the UCNP@TiO_2.

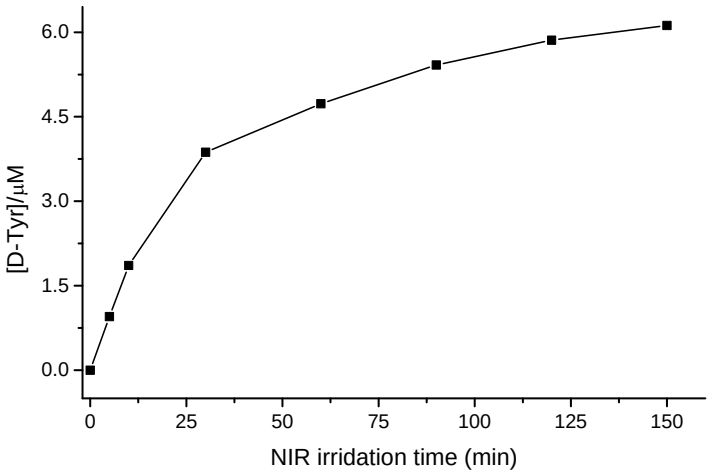


Fig. S3 Time course monitoring of the release of free D-Tyr from UCNP@TiO_2-D-Try under the irradiation of NIR diode laser (5 W).

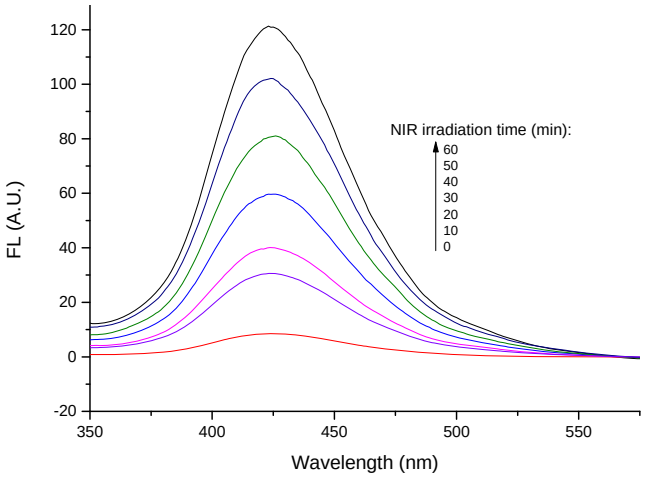


Fig. S4 Time course monitoring of the production of ROS •OH UCNP@TiO_2-D-Try under the irradiation of NIR diode laser (5 W).

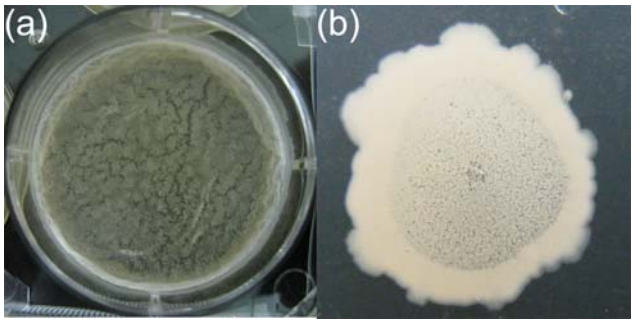


Fig. S5 *B. subtilis* biofilms at (a) the air/liquid interface of standing cultures, and (b) semi-solid surface treated with SiO_2@TiO_2-D-Try + NIR irradiation.

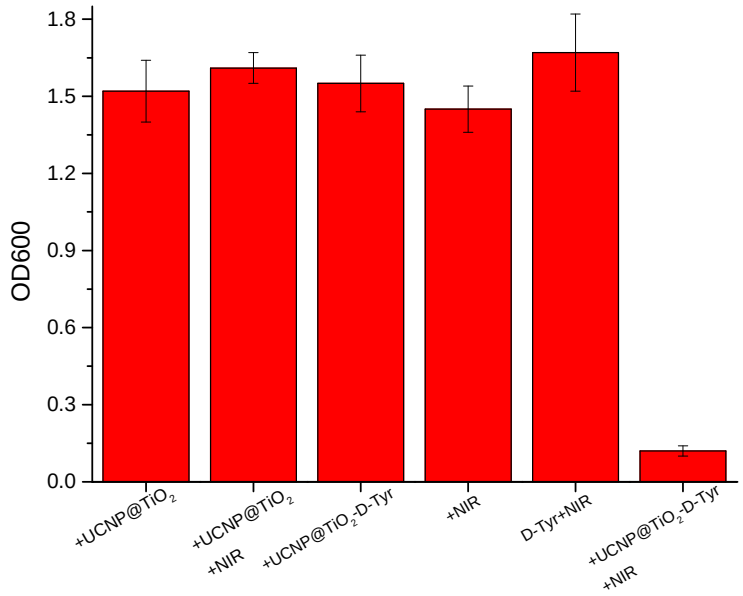


Fig. S6 Live or death assay of the cells within the biofilms after different treatments. After treatment of the biofilms, 10 μL of each biofilm matrix (under the surface) was further cultured in LB medium for 12 h, and the absorbance of each one was then measured und 600 nm. If the bacteria were killed, the OD600 was maintained a low value.