Electronic Supplementary Information

Highly Efficient Photothermal Nanoparticles for Rapid Eradication of Bacterial Biofilms

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Experimental Section

Materials and Instruments.

All chemicals were commercially available and used as supplied without further purification. Deuterated solvents were purchased from J&K. TEP-$B(OH)_2$ was purchased from AIEgen Biotech Co., Ltd. Tetrahydrofuran (THF) was dried by distillation using sodium as drying agent and benzophenone as indicator. Compounds **2**, **3**, **4**, 2TPE-PDI-C8 and 2TPE-PDI-C16 were synthesized according to the published procedures. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ARX 400 NMR spectrometer using tetramethylsilane (TMS; $\delta = 0$) as internal reference. High-resolution mass spectra (HRMS) were obtained on a Finnigan MAT TSQ 7000 Mass Spectrometer operated in a MALDI-TOF mode. Absorption spectra were measured on a JASCO V-570 UV-vis-NIR spectrophotometer. Steady-state photoluminescence (PL) spectra were recorded on an Edinburgh FLS980 fluorescence spectrophotometer. Quantum yield was determined by a Quanta-φ integrating sphere. Particle size analyses were implemented using a ZetaPlus Potential Analyzer (Brookhaven, ZETAPLUS). Transmission electron microscopy (TEM) investigations were carried out on a JEOL-6390 instrument. 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma-Aldrich and used as received. For cell culture, Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), penicillin, streptomycin solution, Live/Dead Baclight™ bacterial viability kit was purchased from Thermo Fisher. Laser confocal scanning microscope images were collected on Zeiss laser scanning confocal microscope (LSM 710) and analyzed using ZEN 2009 software (Carl Zeiss). Single crystal data was collected on a Bruker Smart APEXII CCD diffractometer using graphite monochromated Cu Kα radiation (λ = 1.54178 Å).

Syntheses of 2TPE-2NDTA-02

Synthesis of 3. Compound 2 (NDTA) (117 mg, 0.1 mmol) and Br_2 (160 mg, 0.11 mmol), were dissolved in chloroform (8 mL) under atmosphere and reacted at room temperature for 0.5 h. The reaction was terminated by adding water and extracted with dichloromethane and then purified by silica-gel column chromatography, affording compounds 2 46 mg. yield:37%.

Compound 3. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.67 (s, 1H), 4.15 (dd, *J* = 7.6, 3.9 Hz, 4H), 2.00 (d, *J* = 6.5 Hz, 2H), 1.24 (d, *J* = 6.0 Hz, 64H), 0.86 (d, *J* = 4.2 Hz, 12H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 164.37, 162.10, 161.97, 161.63, 115.76, 115.50, 114.17, 85.36, 70.95, 46.18, 46.08, 36.51, 36.46, 31.95, 31.91, 31.52, 30.11, 30.07, 29.70, 29.67, 29.64, 29.58, 29.38, 29.35, 26.35, 22.70, 14.13.

Synthesis of 4. The compound **3** (150 mg, 0.06 mmol), TPE-$B(OH)_2$ (113 mg, 0.3 mmol), K_2CO_3 (66 mg, 0.48 mmol) and $Pd(PPh_3)_4$ (7 mg, 0.006 mmol) were mixed in THF (10 mL) and deoxygenated H_2O (3 mL) under N_2. The mixture was stirred overnight at 100 °C under N_2. After cooling to room temperature, the mixture was extracted with CH_2Cl_2 and the organic solvent was removed under reduced pressure. The crude product was purified by column chromatography to afford pure 2TPE-2NDTA (79 mg) in a yield of 44%. (Melting point: 299.2−301.2 °C).

Compound 4. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.52 – 6.97 (m, 19H), 5.59 (s, 1H), 4.15 (d, *J* = 7.5 Hz, 4H), 2.01 (d, *J* = 11.5 Hz, 2H), 1.42 – 1.19 (m, 64H), 0.85 (t, *J* = 6.6 Hz, 12H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 162.11, 139.77, 132.33, 131.47, 131.38, 131.30, 128.01, 127.94, 127.72, 127.01, 126.80, 126.63, 124.93, 84.56, 36.46, 36.35, 31.95, 31.92, 31.88, 31.51, 30.08, 30.05, 29.70, 29.67, 29.64, 29.60, 29.55, 29.36, 29.32, 26.35, 22.70, 22.68, 14.13.

Synthesis of 5. To a solution of compound **2** (200 mg, 0.16 mmol) in anhydrous dimethylformamide (15 mL) and dimethyl sulfoxide (3 mL), $Pd(PhCN)_2Cl_2$ (3.1 mg, 0.008 mmol), $AgNO_3$ (81.6 mg, 0.48 mmol) and KF (27.8 mg, 0.48 mmol) were added successively under N_2. The reaction mixture was stirred at 120 °C for 8h under N_2. After cooling to room temperature, saturated NH_4Cl (aq) was added to the mixture and the precipitated product was filtered and collected. The crude product was purified by column chromatography using Hex/DCM (2/3, v/v) to afford pure **3** (159

mg) in a yield of 80%. MS (MALDI-TOF): *m*/*z*: [M]+ calcd for C136H196Br2N8O8S8, 2487.4; found, 2487.3. Elemental analysis: calcd, C: 65.67%, H: 7.94%, N: 4.50%; found, C: 65.39%, H: 7.82%, N: 4.31%.

Compound 5. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.49 – 7.00 (m, 38H), 4.20 (dt, *J* = 34.1, 7.0 Hz, 8H), 2.04 (s, 4H), 1.35 – 1.09 (m, 128H), 0.84 (dd, *J* = 12.4, 5.6 Hz, 24H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 162.30, 145.20, 143.24, 143.10, 132.34, 131.44, 131.37, 131.29, 127.99, 127.92, 127.72, 127.01, 126.80, 126.69, 125.13, 46.15, 31.93, 31.90, 31.87, 30.08, 30.03, 29.68, 29.66, 29.63, 29.60, 29.55, 29.34, 29.27, 26.39, 26.32, 22.70, 22.68, 22.66, 14.15, 14.12, 1.03. MS (MALDI-TOF): *m*/*z*: $[M]^+$ calcd for $C_{172}H_{202}N_8O_8S_8$, 2766.04; found, 2766.23.

Fabrication of AIE NPs

To a THF solution (1 mL), 2TPE-2NDTA-02 (1 mg) was dissolved. Then, DSPE-PEG_{2000} (5 mg) was also added and dissolved in the THF solution. Afterward, the obtained THF solution was added into water (9 mL) accompanied with sonication using a microtip probe sonicator (XL2000, Misonix Incorporated, NY), followed by continuative sonication of the mixture for another 60 s. The THF in the mixture was evaporated by stirring in a fume hood for 12 h. The resulting NP suspension was purified by ultrafiltration (molecule weight cutoff 100 kDa) at 3000×g for 0.5 h, which was subsequently filtered with a 0.22 μm syringe-driven filter.

Photothermal Stability Studies

For photothermal stability studies, the water suspension of 2TPE-2NDTA-02 NPs were irradiated under an 808 nm laser (1 W cm^{-2}), and the absorption spectra were measured at different time points. For anti-photobleaching study, the temperatures of the sample solutions were recorded during five circles of heating and cooling progresses. In one heating-cooling circle, the NIR laser was first used to irradiate the samples for 5 min to reach a steady state, then the laser was removed, and the samples were naturally cooling down to ambient temperature in 10 min.

Photothermal Performance.

The water suspension of 2TPE-2NDTA-02 NPs were continuously exposed to an 808 nm NIR laser (1 W cm^{-2}) for 5 min. The temperature was measured every 30 s using a digital thermometer with a thermocouple probe and stopped until the temperature

nearly reached a plateau. The corresponding IR thermal images of the sample tubes were also recorded.

Photothermal Conversion Efficiency Determination

To evaluate the photothermal conversion efficiency (η), the temperature variations of 2TPE-2NDTA-02 NPs aqueous solution (100 μg mL^{-1}) were recorded upon 808 nm laser irradiation (1 W cm^{-2}) for 10 min to achieve the maximum plateau of temperature, and the 808 nm laser was turned off for natural cooling to room temperature. Thus, η can be calculated referring to the following equation:

$$\eta = \frac{hS(T_{Max} - T_{Surr}) - Q_{Dis}}{I(1 - 10^{-A_{808}})}$$

Where h indexes the heat transfer coefficient; S represents the surface area of the used holder; T_{Max} and T_{Surr} denote the maximum steady-state temperature and room temperature of the ambient environment, respectively; Q_{Dis} is the heat wastage form the light loss of the solvent and holder, and the Q_{Dis} was determined using pure water; I indexes laser intensity (1 W cm^{-2}), and A_{808} represents the 808 nm absorbance of NIR NPs. hS can be calculated referring to the following equation:

$$\tau_S = \frac{m_D C_D}{hS}$$

Where m_D and C_D index the solution mass (0.1 g) and heat capacity (4.2 J g^{-1}) of pure water used as the solvent, respectively. As noted, can be calculated referring to the following equation:

$$t = -\tau_s ln(\frac{T_{RT} - T_{Surr}}{T_{Max} - T_{Surr}})$$

where T_{RT} denotes as the real-time temperature in the cooling period.

Q_{Dis} the heat input due to light absorption by the water and container, which can be described as:

$$Q_{Dis} = hS_{Buff} \times (T_{Max} - T_{Surr})_{Buff}$$

Biofilm Culture.

A single *S. aureus* colony was transferred into 30 mL TSB medium followed by vigorous agitation. 100 μL inoculated medium into each well of 96-well plate, then incubated at 37 °C for 3 days. Mushroom-like biofilm structure could be found on the bottom of the well. For fluorescence imaging and SEM experiment, the biofilms were culture on cover glass by using the same procedures.

Biofilm Dispersion Assay.

100 μL of the NPs at different concentrations was added into the wells. Simultaneously, we used PBS and NPs solution as the control group. Then, the biofilms were incubated with the NPs for 4 h and subsequently irradiated with NIR light for 10 min. The cells were fixed with methanol and then stained with 150 μL of 0.5 % crystal violet (CV) solution. After 30 min, the wells were vigorously rinsed at least four times with sterile PBS to remove unbound dye and 200 μL of 33% acetic acid was added to release the dye. The biofilms were quantified by measuring the absorbance after untreated and treated with the liposomes at 590 nm using a microplate reader. Each concentration of material was tested in five replicates, and three independent experiments of each group were conducted. Simultaneously, the viability of biofilm cells was counted by the plate count method.

Fluorescence Imaging.

The fluorescence imaging of the bacterial biofilm treated with 2TPE-2NDTA-02 NPs under NIR irradiation was characterized by confocal laser scanning microscopy (CLSM). *S. aureus* biofilms were incubated with PBS, and 2TPE-2NDTA-02 NPs with NIR irradiation, then the medium was removed, and the residual biofilm was stained with BacLight Live/Dead kit for 10 min in saline. After being slightly rinsed with saline for 3 times, confocal microscope (ZEISS, LSM 800) was employed to observe the bacteria in biofilm.

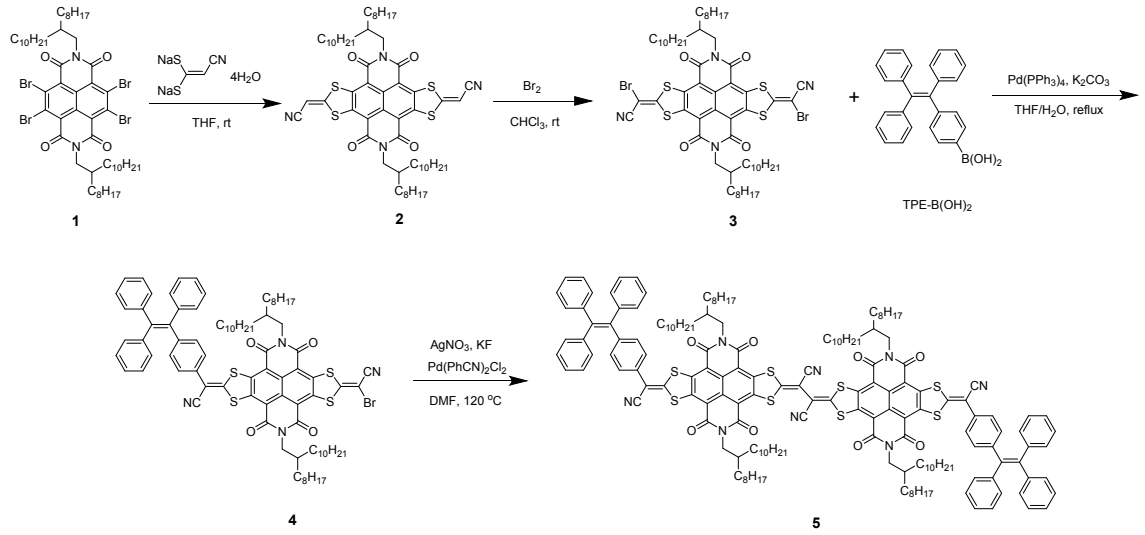
Observation of Bacterial Morphology.

The morphology of the bacterial biofilm treated with 2TPE-2NDTA-02 NPs under NIR irradiation was monitored by scanning electron microscopy (SEM). For preparation of SEM sample, *S. aureus* biofilms were incubated with PBS, and 2TPE-

2NDTA-02 NPs with NIR irradiation, then the medium was removed, and the residual biofilm was fixed with 4% glutaraldehyde in PBS for 8 h at 4 °C. The samples were then dehydrated successively by 25, 50, 75, 100% ethanol. Then, the bacterial biofilms were freeze-dried. The dried samples were further plated with gold for SEM observation.

Bacterial biofilm EPS matrix staining

Blank or treated biofilms were stained with 200μL FilmTracer SYPRO RUBY Biofilm Matrix Stain (Invitrogen, F10318). The staining solution were added very gently so as not to disturb the biofilm. After staining for 30 min, the samples were washed with sterilized water very gently. Then the confocal images were collected by ZEISS LSM 800. Excitation wavelength: 488 nm. Emission wavelength range: 550 nm – 650 nm.



Scheme S1. The synthesis route to 2TPE-2NDTA-02 (**5**).

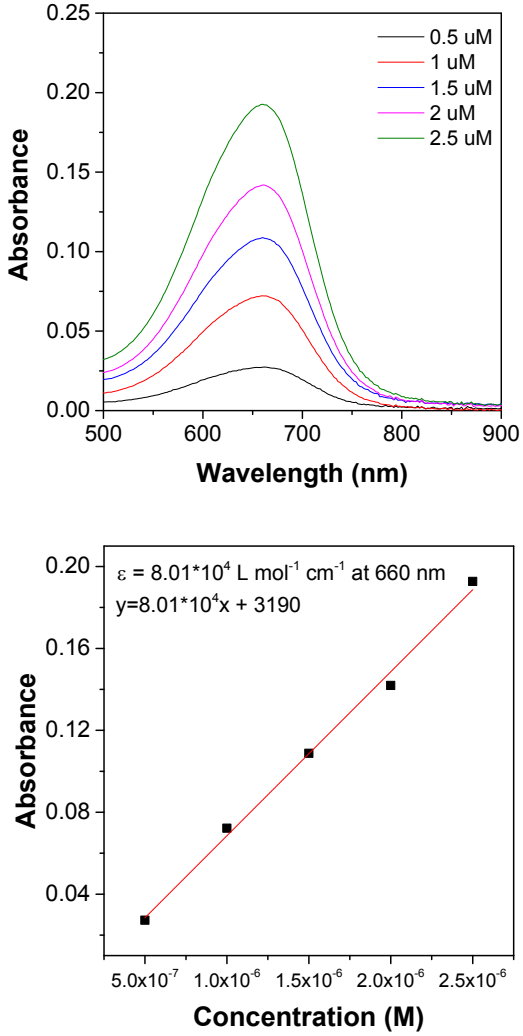


Figure S1. Molar abosorptivity measurement of 2TPE-2NDTA-02.

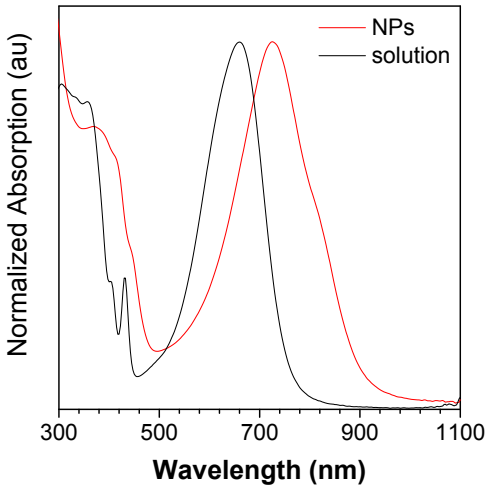


Figure S2. Normalized absorption spectra of 2TPE-2NDTA-02 in solution and TN NPs.

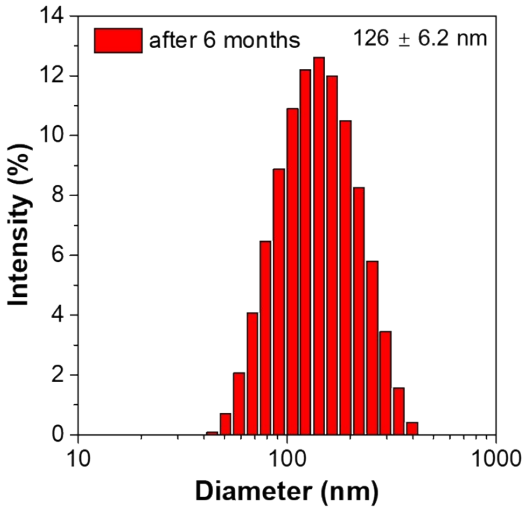


Figure S3. DLS histogram of AIE NPs stored for 6 months.

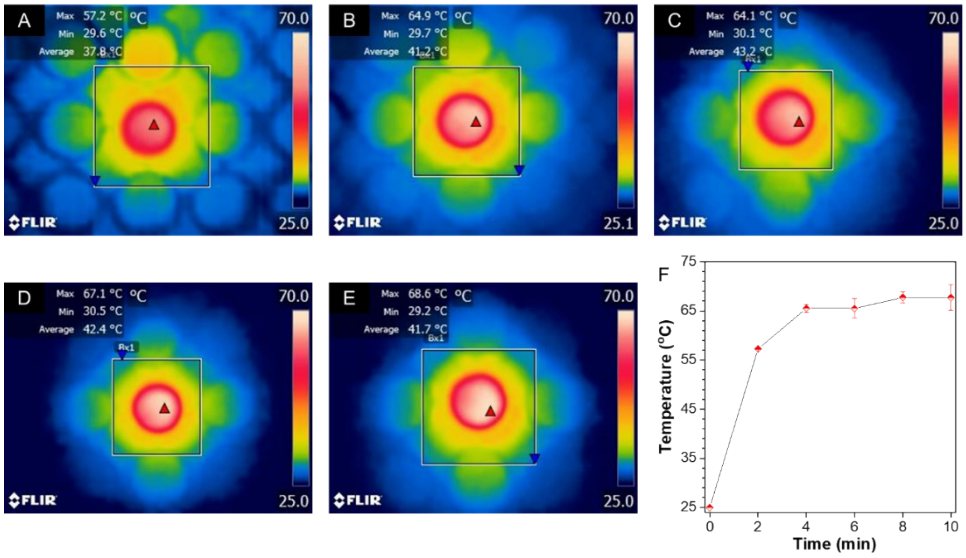


Figure S4. (A–E) IR thermal images of various TN NPs in aqueous solution (100 μM based on 2TPE-2NDTA-02) in a 96-well plate upon exposure to 808 nm laser irradiation (1 W·cm^{-2}) for, (A) 2min, (B) 4 min, (C) 6 min, (D) 8 min and (E) 10 min. (F) The temperature changes of solutions of AIE NPs in 96-well plate as a function of time.