Electronic Supplementary Information

Single nanoparticles as versatile phototheranostics for tri-modal imaging guided photothermal therapy

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1. General information and experimental procedure

General information

NMR spectra were carried out on a 400 MHz NMR spectrometer (Ultra Shield Plus, Bruker). The morphology of NP was determined by HT7700 transmission electron microscope (TEM) and its size was determined by particle size analyzer (Brookhaven Instruments). The absorption and emission spectra were obtained by UV3600UV/vis/NIR spectrophotometer (Shimadzu) and FLSP920 fluorescence spectrophotometer (Edinburgh), respectively. The MTT experiment was carried out by using PowerWave XS/XS2 enzyme labeling instrument (BioTek).

Experimental procedure

Preparation of DPP-TT NPs: DPP-TT in THF (1.0 mg/mL, 1 mL) was quickly added into the aqueous solution (5 mL) of DSPE-mPEG5000 (10 mg) under sonication. The N_2 is blown on the surface of the solution to remove THF. Finally, the water-soluble DPP-TT NPs was obtained by ultrafiltration (3000 r/min).

Photothermal test of DPP-TT NPs: The temperature curves of different concentrations of DPP-TT NPs (0, 20, 40, 60, 80 μg/mL) under 808nm laser (1W/cm^2) were obtained by infrared imager (FLIR E50; Estonia). In addition, 100 μg/mL DPP-TT NPs under irradiation with 808 nm laser at various power densities (0.25, 0.5, 0.75, and 1.0 W/cm^2) were also studied.

Cytotoxicity studies of DPP-TT NPs: MTT assay was used to evaluate the cytotoxicity of DPP-TT NPs in vitro. Hela cells and NIH-3T3 cells were cutured in DMEM medium containing 10% fetal bovine serum and 1% streptomycin/penicillin at 37 ℃ in 5% CO_2 for 24 hours. Then the cells were inoculated in 96-well plate and cultured for 24 hours, the NIH-3T3 cells were treated with fresh medium containing different concentrations of DPP-TT NPs. Hela cells were treated in fresh medium with DPP-BDT NPs, and divided into two groups: (1) without irradiation, (2) with 808 nm laser irradiation (1 W/cm^2, 7 min). Then, MTT (20 μL) was added to each pore, incubated another 4 hours in dark environment, the supernatant was removed, and 150 μL DMSO was added to dissolve the crystals. The absorbance of 490nm was

recorded by PowerWave XS/XS2 microplate spectrophotometer, in which the untreated cells were the control group. The cell viability was expressed as the ratio of the average absorbance of the cells treated with DPP-TT NPs to the average absorbance of the control group.

Live and dead cell assay: Hela cells were treated by the above methods and stained with Calcein AM (2 μM) and propidium iodide PI (8 μM) for 10 minutes. When AM exists in living cells, it produces green fluorescent substance (Ex/Em: 495 nm/520 nm) with strong fluorescence signal, and for damaged cell membrane, PI enters the cell and binds to nucleic acid can produce bright red fluorescence signal (Ex/Em: 530 nm/620 nm). Thus, the fluorescence images of living/dead cells were obtained under inverted fluorescence microscope.

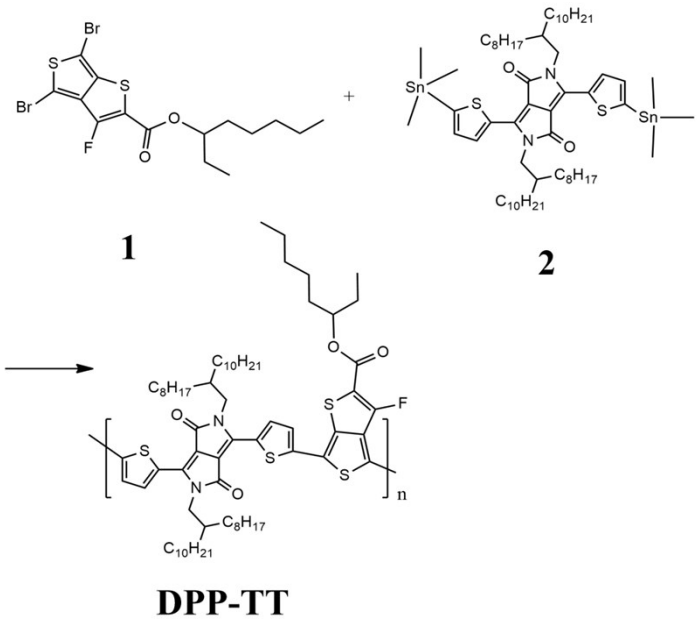
In vitro NIR-II fluorescence imaging and PA imaging: Fluorescence imaging of DPP-TT NPs with different concentrations (0.01, 0.02, 0.05, 0.1 and 0.2 mg/mL) was performed by an NIR-II fluorescence imaging instrument. At the same time, for PA imaging, different concentrations (0.1, 0.2, 0.5, 1 and 2 mg/mL) of DPP-TT NPs were added to the EP tubes for PA signal detection using a photoacoustic instrument (Nexus 128, Endra Inc.).

In vivo NIR-II fluorescence imaging and PA imaging: for *in vivo* NIR-II imaging, Hela tumor mice were injected intravenously with DPP-TT NPs (200 μL, 2 mg/mL), the fluorescence intensity and NIR-II imaging was recorded every 2 hours until the fluorescence disappeared. For *in vivo* PA imaging, the mice were monitored by a PA instrument (Nexus 128, Endra Inc.) at indicated time points after injection.

In vivo combination therapy: All tumor bearing mice were purchased from Jiangsu KeyGEN BioTECH Corp., Ltd. and used according to the guideline of the Laboratory Animal Center of Jiangsu KeyGEN BioTECH Corp., Ltd. The animals were randomly divided into four groups and given following different treatments: (a) PBS; (b) PBS + NIR laser; (c) DPP-TT NPs only (2 mg/mL, 200 μL); (d) DPP-TT NPs (2 mg/mL, 200 μL) + NIR laser. About 10 hours after caudal vein injection, the tumor areas of mice were exposed to laser (808 nm, 1 W/ cm^2) for 10 minutes. Then, the tumor volume and body weight of the mice were recorded every two days within 15 days.

After therapy, the mice were dissected, and the major organs were further studied by H&E staining.

2. *Synthesis of DPP-TT*



Scheme S1 Synthesis of DPP-TT.

In N_2 atmosphere, compound 1 was prepared by 4, 6-dibromo-3-fluorothieno [3,4] thiophene-2-carboxylate octyl ester. Then compound 1 (0.1 mmol, 47.2 mg) and compound 2 (0.1 mmol, 118.7 mg) were added to the reaction bottle. Pd $(PPh_3)_4$ (15 mg) was added as catalyst, 15 mL anhydrous toluene was added, and the reaction was washed with nitrogen for 10 to 20 minutes. Under the condition of avoiding light, the mixture was stirred violently at 110 °C for 24 hours. After the solvent was removed by steaming, the crude product was purified by column chromatography, and the polymer DPP-TT was blue-black solid (74.8 mg, 63% yield).

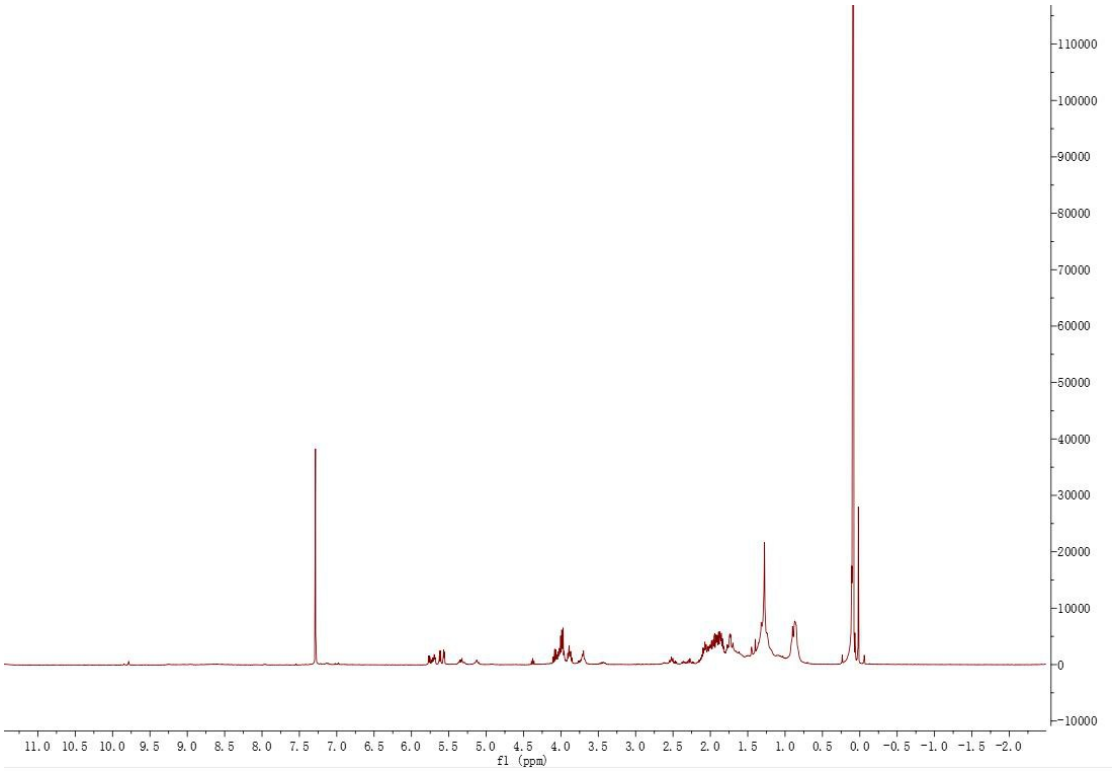


Fig. S1 ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 298 K) of DPP-TT.

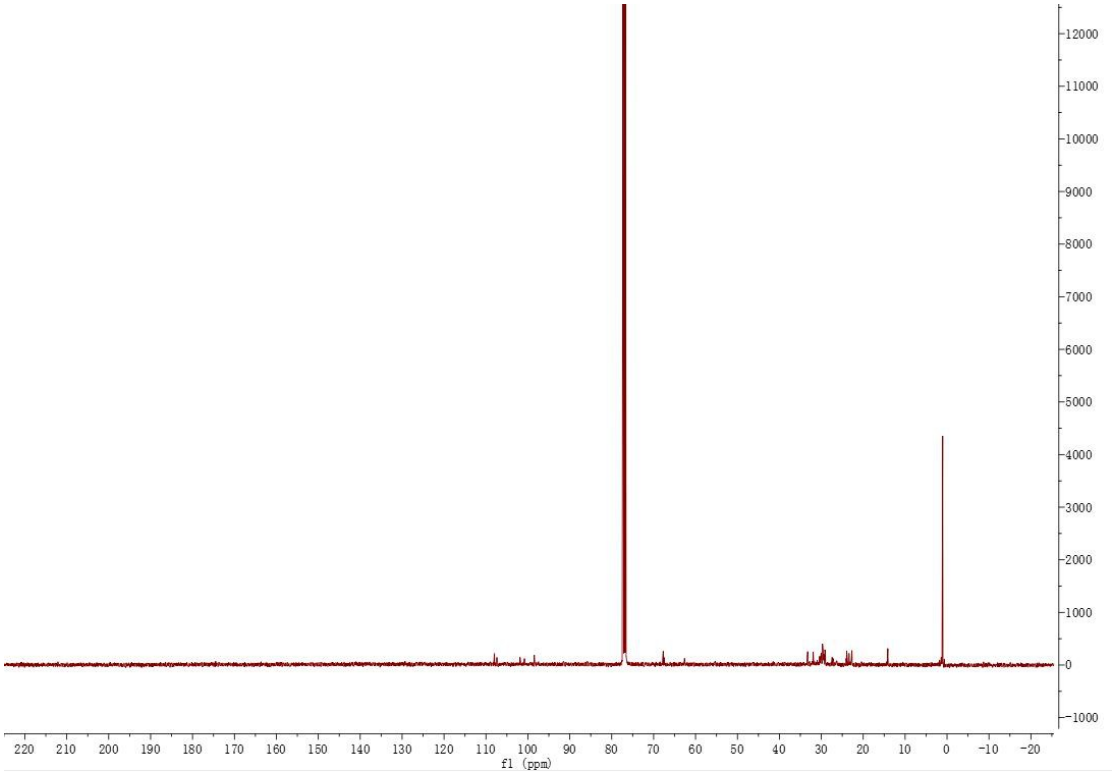


Fig. S2 ^{13}C NMR spectrum (100 MHz, $CDCl_3$, 298 K) of DPP-TT.

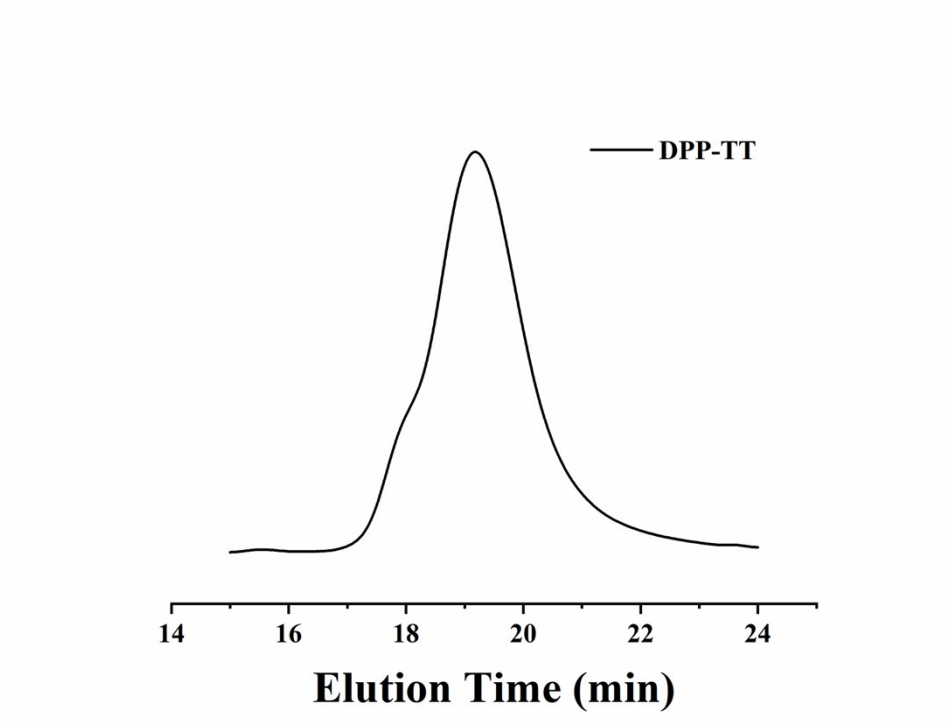


Fig. S3 GPC of DPP-TT.

3. Stability of DPP-TT NPs

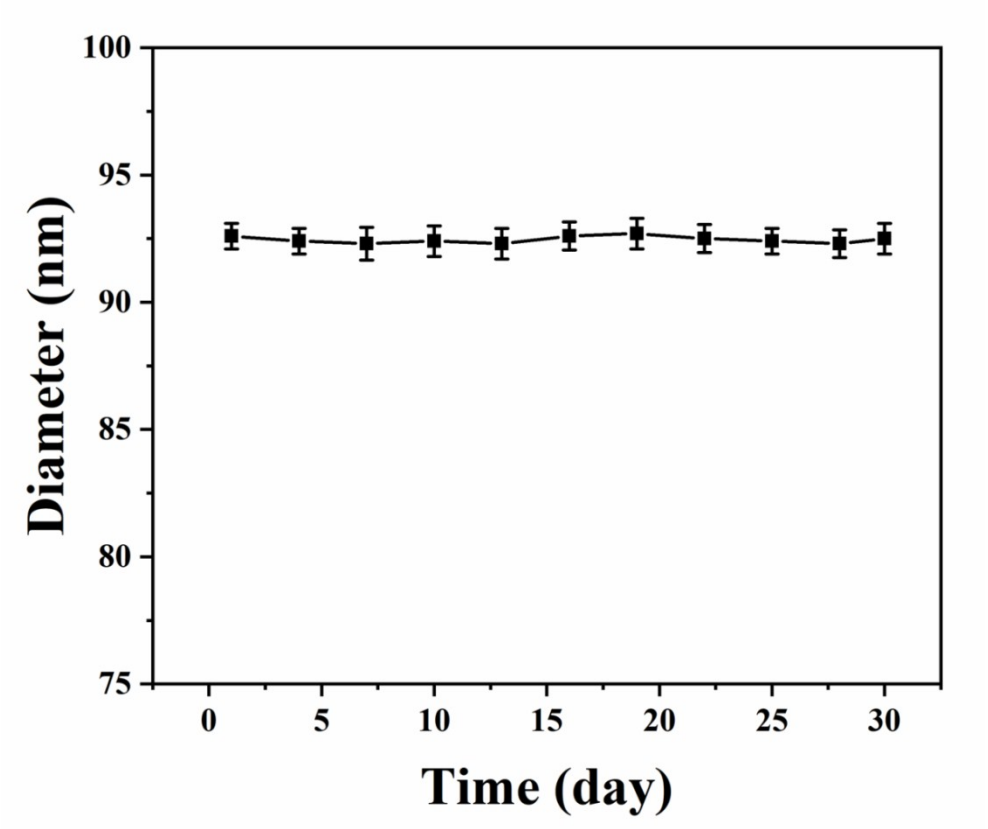


Fig. S4 Stability of DPP-TT NPs with different time.

4. *DLS of DPP-TT NPs at different conditions*

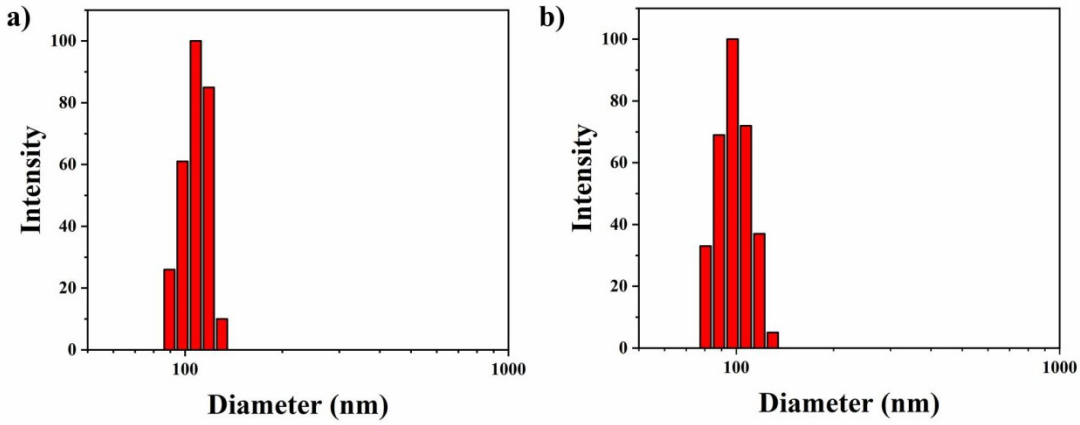


Fig. S5 DLS data of DPP-TT NPs (a) after light irradiation (808 nm, 1 W/cm^2, 5 min) or at high temperature (42 ℃, 3 min).

5. *UV-Vis-NIR absorption of DPP-TT NPs in water*

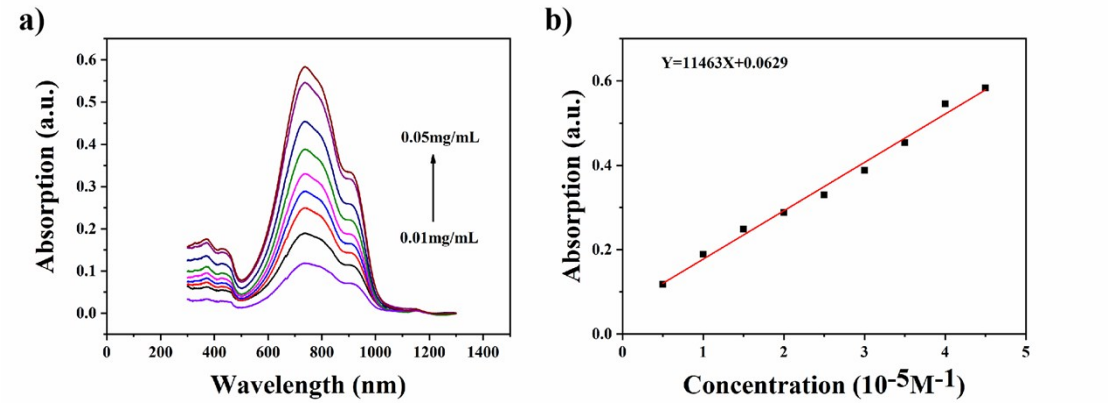


Fig S6 a) Absorption curves of DPP-TT NPs at different concentrations. b) Linear absorbance versus concentration obtained from (a).

6. *Photostability of DPP-TT NPs.*

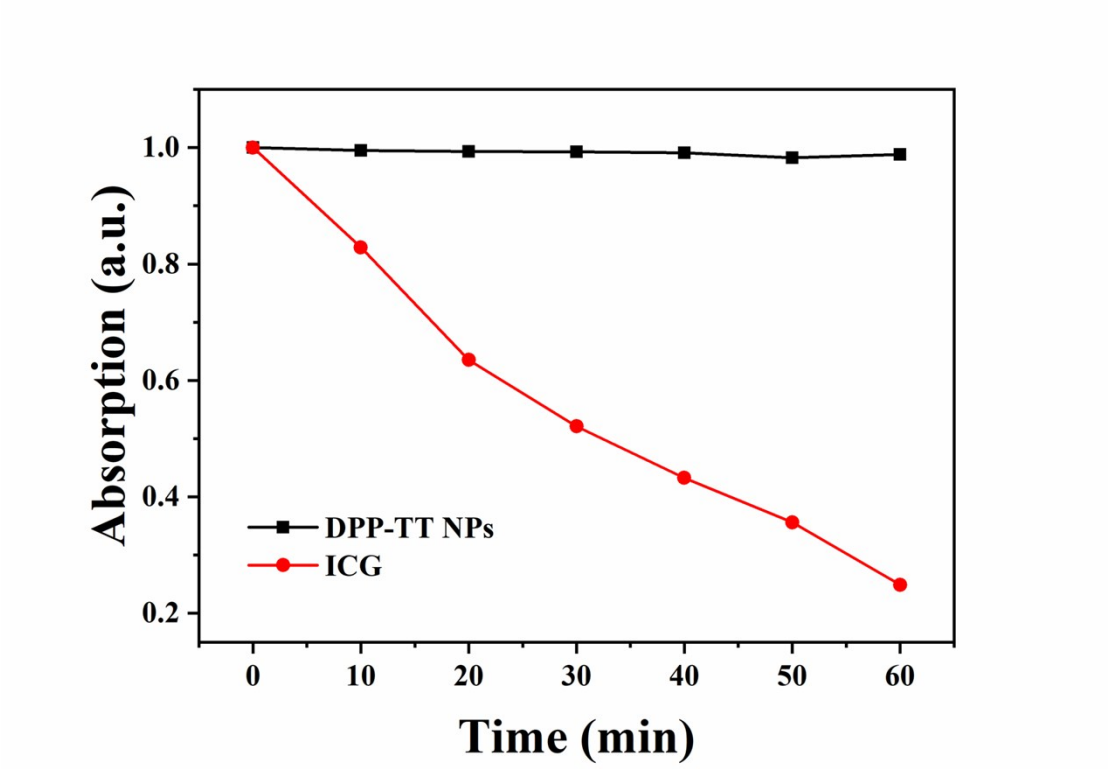


Fig S7 Photostability of DPP-TT NPs.

7. *Penetration depth measurement of DPP-TT NPs*

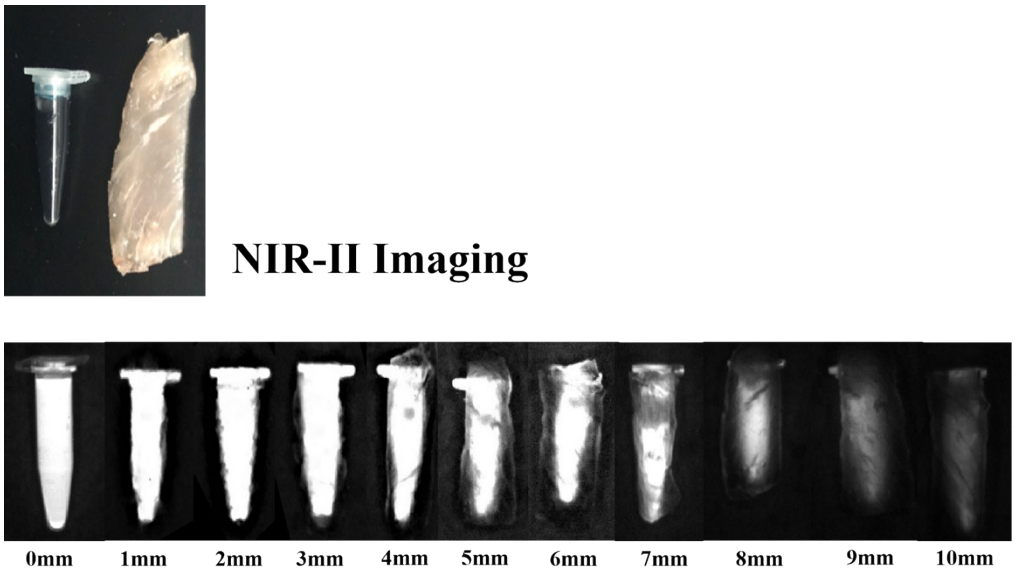


Fig. S8 Penetration depth measurement of DPP-TT NPs in a simulated deep-tissue setting (chicken-breast tissues).

8. *Infrared thermal images of DPP-TT NPs*

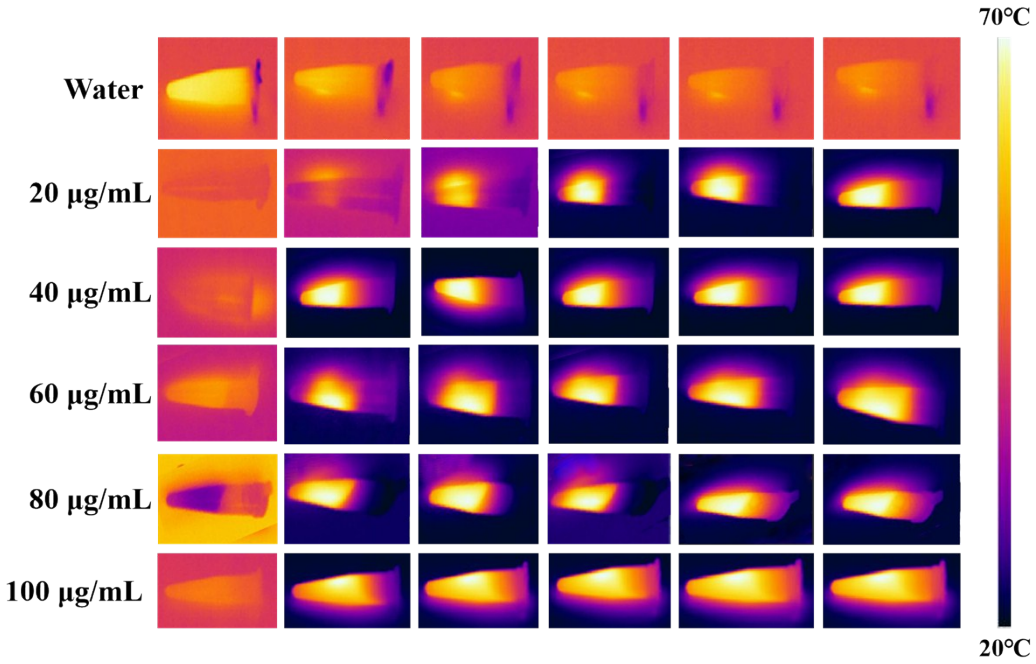


Fig. S9 Infrared thermal images of DPP-TT NPs in water after NIR laser irradiation for different times (808 nm, 1 W/cm^2).

9. *Photothermal stability of DPP-TT NPs*

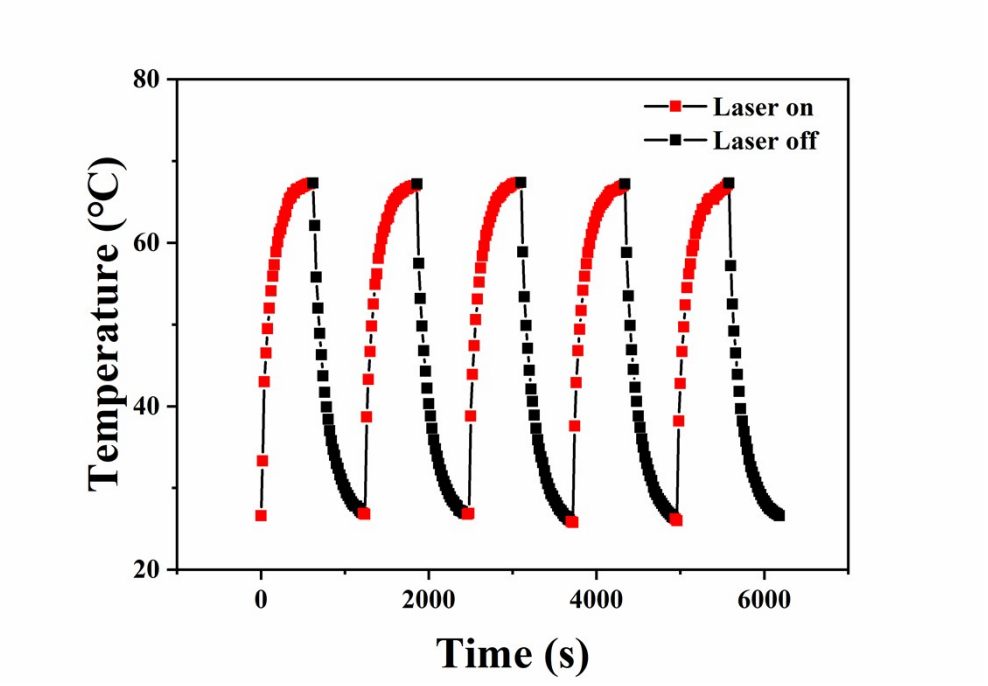


Fig S10 Photothermal stability of DPP-TT NPs.

10. The color image of NIR-II fluorescence imaging

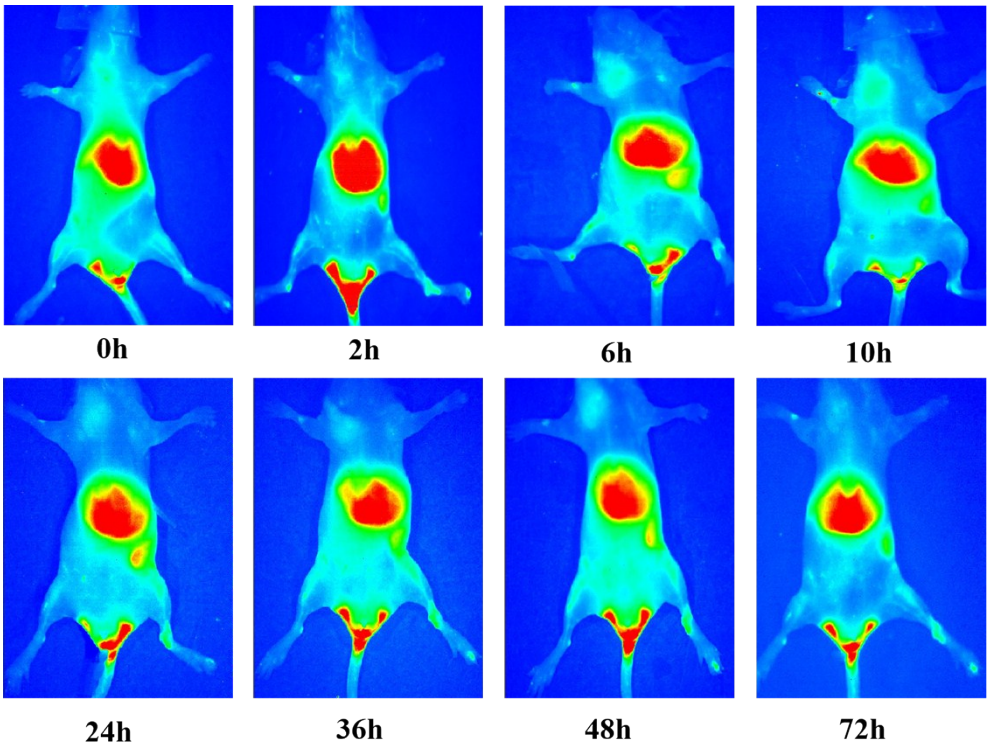


Fig. S11 The color image of NIR-II fluorescence imaging

11. Ex vivo NIR-II fluorescence images of major organs and tumors

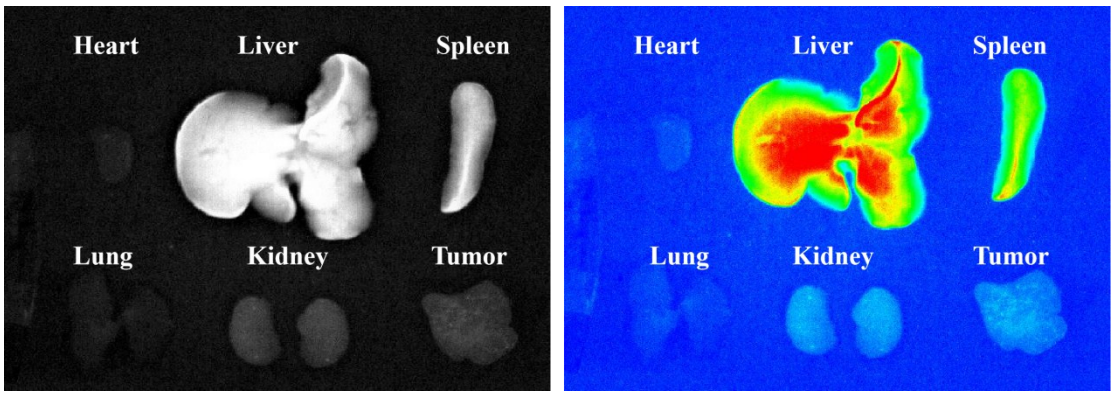


Fig. S12 Ex vivo NIR-II fluorescence images of dissected tumor and major organs after injection of DPP-TT NPs.