# Semiconducting Polymer Nanoparticles for NIR-II Fluorescence Imaging-guided Photothermal/Thermodynamic Combination Therapy

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1. Supporting figures

a

b
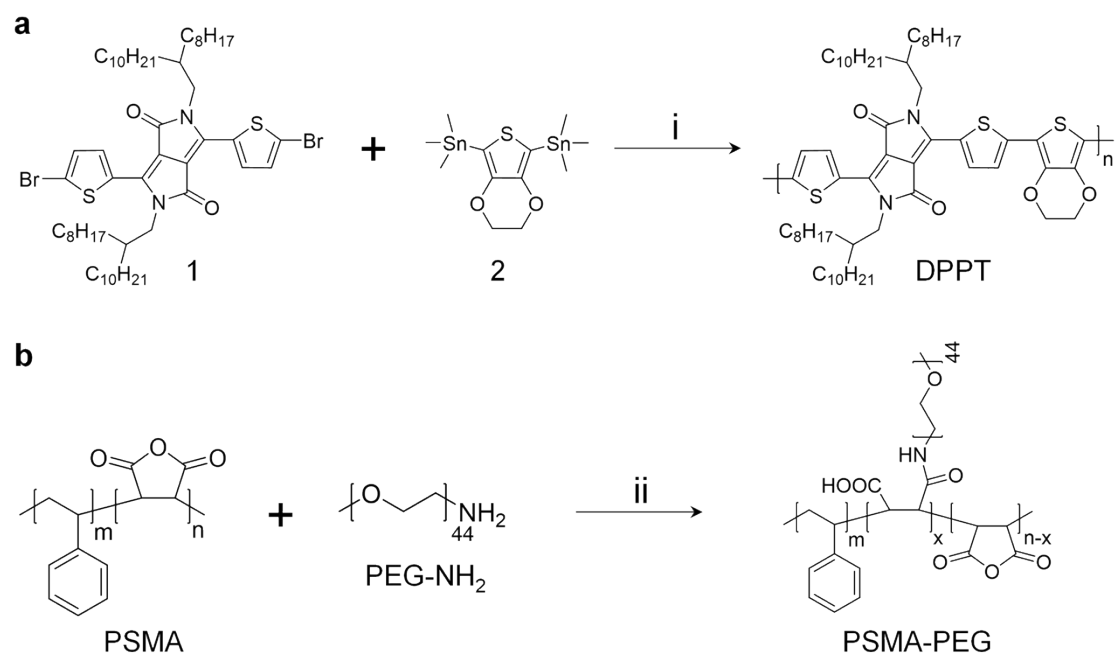
Scheme S1. Synthetic procedures of DPPT (a) and PSMA-PEG (b). Reagents and conditions: i) $Pd_2(dba)_3$, tri(o-tolyl)phosphine, toluene, N_2 atmosphere, 110 °C, 6 h; ii) N,N-diisopropyl-ethylamine, anhydrous tetrahydrofuran, room temperature, 48 h.
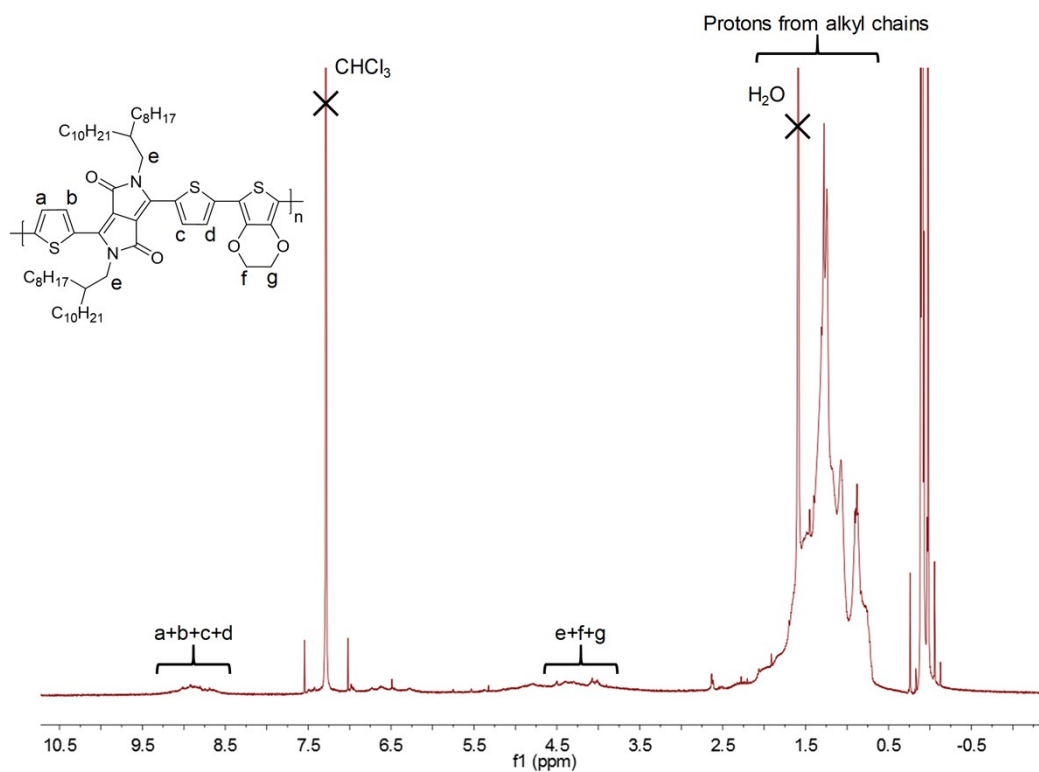

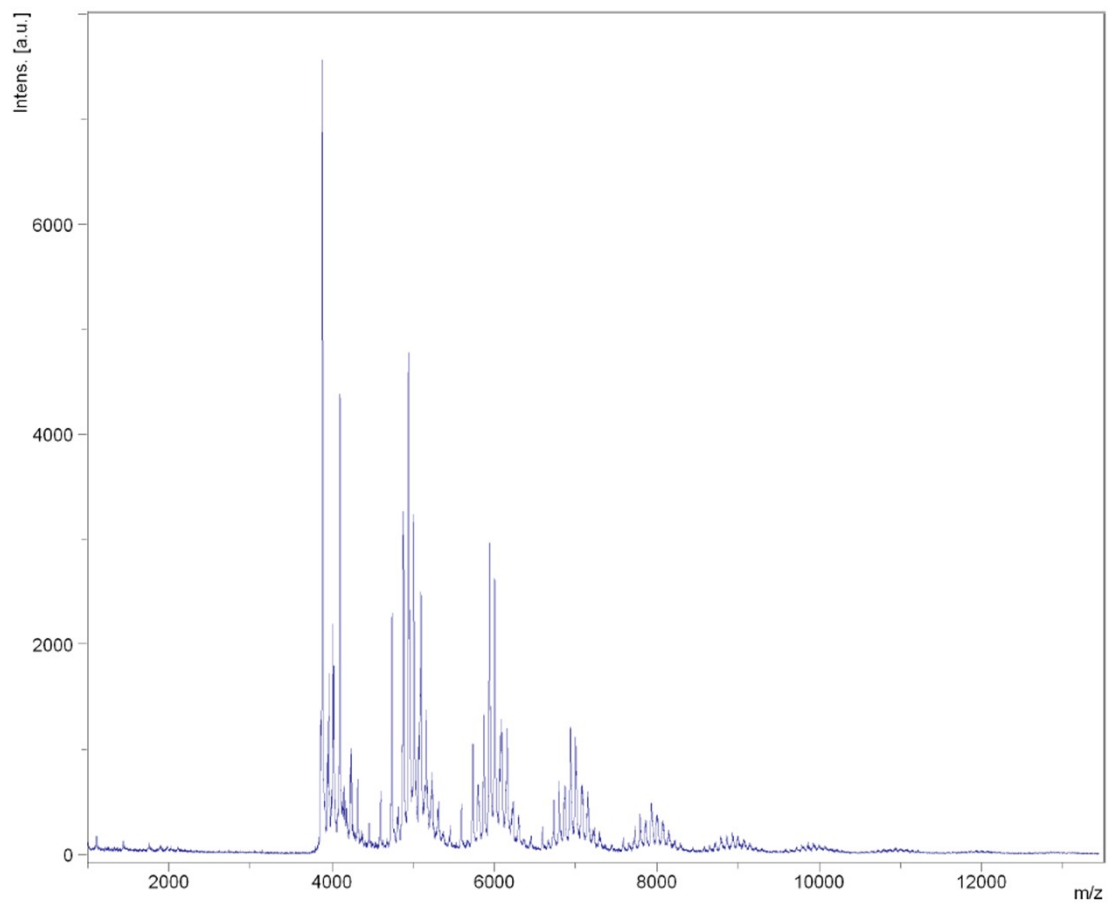**Figure S1**. ^1H NMR spectrum of DPPT. $CDCl_3$ was used as the solvent.

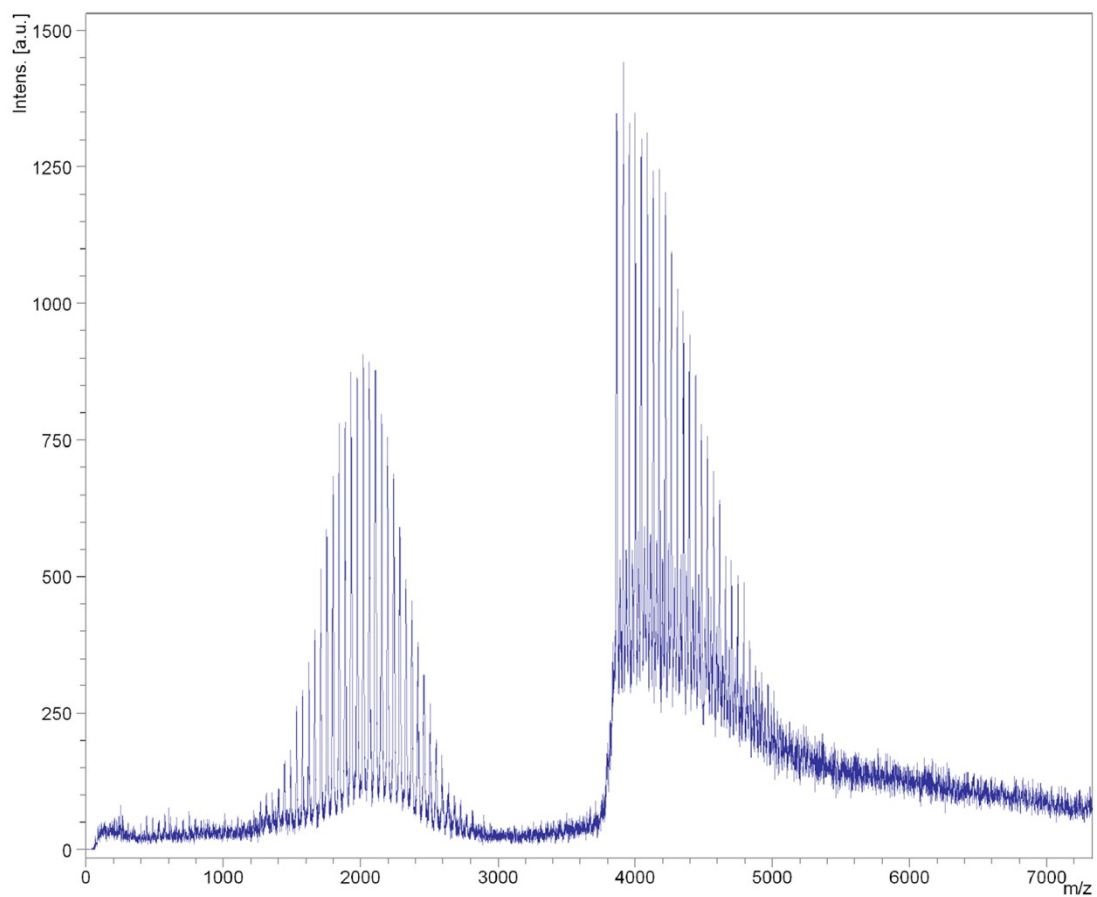**Figure S2**. MALDI-TOF-MS spectrum of DPPT.

Figure S3. MALDI-TOF-MS spectrum of PSMA-PEG.
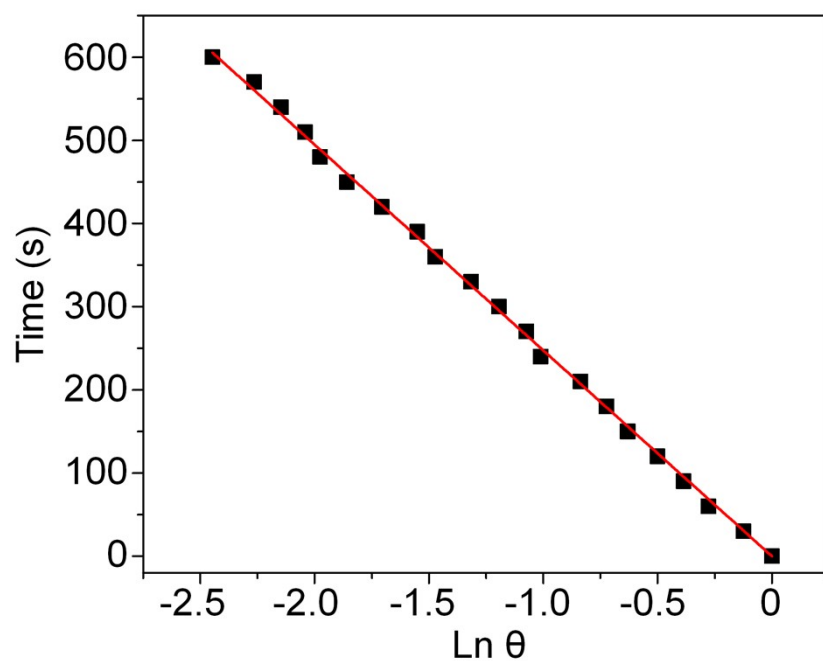

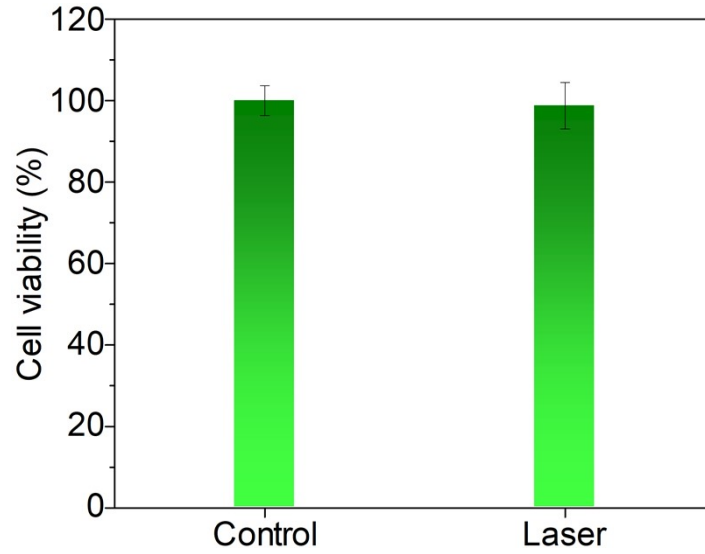**Figure S4**. Calculation of photothermal conversion efficiency for ADPPTN.

Figure S5. Viability of 4T1 cells with or without 808 nm laser irradiation (1 W/cm^2)

for 10 min.
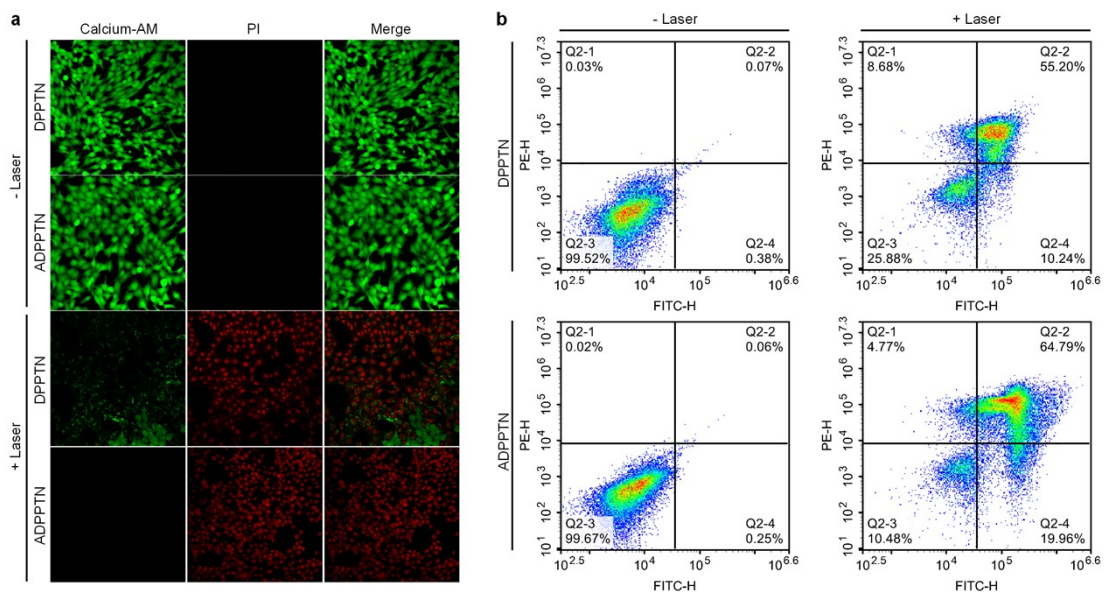
Figure S6. (a) Live/dead assay of 4T1 cells under different treatment with or without

808 nm laser irradiation. (b) Representative flow cytometry plots of 4T1 cell under
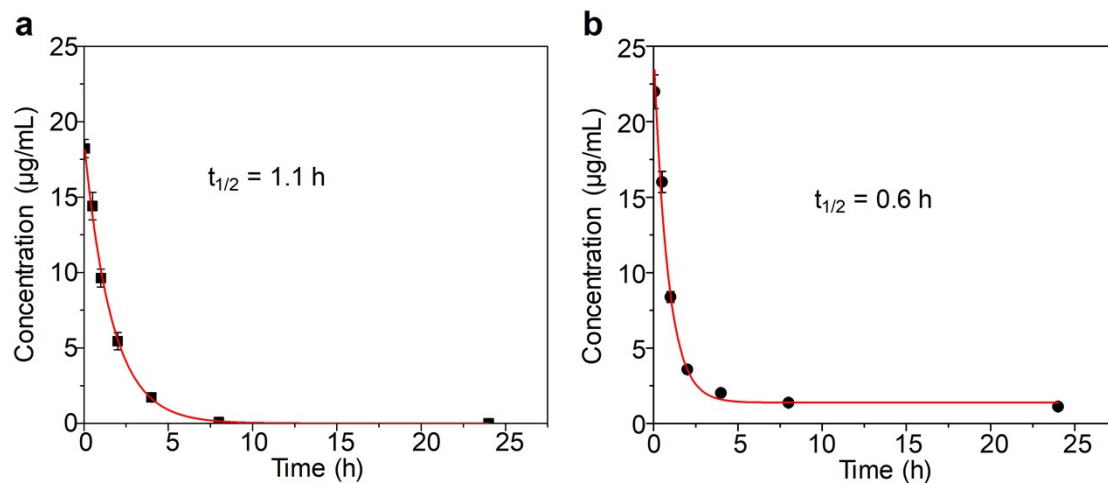
different treatments.

Figure S7. Blood concentration of DPPTN (a) and ADPPTN (b) as a function of post-injection time. The error bars represent standard deviation of three separate measurements (n = 3).
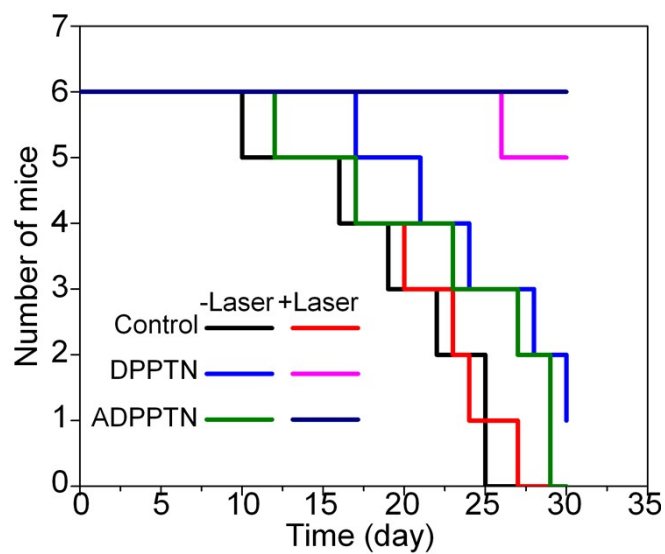
Figure S8. Number of mice survived after different treatments for 30 days.
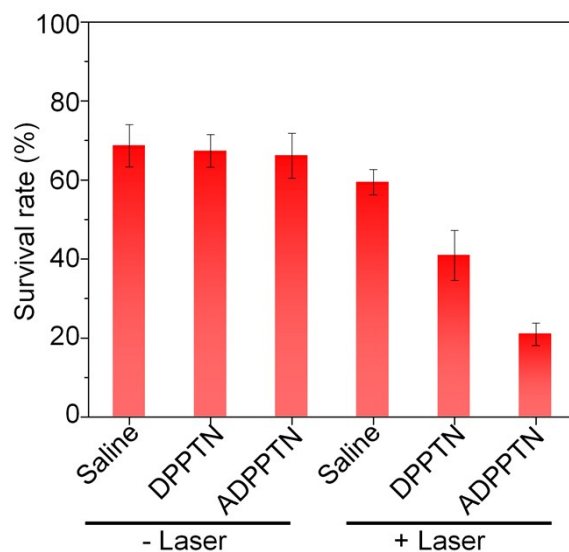
Figure S9. Survival rates of tumor cells under different treatments tested by PCNA staining. The error bars represent the standard deviations of three separate measurements.

2. Experimental Section

2.1 Chemicals

All chemicals and solvents were purchased from Sigma Aldrich and used as received unless otherwise noted. 3,6-bis(5-bromothiophen-2-yl)-2,5-bis(2-octyldodecyl)pyrrolo[3,4-c]pyrrole-1,4(2H,5H)-dione (monomer 1) and 5,7-Bis(trimethylstannyl)-2,3-dihydrothieno[3,4-b][1,4]dioxine (monomer 2) were purchased from SunaTech Inc.

2.2 Characterizations

NMR spectra were acquired on a Bruker Ultra Shield Plus 400 MHz spectrometer and tetramethylsilane (TMS) was used as the internal standard. MALDI-TOF-MS was

performed on a Bruker Daltonics Autoflex equipment. Dynamic light scattering (DLS) and Zeta potential characterization was performed on Brookhaven ZetaPALS. Transmission electron microscopy (TEM) images were acquired through a HT7700 transmission electron microscope (acceleration voltage 100 kV). Absorption spectra were measured on a Shimadzu UV-3600 ultraviolet-visible-near-infrared spectrophotometer. The photoluminescent spectra were measured on a Fluoromax-3 spectrophotometer (JobinYvon). Thermo image and temperature recording were accomplished by FLIR E95 thermal imager. ESR spectra was recorded on a Bruker EMX 10/12 instrumentation.

2.3 Synthesis of DPPT

Monomer 1 (101.9 mg, 0.12 mmol), monomer 2 (46.8 mg, 0.1 mmol), $Pd_2(dba)_3$ (4.6 mg, 0.005 mmol) and tri(o-tolyl)phosphine (12.2 mg, 0.04 mmol) were added into a 20 ml schlenk tube, and the tube was purged with argon. 10 mL degassed toluene was then added into the tube by using a syringe, and the reaction was conducted under 110 °C for 6 h. After that, the reaction mixture was cooled to room temperature and repeatedly precipitated against methanol for 3 times, the precipitate was collected and dried under vacuum to give the product as a dark-green solid (yield: 74 %).

2.4 Synthesis of PSMA-PEG

To a dried flask was added amino-capped methyl poly(ethylene glycol) (mPEG-NH$_2$) (Mn = 2000, 0.2 mmol, 400 mg), poly(styrene-co-maleic anhydride) (PSMA) (Mn = 1900, styrene 75%, 0.2 mmol, 380 mg), N,N-diisopropyl-ethylamine (0.1 ml), anhydrous tetrahydrofuran (THF) (25 ml). The reaction was conducted at room

temperature for 48 h. After that, the solution was dropped into excess anhydrous ether to give the white precipitate. The precipitate was collected and dried under vacuum to yield the product as a white solid (yield: 90 %).

2.5 Preparation of DPPTN

DPPT (0.2 mg/ml) and PSMA-PEG (2 mg/ml) was dissolved into 1 mL of THF. The solution was rapidly injected into 9 mL of water under vigorous ultrasonication for 1 min. After that, the THF in the solution was removed by gentle nitrogen flow, and the resulting solution was stored for 48 h at room temperature. The nanoparticles solution was filtered through a 220 nm syringe driven filter to give the desired DPPTN solution.

2.6 Preparation of ADPPTN

1 mL aqueous solution of 2,2′-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (AIPH) (1 mg/ml) was added into 10 mL of DPPTN solution (0.25 mg/mL), and the resulting solution was stirred at room temperature for 1 h. The free AIPH was removed by ultrafiltration to give the final ADPPTN solution.

2.7 Determination of photothermal conversion efficiency

1 mL ADPPTN solution (15 μg/mL) and 1 mL ICG solution (65 μg/mL) was added into quartz cuvettes, respectively. The solutions were irradiated by 808 nm laser (1 W/cm^2), and the solution temperature was monitored by a thermo camera. Photothermal conversion efficiency was calculated as steps described below.

During cooling phase of the photothermal cycles, the cooling law gives the relationship between temperature and time:

$$t = -\frac{mC}{hS}\ln\theta$$

$$\theta = \frac{T_t - T_{surr}}{T_{max} - T_{surr}}$$

which $\theta = \frac{T_t - T_{surr}}{T_{max} - T_{surr}}$, T_t is the temperature at time t, T_{surr} is the ambient temperature, T_{max} is the highest temperature reached during heating phase. m is the mass of the irradiated solution, C is the heat capacity of the solution, hS is the heat transfer rate, which is an intrinsic constant related with the container and the solution. The photothermal conversion efficiency can be calculated by the following equation.

$$\eta = \frac{E_{heat}}{E_{laser}} = \frac{hS(T_{max} - T_{surr}) - hS(T_{max,water} - T_{surr})}{I(1 - 10^{-Abs808})}$$

E_{heat} represents the heat energy generated from nanoparticles upon irradiation. E_{laser} represents the actual input energy which is the absorbed light energy.

2.8 AIPH radicals determined by UV absorption

A solution containing ADPPTN (33 μg/ml), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (1 mg/ml) was prepared and divided into two portions. The two solutions were incubated at 37 °C and 44 °C for 6 h, respectively. The UV absorption of solutions were recorded every 2 h.

2.9 Cellular uptake studies

Poly[(9,9-dioctyl-2,7-divinylenefluorenylene)-alt-{2-methoxy-5-(2-ethylhexyloxy)-1,4-phenylene}] (PEPV) (5% w/w) was used to encapsulated into DPPTN and ADPPTN, respectively, by using similar nanoprecipitation method mentioned above. 4T1 cells were received from Jiangsu KeyGEN Biotech Corp., Ltd. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with antibiotics (10 mg/mL of streptomycin and 10 U/mL of penicillin) and 10% fetal bovine serum (FBS) at 37

°C in an atmosphere of 5% carbon dioxide and 95% humidified air. The cells were cultured in confocal dishes and incubated with PEPV-loaded DPPTN or ADPPTN (4 μg/mL) for 8 h. Before imaging, the cells were washed with fresh DMEM, and cell nuclei were stained with DAPI. Confocal images were acquired on LSM880 confocal laser scanning microscope (Carl Zeiss, Germany) with excitation wavelength of 405 nm for DAPI and 488 nm for PEPV-loaded DPPTN and ADPPTN. For flow cytometry studies, 4T1 cells were cultured in 12-well plates (2×10^5 cells per well) and cultured for 24 h under 37 °C with 5% carbon dioxide and 95% humidified air. PEPV-loaded DPPTN or ADPPTN was then added into wells at the final concentration of 4 μg/mL and cultured for another 8 h. The cells were then washed with PBS and trypsinized, and collected into 5 mL tubes. The fluorescence intensity of cells was measured on a NovoCyte flow cytometer.

2.10 Cytotoxicity studies

4T1 cells were cultured in 96-well plates (10^4 cells in 200 μL DMEM per well) for 24 h, and DPPTN or ADPPTN (final concentration 0, 0.5, 1, 2, 4, 8, 16 μg/mL for both samples) were added into the cultured cells. After culturing for another 8 h, cells were treated with or without 808 nm laser irradiation (1 W/cm) for 10 min. MTT assay was utilized to determine the cell viabilities.

2.11 Live/Dead staining

4T1 cells were seeded in confocal dishes for 24 h, followed by four different treatments: DPPTN with or without laser, ADPPTN with or without laser. After that, the cells were stained with Calcium-AM and PI according to the standard protocol.

After 30 min, the cells were washed with PBS three times, and fresh medium was added to the dishes for confocal fluorescence imaging.

2.12 Tumor mouse model

All the mice experiments were carried out in accordance with the guidelines of the Laboratory Animal Center of Jiangsu KeyGEN Biotech Corp., Ltd. 1 million 4T1 cells were suspended in 50% v/v mixture of Matrigel in supplemented DMEM were subcutaneously injected into the left axilla of balb/c female mouse (20 g) to establish the tumor model. The tumor volume was calculated based on the following formula:

$$V = \frac{1}{2}Dd^2$$

V is the volume of tumor, and D is the maximum diameter of tumor, and d is the minimum diameter of tumor.

2.13 Pharmacokinetic studies

The end of balb/c mice tail was cut for blood collection, and the blood was sampled by heparinized capillary tubes. Blood before injection was collected as the reference. The mice were then i.v. injected with ADPPTN or DPPTN (200 μg/mL, 200 μL). Blood was collected at 3 min, 0.5, 1, 2, 4, 8, and 24 h post-injection. The NIR-II fluorescence intensity of blood sample in tubes were then measured by an In Vivo NIR-II fluorescence imaging system. The concentration of nanoparticles in the blood sample were calculated by the standard curve of ADPPTN and DPPTN. The results were presented as a bi-exponential decay curve to estimate the circulation half-life values.

2.14 In vivo NIR-II fluorescence imaging

ADPPTN (200 μg/mL, 200 μL) was intravenously injected into 4T1 tumor bearing mice. NIR-II fluorescence imaging were then conducted by an In Vivo NIR-II fluorescence imaging system at different time points. Images were acquired for 100 ms under 808 nm excitation with 1064 nm LP filter. For ex vivo biodistribution studies, the injected mice were sacrificed and the tumor, liver, spleen, lung, heart, kidney and intestine were collected for NIR-II fluorescence imaging to evaluate the content of ADPPTN in these tissues.

2.15 In vivo anticancer study

4T1 tumor bearing mice were randomly divided into 6 groups, each group had 6 mice. For the groups without laser irradiation, the mice were intratumorally injected with saline, DPPTN or ADPPTN (200 μg/mL, 200 μL), respectively. For the groups with laser irradiation, the tumor region of mice were exposed to 808 nm laser (0.3 W/cm^2) for 10 min after intratumoral injection of saline, DPPTN or ADPPTN (200 μg/mL, 200 μL), respectively. For the laser irradiation groups, the tumor temperature was monitored by thermo camera. Tumor volume and body weight of each mouse were measured every other day. The tumor inhibition rate was calculated by the following equation:

$$Inhibition\ rate = \left(1 - \frac{tumor\ volume\ of\ tested\ group}{tumor\ volume\ of\ saline\ group\ without\ laser}\right) * 100\%$$

2.16 Histological studies

The tumor bearing mice for in vivo anticancer studies were sacrificed after 14 days of treatments. For all groups, the tumors of mice were collected and fixed in 4% formalin. The tumor tissues were then tested for proliferating cell nuclear antigen

(PCNA) staining based on the standard protocol. For ADPPTN-treated mice with laser irradiation, the major organs including heart, lung, kidney, liver, spleen and intestine were harvested and fixed in 4% formalin. Paraffin embedded sectioning was then performed for hematoxylin and eosin (H&E) staining according to standard protocol. The images of slices were captured by using a digital microscope for both PCNA and H&E staining.

2.17 Data analysis

Intensities of NIR-II fluorescence images were determined by ROI analysis. Results were exhibited as mean ± SD unless otherwise stated. All the statistical calculations were conducted by Graphpad Prism v.6 software (GraphPad Software, Inc., CA, USA).