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Electronic Supplementary Information (ESI)

NIR-II Absorbing Conjugated Polymer Based on Tetra-Fused Isoindigo with Ultrahigh Photothermal Conversion Efficiency for Cancer Therapy

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Supporting Experimental Section

Materials: Chemical reagents were purchased from Energy Chemical, Aldrich, Laysan Bio or Alfa Aesar and used as received. All air and water sensitive reactions were performed under argon atmosphere. Toluene was distilled in standard method prior to use. 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[biotin(polyethylene glycol)] (DSPE-MPEG$_{2000}$-Biotin) was purchased from Laysan Bio, Inc. (Arab, AL). The compound **4TTD-2Br** were prepared according to the previously reported literatures.[1]

Characterization: ^{1}H (400 MHz) NMR spectrum of P4TTD-DPP was recorded on a Bruker spectrometer using $CDCl_3$ (tetramethylsilane as internal standard) as the solvent. Elemental analysis was conducted on a VarioEL elemental analysis system. UV-vis-NIR absorption spectra were recorded on a Shimadzu UV-3600Plus UV-vis-NIR spectrometer. Number-average (M_n) and weight-average molecular weight (M_w) were measured at 150 °C on a PL-GPC 220 system with polystyrene as standard and 1,2,4-trichlorobenzene as eluent. Cyclic voltammograms (CV) were measured on a CHI660a electrochemical analyzer with a three-electrode cell at a scan rate of 100 mV/s. Bu_4NPF_6 (0.1 mol/L) in anhydrous acetonitrile was used as electrolyte. A Pt disk with 2 mm diameter, a Pt wire, and a saturated calomel electrode

(SCE) were used as the working, counter and reference electrodes, respectively. E_{onset}^{ox} and E_{onset}^{re} are oxidation and reduction onsets, respectively, against the half potential of ferrocene/ferrocenium (Fc/Fc^+) (E^o = 0.39 V), as determined in CV curves. The highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) energy levels were calculated according to the equations $E_{\mathrm{HOMO}} = -(4.80 + E_{onset}^{ox})$ eV and $E_{\mathrm{LUMO}} = -(4.80 + E_{onset}^{re})$ eV, respectively. Femtosecond transient visible and NIR absorption spectroscopy was carried out by using an Astrella (Coherent Corporation, USA) Ti:sapphire regenerative amplifier (1 kHz repetition rate, and 800 nm laser pulse with 100 fs pulse width). The output of the amplifier was split with one beam used to generate the pump pulse with a TOPAS-Prime (Light Conversion) optical parametric amplifier and the second to generate a white light probe using either a sapphire crystal for the visible range or a proprietary crystal for the NIR spectral region (Helios fire, Ultrafast Systems, USA). The spectra and decays were detected by a Helios fire TA spectrometer (830–1500 nm) with a 6 ns delay stage. Femtosecond TA measurements were performed using low excitation fluences of 60.0 nJ cm^{-2} at 800 nm to avoid nonlinear photophysics. The TA spectrum was processed by removing noise, subtracting the background and scattered light before dynamics analysis. Photoluminescence spectra of the CPs were measured on an Edinburgh Instruments FLS980 Fluorescence Spectrofluorometer with a solution of P4TTD-DPP in deoxygenated $CHCl_3$, the excitation wavelength is 1050 nm. P4TTD-DPP has no fluorescence property, which is related to its ultrafast intramolecular conversion process of the excited state. The size of the NPs of the polymers were measured by dynamic light scattering (DLS) carried with a particle size analyzer NanoBrook 90Plus instrument (Brookhaven Instruments Co. USA). Transmission electron microscopy (TEM) images were acquired from a TALOSF200X transmission electron microscope with an accelerating voltage of 200 kV.

DFT Calculation: Density functional theory (DFT) calculations using the Gaussian 09 program at the B3LYP/6-31G(d,p) level were applied to obtain energy minimized conformations of model compounds of polymers. Long alkyl chains were replaced by methyl groups to simplify the calculations. Frontier molecular orbitals and energy levels were calculated based on the

optimized geometries. To assign the oscillator strength (*f*) of the transition from S_0 to S_1, time-dependent density functional theory (TD-DFT) calculations were performed on the optimized geometries using Gaussian 09 program at the B3LYP/6-31G (d,p) level.

Preparation of CP NPs: P4TTD-DPP (3 mg) and amphiphilic lipid-PEG 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[biotin(polyethylene glycol)] (DSPE-$MPEG_{2000}$-Biotin, 12 mg) were dissolved in tetrahydrofuran (THF, 10 mL). The resulting tetrahydrofuran (THF) solution was added dropwise into ultra-purified water (40.0 mL), with sonication being used to aid in stirring at a constant rate for 24 hours. This was followed by a seven-day dialysis process at room temperature to eliminate any remaining THF. The NPs suspension were purified by ultrafiltration and filtered through a 0.2 μm syringe driven filter. Then the obtained NPs were subsequently concentrated to 1.0 mg mL^{-1} and stored at 4 °C for further use.

Photothermal Performance: The photothermal conversion efficiency (η) values were calculated with the equation $\eta = (hA_{\triangle}T_{max}\text{-}Q_s)/[I(1\text{-}10^{-A_\lambda})]$ where h is the heat transfer coefficient, A is the surface area of the system, $_{\triangle}T_{max}$ is the temperature difference between the maximum steady-state temperature and ambient temperature, I is the laser power, A_λ is the absorbance of the solution at the irradiation wavelength, Q_s is the heat change of the pure solvent. The unknown hA value was calculated by the linear data of time versus –ln θ curve according to equation: $t = -(\sum_i m_i C_{p,i})\ln\theta/(hA)$.[2]

Animals and Tumor Xenograft Model: All animal experiments were performed under the guidelines set by Nantong Committee of Use and Care of Laboratory Animals and the overall project protocols were approved by the Animal Ethics Committee of Nantong University. To established tumor-bearing mouse model, about 5-week-old female BALB/c mice were purchased from the Laboratory Animal Center of Nantong University (Nantong, China) for the experiment of photothermal therapy (PTT). We selected the murine 4T1 breast cancer cells in this study. To establish tumors in six-week-old BALB/c, 100 μL of cell culture medium containing 2-3 million murine 4T1 breast cancer cells were inoculated into the abdomen of mice of the BALB/c mouse. After about 7 days, the tumor-bearing mice were used for PTT.

In Vivo PTT: The xenograft 4T1 tumor-bearing mice were randomly separated into 4 groups (5 mice per group) when the tumor volumes reached about 50-100 mm^3: "Saline", "Saline + laser", "P4TTD-DPP NPs", and "P4TTD-DPP NPs + laser", respectively. The NPs (100 μL; 350 μg/mL based on CPs) or Saline (100 μL) were intravenously injected into the tumor-bearing mice, respectively. For the "Saline + laser", "Saline + laser", and "P4TTD-DPP NPs + laser" groups, the tumor areas of mice were irradiated by a 1064 nm laser at 1.0 W/cm^2 for 5 min at 6 h post-injection. After treatments, the mice weight and tumor volumes were measured every two days for 20 days. The tumor volume was calculated using the following formula: length × $width^2/2$ (length and width were the longest and shortest diameters of tumors, respectively). Relative tumor size was calculated as V/V_0 (V_0 was the tumor volume of day 0). The survival rates of mice in each group were also monitored.

Biodistribution of PDPP-4TTD NPs in vivo: To investigate the biodistribution of PDPP-4TTD NPs in vivo, IR780 was encapsulated within PDPP-4TTD NPs, and their behavior was studied using ex vivo near-infrared (NIR) fluorescence imaging method. As depicted in the figure S10, following intravenous administration, the NPs predominantly accumulated in the liver and lungs of the mice. Of note, the fluorescence intensity of the NPs in the tumor exhibited a gradual increase during the first 6 hours post-injection, after which it began to decrease. This finding suggests that the optimal time for light irradiation after systemic administration is 6 hours, indicating a window for maximizing therapeutic efficacy.

Cell Culture and Cytotoxicity Assay: The murine 4T1 breast cancer cells were supplied by School of Pharmacy, Nantong University. The cancer cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin−streptomycin (PS). The cells were maintained in an atmosphere of 5% CO_2 and 95% humidified air at 37 °C. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was conducted to evaluate the biocompatibility of P4TTD-DPP NPs. First, 4T1 cancer cells were seeded in 96-well plates (Costar, IL, USA) at a density of 5×10^3 cells/well and incubated in complete DMEM (100 μL). After 24 h incubation, when the cell number of each well reached an appropriate density, both cells were added to a series of concentrations of NPs (0, 5, 7.5, 10, and 15 μg/mL based on CPs). Six hours following the endocytosis process

of the NPs, the cellular samples were subjected to illumination using a laser with a wavelength of 1064 nm (1 W/cm², 5 minutes). After incubation for another 24 h, 20 μL of freshly prepared MTT solution (5 mg/mL) in PBS was added into each well. After 4 h, the MTT medium solution was carefully removed and then DMSO (150 μL) was added into each well and the plate was gently shaken for 5 min at room temperature to completely dissolve all the precipitates. The maximum absorbance of the precipitation mentioned above at 490 nm was then measured by the microplate Reader (INFINITE M NANO). The cell viability was expressed by the ratio of the absorbance of the cells incubated with 5, 7.5, 10, and 15 μg/mL of samples to that of the cells incubated with the sample concentration of 0 μg/mL.

Histological Studies: After 20 days of mouse experiment, all groups of mice were sacrificed. The tumors and important normal organs (kidney, spleens and livers) were excised and fixed in 4% paraformaldehyde for two days before dehydrated overnight, and embedded in paraffin on the next day for H&E staining.

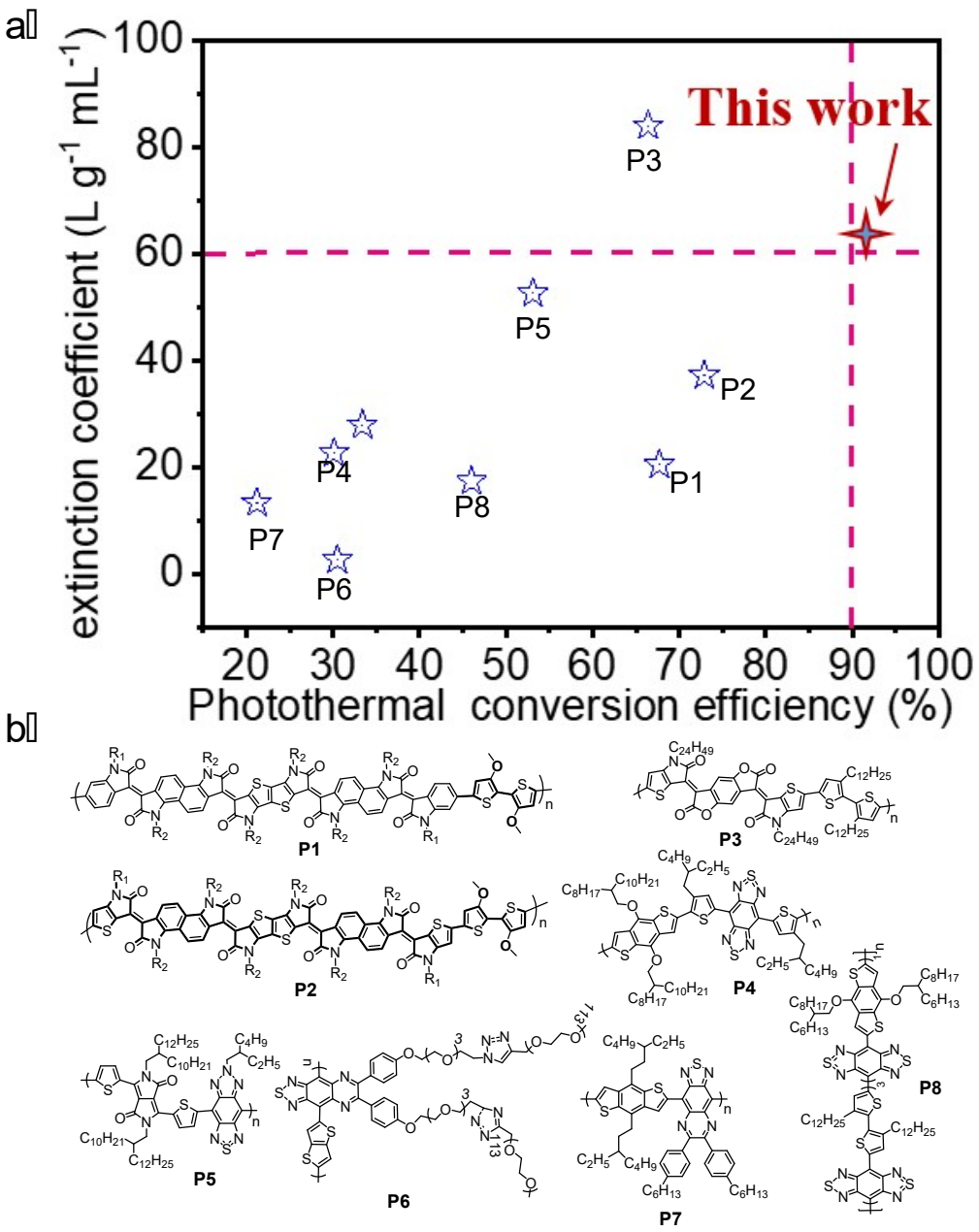

Figure S1. (a) The photothermal conversion efficiencies and absorption coefficients at 1064 nm of reported conjugated polymers, the values of P4TTD-DPP reported in this manuscript is also shown; (b) the structures of NIR-II CPs (P1-P8) in figure S1a.[1,3-12]

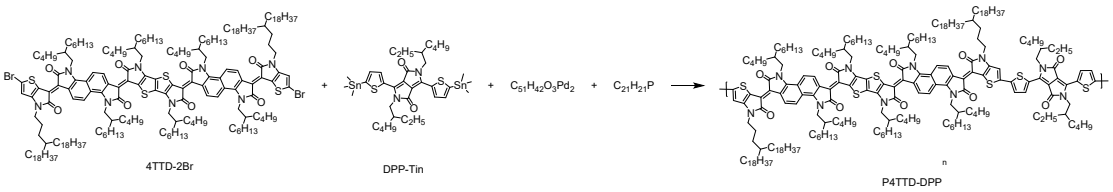

Figure S2. Synthetic routes to **P4TTD-DPP**.

P4TTD-DPP. In a Schlenk tube was charged with **4TTD-2Br** (140 mg, 0.043 mmol), **DPP-Tin** (36.7 mg, 0.043 mmol), tris(dibenzylideneacetone)dipalladium (0.8 mg, 1.0×10^{-4} mmol), tri-*o*-tolylphosphine (2.4 mg, 6.8 x 10^{-4} mmol) and toluene (10.0 mL). The mixture was evacuated and backfilled with argon three times and stirred at 120 °C for 10 h. Then 0.50 mL bromobenzene was added, the reaction was continued for another 12 h. After cooled to room temperature, the mixture was added dropwise into 80 mL methanol. The precipitate was filtered and then dissolved in 30 mL *o*-DCB at 100 °C. After cooling down, 80 mL aqueous solution of sodium diethyldithiocarbamate trihydrate (2.5 mg/mL) was added and then the mixture was stirred over night to remove residual catalyst. The separated organic phase was added dropwise into 80 mL ethanol and then separated the polymer. Then polymer was extracted with acetone and hexane on a Soxhlet's extractor before dried in vacuum. The final product was obtained as a black solid in a yield of 93% (146 mg). Elemental Anal. Calcd for $(C_{232}H_{362}N_{10}O_{10}S_6)_n$ (%): C, 76.47; H, 10.01; N, 3.84; S, 5.28; Found: C, 76.28; H, 9.981; N, 3.75; S, 5.19. GPC (1,2,4-trichlorobenzene, polystyrene standard, 130 °C), M_n: 1.09 x 10^4 g/mol, PDI: 1.56.

Cirrus GPC Sample Peak Report

Generated by: GPC 2023年7月6日 19:45
Workbook: C:\Cirrus Workbooks\20220809\20220809.plw

Sample Details
Sample Name: 1
Acquired: 2023/7/4 21:22:54 By Analyst: GPC
Batch Name: 20230704
Filename: C:\Cirrus Workbooks\20220809\20230704-0002.cgrm
Concentration: 0.10 mg/ml K of Sample: 14.1000
Injection Volume: 200.0 ul Alpha of Sample: 0.7000
LIMS ID: Bottle ID:

Workbook Details
Eluent: TCB Flow Rate: 1.00 ml/min
Column Set: Column Set Length: 950 mm
Detector: RI Temperature: 150

Analysis Using Method: 20230518
Comments:

Results File: C:\Cirrus Workbooks\20220809\20230704-0002-Repeat (01).rst

Calibration Used: 2023/5/17 14:22:39
Calibration Type: Narrow Standard Curve Fit Used: 1
Calibration Curve: y = 13.711366 - 0.426198x^1

High Limit MW RT: 16.25 mins Low Limit MW RT: 25.72 mins
High Limit MW: 6104485 Low Limit MW: 563
K: 17.5000 FRM Name:
Alpha: 0.6700 Flow Marker RT: 0.00 mins
FRCF: 1.0000

MW Averages

Mp: 14369	Mn: 10934	Mv: 15883	Mw: 17060
Mz: 25167	Mz+1: 33188	PD: 1.5603	

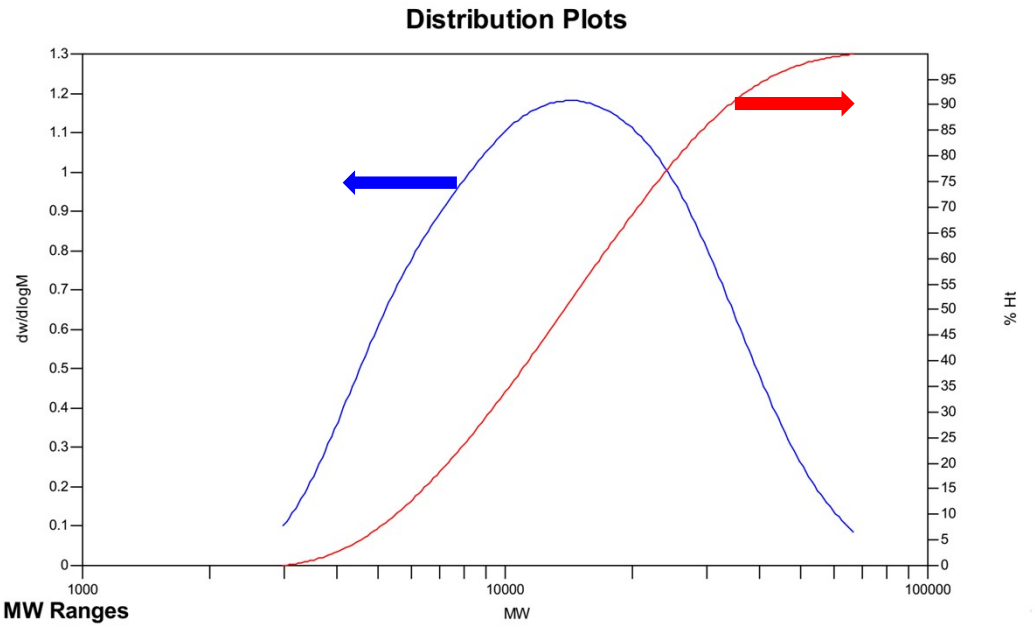

Figure S3. GPC measurement report of **P4TTD-DPP** with with polystyrene as standard and 1,2,4-trichlorobenzene as eluent.

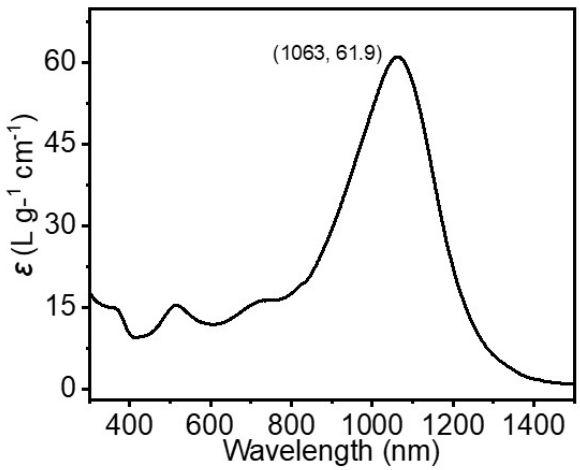

Figure S4. Absorption spectrum of P4TTD-DPP in THF.

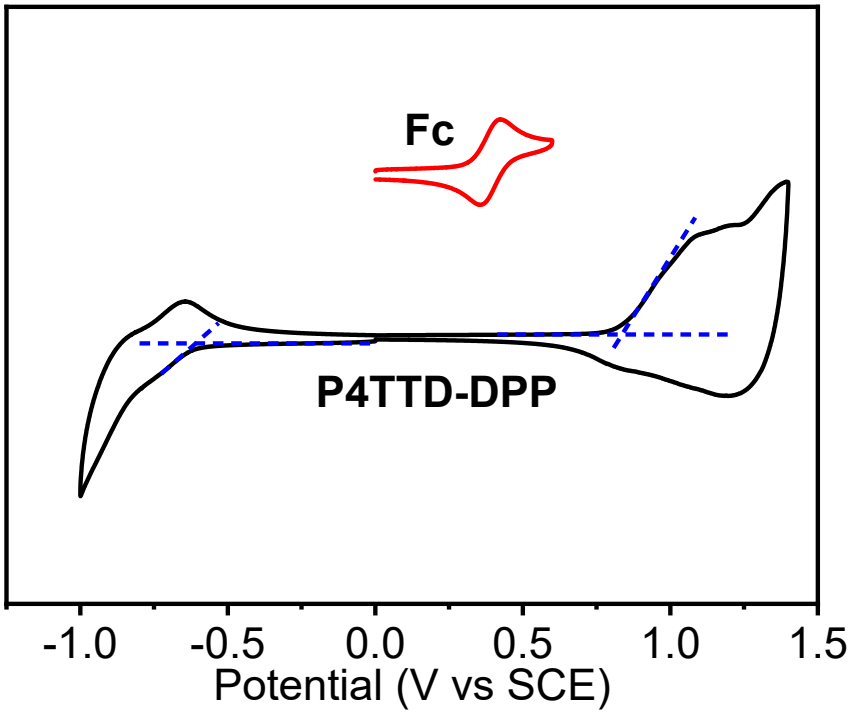

Figure S5. Film cyclic voltammograms (CV) of four CPs. The measurements were conducted in anhydrous acetonitrile with Bu_4NPF_6 (0.1 mol/L) as electrolyte.

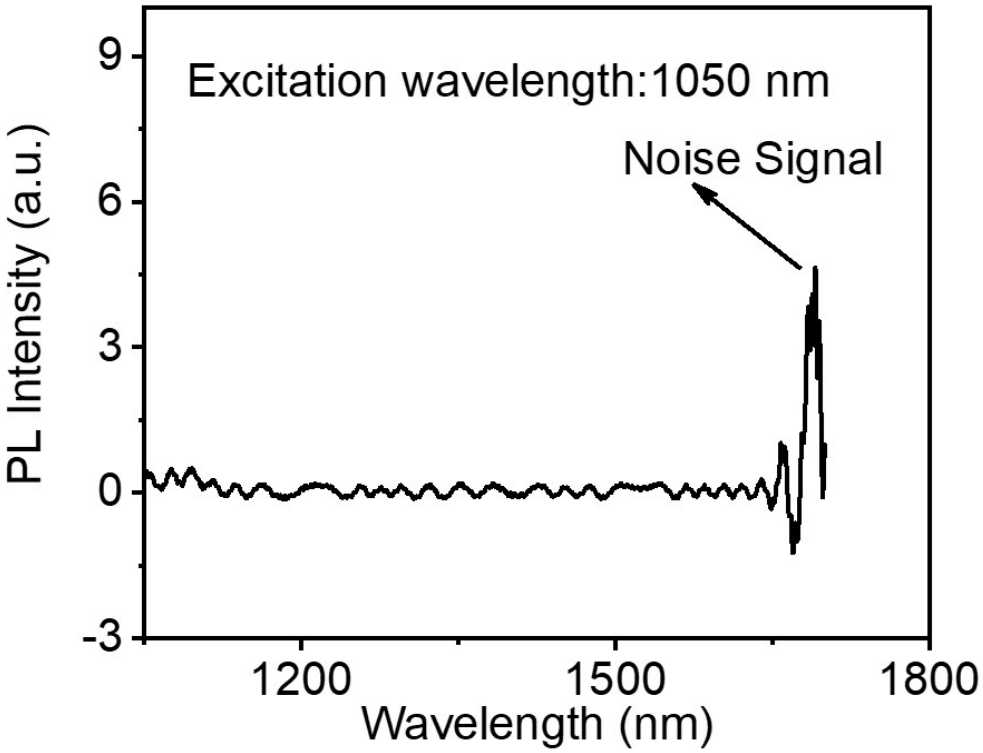

Figure S6. Emission spectrum of P4TTD-DPP in deoxygenated $CHCl_3$ solution at room temperature with excitation wavelength at 1050 nm.

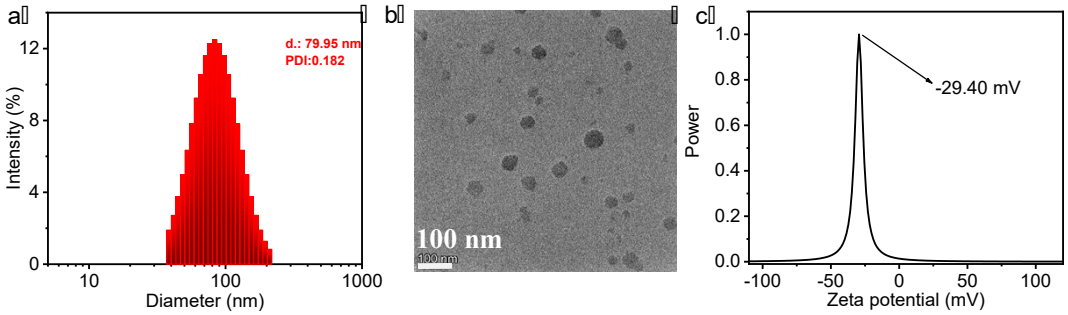

Figure S7. (a) Size distribution of P4TTD-DPP NPs measured by dynamic light scattering (DLS); (b) TEM image of P4TTD-DPP NPs, scale bars:100 nm; (c) zeta potential of P4TTD-DPP NPs.

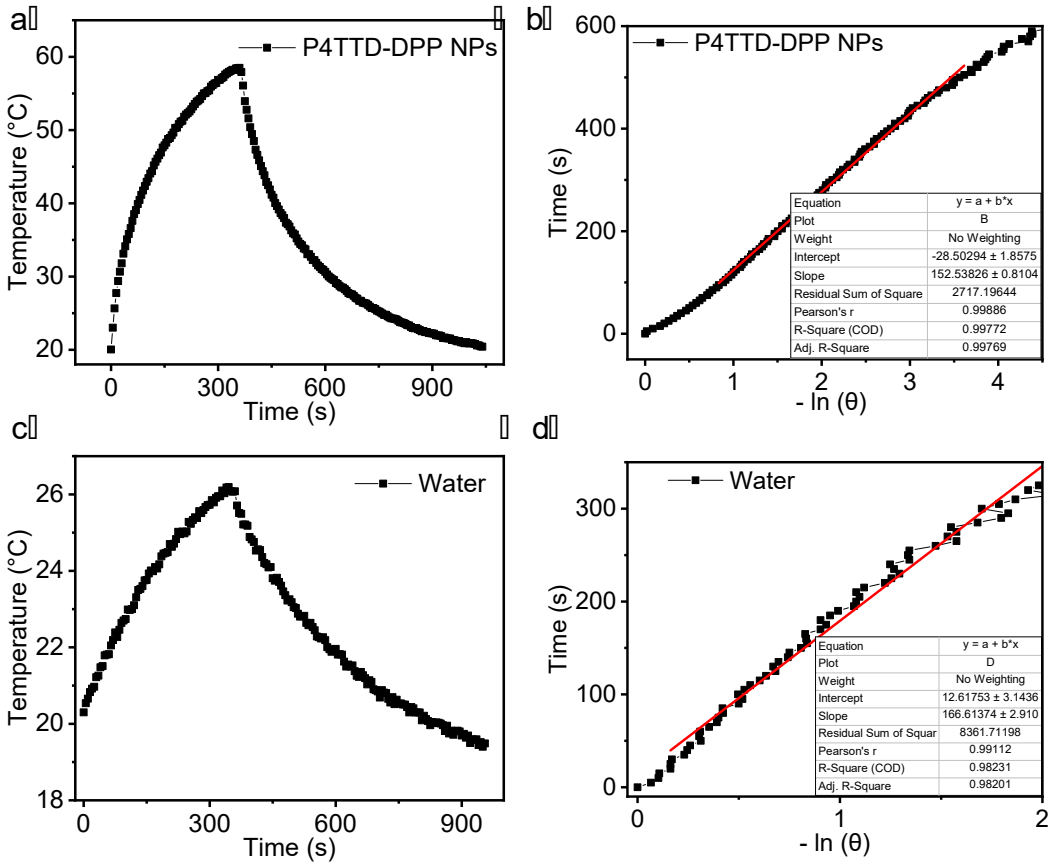

Figure S8. Temperature change curves of P4TTD-DPP NPs (a) in water (50 μg/mL) and water under 1064 nm NIR laser irradiation (1.0 W/cm^2, 6 min) and cooling time in correlation with -ln θ obtained from the cooling period of P4TTD-DPP-NPs (b) aqueous solutions and water (d) after 1064 nm NIR laser irradiation for 6 min.

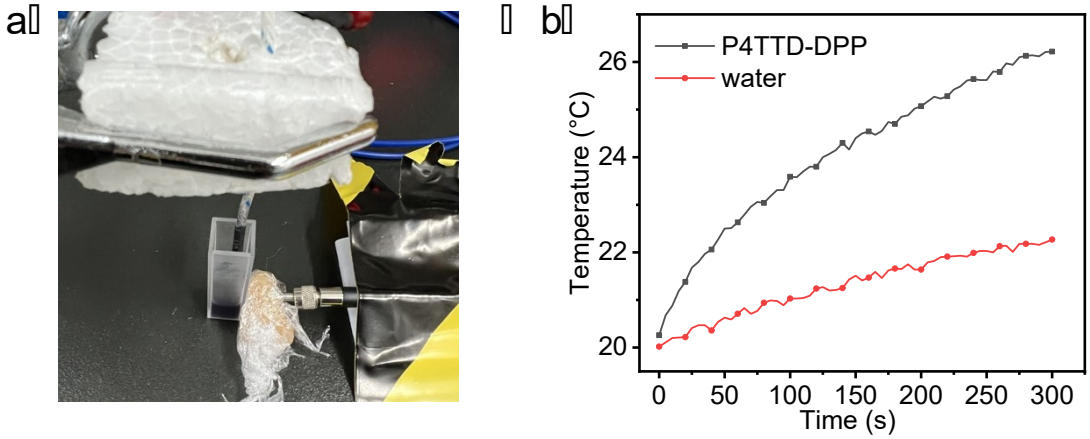

Figure S9. (a) Experimental setup for comparative study of deep-tissue photothermal heating capacities of P4TTD-DPP NPs and water using a 1064 nm wavelength pulse laser (Laser fluence 1.0 W/cm^2); (b) Temperature elevation curves of P4TTD-DPP NPs aqueous solutions (50 μg/mL) and water under 1064 nm laser irradiation (1.0 W/cm^2, 5 min).

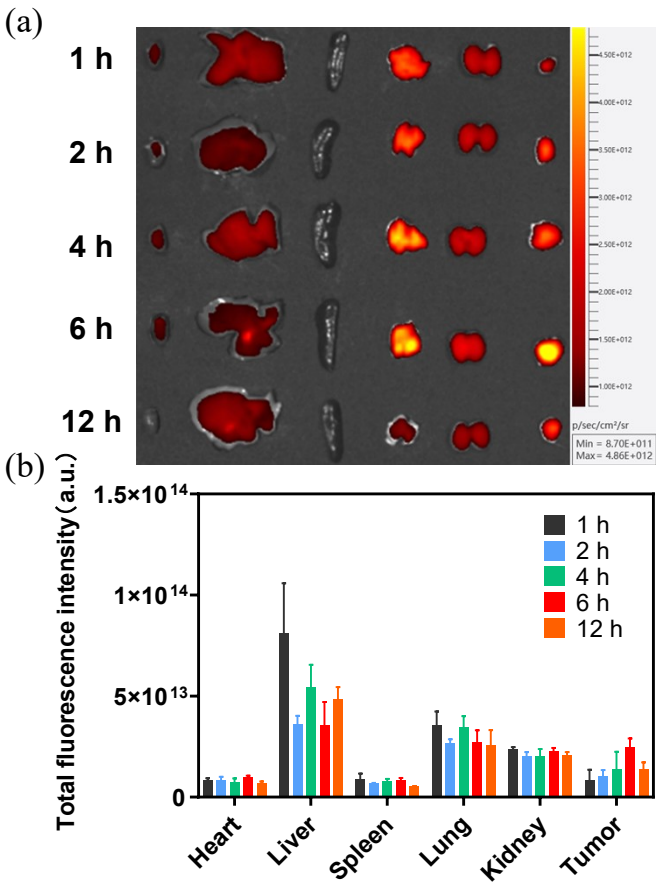

Figure S10. (a) Ex vivo NIR fluorescence images and (b) total fluorescence intensity of heart, liver, spleen, lung, kidney and tumor for different time.

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