Electronic Supplementary Information

D-A polymers for fluorescence/photoacoustic imaging and characterization of their photothermal properties

Yaowei Zhu†[a], Chuantao Gu†[a], Yawei Miao[a], Bing Yu[a,b], Youqing Shen[a,c], Hailin Cong*[a,b]

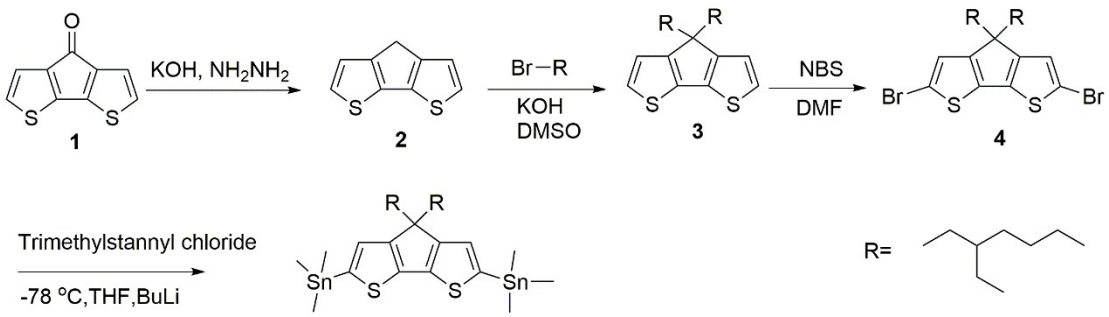
a. Institute of Biomedical Materials and Engineering, College of Materials Science and Engineering, Qingdao University, Qingdao 266071, China.

b. State Key Laboratory of Bio-Fibers and Eco-Textiles, College of Chemistry and Chemical Engineering, Qingdao University, Qingdao 266071, China.

c. Center for Bionanoengineering and Key Laboratory of Biomass Chemical Engineering of Ministry of Education, College of Chemical and Biological Engineering, Zhejiang University, Hangzhou 310027, China.

† Yaowei Zhu and Chuantao Gu contributed equally to this work.

E-mail: hailincong@yahoo.com; Fax: +86-532-85955529; Tel: +86-532-85953995



Scheme S1. Synthetic route of donor unit

Synthesis of cyclopenta[2,1-b:3,4-b']dithiophene

4H-Cyclopenta[2,1-b:3,4-b']dithiophen-4-one (4.97 g, 25.8 mmol) was dissolved in 100 mL of ethylene glycol and the solution was added potassium hydroxide (4.95 g). , 88.4 mmol) powder, after the potassium hydroxide powder was completely dissolved, hydrazine hydrate (9.9 mL, 204.2 mmol) was added to the solution under nitrogen, and then the solution was gradually raised to 180 °C. The reaction was carried out under magnetic stirring for 8 hours, and naturally dropped to room temperature after completion of the reaction. The reaction solution was extracted three times with water and diethyl ether. The organic phase was taken and dried over anhydrous Na_2SO_4. The solid was removed by filtration, and the remaining solution was treated by distillation under reduced pressure, and the organic solvent was removed to give a crude product. The crude product was purified using a silica gel column eluting with hexane. After removing n-hexane by distillation under reduced pressure, 4.34 g of white crystals was obtained. The yield of the reaction was 94%.

^{1}H NMR (600 MHz, CDCl3) δ 7.18 (d, J = 4.9 Hz, 2H), 7.09 (d, J = 4.8 Hz, 2H),3.54 (s, 2H). (Figure S1)

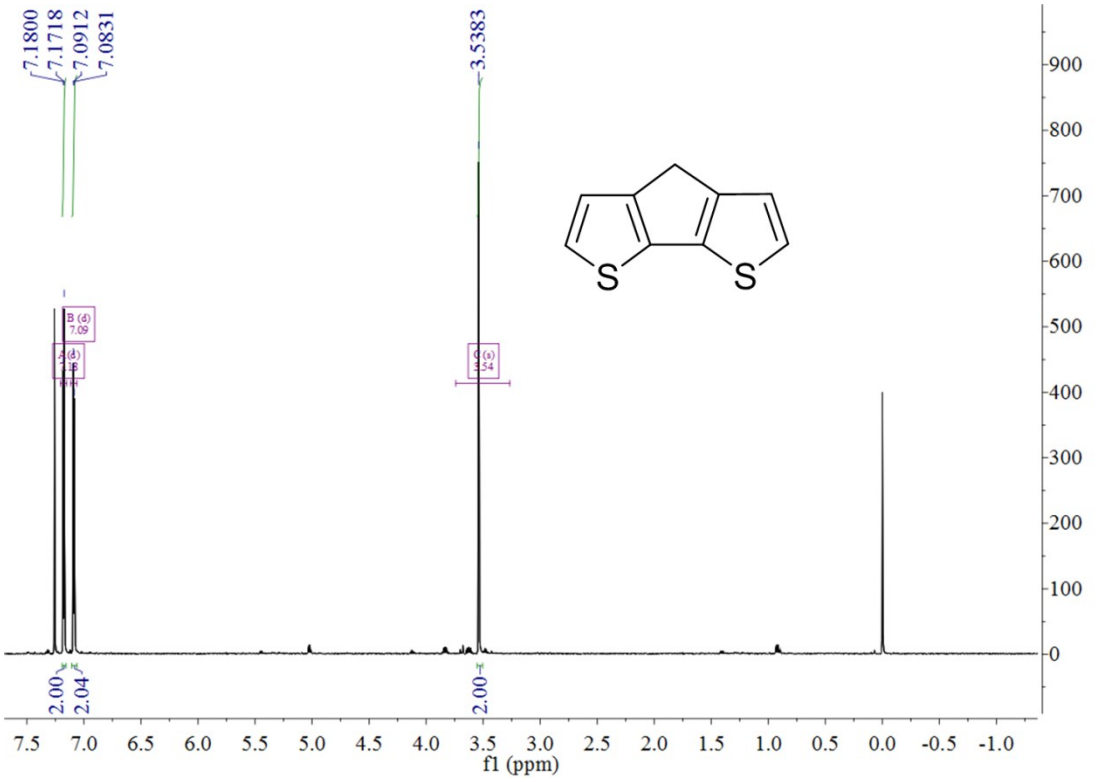


Figure S1. ^{1}HNMR spectrum of cyclopenta[2,1-b:3,4-b']dithiophene.

Synthesis of 4,4-Di(2-ethylhexyl)-cyclopenta[2,1-b:3,4-b']dithiophene

Dissolve cyclopenta[2,1-b:3,4-b']dithiophene (3.10 g, 17.4 mmol), bromoisooctane (6.8 g, 34.8 mmol) and potassium iodide (40 mg) in 60 mL DMSO, then the solution was lowered to 0 °C in an ice-water bath. After adding potassium hydroxide (3.10 g, 55.2 mmol) powder to the reaction solution, the reaction solution was naturally stirred to room temperature and stirred for 16 hours. After the reaction was completed, the reaction solution was extracted three times with water and diethyl ether. The organic phase is extracted and dried over anhydrous Na_2SO_4, then filtered to remove solids. The organic solvent was removed by distillation under reduced pressure to give a crude produce. The crude product was purified on a silica gel column eluting with n-hexane. Finally, n-hexane was distilled off under reduced pressure to give 5.73 g of pale yellow oil. The reaction yield was 82%.

^{1}H NMR (600 MHz, CDCl3) δ 7.11 (d, J = 5.0 Hz, 2H), 6.93-6.91 (m, 2H),1.82-1.90 (m, 4H), 1.01-0.84 (m, 18H), 0.75 (t, J = 7.0 Hz, 6H), 0.58 (t, J = 7.4 Hz,6H). (Figure S2)

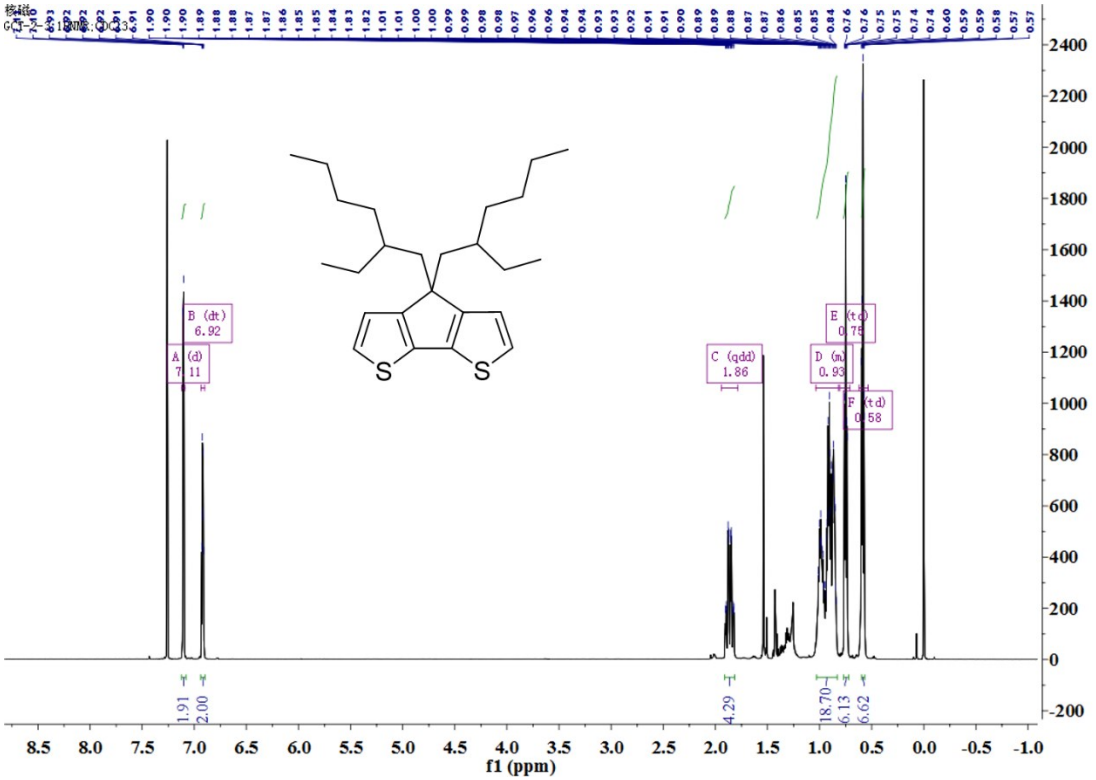


Figure S2. ^{1}HNMR spectrum of 4,4-Di(2-ethylhexyl)-cyclopenta[2,1-b:3,4-b']dithiophene

Synthesis of 2,6-Dibromo-4,4-bis(2-ethylhexyl)-4H-cyclopenta[1,2-b:5,4-b']dithiophene

4,4-Di(2-ethylhexyl)-cyclopenta[2,1-b:3,4-b']dithiophene (3.0 g, 7.4 mmol) was dissolved in 75 mL of THF. NBS (2.66 g, 14.90 mmol) was added in portions to a nitrogen-protected solution in the dark. The solution was stirred under magnetic stirring overnight, and the reaction temperature was room temperature. After the reaction was completed, the reaction solution was extracted three times with water and diethyl ether. The organic phase is extracted and dried over anhydrous Na_2SO_4, then filtered to remove solids. The organic solvent was removed by distillation under reduced pressure to give a crude product. The crude product was purified on a

silica gel column eluting with n-hexane. Finally, n-hexane was distilled off under reduced pressure to give 3.44 g of pale yellow oil. The reaction yield was 82%.

^{1}H NMR (600 MHz, CDCl3) δ 6.93 (t, J = 5.6 Hz, 2H), 1.82-1.78 (m, 4H), 1.05-0.86 (m, 18H), 0.78 (t, J = 7.0 Hz, 6H), 0.62 (t, J = 7.4 Hz, 6H). (Figure S3)

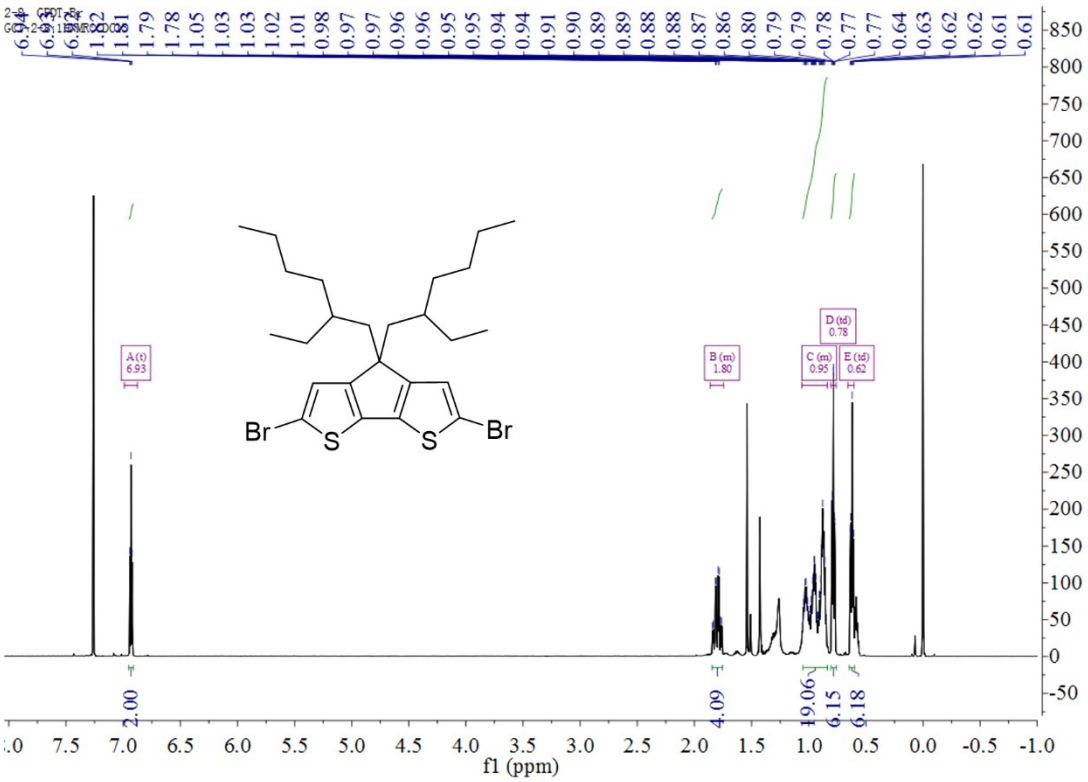


Figure S3. ^{1}H NMR spectrum of 2,6-Dibromo-4,4-bis(2-ethylhexyl)-4H-cyclopenta[1,2-b:5,4-b']dithiophene

Synthesis of 2,6-Bis(triMethyltin)-4,4-bis(2-ethylhexyl)-4H-cyclopenta[2,1-b:3,4-b']dithiophene

2,6-Dibromo-4,4-di(2-ethylhexyl)-cyclopenta[2,1-b:3,4-b']dithiophene (3.28 g, 5.85 mmol) was dissolved in 50 In anhydrous ethyl ether, The reaction solution was cooled to -78 °C in a liquid nitrogen-acetone bath under argon atmosphere, and 7.7 mL of n-BuLi (1.6 M, 12.32 mmol) solution was slowly added dropwise to the solution. After the addition was completed, the -78 °C was maintained for 1 hour. Then, 12.90 mL of a solution of trimethyltin chloride (1.0 M, 12.90 mmol) was added to the reaction solution through a long steel needle. After the addition was completed, the solution was naturally warmed to room temperature and stirred under magnetic stirring overnight.

After the reaction was completed, the reaction solution was poured into water and extracted with diethyl ether three times. The organic phase is extracted and dried by anhydrous Na_2SO_4. The solvent is removed by distillation under reduced pressure to give a crude product. The crude product was quickly passed through an alumina column pretreated with triethylamine. The organic solvent was then distilled off under reduced pressure to give 3.08 g of pale yellow oil. The reaction yield was 94%.

^{1}H NMR (600 MHz, CDCl3) δ 6.95-6.93 (m, 2H), 1.88-1.80 (m, 4H), 1.33-1.24 (m, 2H), 1.04-0.81 (m, 16H), 0.74 (t, J = 7.1 Hz, 6H), 0.59 (t, J = 7.3 Hz, 6H), 0.38 (s, 18H). (Figure S4)

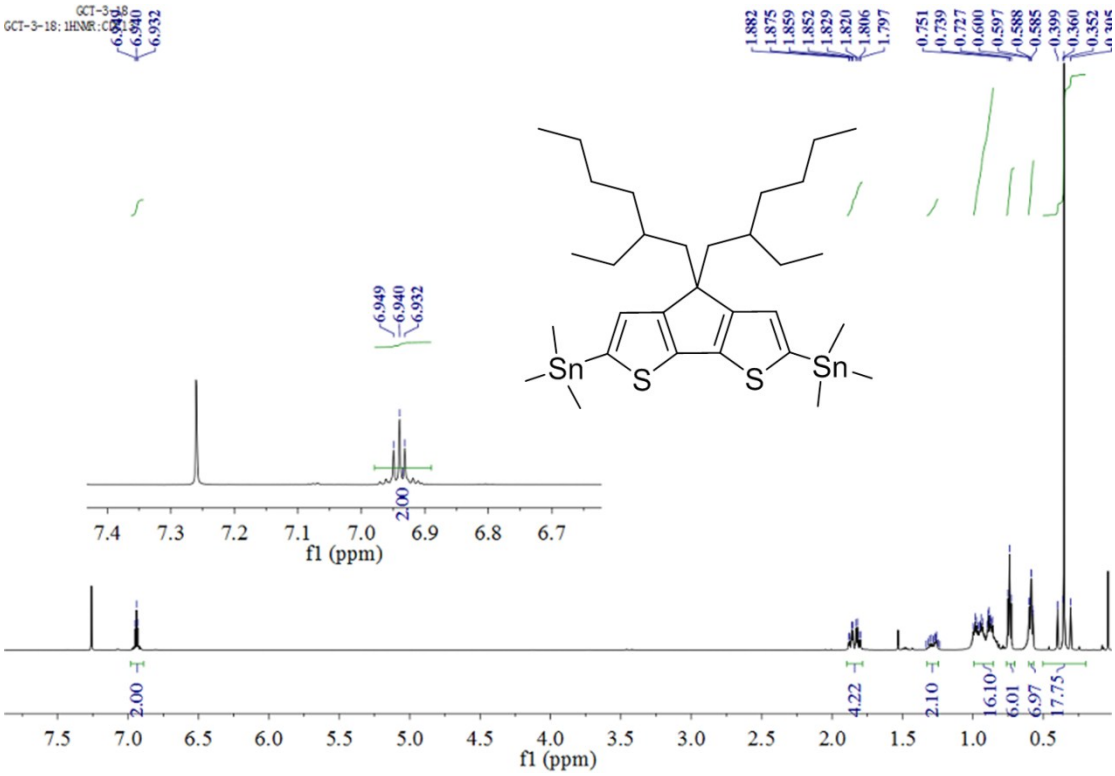


Figure S4. ^{1}H NMR spectrum of 2,6-Bis(triMethyltin)-4,4-bis(2-ethylhexyl)-4H-cyclopenta[2,1-b:3,4-b'] dithiophene

Synthesis of P-TT

2,6-bis(trimethyltin)-4,4-di(2-ethylhexyl)- cyclopenta[2,1-b:3,4-b'] Thiophene (145 mg, 0.20 mmol) and Octyl 4,6-DibroMothieno [3,4-b]thiophene-2-carboxylate (90.984 mg, 0.20 mmol) were dissolved in toluene (8 mL).The reaction was carried out under the catalysis of tetrakis (triphenylphosphine) palladium (5 mg, 0.0026 mmol). The reaction temperature was 120 °C and the reaction time was 36 h. After the reaction was completed, the reaction solution was cooled to room temperature. The reaction solution was poured into 150 mL of anhydrous methanol, followed by suction filtration to give a crude product. The crude product was dissolved in chloroform and purified on silica gel column (silica gel 80-100 mesh). The collected chloroform solution was distilled under reduced pressure, and the obtained solid was dissolved in chloroform as little as possible, and then added to 120 mL of anhydrous methanol, and then subjected to suction filtration and vacuum drying to obtain 174.64 mg of a dark blue solid (P-TT, yield 74%).

^{1}HNMR (600 MHz, CDCl3) δ 8.18-7.96 (m, 1H), 7.19-7.00 (m, 2H), 4.41 (br, 2H), 1.90 (d, 6H), 1.36 (t, 10H), 1.05-0.90 (m, 18H), 0.72 (d, 15H). (Figure S5)

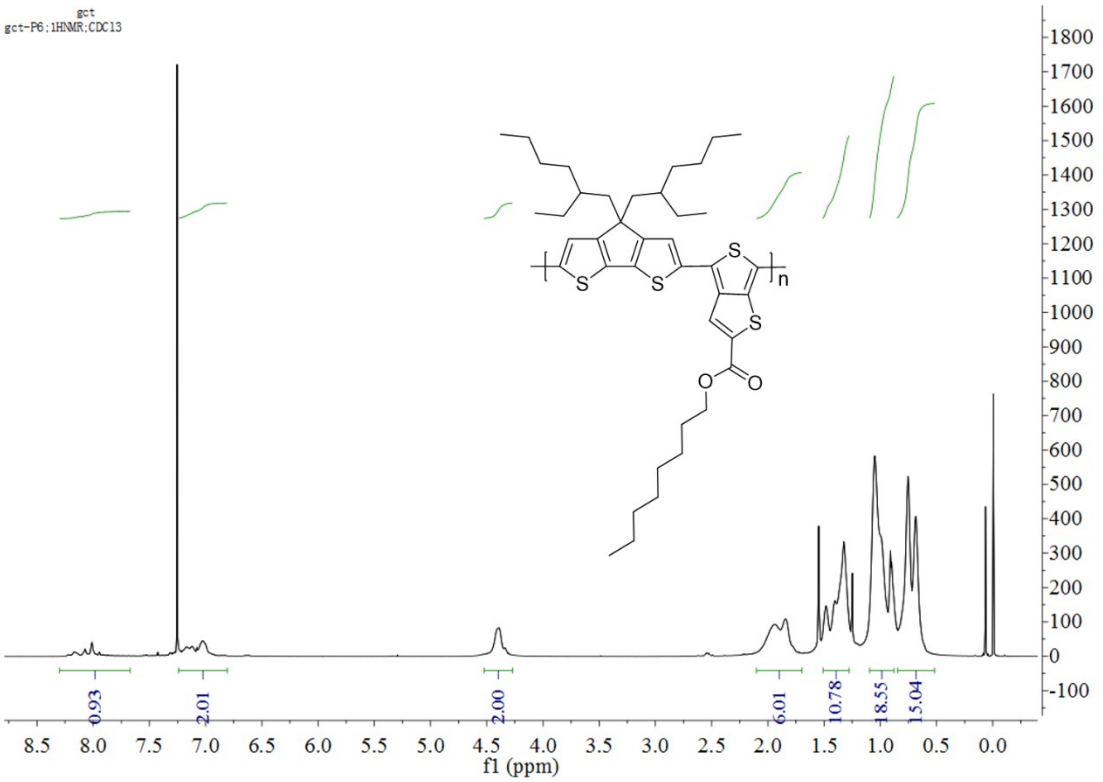


Figure S5. ^{1}HNMR spectrum of P-TT.

Synthesis of P-DPP

2,6-bis(trimethyltin)-4,4-di(2-ethylhexyl)-cyclopenta[2,1-b:3,4-b'] dithiophene (153 mg, 0.21 mml) and 3,6-Bis(5-bromothiophen-2-yl)-2,5-bis(2-ethylhexyl)pyrrolo[3,4-c]pyrrole -1,4(2H,5H) -dione (143 mg, 0.21 mmol) was dissolved in toluene (8 mL). The reaction was carried out under the catalysis of tetrakis (triphenylphosphine) palladium (5 mg, 0.0026 mmol). The reaction temperature is 120 °C, and the reaction time is 36 h. After the reaction was completed, the reaction solution was cooled to room temperature. The reaction solution was poured into 150 mL of anhydrous methanol, followed by suction filtration to give a crude product. The crude product was dissolved in chloroform and purified on silica gel column (silica gel 80-100 mesh). The collected chloroform solution was distilled under reduced pressure, and the obtained solid was dissolved in chloroform as little as possible, and then added to 120 mL of anhydrous methanol, and then subjected to suction filtration and vacuum drying to obtain 201.28mg f a dark Green solid (P-DPP, yield 68%).

^{1}HNMR (600 MHz, CDCl3) δ 8.96 (br, 2H), 7.39 (br, 1H), 7.21-6.95 (m, 3H) 4.07 (br, 4H), 1.90 (br, 6H), 1.40-1.25 (m, 14H), 1.03-0.91 (m, 32H), 0.71 (d, 12H). (Figure S6)

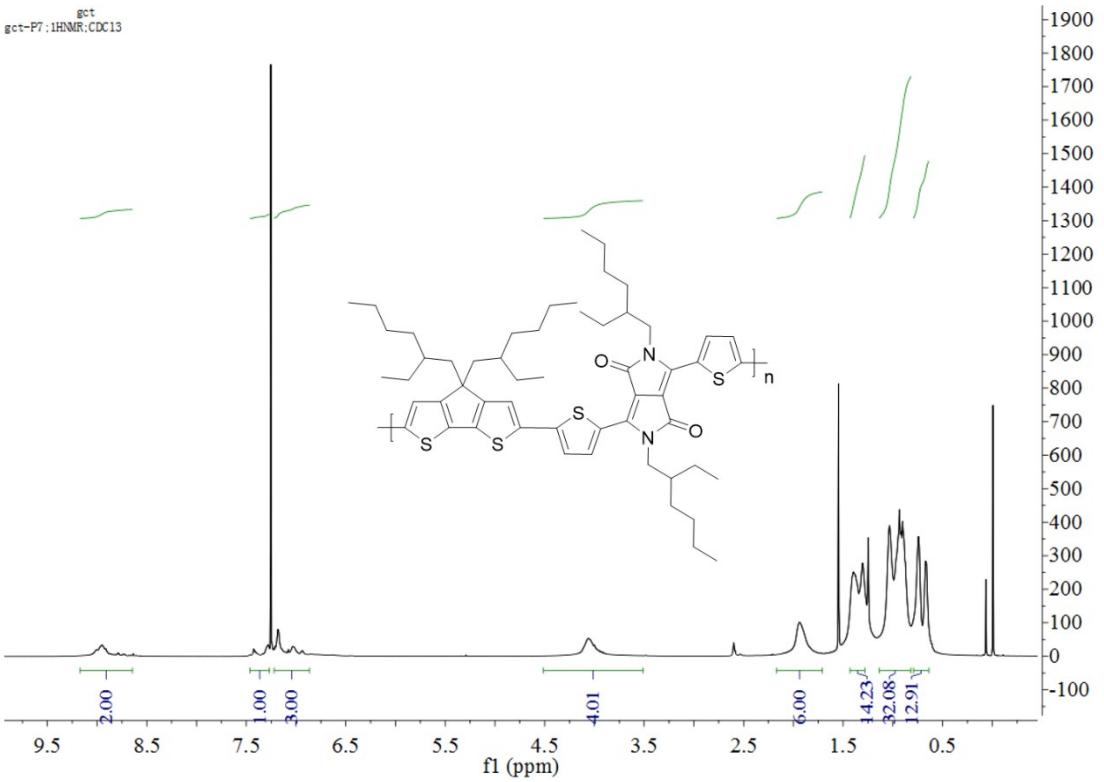


Figure S6. ^{1}HNMR spectrum of P-DPP.

Preparation of nanoparticles

CPNs were prepared by nanoprecipitation. First, 1 mg of the polymer and 5 mg of DSPE-PEG2000 were dissolved in 4 mL of tetrahydrofuran, and the solution was quickly added to 36 mL of distilled water under strong ultrasonication, followed by distillation under reduced pressure to remove tetrahydrofuran and most of the water to obtain 2 mL of a concentrated CPNs solution at a concentration of 500μg·mL^{-1}.

Calculation of quantum yield

IR-26 was used as a standard sample, and its absorption spectrum was measured by the UV-Vis-NIR to obtain five concentrations of solutions having absorbances at 808 nm of 0.023, 0.040, 0.053, 0.062, and 0.083. (Figure S2a) The above five concentrations of IR-26 were excited by laser at 808 nm to obtain their fluorescence spectra. (Figure S2b). The range of 900-1500 nm in the fluorescence spectrum was integrated using Origin Lab, and the results were plotted according to the absorbance-integral area to obtain the intercept and slope of the curve. （Figure 3a）。

Similarly, different absorbance solutions (Figure 2c, e) of CPNs of P-TT and P-DPP were excited at 808 nm, and their fluorescence spectra were obtained using the Origin Lab versus fluorescence spectra (Figure 2 d, f) 900- The range of 1500 nm is integrated, and the obtained structure is plotted according to the absorbance-integral area to obtain the intercept and slope of the curve (Figure 3b, c)

Quantum yield is calculated by the following formula

$$QY_{sample} = QY_{IR26} \times \frac{Slope_{sample}}{Slope_{IR26}} \times \frac{{n_{sample}}^2}{{n_{IR26}}^2}$$

Calculation result: $QY_{P\text{-}TT}$=0.5%, $QY_{P\text{-}DPP}$=1.5%

Cytotoxicity test

Hela cells were used to test for cytotoxicity. The CPNs of P-TT and P-DPP were dissolved in the culture medium at a concentration of 10 μg·mL^{-1}, 20 μg·mL^{-1}, 30 μg·mL^{-1}, 40 μg·mL^{-1} and 50 μg·mL^{-1}. Five concentrations of the solution were used to test cytotoxicity. Ten groups were tested in parallel for each concentration. After the highest and lowest values were removed, the average was obtained and the final result was obtained.

Vascular fluorescence imaging of nude mice

The nude mice used were 4 weeks old, and a laser of 808 nm was used as an excitation light source with a laser intensity of 885 mW·cm-2. The signal filter in front of the camera lens was 850 nm long, and the volume of the CPNs solution injected into the nude mice was 50 μL. The concentration was 500 μg·mL^{-1}. Nude mice were anesthetized with ether after injection of CPNs and then placed under a camera lens for imaging.

Photothermal therapy

A 500 ug/ml CPNs solution was added to a 1 mL centrifuge tube, and photothermal data (power: 0.9 W·cm^{-2}) was measured under laser irradiation at 660 nm.

The nude mice used were 4 weeks old, HeLa cells were used for tumour inoculation. Orthotopic injection was used at a dose of 20 microliters (500 micrograms per milliliter). The formula for calculating tumour volume (V) is as follows:

$$V = \frac{a \times b^2}{2}$$

a represents the length of the tumour and *b* represents the width.

The relative tumour volume (V_{RTV}) is calculated as follows:

$$V_{RTV} = \frac{V}{V_0}$$

V_0 represents the initial volume of tumour.

Calculation of photothermal conversion efficiency

The photothermal conversion efficiency (η) is calculated by the following formula:

$$\eta = \frac{(hs(T_{Max} - T_{Surr}) - Q_{Dis})}{I(1 - 10^{-A_{660}})} \qquad S1$$

h represents the heat transfer coefficient, *s* represents the surface area of the container, and the value of *hs* is determined by the equation (S2). T_{Max} represents the highest steady state temperature of the solution, and T_{Surr} represents the surrounding temperature. Q_{Dis} represents the amount of heat emitted by the laser mediated by the solvent and the container. *I* represent the laser power and A_{660} represents the absorbance of the solution at 660 nm.

$$hs = \frac{mC}{\tau_s} \qquad S2$$

m represents the mass of the solution, *C* represents the specific heat capacity of the solvent, and the value of τ_s can be determined by the formula (S3).

$$\tau_s = -\frac{t}{ln(\theta)} \qquad S3$$

Θ is a dimensionless constant about time and *t* is time. Θ can be calculated by the following formula (S4).

$$\theta = \frac{T - T_{Surr}}{T_{Max} - T_{Surr}} \qquad S4$$

T represents the solution temperature at time *t*.[1]

The calculation results show that $\eta_{P\text{-}TT}$ = 16%, $\eta_{P\text{-}DPP}$ = 15%.

Photoacoustic imaging of the liver of nude mice

The nude mice used were 4 weeks old, the pulsed laser had a wavelength of 530 nm, the laser intensity was 1 W·cm^{-2}, and the volume of the CPNs solution injected into the nude mice was 50 μL. The concentration of SPN nanoparticles was 500 μg·mL^{-1}. Nude mice were anesthetized with ether after injection of CPNs solution, and then their limbs were fixed on a wooden board. The liver part of the nude mouse is coated with ultrasonic glue, the ultrasonic glue is in contact with the lower surface of the plastic wrap, the water is directly contacted above the wrap film, and the signal receiver receives the acoustic signal in the water.

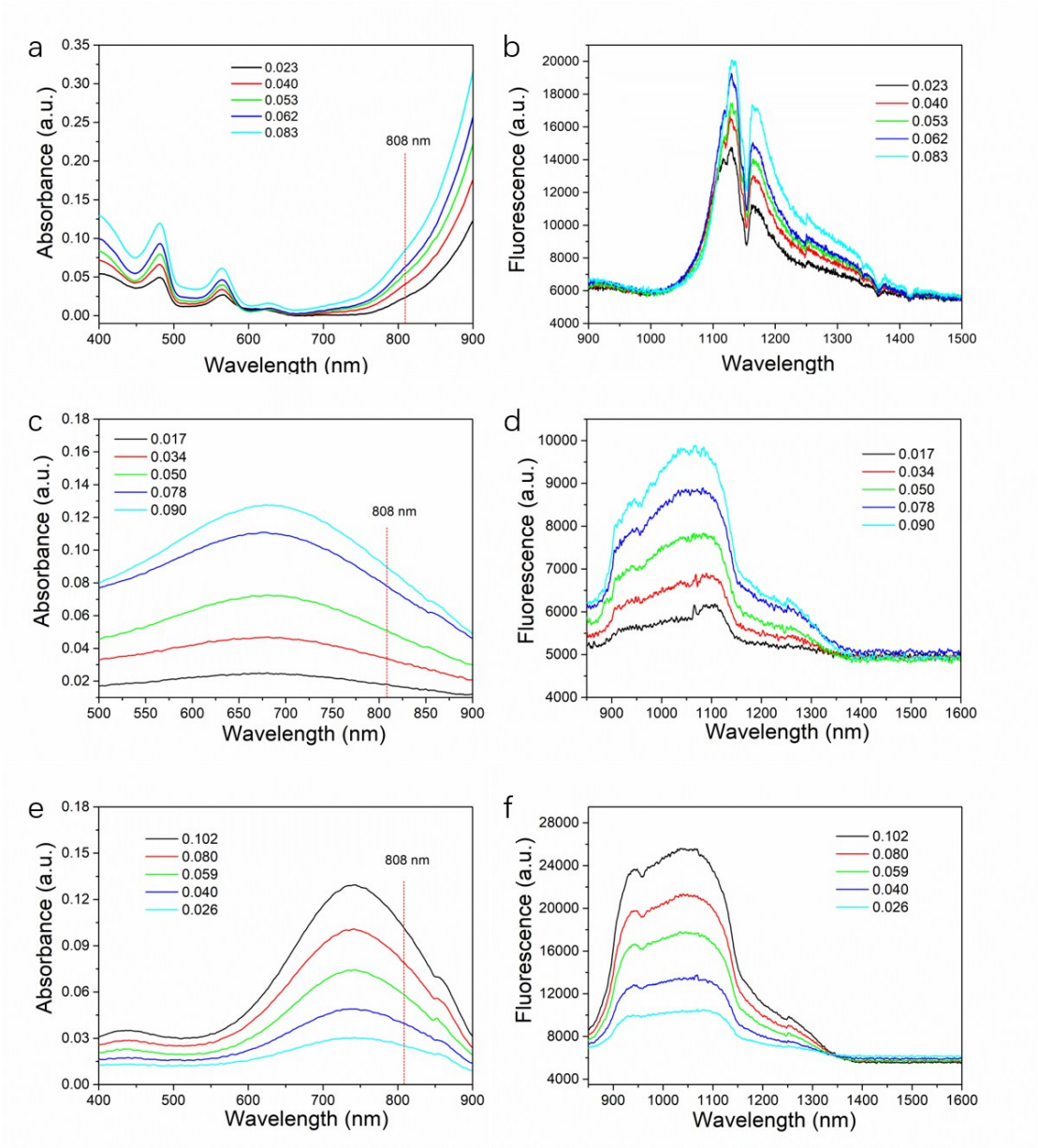


Figure S7. a, c, e) Absorption spectrum of IR26, P-TT and P-DPP. b, d, f) Fluorescence spectrum of

IR26, P-TT and P-DPP excited at 808 nm.

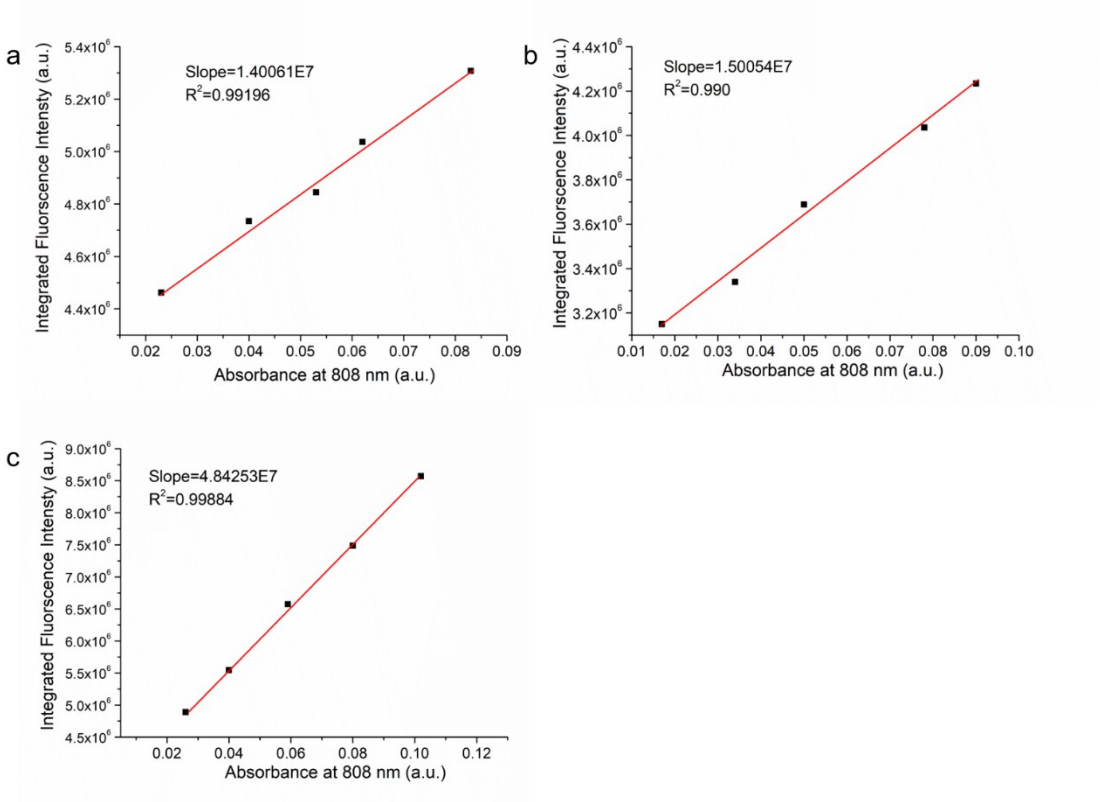


Figure S8. Fit curve of absorbance at 808 nm with integrated fluorescence intensity. a, b, c) the curve of IR26,P-TT and P-DPP

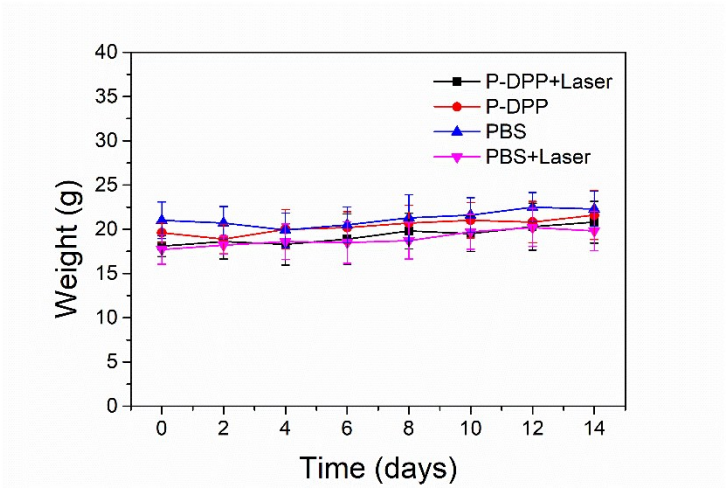


Figure S9. Body weight changes in nude mice within 14 days.

Reference

1. H. Wang, J. Chang, M. Shi, W. Pan, N. Li, and B. Tang, Angew. Chem. 2019, 131(4), 1069-1073.