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Electronic Supplementary Information

Rational design of polymers through donor modulation to weaken aggregation-caused quenching effect for NIR-II fluorescence imaging

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Materials

All reagents were purchased from commercial sources. 4-Bromoaniline (Aladdin, Shanghai, China.) paraformaldehyde (Aladdin, Shanghai, China.), trifluoroacetic acid (Macklin, Shanghai, China.), Mg_2SO_4 (Macklin, Shanghai, China.), toluene (Xihua, Guangdong, China), chloroform (Xihua, Guangdong, China), dichloromethane (Xihua, Guangdong, China), petroleum ether (Xihua, Guangdong, China), ethyl acetate (Xihua, Guangdong, China), n-hexane (Xihua, Guangdong, China), tetrahydrofuran (Xihua, Guangdong, China), calcium hydride (Macklin, Shanghai, China.)，2,5-bis(2-ethylhexyl)-3,6-bis(5-(trimethylstannyl)thiophen-2-yl)-2,5- dihydropyrrolo[3,4-c]pyrrole-1,4-dione (Zhiyan, shanghai, China), tetrakis (triphenylphosphine)palladium (Macklin, Shanghai, China.), 3,4-Ethylenedioxythiophene (Aladdin, Shanghai, China.), n-Butyllithium solution (Aladdin, Shanghai, China.), N-bromosuccinimide (Macklin, Shanghai, China.), DSPE-PEG2000 (Macklin, Shanghai, China.). Unless otherwise specified, it can be used directly without purification.

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Fourier 300 MHz. Absorption spectra were tested by UV-Vis-NIR Absorption Spectrometer (L8, Shanghai, China). Fluorescence spectra were tested by Steady-state/transient fluorescence spectrometer (FLS1000, The United Kingdom). The size of nanoparticles was measured by Dynamic Light Scattering Particle Size Analyzer (Malvern Panalytical, Shanghai, China). The NIR-II *in vivo* imager was built by Joint Laboratory of Opto-Functional Theranostics in Medicine and Chemistry, the First Hospital of Jilin University, for the *in vivo* imaging experiments.

Chemical synthesis

Synthesis of 2,8-dibromo-6H,12H-5,11-methanodibenzo[b, f][1,5]diazocine. (3, Donor A)

4-Bromoaniline (10.7 g, 60 mmol) and paraformaldehyde (3.6 g, 120 mmol) were added to a 250 ml round bottom flask, trifluoroacetic acid (TFA, 120 mL) was added dropwise to the above mixture and stirred at -15 ºC for 1h. Subsequently, the reaction solution was raised to room temperature and stirring was continued for 144 h. The reaction solution was slowly poured into a mixture of ice and ammonia. The reaction solution was extracted three times with water and dichloromethane (30 mL), and then dried over anhydrous Mg_2SO_4 for 1 h. The solid residue was filtered and the solution was collected. The filtrate was evaporated to dryness on a rotary evaporator under reduced pressure to obtain a crude product. The crude product was further purified by column chromatography (SiO_2, petroleum ether/ethyl acetate, 6:1, v/v) to give white crystals. The product was vacuum dried and had a mass of 11.4 g. (Yield: 51 %)

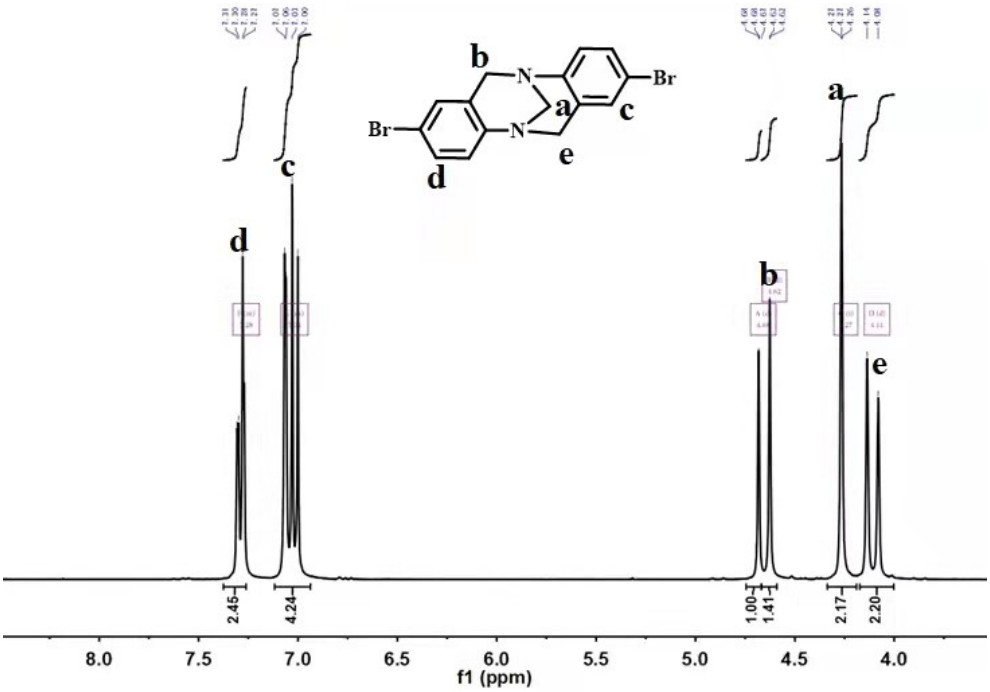

Figure S1. [1]HNMR spectroscopy of 2,8-dibromo-6H,12H-5,11-methanodibenzo [b, f] [1,5] diazocine.

Synthesis of Polymer P-TB (5)

Refining of toluene：Measure 150 mL of toluene into a 250 mL round-bottomed flask, and add a small amount of calcium hydride. The mixture was stirred and refluxed for 2 h at 120 ºC. Subsequently, the reflux unit was changed to a distillation unit. The distillate was collected and nitrogen gas was continuously passed through to obtain 100 mL of purified toluene.

2,8-dibromo-6H,12H-5,11-methanodibenzo[b, f][1,5]diazocine. (3) (40 mg, 0.105 mmol), 2,5-bis(2-ethylhexyl)-3,6-bis(5-(trimethylstannyl)thiophen-2-yl)-2,5-dihydropyrrolo[3,4-c]pyrrole-1,4-dione (62 mg, 0.072 mmol) and tetrakis (triphenylphosphine)palladium (10 mg, 0.007 mmol) were dissolved in dry toluene (20 mL). The reaction was carried out at 115 °C for 48 h under nitrogen atmosphere. The reaction solution was cooled to room temperature. The reaction solution was extracted three times with water and dichloromethane (30 ml), the organic phase was collected and dried with anhydrous Mg_2SO_4 for 3 h. The solid residue was filtered and the solution was collected. The filtrate was evaporated to dryness on a rotary evaporator under reduced pressure to obtain a crude product. The crude product was further purified by column chromatography (SiO_2, chloroform). The product was washed with ice methanol, filtered with suction, and dried under vacuum for 24 h to obtain black polymer P-TB. (40 mg, yield: 78 %)

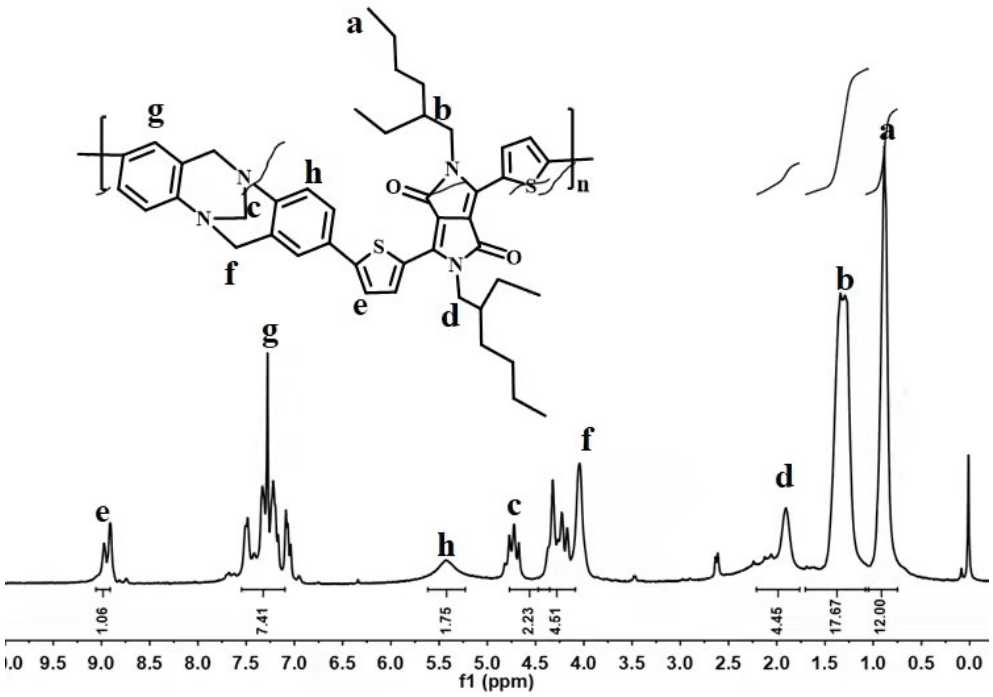

Figure S2. ^{1}HNMR spectroscopy of polymer P-TB.

Synthesis of tributyl(2,3-dihydrothieno[3,4-b][1,4]dioxin-5-yl)stannane (7)

Refinement of tetrahydrofuran (THF): Measure 150 mL of THF solution into a 250 mL round-bottomed flask, and add a small amount of calcium hydride. Reflux at 66 ºC for 2 h. Change the reflux unit to a distillation unit. The distillate was collected and nitrogen gas was continuously purged to obtain 100 mL of refined THF.

3,4-Ethylenedioxythiophene (995 mg, 7.02 mmol) was dissolved in 30 mL of THF and the reaction solution was warmed to -78ºC under nitrogen protection. 4.388 mL of n-butyllithium in n-hexane solution (1.6 M, 7.02 mmol) was measured and added dropwise to the reaction solution. Subsequently, the reaction was carried out at -78ºC for 1 h under nitrogen atmosphere. Tributyltin chloride (2.285 g, 7.02 mmol) was added to the reaction solution. The reaction solution was warmed to room temperature and stirred overnight. After the reaction was completed, the reaction solution was extracted three times with n-hexane and water. The organic phase was collected and dried with anhydrous $MgSO_4$ for 3 h. The solid residue was removed by suction filtration, and the filtrate was evaporated to dryness by a rotary evaporator under

reduced pressure. Obtained 2.57 g of oily yellow liquid. The product was directly carried to the next step without further purification.

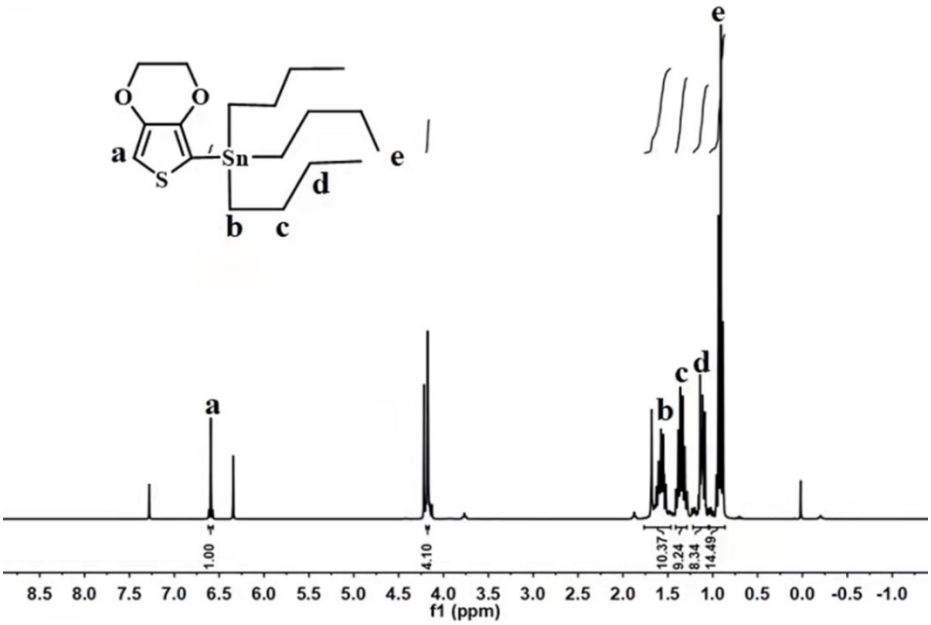

Figure S3. [1]HNMR spectroscopy of tributyl(2,3-dihydrothieno[3,4-b][1,4]dioxin-5-yl)stannane.

Synthesis of 2,5-bis(2,3-dihydrothieno[3,4-b][1,4]dioxin-5-yl)thiophene (9, Donor B)

Refining of toluene: Measure 150 mL of toluene solution into a 250 mL round-bottomed flask, and add a small amount of calcium hydride. Reflux at 115 ºC for 2 h. Change the reflux unit to a distillation unit. The distillate was collected and nitrogen gas was continuously purged to obtain 100 mL of refined toluene.

Tributyl(2,3-dihydrothieno[3,4-b][1,4]dioxin-5-yl)stannane (1.0 g, 2.56 mmol), 2,5-dibromothiophene (281 mg, 1.16 mmol) and Tetrakis(triphenylphosphine) palladium (67 mg, 0.058 mmol) was dissolved in 20 mL of toluene. The reaction was carried out at 115ºC for 48 h under nitrogen protection. After the reaction solution was cooled to room temperature, the reaction solution was extracted three times with water and 30 ml of n-hexane. The organic phase was collected and dried over anhydrous Mg_2SO_4 for 1 h. The solid residue was removed by suction filtration, and the filtrate was evaporated to dryness to obtain a crude product. The crude product was further

purified by column chromatography (SiO_2, n-hexane/ethyl acetate, 1:9, v/v) The product is 238 mg of wine red oily liquid (Yield: 56 %).

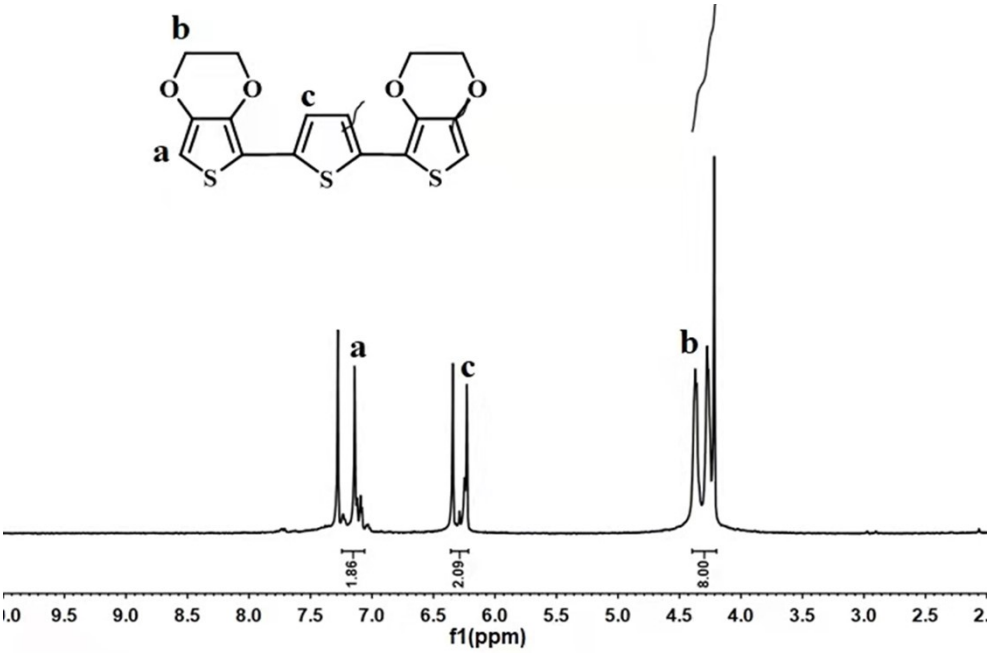

Figure S4. [1]HNMR spectroscopy of 2,5-bis(2,3-dihydrothieno[3,4-b][1,4]dioxin-5-yl)thiophene.

Synthesis of 2,5-bis(7-bromo-2,3-dihydrothieno[3,4-b][1,4]dioxin-5-yl)thiophene (10)

2,5-Bis(2,3-dihydrothieno[3,4-b][1,4]dioxin-5-yl)thiophene (238 mg, 0.63 mmol) was dissolved in 30 mL of purified tetrahydrofuran, protected from light, *N*-bromosuccinimide (NBS) was added to the solution in portions at 0 ºC. The reaction was carried out for 16 h. After the reaction solution was cooled to room temperature, the reaction solution was extracted three times with water and 30 mL of n-hexane. The organic phase was collected and dried over anhydrous Mg_2SO_4 for 1 h. The solid residue was removed by suction filtration, and the filtrate was evaporated to dryness to obtain a crude product. The crude product was further purified by column chromatography (SiO_2, n-hexane/ethyl acetate, 2:1, v/v). The product is 210 mg of pale yellow solid. (Yield: 64 %)

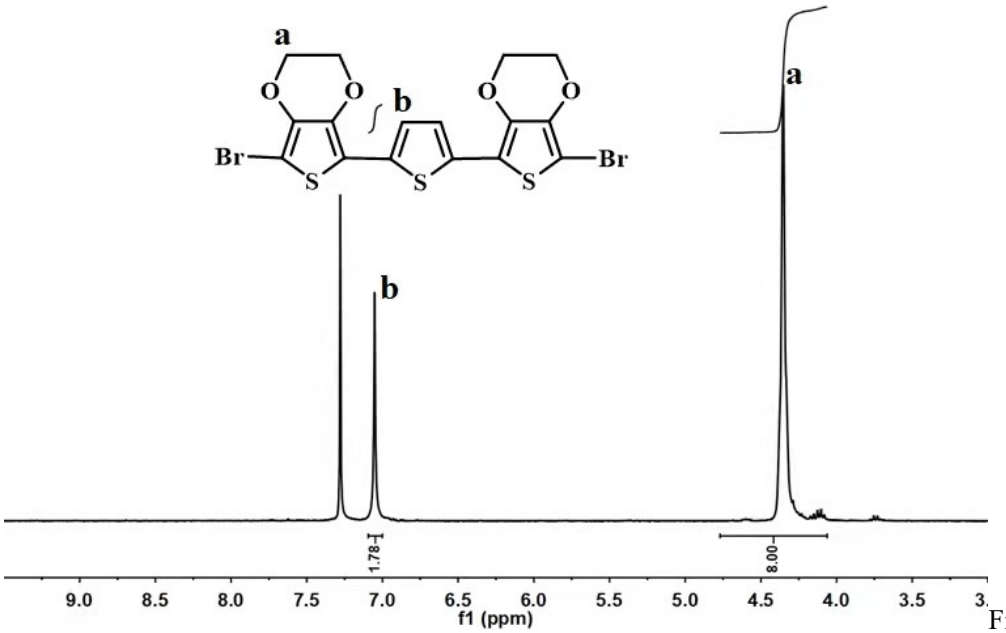

Figure S5. ^{1}HNMR spectroscopy of 2,5-bis(7-bromo-2,3-dihydrothieno[3,4-b][1,4]dioxin-5-yl)thiophene.

Synthesis of P-TP

2,5-Bis(7-bromo-2,3-dihydrothieno[3,4-b][1,4]dioxin-5-yl)thiophene (10) (31 mg，0.059 mmol), 2,5-bis(2-ethylhexyl)-3,6-bis(5-(trimethylstannyl)thiophen-2-yl)-2,5-dihydropyrrolo[3,4-c]pyrrole-1,4-dione (50 mg, 0.059 mmol) and tetrakis (triphenylphosphine)palladium (8.0 mg, 0.0059 mmol) were dissolved in dry toluene (20 mL). The reaction was carried out at 115 °C for 48 h under nitrogen atmosphere. The reaction solution was cooled to room temperature. The reaction solution was extracted three times with water and dichloromethane (30 mL), the organic phase was collected and dried with anhydrous Mg_2SO_4 for 3 h. The solid residue was filtered and the solution was collected. The filtrate was evaporated to dryness on a rotary evaporator under reduced pressure to obtain a crude product. The crude product was further purified by column chromatography (SiO_2, chloroform). The product was washed with ice methanol, filtered with suction, and dried under vacuum for 24 h to obtain black polymer P-TB. (30 mg, yield: 58 %)

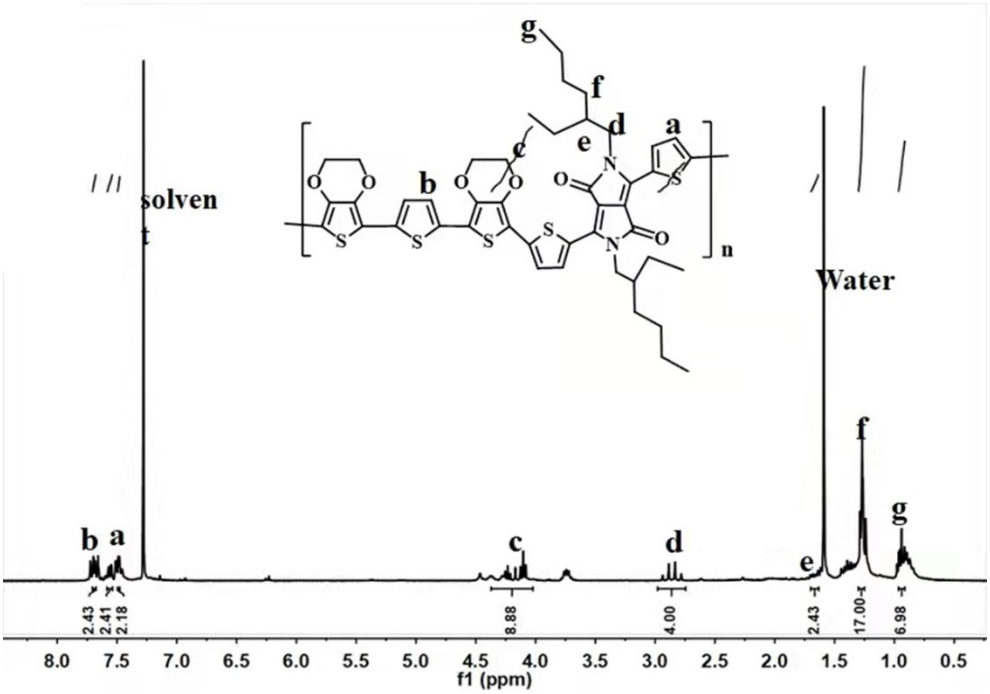

Figure S6. [1]HNMR spectroscopy of polymer P-TP.

Theoretical Simulation

The conformations of the donor unit, acceptor unit, one repeat unit and two repeat units were all modeled to replicate the contribution from the adjacent groups in polymer P-TB and P-TP. All the systems shown in this study are full optimized to investigate the optimal structures and reach their energy minimums where only the bonding connections in these simulated system were kept and all various spatial stretching of each unit and the potential twisted possibility among each unit are fully considered. Finally we identify their global energy minimums and the corresponding conformations. All the simulation are conducted using the hybrid exchange correlation B3LYP functional together with 6-31G(d) basis set by Gaussian09 D.01 programs.[S1]

Preparation of nanoparticles

Nanoparticles 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 500 $\mu g \cdot mL^{-1}$.

The size of the nanoparticles was measured by dynamic light scattering. The size of P-TBNPs and P-TPNPs is the average of the results of three parallel tests.

Calculation of molar extinction coefficient

Chloroform solutions of polymers P-TB and P-TP at a concentration of 10 mg/mL were used for UV-Vis-NIR absorption spectroscopic characterization. Based on one repeating unit, the molar concentrations of P-TB and P-TP were 1.3×10^{-5} $mol \cdot L^{-1}$ and 1.13×10^{-5} $mol \cdot L^{-1}$, respectively. The absorbances of P-TB and P-TP at the absorption peaks were 1 and 1.34, respectively; and the absorbances of P-TB and P-TP at 808nm is 0.025 and 0.16, respectively. Similarly, PBS aqueous solutions of P-TBNPs and P-TPNPs are configured with concentrations of 1.3×10^{-5} mol·L-1 and 1.13×10^{-5} $mol \cdot L^{-1}$, respectively. The absorbances of P-TBNPs and P-TPNPs at the absorption peaks were 0.39 and 1.02, respectively; and the absorbances of P-TBNPs and P-TPNPs at 808nm is 0.16 and 0.35, respectively.

The molar extinction coefficient of P-TB and P-TP is calculated according to the following formula:

$$A = \varepsilon \times b \times c$$

Where ε is the molar extinction coefficient, A for absorbance, b for the thickness of the liquid layer (1 cm), and c for the solution concentration ($10^{-5} \cdot mol \cdot L^{-1}$).

The calculation result in main peak is: $\varepsilon_{P\text{-}TB} = 7.7\times10^{4}$ $L \cdot mol^{-1} \cdot cm^{-1}$, $\varepsilon_{P\text{-}TP} = 1.2\times10^{5}$

$L \cdot mol^{-1} \cdot cm^{-1}$; $\varepsilon_{P\text{-}TBNPs} = 3.0\times10^4$ $L \cdot mol^{-1} \cdot cm^{-1}$, $\varepsilon_{P\text{-}TPNPs} = 9.2\times10^4$ $L \cdot mol^{-1} \cdot cm^{-1}$

The calculation result in 808 nm is: $\varepsilon_{P\text{-}TB808} = 2.3\times10^3$ $L \cdot mol^{-1} \cdot cm^{-1}$, $\varepsilon_{P\text{-}TP808} = 1.4\times10^4$ $L \cdot mol^{-1} \cdot cm^{-1}$; $\varepsilon_{P\text{-}TBNPs808} = 1.2\times10^4$ $L \cdot mol^{-1} \cdot cm^{-1}$, $\varepsilon_{P\text{-}TPNPs808} = 3.1\times10^4$ $L \cdot mol^{-1} \cdot cm^{-1}$.

Calculation of quantum yield

IR-26 was used as a standard sample, and its absorption spectrum was measured by the *UV-Vis* to obtain five concentrations of chloroform solutions having absorbances at 808 nm. The above five concentrations of IR-26 were excited by laser at 808 nm to obtain their fluorescence spectra. (Figure S7 a). 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.

Similarly, different absorbance chloroform solutions of P-TB and P-TP were excited at 808 nm, and their fluorescence spectra were obtained using the Origin Lab versus fluorescence spectra The range of 900-1500 nm is integrated, and the obtained structure is plotted according to the absorbance-integral area to obtain the intercept and slope of the curve.

With IR26 (QY=0.5%)[S2] as the standard sample, the quantum yield was calculated using the following formula:

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

Calculation result: $QY_{P\text{-}TT}$=0.4%, $QY_{P\text{-}DPP}$=0.7%

The photothermal conversion efficiency

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

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

h represents the heat transfer coefficient, *s* represents the surface area of the container, and the value of *hs* is determined by the equation. 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.

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

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.

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

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

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

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

The calculation results show that $\eta_{P\text{-}TB}$ = 19%, $\eta_{P\text{-}TP}$ = 17%.

In vitro solution imaging experiments

Two fluorescent polymers in PBS were used for *in vitro* imaging at concentrations of 50 μM, 100 μM, 200 μM, 400 μM, 600 μM and 1000 μM, respectively. A 1000 nm filter was used, the laser current was 7.17 mA, and the exposure time for imaging was 100 ms.

In vitro imaging of P-TB and P-TB in dichloromethane under the same conditions was also tested.

Cytotoxicity test

L929 cells were used to test for cytotoxicity. The P-TBNPs and P-TPNPs 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.

In vivo mouse imaging experiments

Healthy female mice (aged 5-6 weeks) were used for *in vivo* imaging experiments. The mice subjected to the imaging experiments were divided into two groups of three mice each. Breast cancer cells (4T1) were suspended in culture medium, and the cell suspension was injected subcutaneously into the left axilla of mice (100 μL, the number of cells was counted by a counting plate, about 2 million cells). The tumor volume was observed daily, and the tumor volume was calculated according to the formula:

$$V = \frac{L \times W^2}{2}$$

Where L and W represent the longitudinal and transverse diameters of the tumor, respectively. When the tumor in the mouse grows to about 100 mm^3, it can be used for *in vivo* imaging experiments.

First, tumor-bearing mice were injected with 100 μL of P-TBNP or P-TPNPs *via* the tail vein, and the concentration of nanoparticles was 600 μM. Then, the mice were anesthetized with isoflurane and NIR-II fluorescence imaging was performed in an imager under excitation by a laser with a wavelength of 808 nm. A 1000 nm filter was used, the laser current was 7.17 mA, and the exposure time for imaging was 100 ms.

Live subject statement

All animal experiments were performed according to the Guide for the Care and Use of Laboratory. Animals and were approved by the Animal Care and Use Committee of Jilin University (Approval No. SY202103013).

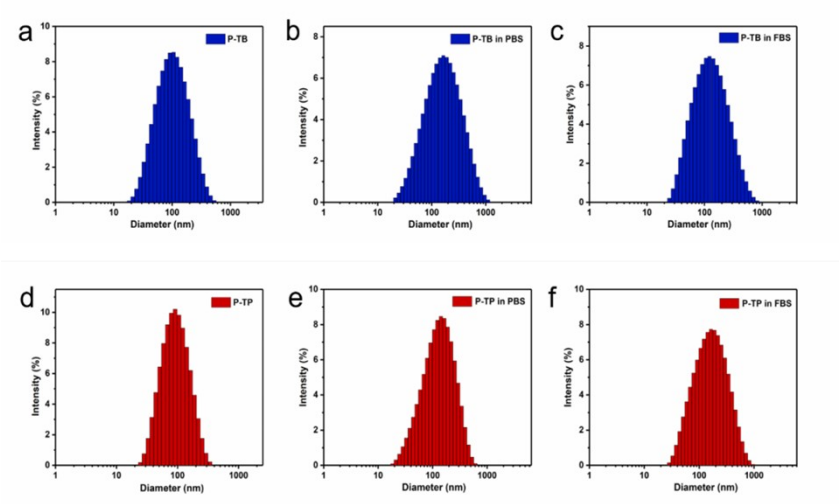

Figure S7. The diameter of P-TBNPs (a, b, c) and P-TPNPs (d, e, f) in different physiological environments.

Table S1: The sizes of polymer nanoparicles in different physiological environments

Polymer nonoparticles	Physiological environments		
	Water (nm)	Phosphate buffered saline (PBS) (nm)	Fetal bovine serum (FBS) (nm)
P-TB NPs	89	121	127
P-TP NPs	82	102	102

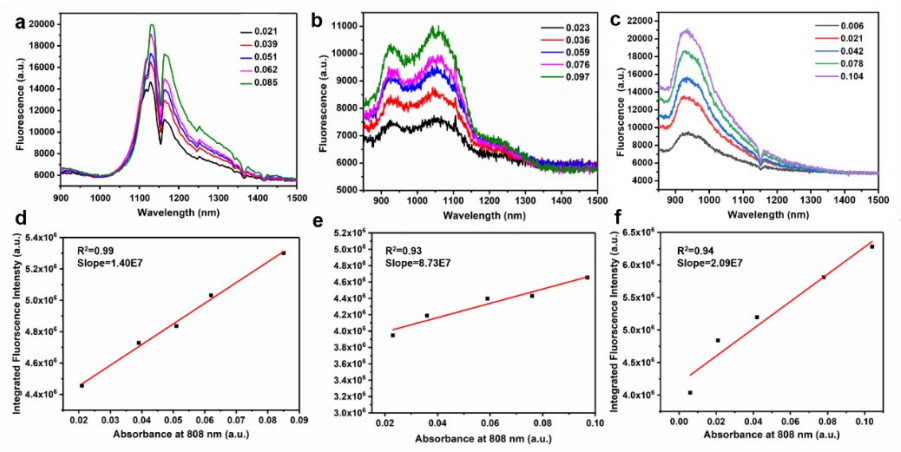

Figure S8. a, b, c) Fluorescence spectrum of IR26, P-TB and P-TP excited at 808 nm. d, e, f) Fit curve of absorbance at 808 nm with integrated fluorescence intensity, the curve of IR26, P-TB and P-TP.

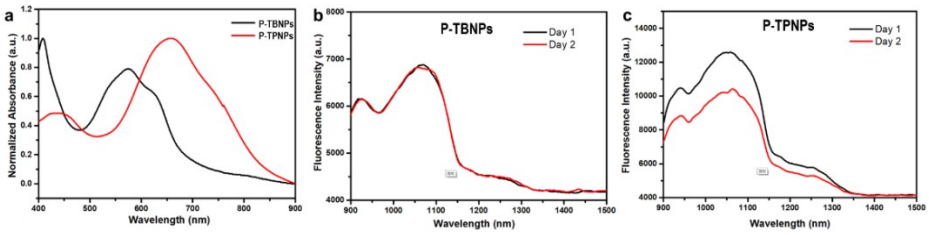

Figure S9. (a) The normalized absorbance spectrum of P-TBNPs and P-TPNPs (b) Fluorescence spectrum of P-TBNPs in dichloromethane. After 24 hours of exposure to sunlight, the fluorescence spectrum of P-TBNPs was measured again. (c) Fluorescence spectrum of P-TBNPs in dichloromethane. After 24 hours of exposure to sunlight, the fluorescence spectrum of P-TPNPs was measured again.

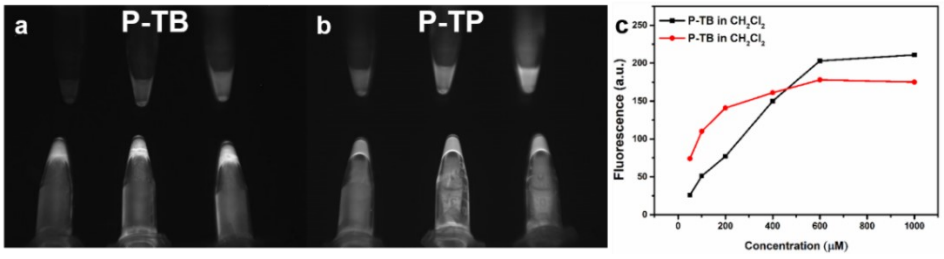

Figure S10. (a), (b)NIR-II imaging photographs of P-TB and P-TP in dichloromethane. (c) Variation curve of fluorescence intensity with solution concentration. The concentration is 50 μM, 100 μM, 200 μM, 400 μM, 600 μM and 1000 μM, respectively.

Table S2: Fundamental optical data for P-TB and P-TP.

Polymer	ε (Main peak, $L \cdot mol^{-1} \cdot cm^{-1}$)	ε (808 nm, $L \cdot mol^{-1} \cdot cm^{-1}$)	Absorption peak (nm)	Emission peak (nm)	Stokes shift (nm)	QY (%)
P-TB	7.7×10^4	2.3×10^3	610	1060	450	0.4
P-TP	1.2×10^5	1.4×10^4	640	930	290	0.7
P-TBNPs	3.0×10^4	1.2×10^4	575	1057	482	
P-TBNPs	9.2×10^4	3.1×10^4	660	1070	410	

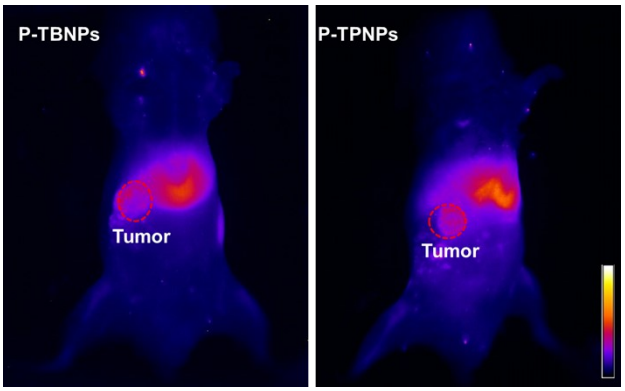

Figure S11. NIR-II imaging photographs of mice in the supine position at 24 h.

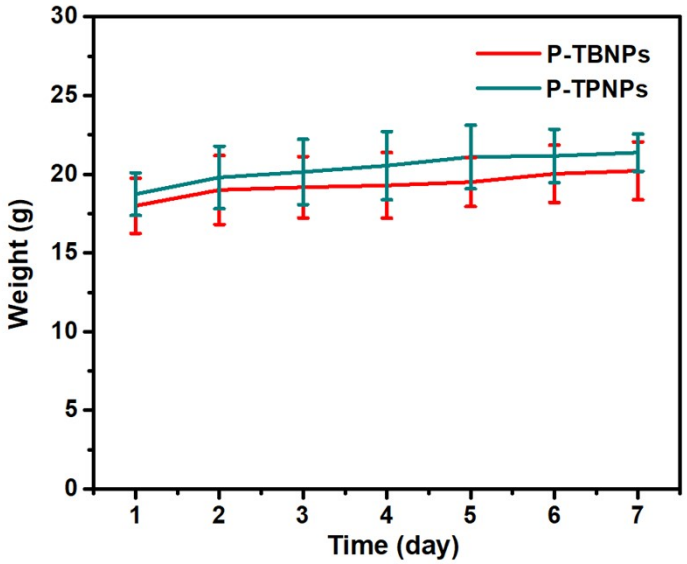

Figure S12. Body weight changes in mice within 7 days.

Reference

[S1] Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Keith, T.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. Gaussian 09, Revision D.01; Gaussian, Inc. Wallingford, CT, 2013.

[S2] J. E. Murphy , M. C. Beard , A. G. Norman , S. P. Ahrenkiel , J. C. Johnson , P. Yu , O. I. Mićić , R. J. Ellingson and A. J. Nozik , J. Am. Chem. Soc., 2006, 128 , 3241 —3247.