

Unimolecular micelles from star-shaped block polymers by photocontrolled BIT-RDRP for PTT/PDT synergistic therapy

Supporting Information

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Experimental section

Materials

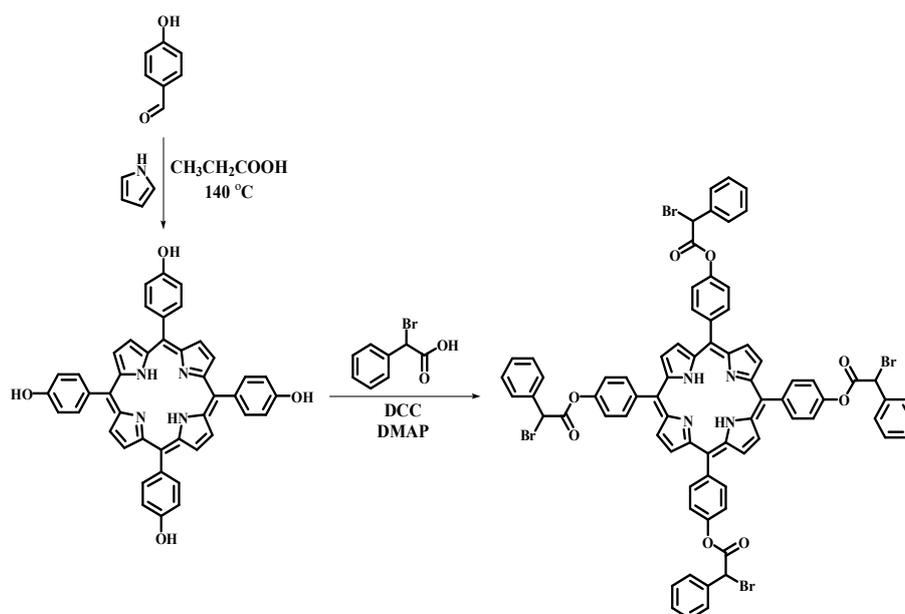
Monomers methyl methacrylate (MMA, 98%, Shanghai Chemical Reagents Co. Ltd) and Polyethylene glycol methacrylate (PEGMA, average molar mass 500 g mol⁻¹, 99%, Sigma-Aldrich) were passed

through neutral alumina column before use. Pyrrole (97%), 4-hydroxybenzaldehyde (98%), 2-bromo-2-phenylacetic acid (EBP, 97%), methyl isonipecotate (98%), methoxy polyethylene glycol amine (mPEG-NH₂, 99%, $M_n = 1000 \text{ g mol}^{-1}$), 1-hydroxybenzotriazole (HOBt, 97%), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 99%), tert-butyl 3-hydroxypropylcarbamate (98%), propionic acid (99%) and *N, N'*-dicyclohexylcarbodiimide (DCC, 98%) were obtained from Energy Chemical (Shanghai) and used as received. α -bromophenylacetic acid (EBP, 97%), 4-dimethylaminopyridine (4-DMAP, 98%), triethylamine (TEA, 99%), NaI (99%), pyrene (99%) and thiophenethiol (95%) were obtained from Macklin (Shanghai) and used as received. Croconic acid (99%) and trifluoroacetic acid (99%) were obtained from Sigma-Aldrich and used as received. Phosphate buffered saline (PBS, pH = 7.2-7.4) was obtained from Procell Life Science & Technology Co. Ltd and used as received. Fetal bovine serum (FBS) was obtained from Tianhang Biotechnology Co. Ltd and used as received. Acetic acid (HOAc, 99%), 1-butanol (99%), *N, N*-diisopropylethylamine (DIPEA, 97%), diethyl ether (99%), methacryloyl chloride (99%), dimethyl sulfoxide (DMSO, analytical reagent), tetrahydrofuran (THF, analytical reagent) and all other organic solvents were obtained from Shanghai Chemical Reagents Co. Ltd and used as received unless mentioned.

Characterization

The number average molar mass ($M_{n,\text{GPC}}$) and molar mass dispersity (D) values of the resulting polymers were determined using a TOSOH HLC-8320 gel permeation chromatograph (GPC) equipped with a refractive-index detector (TOSOH), using TSK gel guard column Super MP-N ($4.6 \times 20 \text{ mm}$) and two TSK gel Supermulti pore HZ-N ($4.6 \times 150 \text{ mm}$) with measurable molar mass ranging from 5×10^2 to $5 \times 10^5 \text{ g mol}^{-1}$. THF or DMF + 0.01 mol mL⁻¹ LiBr were used as an eluent at a flow rate of 0.6 mL min⁻¹ operated at 40 °C. GPC samples were injected using a TOSOH plus autosampler and calibrated with the linear PMMA or PS standard purchased from TOSOH. ¹H NMR spectra were recorded on a Bruker 300 MHz nuclear magnetic resonance instrument using CDCl₃ or DMSO-*d*₆ as the solvent. UV-*vis* absorption spectra of small and large molecules were measured using an ultraviolet spectrophotometer (UV-2600 spectrophotometer, Shimadzu Corporation, Kyoto, Japan). Emission spectra were recorded on a FluoroMax-4 fluorescence spectrometer (Horiba Jobin Yvon). Transmission electron micrographs (TEM) were taken using a FEI TecnaiG220 (120kV). The sample was prepared by depositing a drop of micellar solution onto a 200 mesh copper grid coated with a carbon film. The hydrodynamic diameter of the micelles was measured by a NanoBrook 90Plus dynamic light scattering instrument. The equilibration time was 2 minutes, the test temperature was 25 °C, and each sample was measured 5 times and averaged. The intensity weighted mean hydrodynamic diameter and the particle size distribution were obtained from the analysis of the autocorrelation functions using the method of cumulants. The NIR light source used for the light warming experiment was purchased from Suzhou Monkey Robotics Co. Ltd. with a maximum absorption wavelength of 810 nm and a light power density of 100 mW cm⁻². The NIR light source used in the cell therapy experiment was MW-RIR-808, which was purchased from Changchun Leshi Photoelectric Technology Co. Ltd. with a maximum absorption wavelength of 808 nm and an illumination power density of 250 mW cm⁻². Multifunctional enzyme marker (SynergyNEO, BioTek) and flow cytometry (FACSVerse, BD) were used for cell counting assays. Confocal laser scanning microscopy (FV1200, OLYMPUS) was used for confocal imaging.

Synthesis of THPP-4Br



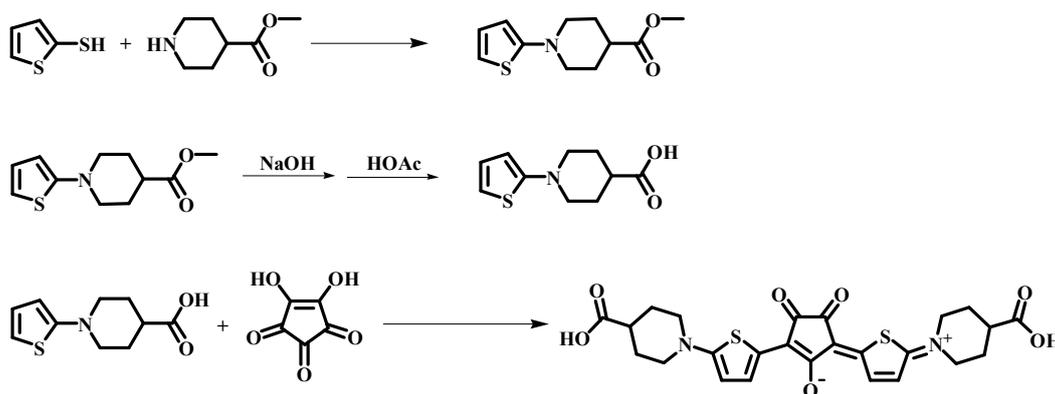
Scheme S1. Synthetic route of the star initiator THPP-4Br.

Referring to the literature method, 9.15 g (75.0 mmol) of 4-hydroxybenzaldehyde was dissolved in 200 mL of propionic acid ($\text{CH}_3\text{CH}_2\text{COOH}$) and the mixture was placed in a 500 mL three-necked flask and heated to 140 °C. 5.25 g (78.3 mmol) pyrrole was slowly added to the flask within 2 h and continued to reflux for 4 h. After cooling to room temperature, 125 mL of ethanol ($\text{C}_2\text{H}_5\text{OH}$) was added, mixed well and then refrigerated at 4 °C and left overnight. The filter was filtered under reduced pressure and the filter cake was washed with $\text{CH}_3\text{CH}_2\text{COOH}$ and chloroform (CHCl_3) in turn, and then the filter cake was dissolved in $\text{C}_2\text{H}_5\text{OH}$. The solid impurities insoluble in $\text{C}_2\text{H}_5\text{OH}$ were removed by filtration under reduced pressure and distilled under reduced pressure to obtain 7.68 g purple powder solid (5, 10, 15, 20-tetrakis (4-hydroxyphenyl)-porphyrin, THPP, yield: 47.7%). Then, 4.53 g (21.1 mmol) EBP and 5.07 g (24.5 mmol) DCC were dissolved in 70 mL of dichloromethane (CH_2Cl_2) and stirred for 30 min. 0.31 g (2.5 mmol) DMAP and 0.67 g (1.0 mmol) THPP were added and continue stirred for 48 h at room temperature. The filtrate was filtered under reduced pressure to remove insoluble impurities (DCU) and the first purple band was collected as crude product by column chromatography using pure CH_2Cl_2 as eluent, and then the crude product was subjected to a second column chromatography using petroleum ether/ethyl acetate (1:4, v/v) as eluent to obtain THPP-4Br (0.52 g, yield: 36.2%). ^1H NMR (300 MHz, CDCl_3 , TMS, δ , ppm): 5.76 (s, 4H), 7.48–7.53 (m, 20H), 7.78–7.81 (m, 8H), 8.19–8.21 (d, 8H), 8.83 (s, 8H).

Synthesis of ketocyanine dye NIR-800

5.38 g (37.5 mmol) methyl isonipecotate and 2.90 g (25.0 mmol) thiophenethiol were dissolved in 40 mL toluene and refluxed at 110 °C for 2 h. After the reaction was cooled to room temperature, the excess toluene was removed by rotary evaporation, and the crude product was separated by column chromatography using petroleum ether/ethyl acetate (4:1, v/v) as eluent to obtain light yellow crystals of methyl 1-thiophene-2-piperidinecarboxylate. Then, 2.70 g (12.0 mmol) methyl-1-thiophene-2-piperidinecarboxylate was added to 60 mL aqueous sodium hydroxide (0.5 mol L^{-1}) and stirred at 90 °C for 3 h. After the reaction was cooled to room temperature, 10% HOAc was added to the reaction solution

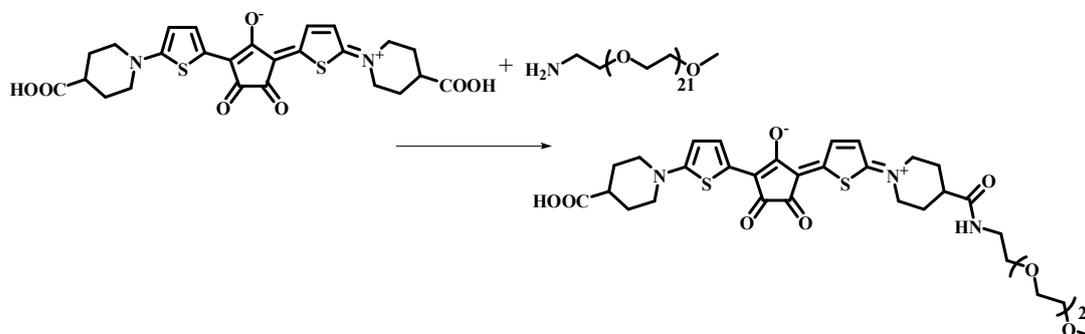
and the pH of the reaction solution was adjusted to 2.0. The reaction solution was filtered under reduced pressure and washed with 100 mL deionized water and dried under vacuum to a constant weight to yield a light blue solid powder of 1-thiophene-2-piperidinecarboxylic acid. Finally, 0.66 g (3.0 mmol) 1-thiophene-2-piperidinecarboxylic acid and 0.21 g (1.5 mmol) ketonic acid were dissolved in the solvent mixture of 10 mL toluene and 10 mL n-butanol and refluxed at 120 °C for 1.5 h under light-proof conditions. The reaction was cooled to room temperature, and the black filter cake was collected by filtration under reduced pressure, washed with methanol 4 to 5 times, and dried under vacuum to constant weight to obtain the black-brown powder NIR-800. The total yield of the reaction was 52.9%. ¹H NMR (300 MHz, DMSO-*d*₆, TMS, δ, ppm): 1.54-1.90 (m, 4H), 1.94-2.08 (m, 4H), 2.17-2.37 (m, 2H), 3.31-3.59 (t, 4H), 3.92-4.07 (t, 4H), 7.02 (d, 2H), 8.47 (d, 2H).



Scheme S2. Synthetic route of NIR-800.

Synthesis of polyethylene glycol-modified ketocyanine dye mPEG-NIR-800

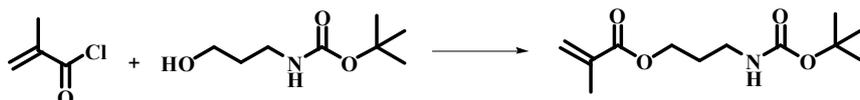
0.144 g (0.29 mmol) NIR-800 and 0.225 g (1.15 mmol) EDC were placed in a flask to which 0.162 g (1.15 mmol) 1-hydroxybenzotriazole (HOBt) was added and dissolved with 3.0 mL DMF and stirred for 1 h at room temperature. 0.45 mL (2.70 mmol) DIPEA was added to the mixture and stirred for 30 min. Then, 0.29 g (0.29 mmol) mPEG-NH₂ was added and stirred for 72 h at room temperature. At the end of the reaction, the crude product was dissolved in 5.0 mL methanol and settled into anhydrous ether to collect the black solid insoluble in ether and repeated 3 to 4 times. It was then dried under vacuum to a constant weight to finally obtain a black viscous solid mPEG-NIR-800.



Scheme S3. Synthetic route of mPEG-NIR-800.

Synthesis of functional monomer BAPMA

6.4 g (36.5 mmol) tert-butyl 3-hydroxypropylcarbamate and 5.1 mL triethylamine were dissolved in 20.0 mL dichloromethane, and 3.55 mL (36.5 mmol) methacryloyl chloride was added slowly dropwise in an ice-water bath over 30 min with continued stirring for 12 h. The insoluble white solid was removed by filtration under reduced pressure, the excess dichloromethane was removed by rotary evaporation, and the crude product was separated by column chromatography using petroleum ether/ethyl acetate (3:1, v/v) as eluent to obtain BAPMA as a yellow oily liquid. the yield was 75.6%. ¹H NMR (300 MHz, CDCl₃, TMS, δ, ppm): 1.42 (s, 9H), 1.84 (m, 2H), 1.97 (s, 3H), 3.21 (m, 2H), 4.20 (t, 2H), 5.00 (s, 1H), 5.57 (s, 1H), 6.08 (s, 1H).



Scheme S4. Synthetic route of BAPMA.

Calculation of the photothermal conversion efficiency of THPP-4PMMA-*b*-4P(PEGMA-*co*-APMA)@NIR-800

The photothermal conversion efficiency of THPP-4PMMA-*b*-4P(PEGMA-*co*-APMA)@NIR-800 was calculated by the following equation,

$$\sum_i m_i C_{pi} * \frac{dT}{dt} = Q_{in,NPs} + Q_{in,buffer} - Q_{in,out} \quad (1)$$

$$\eta = \frac{h_s(T_{max} - T_{surr}) - Q_{Dis}}{I(1 - 10^{-A_{808}})} \quad (2)$$

$$Q_{Dis} = h_s(T_{max} - T_{surr}) = Q_{in,buffer} - Q_{in,out} \quad (3)$$

$$h_s = \frac{m_{solvent} C_{solvent}}{\tau_s} \quad (4)$$

$$t = \tau_s \ln(\theta) \quad (5)$$

$$\theta = \frac{T - T_{surr}}{T_{max} - T_{surr}} \quad (6)$$

in which, η is the photothermal conversion efficiency, h_s is the heat transfer coefficient, T_{max} and T_{surr} are the temperature of the aqueous solution of polymers THPP-4PMMA-*b*-4P(PEGMA-*co*-APMA)@NIR-800 and the pure solvent (water) at the maximum steady-state temperature, respectively, I is the near-infrared optical power and A_{808} is the absorbance of the toluene solution of polymers THPP-4PMMA-*b*-4P(PEGMA-*co*-APMA)@NIR-800 at 808nm. By drawing the t - $(\ln\theta)$ curve, the τ_s of the aqueous polymer solution and pure water was obtained, and the heat transfer coefficient of the two solutions is

calculated by substituting into the heat transfer coefficient formula (4), and the photothermal conversion efficiency of the aqueous polymer solution was 20.0 % through the photothermal conversion efficiency calculation formula (2).

Additional Table and Figures

Table S1. Synthesis of THPP-4PMMA^a.

Entry	t (h)	R	^b Conv. (%)	$M_{n,th}$ (g/mol)	^c $M_{n,GPC}$ (g/mol)	^d $M_{n,NMR}$ (g/mol)	^e \mathcal{D}
1	14	100/1/12/2	64.5	7900	9000	10800	1.19
2	14	200/1/12/2	58.2	13000	10300	14600	1.15

^aPolymerization conditions: R = [MMA]₀/[THPP-4Br]₀/[NaI]₀/[TEA]₀, V_{MMA} = 0.5 mL, $V_{solvent}$ = 1.0 mL, under irradiation with blue LED light (λ_{max} = 460 nm, 15 mW/cm²) at 25 °C. ^bDetermined by gravimetry.

^cDetermined by GPC using linear PMMA as the standard in THF. ^dCalculated by ¹H NMR.

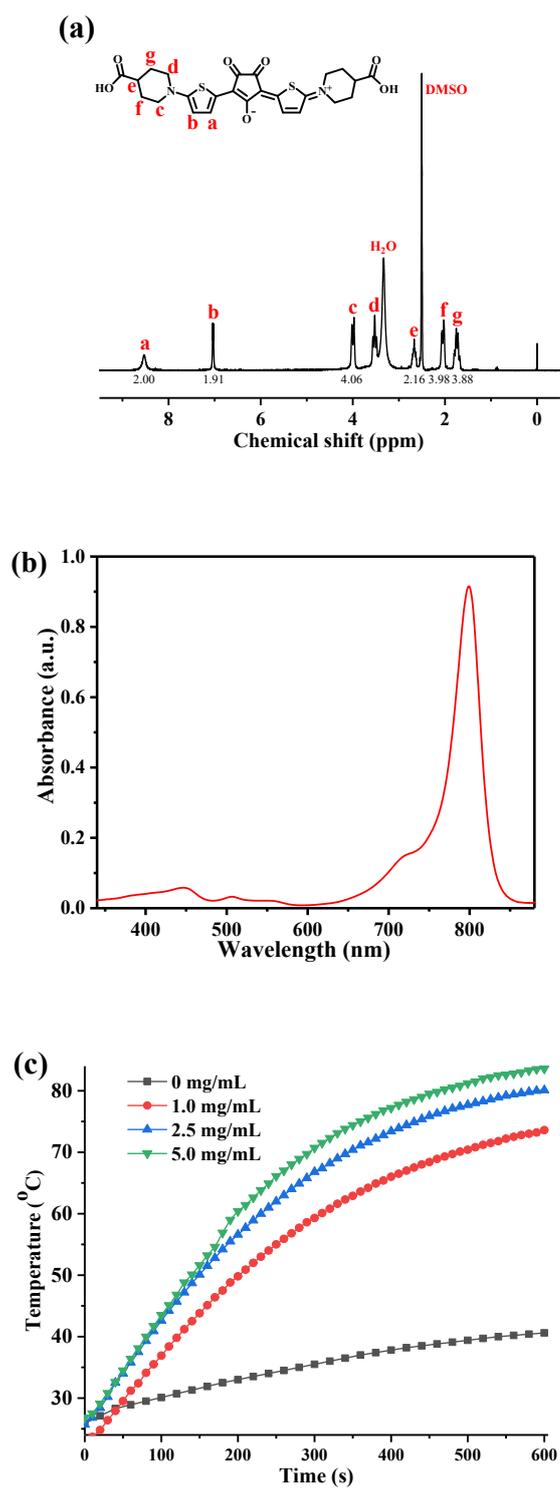


Figure S1. ^1H NMR spectrum in $\text{DMSO-}d_6$ (a), UV-*vis* absorption spectrum in DMF (b), temperature change curves (c) of NIR-800 in H_2O . NIR irradiation conditions: $V_{\text{solution}} = 2.0 \text{ mL}$, $\lambda_{\text{max}} = 810 \text{ nm}$, 100 mW/cm^2 .

NIR-800, containing a ketocyanine group and two carboxyl functional groups, was successfully synthesized by the chemical shifts and integrated areas of the signal peaks in ^1H NMR spectrum as shown in Figure S1a. The UV-*vis* absorption spectrum was shown in Figure S1b. The results of the photothermal conversion ability test were shown in Figure S1c, which prove that the compound exhibited good photothermal conversion ability under NIR light, and its aqueous solution could be warmed up to 70 °C or even above in a short period of time. Common photosensitive molecules containing large π -bonds first leaped to the excited state when absorbing light energy, followed by fluorescence. However, some photothermal reagents such as ketocyanine do not fluoresce after absorbing light energy and can convert most of the absorbed light energy into thermal energy, which may be one of the reasons for their remarkable photothermal conversion ability.

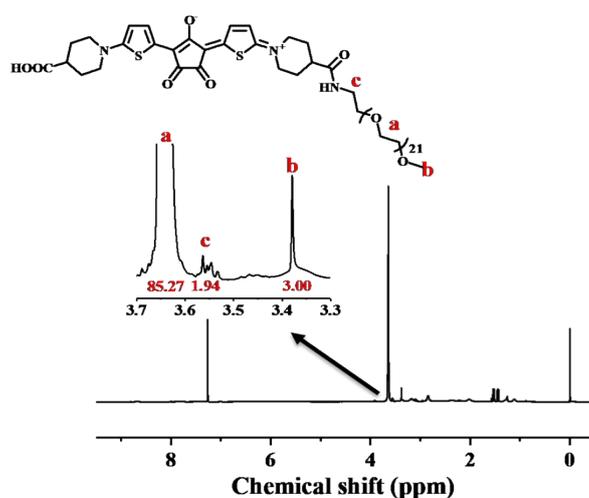


Figure S2. ^1H NMR spectrum of mPEG-NIR-800 in CDCl_3 .

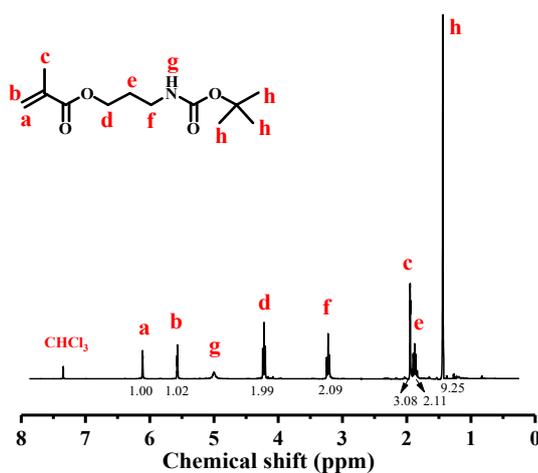


Figure S3. ^1H NMR spectrum of BAPMA in CDCl_3 .

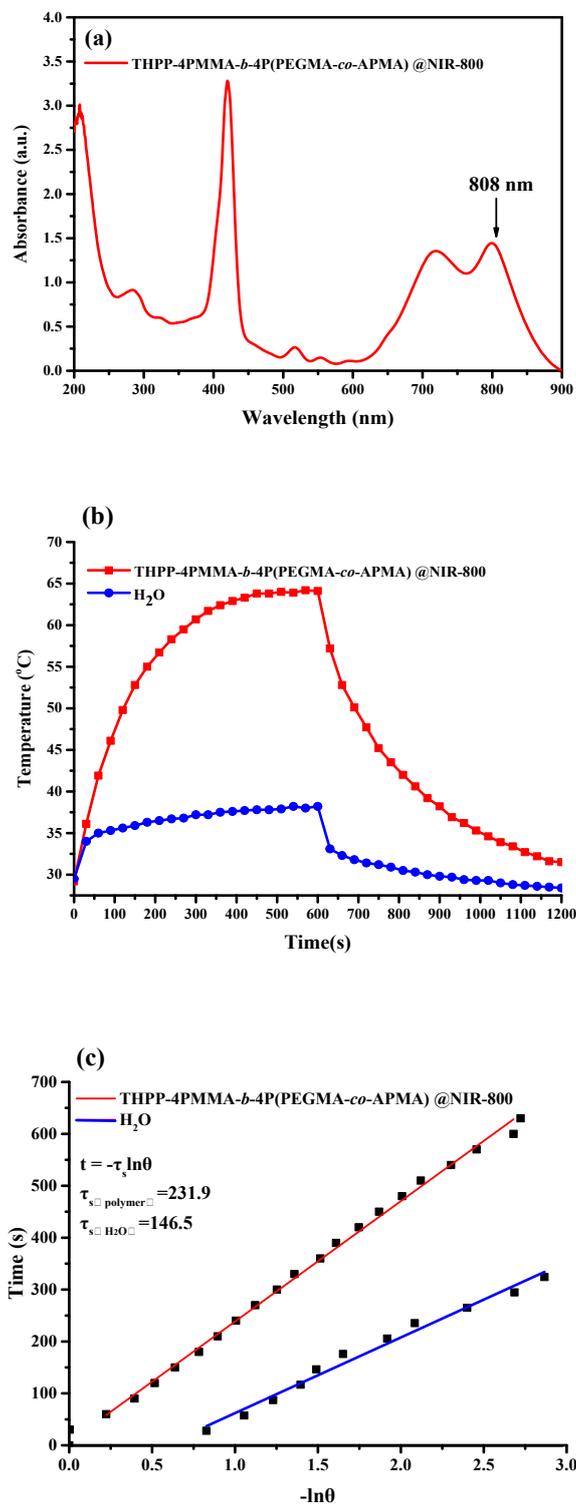
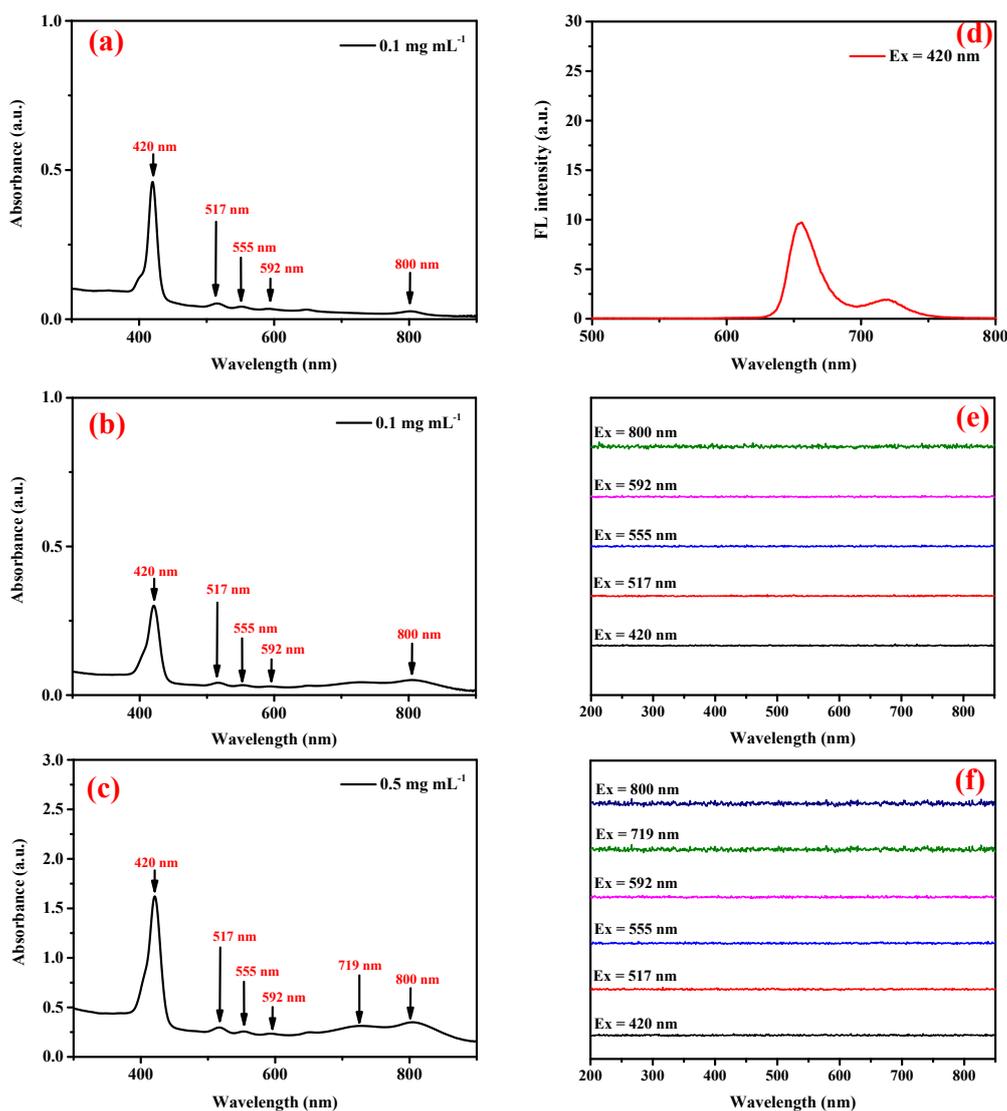


Figure S4. The UV-*vis* absorption spectrum (a) and the cooling curve (b) of THPP-4PMMA-*b*-4P(PEGMA-*co*-APMA)@NIR-800 in H₂O after irradiation with annular NIR laser and its corresponding time-(-lnθ) linear curve(c).

Similar peaks emerged when using either DMF or H₂O as the solvent in UV-*vis* absorption spectra as shown in Figures S5a, b and c. THPP-4PMMA-*b*-4P(PEGMA-*co*-APMA)@NIR-800 showed emission peaks at 620-780 nm under excitation of light at 420 nm (Figure S5d) when DMF was used as solvent, while there were no emission peaks with various exciting wavelengths (420-800 nm) when H₂O was used as solvent probably due to the characteristics of photothermal conversion



of NIR-800 in water (Figures S5e, f).

Figure S5. The UV-*vis* absorption spectra (a, b, c) and fluorescence spectra (d, e, f) of the polymer THPP-4PMMA-*b*-4P(PEGMA-*co*-APMA)@NIR-800 in DMF (0.1 mg/mL, a, d) and H₂O (0.1 mg/mL (b, e) and 0.5 mg/mL (c, f)).

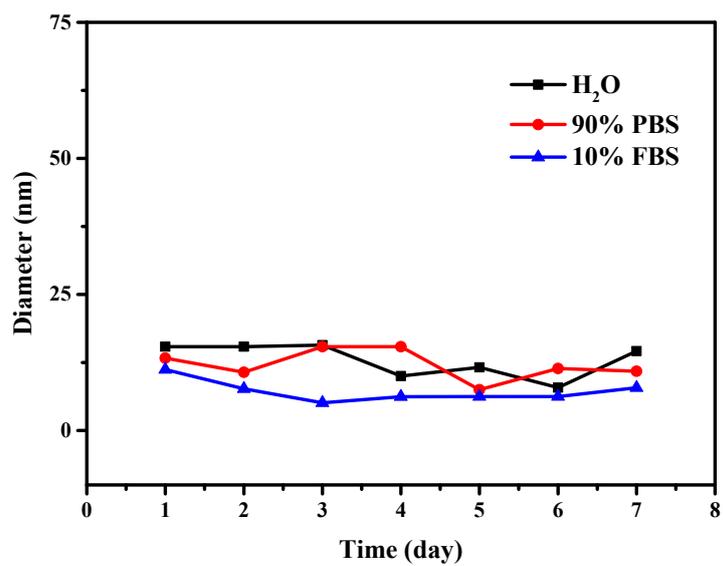


Figure S6. Size distribution of THPP-4PMMA-*b*-4P(PEGMA-*co*-APMA)@NIR-800 at different solvent (H₂O, 90% PBS, 10% FBS), $C = 0.1$ mg/mL.