Supporting Information

In-situ Catalytic Reaction for Solving the Aggregation of Hydrophobic Photosensitizers in Tumor

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1. Experimental Section

1.1 Chemicals and reagents.

Triethylamine (TEA), dimethyl formamide (DMF), dioxane (Diox), dichloromethane (DCM) and dimethylsulfoxide (DMSO) were dried over calcium hydride before use. Tetrahydrofuran (THF) was refluxed with sodium chips under N_2 before use. Ferrocenecarboxylic acid (FA), 2-hydroxyethyl methacrylate (HEMA), 2-bromo-2-methylpropionyl bromide, PEG (5000), 4-hydroxybenzaldehyde, pyrrole, 4-(dimethyl amino) pyridine (DMAP), *N*, *N*-dicyclohexylcarbodiimide (DCC), 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) and 4′, 6-diamidino-2-phenylindole (DAPI), 2′,7′-dichlorofluorescein diacetate (DCHF-DA) were all purchased from Aladdin Reagents of China and used directly as received. All other reagents were purchased from commercial resources and used as received unless otherwise noted.

1.2. Characterizations

^{1}H NMR spectra were recorded at 400 MHz, using BRUKER AV400 Spectrophotometer with tetramethylsilane (TMS) as an internal reference. The fluorescence spectra were recorded on a F-4500 fluorescence spectrophotometer at room temperature. The UV-Vis spectra of the samples were measured over different irradiation time intervals by using a Thermo Scientific Evolution 220 spectrophotometer. Dynamic light scattering (DLS) measurements were carried out at BECKMAN COULTER Delasa Nano C particle analyzer, and all the measurements were carried out at room temperature. Transmission electron microscopy (TEM)

analysis was performed on a JEOL JEM1400 electron microscopes operated at 100 kV. Samples for TEM were prepared by dropping the nanoparticles solution onto a carbon-coated copper grid and then dried at room temperature.

1.3. Synthesis of 5,10,15,20-Tetrakis(4-methacrylate phenyl)porphyrin (THPP).

4-hydroxybenzaldehyde (3.66 g, 30 mmol) was dissolved in 240 mL propionic acid. After three cycles of purging with nitrogen for 15 min and vacuumizing for 5 min, pyrrole (2.01 g, 30 mmol) was added slowly to the propionic acid at 135℃. After 4 h, the propionic acid was evaporated in a vacuum. The residual product was purified by silica gel column chromatography eluted with ethyl acetate and petroleum ether (v/v = 1:3) to give a purple powder with 10% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.1 (s, 4H, Ar-OH), 8.90 (m, 8H, β-H), 8.24 (m, 8H, 5, 10, 15, 20-Ar-o-H), 7.55 (m, 8H, 5, 10, 15, 20-Ar-m-H), -2.81 (s, 2H, -NH-).

1.4. Synthesis of 5,10,15,20-Tetrakis (4-methacrylate phenyl) porphyrin (TMPP).

THPP (0.678 g, 1.0 mmol) was dissolved in 10 mL THF with argon, and methacryloyl chloride (2.08 g, 20.0 mmol) was added slowly at 0 °C. After reacting for 1 h, the mixture continued reacting at room temperature for 23 h. Sodium bicarbonate solution (50 mL) was added into the mixture to stop the reaction, and then the product was extracted with ethyl acetate (50 mL), washed with water (50 mL*2), and dried with anhydrous sodium sulfate. Ethyl acetate was evaporated, and the crude product was obtained. The crude product was further purified by column chromatography eluted with ethyl acetate and petroleum ether (v/v = 1:2), and a purple solid was gained with 30% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.90 (m, 8H,

β-H), 8.24 (m, 8H, 5,10,15,20-Ar-o-H), 7.55 (m, 8H, 5,10, 15, 20-Ar-m-H), 6.53 (m, 4H, H-CH-C-), 5.89 (m, 4H, H-CH-C-), 2.21 (m, 12H, CH_3-C-), -2.81 (s, 2H, -NH-).

1.5. Synthesis of the monomer 2-(methacryloyloxy) ethyl ferrocene carboxylate (MAEFc).

FA (2.02 g, 8.78 mmol), 4-(dimethylamino) pyridine (0.13 g, 1.06 mmol) and 2-hydroxyethyl methacrylate (HEMA) (0.85 g, 10.72 mmol) were dissolved in 30 mL of anhydrous dichloromethane (DCM). The mixture was cooled to 0 °C in an ice-water bath under argon flow and stirred for 20 min. Then, DCC (2.15 g, 10.43 mmol) in anhydrous DCM (20 mL) was added to the above solution at 0 °C over 15 min. After the addition was completed, the mixture was stirred at 0 °C for another 1 h and then 24 h at room temperature. After removing the insoluble salts by suction filtration, the filtrate was concentrated and further purified by silica gel column chromatography using petroleum ether/ethyl acetate (10/1, v/v) as the eluent. After removing the solvents using a rotary evaporator, the final product MAEFc was obtained as a yellow solid (1.91 g, yield: 95%). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 1.92 (s, 3H, -CH_3), 4.22 (m, 5H, -CH- in the ferrocene group), 4.42 (s, 4H, -OCH_2CH_2O-), 4.50 (m, 2H, -CH- in the ferrocene group), 4.75 (m, 2H, -CH- in the ferrocene group), 5.74 (m, -CH), 6.10 (m, -CH).

1.6. Synthesis of the macro-ATRP initiator PEG-Br.

PEG (4 g, 0.8 mmol) was dissolved in 15 mL anhydrous DCM. After purging with nitrogen for 20 min to eliminate the oxygen, 2-bromo-2-methylpropionyl bromide (0.92 g, 4.0 mmol) was introduced slowly over 15 min at 0 °C. After 1 h, the mixture

was allowed to react at room temperature for another 23 h. After removing the solvents by using a rotary evaporator, the residues were dissolved in DCM and precipitated three times into an excess of diethyl ether to remove any residual monomer. The final product was dried in a vacuum for 48 h at 30 °C, yielding an white solid (4.01 g, yield: 95.0%, $M_{n,GPC}$ = 6238, M_w/M_n = 1.04). (^{1}HNMR (400 MHz, $CDCl_3$, δ):1.85 (s, 6H, -CH_3), 3.40 (s, 3H, CH_3O-), 3.67 (t, -OCH_2CH_2O-).

1.7. Synthesis of the Amphiphilic Block Copolymers PEG-b-PMAEFc.

The amphiphilic copolymers PEG-*b*-PMAEFc was synthesized according to the previous literature [43]. The concrete synthesis of PEG-*b*-PMAEFc is described below: MAEFc (0.60 g, 1.76 mmol), PEG-Br (0.2 g, 0.04 mmol), and PMDETA (34.8 mg, 0.20 mmol) were dissolved in anisole (4 mL). After purging with argon for 0.5 h, CuBr (34.5 mg, 0.24 mmol) was quickly added into the above solution under argon at room temperature. Then the reaction system was heated to 90 °C and kept for 8 h. At the end, the reaction was stopped by exposure to air, and diluted with DCM. The reaction mixture was washed with water to remove the copper, and concentrated to 1.5 mL and precipitated twice in diethyl ether (100 mL). The final orange solid product was gained, and dried in a vacuum for 48 h at 30 °C (0.41 g, yield: 50.0%, $M_{n,GPC}$ = 9950, M_w /M_n = 1.18). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 0.91-1.43 (m, -CH_3 in PMAEFc), 1.80-2.11 (m, -CH_2-), 2.24 (s, -CH_3), 3.40 (s, CH_3O-), 3.67 (s, -OCH_2CH_2O-), 4.22 (s, -CH- in the ferrocene group), 4.32-4.50 (m, -CH- in the ferrocene group and -OCH_2CH_2O-), 4.22 (s, -CH- in the ferrocene group).

2. Supplementary Figures

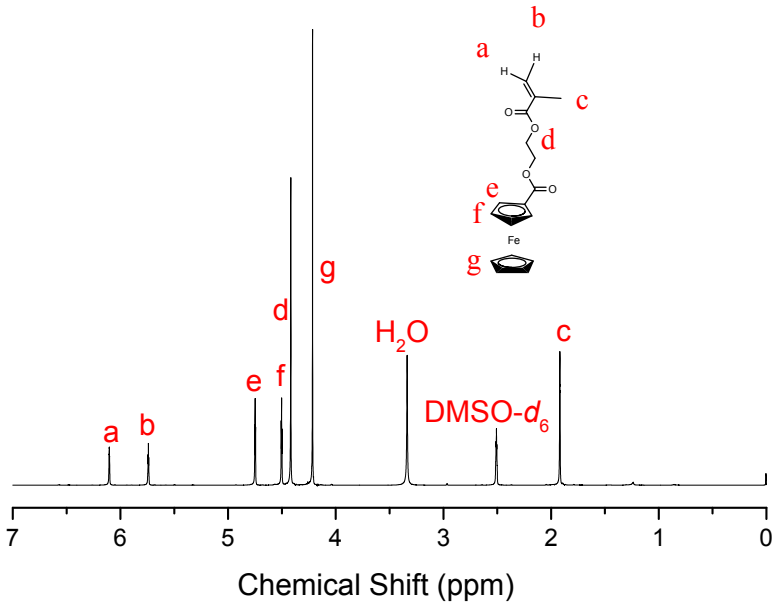

Figure S1. ^{1}H NMR spectrum of MAEFc in DMSO-d_6

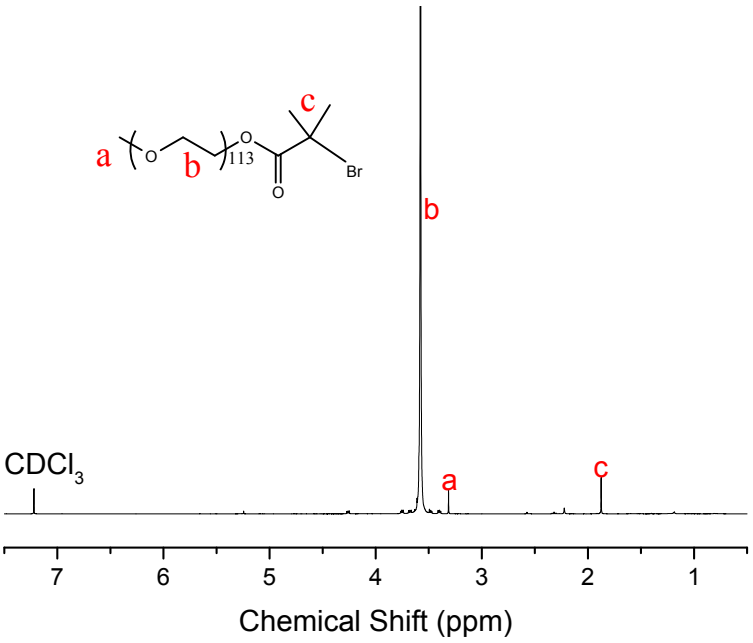

Figure S2. ^{1}H NMR spectrum of PEG-Br in $CDCl_3$

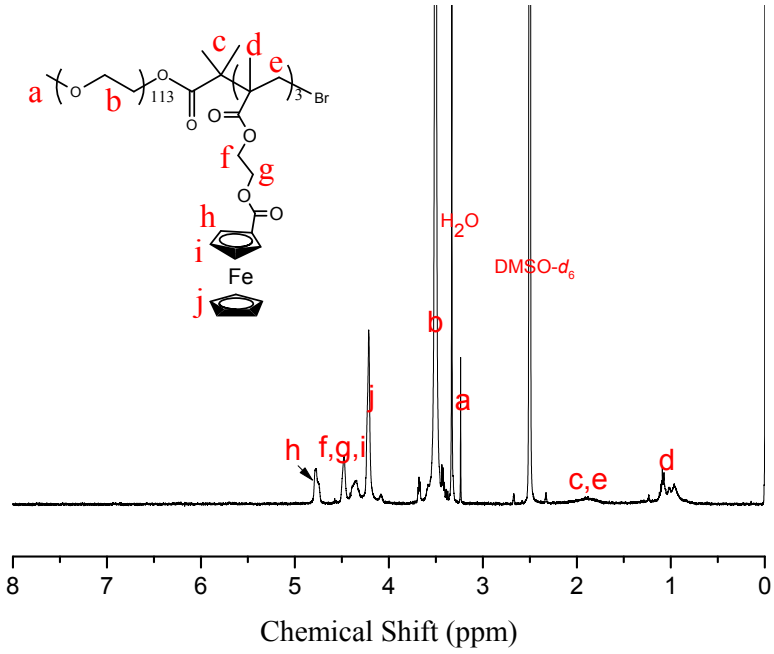

Figure S3. ^{1}H NMR spectrum of PEG-*b*-PMAEFc$_3$ in DMSO-d_6

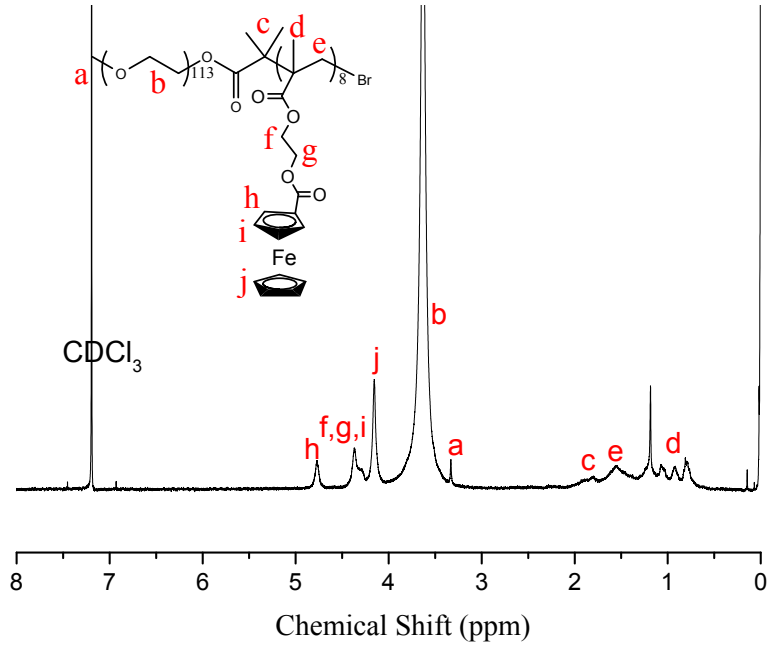

Figure S4. ^{1}H NMR spectrum of PEG-*b*-PMAEFc$_8$ in $CDCl_3$

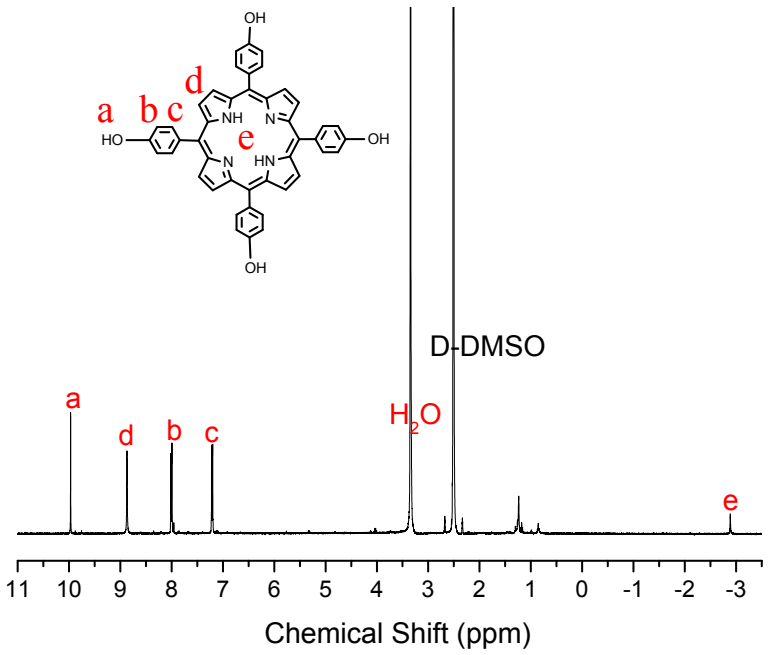

Figure S5. ^{1}H NMR spectrum of p-THPP in DMSO-d_6

Table S1. The results of amphiphilic block polymers via ATRP.

Samples	$M_{n,GPC}$ [a]	$M_{n,NMR}$ [b]	M_w/M_n
PEG-Br	6240	5593	1.04
PEG-*b*-PMAEFc$_3$	6840	6619	1.06
PEG-*b*-PMAEFc$_8$	7950	8329	1.13
PEG-*b*-PMAEFc$_{20}$	9950	12433	1.18

[a]Molecular weights ($M_{n,GPC}$) and molecular weight distributions (PDI) were evaluated by GPC with polystyrene standards.

[b]The final composition of the block polymer and $M_{n,\ NMR}$ were determined from the intergration of ^{1}H NMR spectra. $M_{n,\ NMR\ PEG\text{-}b\text{-}PMAEFc} = DP_{MAEFc} * M_{MAEFc} + M_{n,\ NMR,\ PEG}$, where DPMAEFc, $DP_{MAEFc,}$ and $M_{n,\ NMR,\ PEG}$ were the degree of the polymerization of MAEFc, and the molecular weight of MAEFc and PEG, respectively.

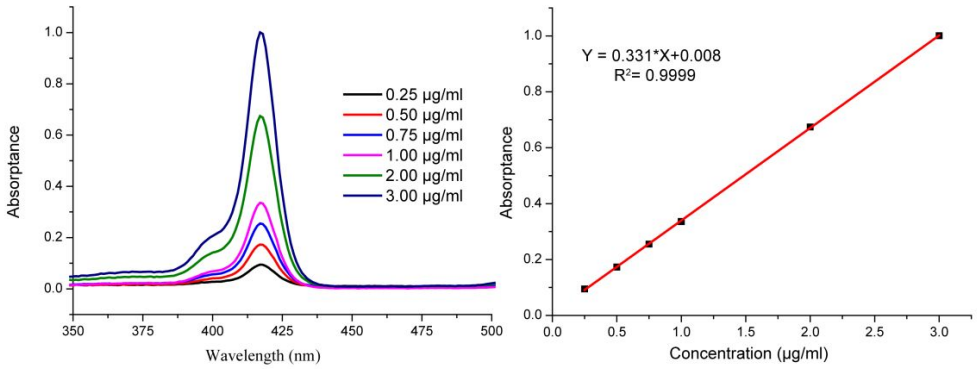

Figure S6. The ultraviolet absorption spectrum of TMPP

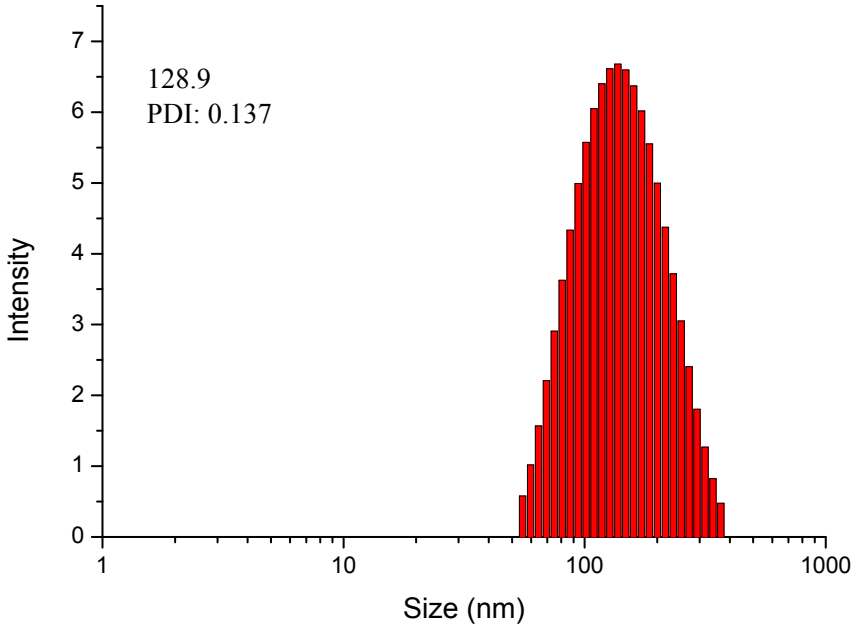

Figure S7. Size distribution of TPFcNP

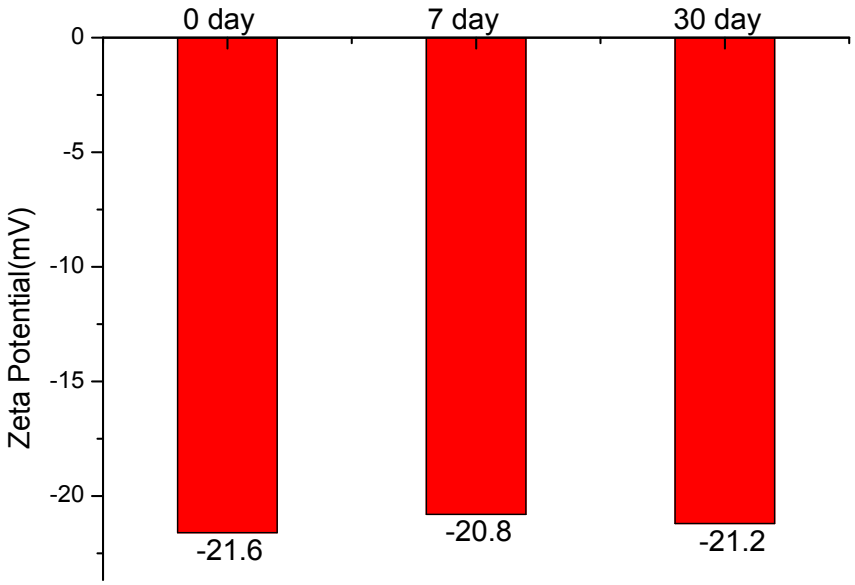

Figure S8. Zeta potential of TPFcNP

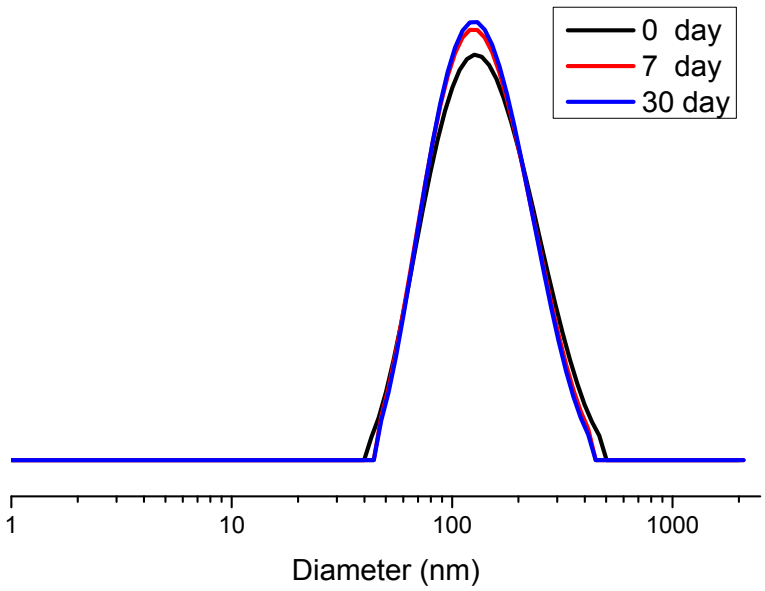

Figure S9. Size distributions of TPFcNP for long time.

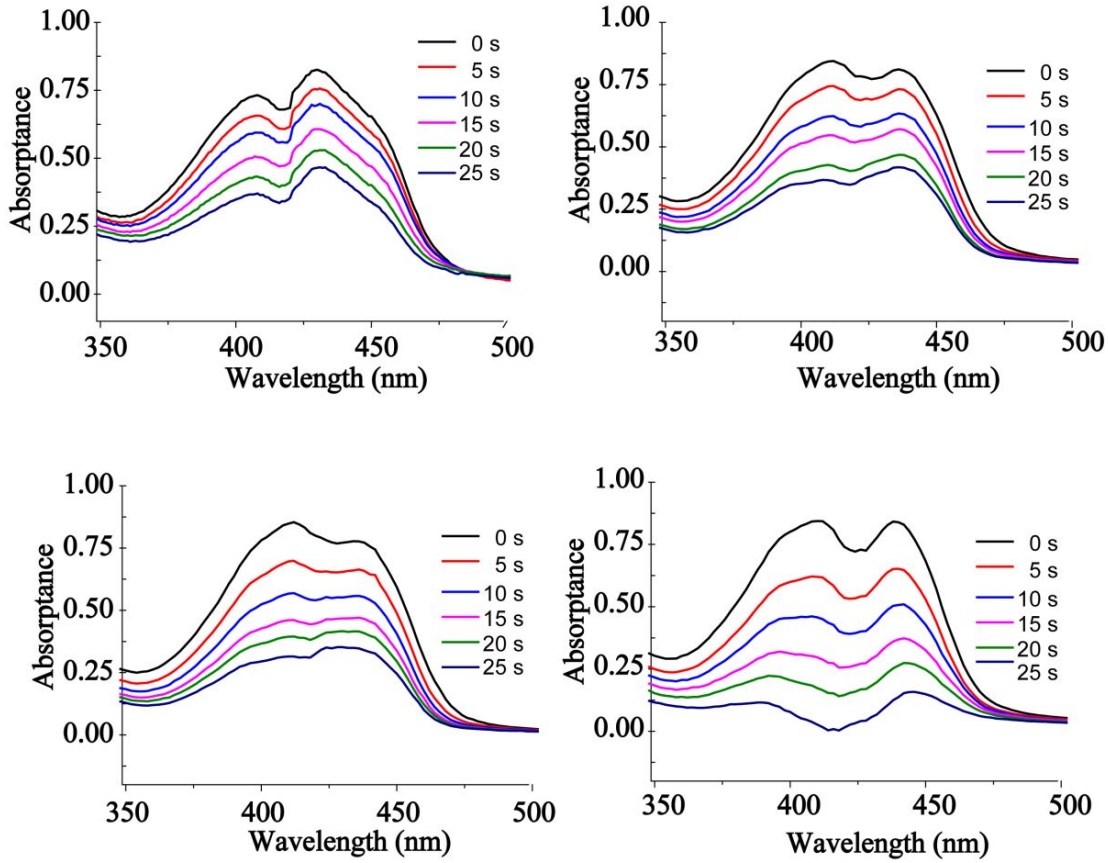

Figure S10. Ultraviolet absorption of DPBF under illumination in different conditions.

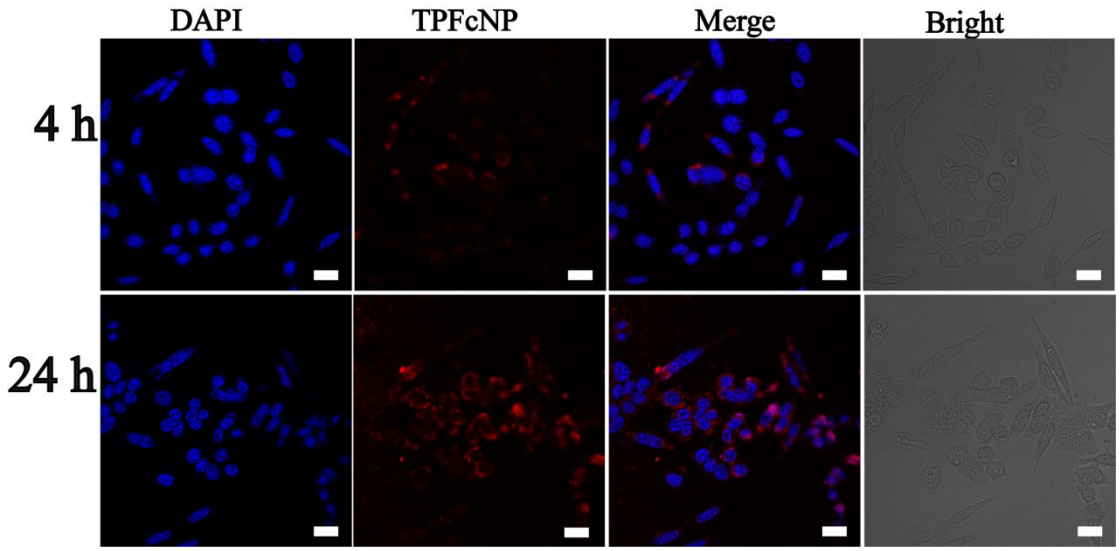

Figure S11. Cellular uptake of TPFcNP for different time.

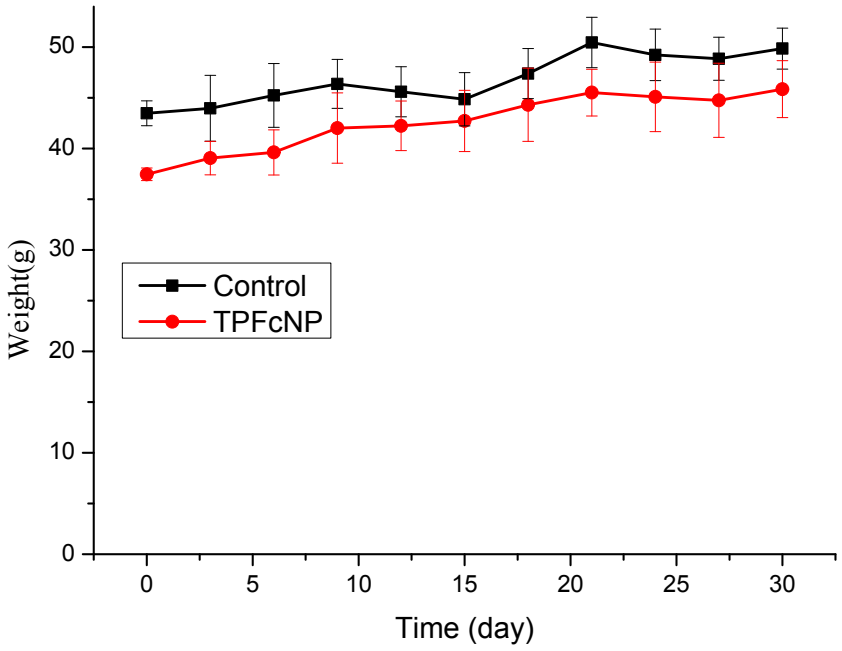

Figure S12. Body weights of K.M. mice in different group

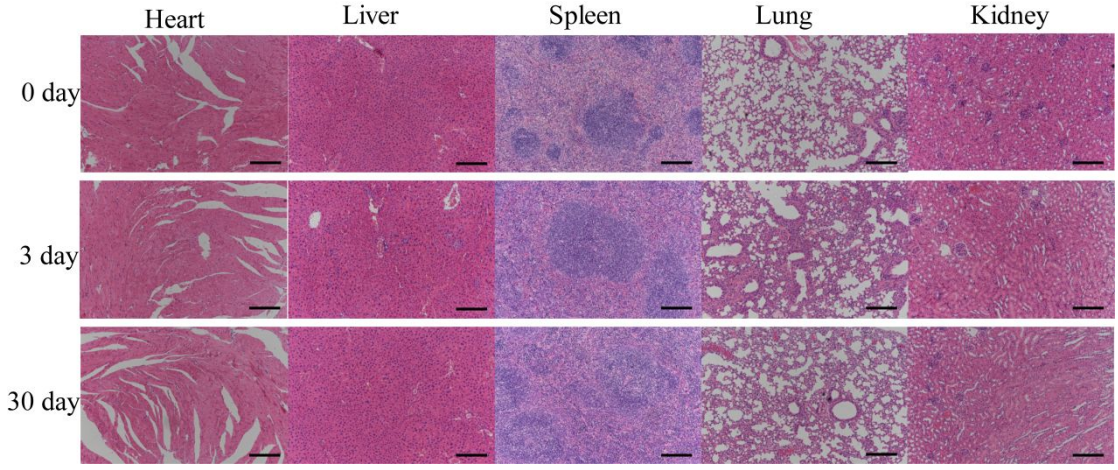

Figure S13. Pathological H&E stained images of tissue sections from heart, liver, spleen, lung and kidney of the mice treated with TPFcNP. The tissue sections were harvested in 3 and 30 days after the intravenous injection of 8 mg porphyrin kg^{-1} dosage, showing no significant change of H&E tissue sections. Scale bars, 200 μm.

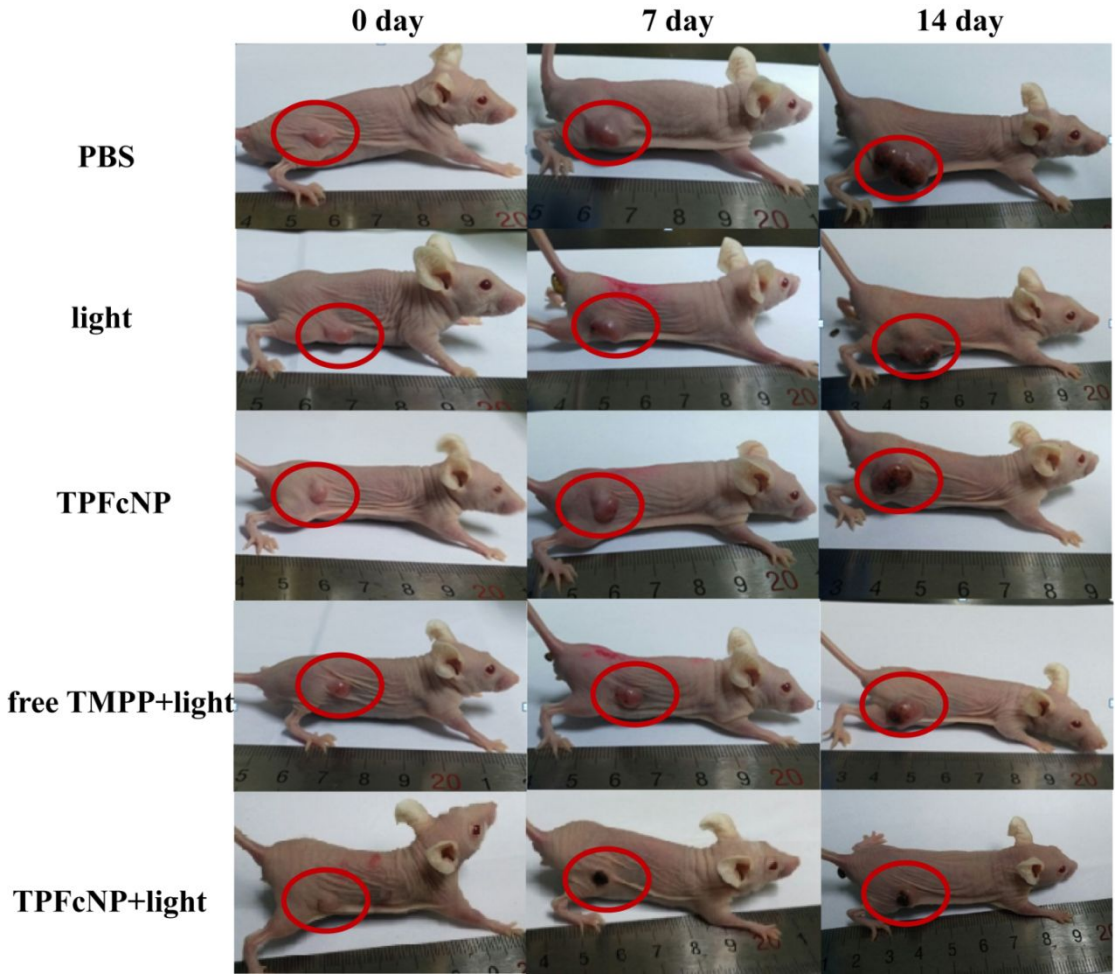

Figure S14. Images of tumor growth for different groups after treatments (0, 7, and 14 day)