Supporting Information

Porphyrin-Based Metallacage for Enhanced Photodynamic Therapy

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Contents

1. Materials and Reagents

Solvents and raw materials are available on the market without further processing and purification. Nitrobenzaldehyde was purchased from TCL. Acetic anhydride, pyridine and hydrated zinc acetate were purchased from Greagent. Stannous chloride, 2-pyridinecarboxaldehyde and trifluoromethanesulfonate were purchased from Adamas. Trypsin-EDTA, PBS, DMEM high glucose, fetal bovine serum (FBS) and RPMI-1640 medium were purchased from Adamas. 4T1 and RAW 264.7 cells were obtained from ATCC. Calcein AM, propidium iodide (PI) were purchased from Shanghai Yeasen BioTechnologies co.,Ltd. 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) was purchased from APExBIO.

2. Characterization

^{1}H NMR spectra were probed on 400 MHz Bruker spectrometer using deuterated DMSO or DMF as the solvent. The fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) was measured by SolariX 7.0T. Scanning electron microscope (SEM) images were collected on a Zeiss EVO MA 25/LS 25. Zeta potential and dynamic light scattering (DLS) measurements were measured using ZEN3690, Malvern Instrument Ltd. UV-vis measurements were obtained on a DU730. Inductively coupled plasma (ICP, Vista MPX ICP) was used for quantitative determination of zinc. Fluorescent measurements were obtained using Shimadzu F-4500 spectrofluorophotometer. Confocal laser scanning microscopy (CLSM) images were acquired by a SP5 II Leica confocal microscope. Hematoxylin and eosin (H&E) staining images were obtained with an ECLIPSE Ci-L microscope. Tunel, CD31, HIF-1a staining images were obtained with a panoramic MIDI digital slice scanner.

3. Methods

3.1 Synthesis of TAPP, Zn-TAPP and PM

Synthesis of 5,10,15,20-tetraphenyl (4-aminophenyl) porphyrin (TAPP).

4-nitrobenzaldehyde (3.6 g, 23.8 mmol) and 4 mL acetic anhydride were dissolved in 100 mL propionic acid. The mixture was heated to reflux, and 1.6 mL pyrrole was slowly dripped into the solution, then the solution kept reflux for 1 h. After the mixture cooled to room temperature, the filter residue was washed with methanol and vacuum dried to get black powder. The powder was dissolved in 100 mL pyridine and the solution was refluxed for 1.5 h. The product was filtered and vacuum dried, purple powder tetra-nitrophenyl porphyrin (TNPP) was obtained. At last, TNPP was dissolved in hot HCl solution and reduced by $SnCl_2{\cdot}2H_2O$. After cooling, the precipitation was neutralized with NaOH affording to the final product as purple powder (yield:69.9%).^{1}H-NMR (DMSO-d_6, 400 MHz): 8.87 (8H, s, H4), 7.83 (8H, d, H3), 7.00 (8H, d, H2), 5.55(8H, s, H1), -2.75(2H, s, H5). ^{13}C NMR (DMSO-d_6, 101 MHz):148.65, 135.61, 131.03, 128.86, 120.72, 112.67.

Synthesis of Zinc (II) 5,10,15,20-tetrakis(4-aminophenyl)-21H, 23H-porphine (Zn-TAPP).

TAPP (100 mg, 148 μmol) was dissolved in mixed solvent DMF/$CHCl_3$ (15 mL/45 mL) and methanol solution of hydrated zinc acetate (130 mg, 593 μmol) was added. The mixture was refluxed for 24 h, and cooled to room temperature, washed with water and pumped to obtain 77 mg green solid product (yield: 70.2%). ^{1}H-NMR (DMSO-d_6, 400 MHz): 8.82 (8H, s, H1), 7.78 (8H, d, H2), 6.94 (8H, d, H3), 5.44(8H, s, H4). ^{13}C NMR (DMSO-d_6, 101 MHz):149.75, 148.00, 135.25, 131.29, 130.45, 120.88, 112.32.

Synthesis of porphyrin-based metallacage (PM).

A mixture of Zn-TAPP (30 mg, 0.04 mmol), 2-pyridinaldehyde (16.5 μL, 0.16 mmol), zinc trifluoromethesulfonic acid (20 mg, 0.56 mmol) and 5 mL DMF was heated at 70 °C for 12 h. PM was obtained by addition of ethyl ether into the reaction mixture as a purple solid 27 mg, about 43% yield. ^{1}H-NMR (DMF-d_7, 400 MHz): 10.14 (24H, s, H6), 9.04 (24H, H13), 8.97 (24H, H1), 8.78 (24H, d, H13*), 8.37 (24H, t, H2), 8.31 (24H, H9), 8.20 (24H, H3), 8.00 (24H, d, H4), 7.85 (24H, d, H9*), 7.21 (24H, d, H8), 5.69 (24H, br, H8*).

3.2 Synthesis of PM@mPEG.

The nanoparticles of PM@mPEG were prepared through matrix-encapsulation method. Briefly, 400 μL of DMF solution containing PM (4 mg) was injected into 4 mL deionized water containing DSPE-mPEG2000 (24 mg). Then the mixture was stirred at 600 rpm for 15 min. After dialysis for 48 h, an aqueous solution of PM@mPEG nanoparticles was obtained.

3.3 Therapeutic effect of PDT *in vivo*.

A 4T1 tumor bearing mouse model was established by subcutaneously inoculating 100 μL 4T1 cells with a density of 3×10^7 cells/mL into the right hindquarters of the mice. After the tumor grew to the size of mung bean, the mice were randomly divided into six groups (n = 3 for each group): (1) PBS; (2) L; (3) PM@mPEG; (4) PM@HAase-mPEG; (5) PM@mPEG + L; (6) PM@HAase-mPEG + L. The nanoparticles were injected into the mice at a dose of 20 mg/kg PM by caudal vein every four days and irradiated with a 660 nm laser for 10 min after 12 h injection each time. Mice weight and tumor volume were monitored daily. After 14 days, the mice were necked and sacrificed, and the tumors, heart, liver, spleen, lungs and kidneys were collected. Tumors from each group were weighed and photographed to show the inhibitory effect of each treatment on the tumors. Tumors and important organs sliced were collected for H&E, TUNEL, CD31 and HIF-1a staining, respectively.

4. Figures and Table

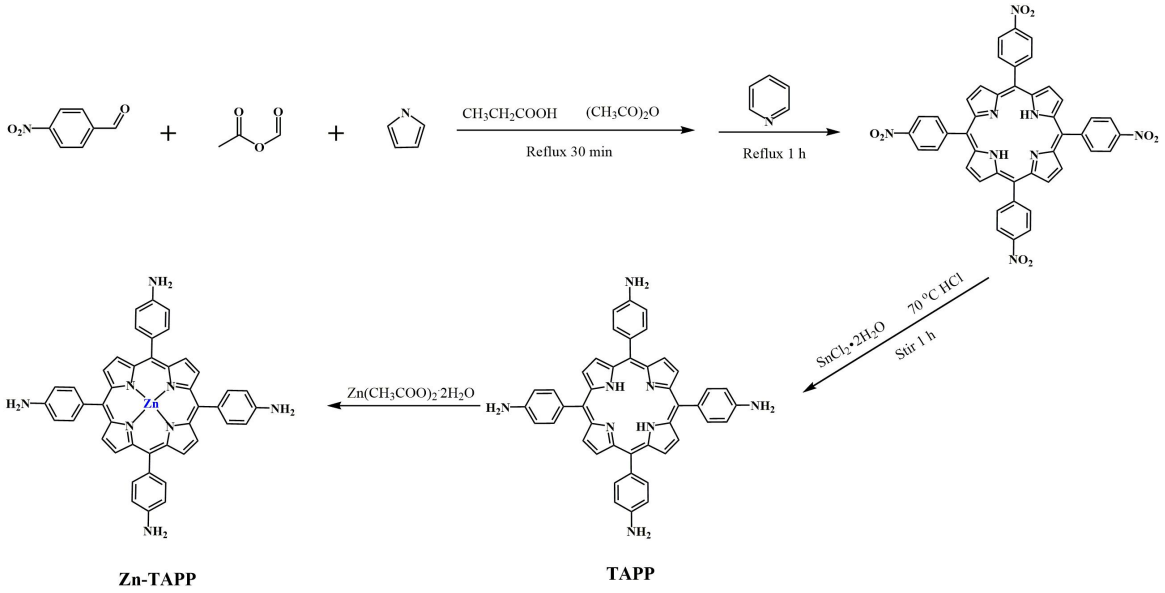
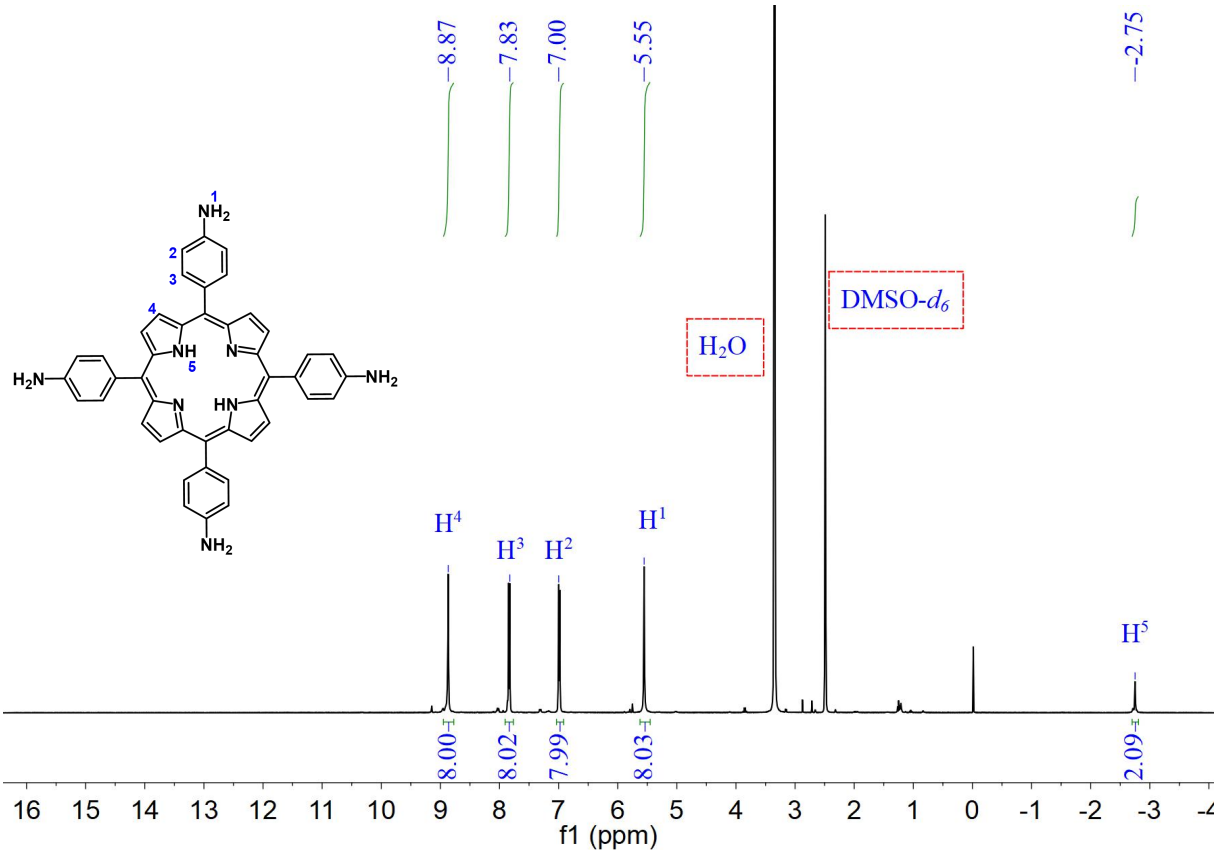


Fig. S1 Synthesis route of ligand.



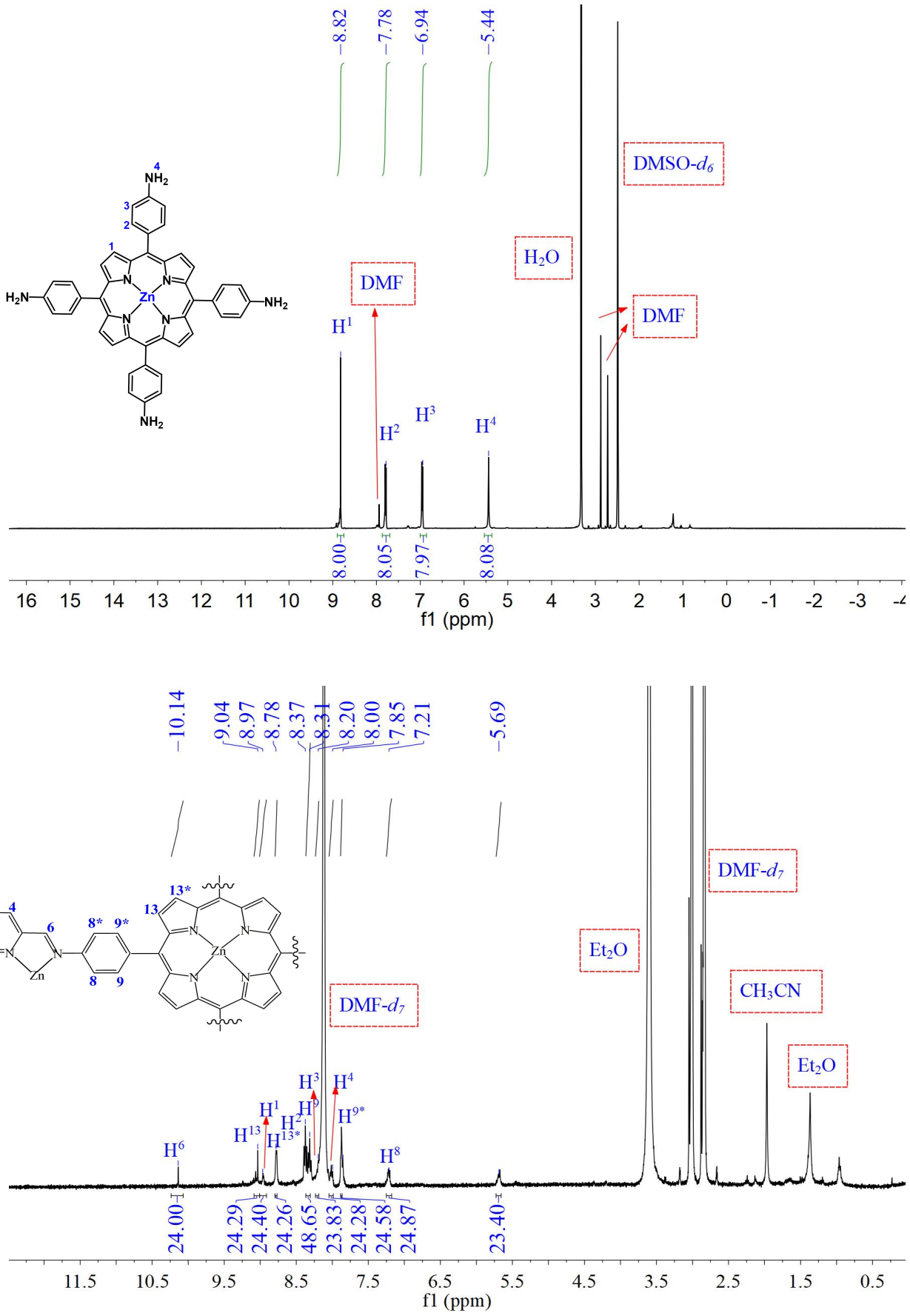
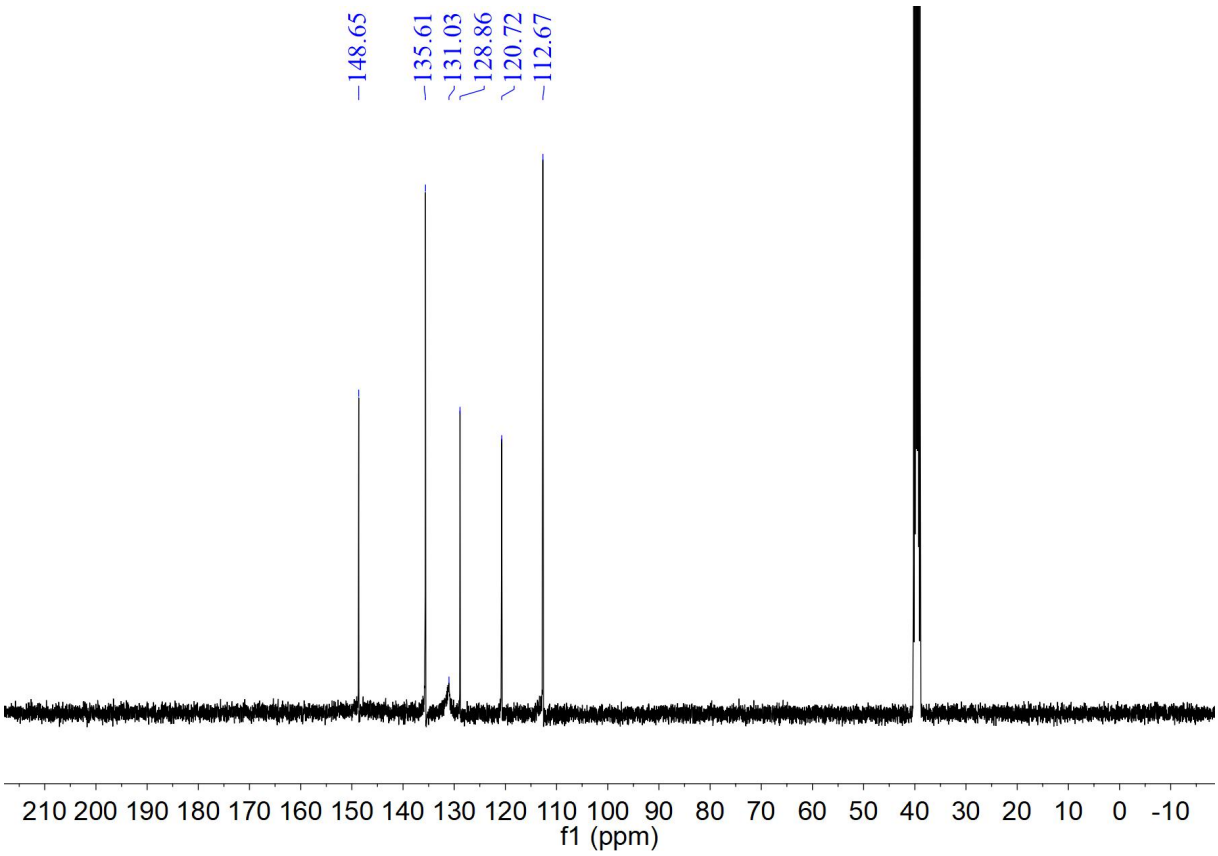


Fig. S2 ^{1}H NMR spectra of TAPP, Zn-TAPP and PM.



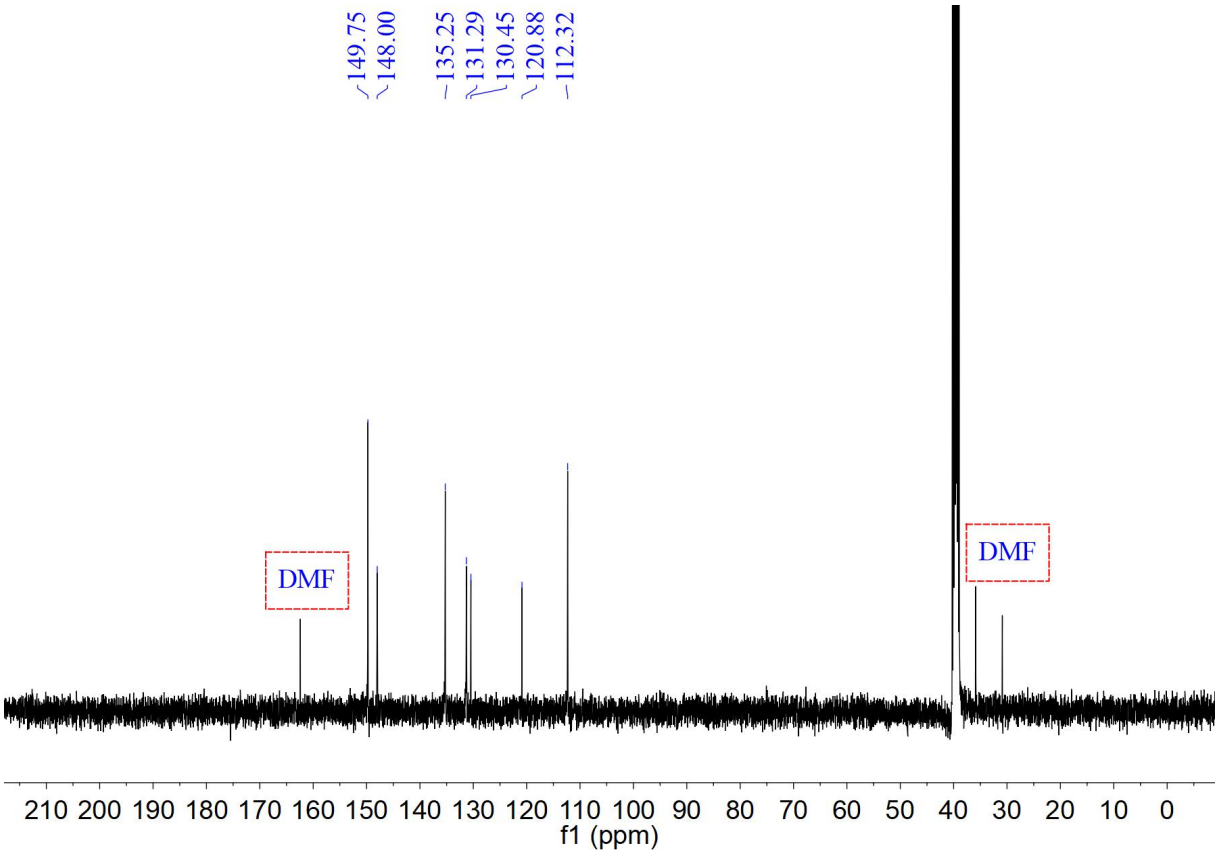
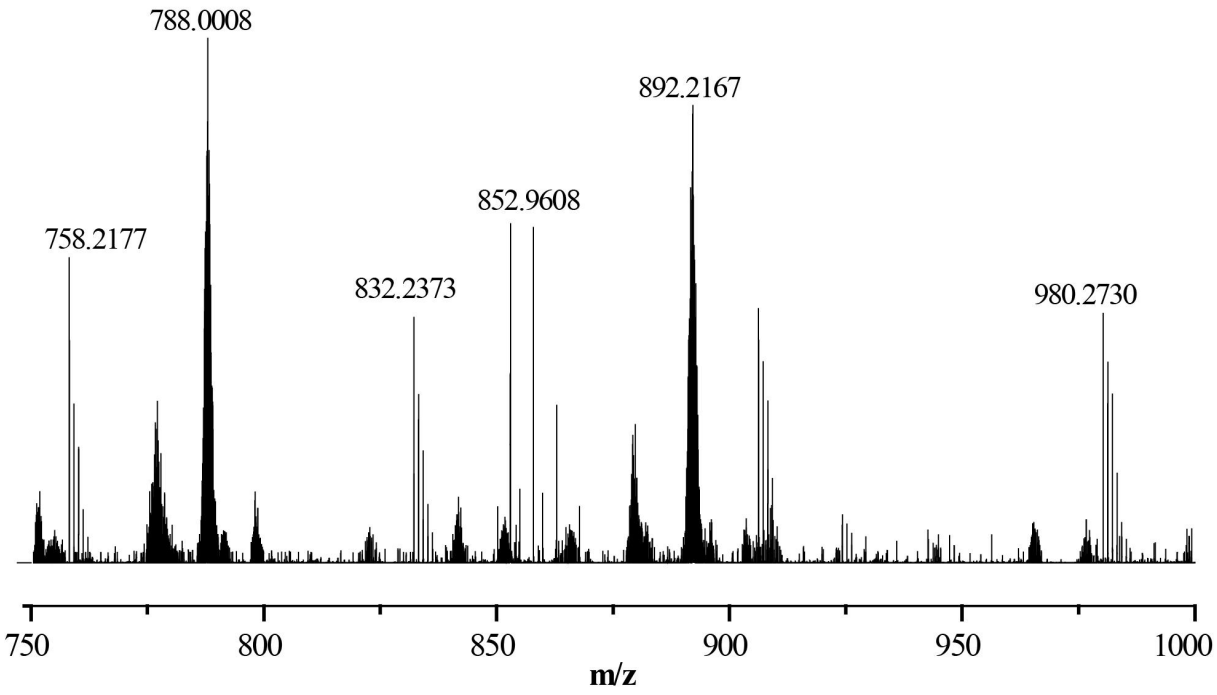


Fig. S3 ^{13}C NMR spectra of TAPP and Zn-TAPP in DMSO-d_6.



m/z	fragment
758.22	$[PM(OTf)_5 \cdot 7DMF]^{11+}$
788.00	$[PM(OTf)_4 \cdot 24DMF \cdot H_2O]^{12+}$
832.24	$[PM(OTf)_6 \cdot 4DMF \cdot 2H_2O]^{10+}$
852.96	$[PM(OTf)_6 \cdot 3DMF \cdot 21H_2O]^{10+}$
892.22	$[PM(OTf)_6 \cdot 12DMF \cdot 3H_2O]^{10+}$
980.27	$[PM(OTf)_7 \cdot DMF]^{9+}$

Fig. S4 Mass spectrum of PM.

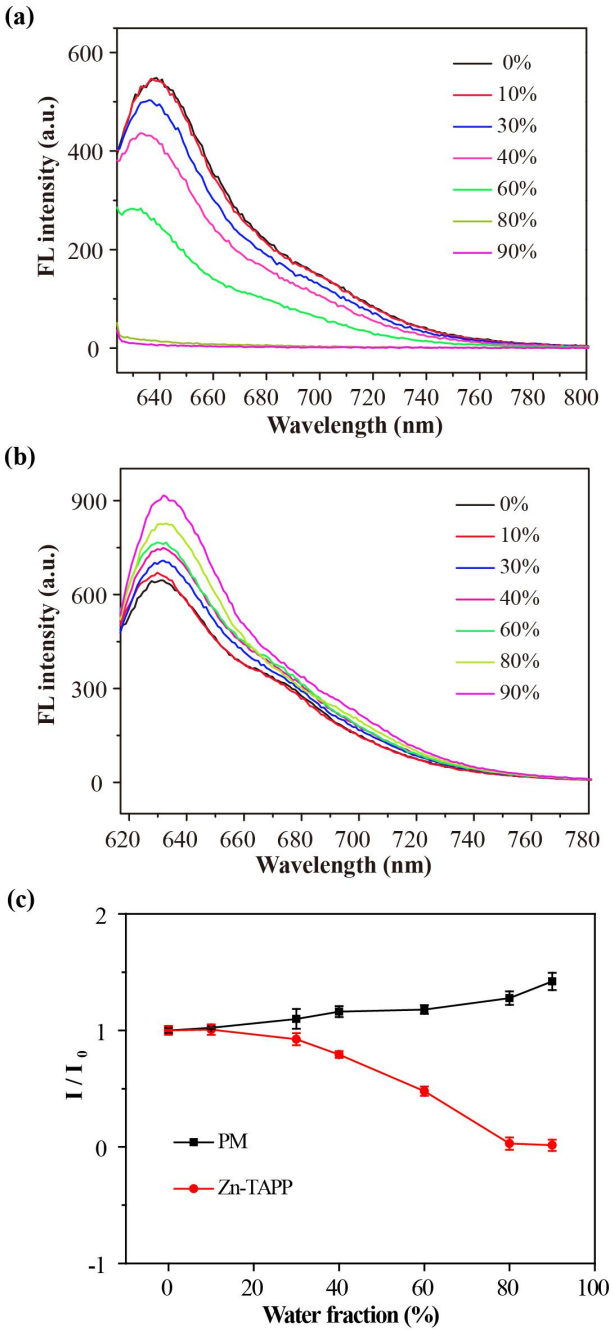


Fig. S5 The fluorescence spectra of Zn-TAPP (a) and PM (b) at the same concentration of Zn-TAPP (84 μM) in DMF/H_2O mixtures with water fractions range from 0% to 90%. (c) The fluorescence intensity ratios of Zn-TAPP and PM in DMF/H_2O mixtures. I_0 and I are intensities in pure DMF and DMF/H_2O mixtures, respectively. Data are presented as means±S.D (n=3).

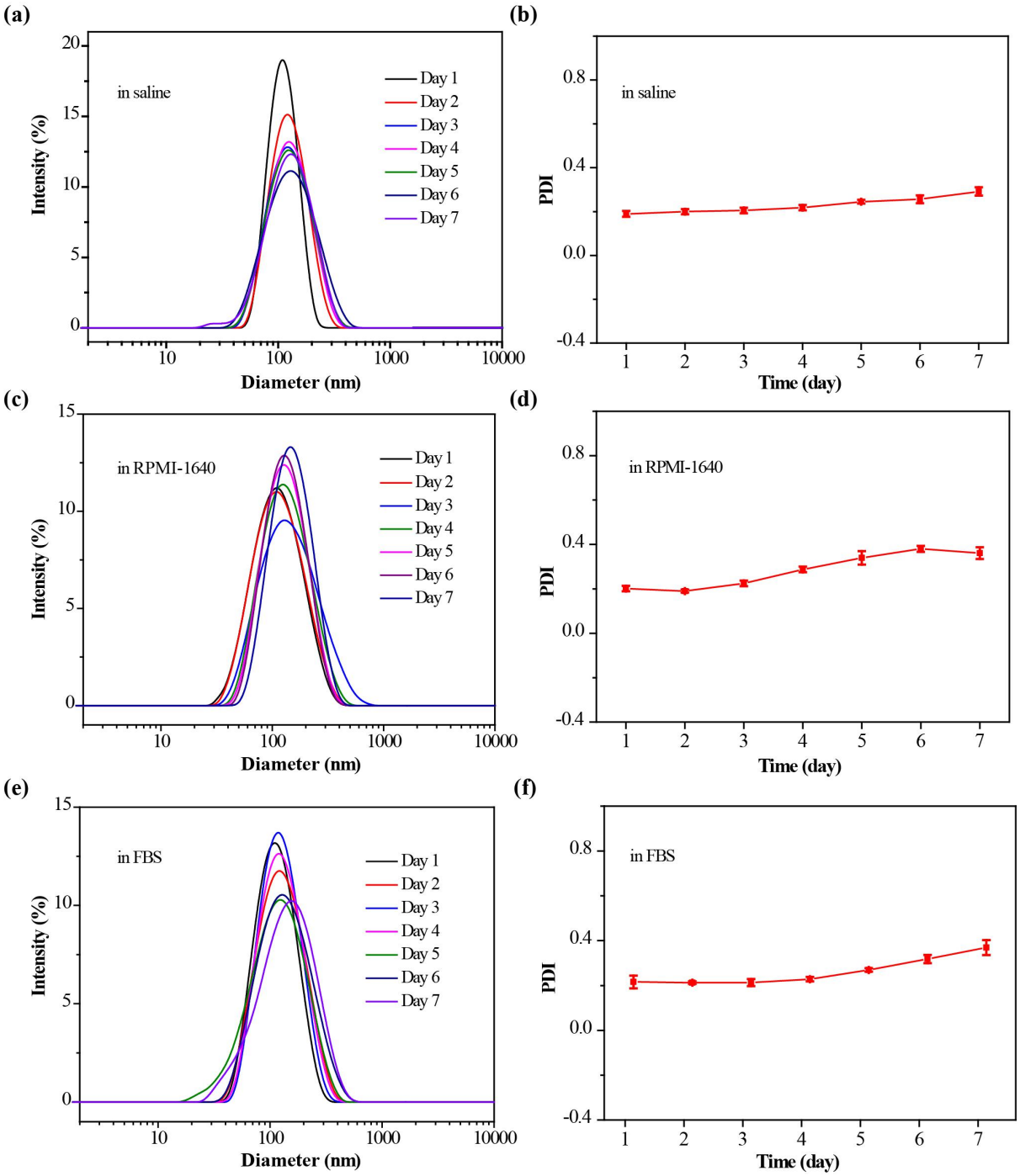


Fig. S6 PM@HAase-mPEG particle size distribution and PDI changes in (a, b) saline, (c, d) RPMI-1640 and (e, f) FBS within 7 days. Data are presented as means±S.D (n=3).

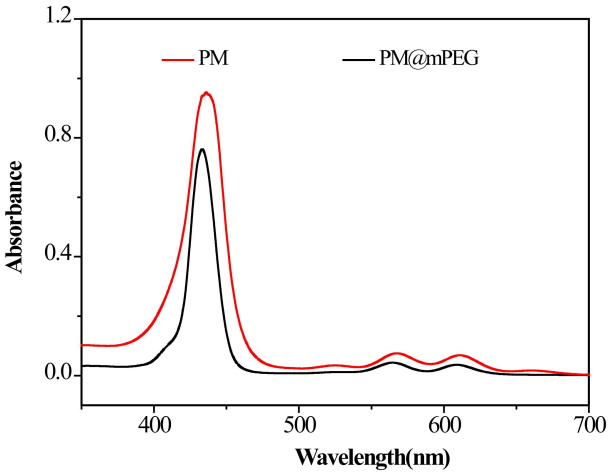


Fig. S7 UV-vis spectra of PM and PM@mPEG.

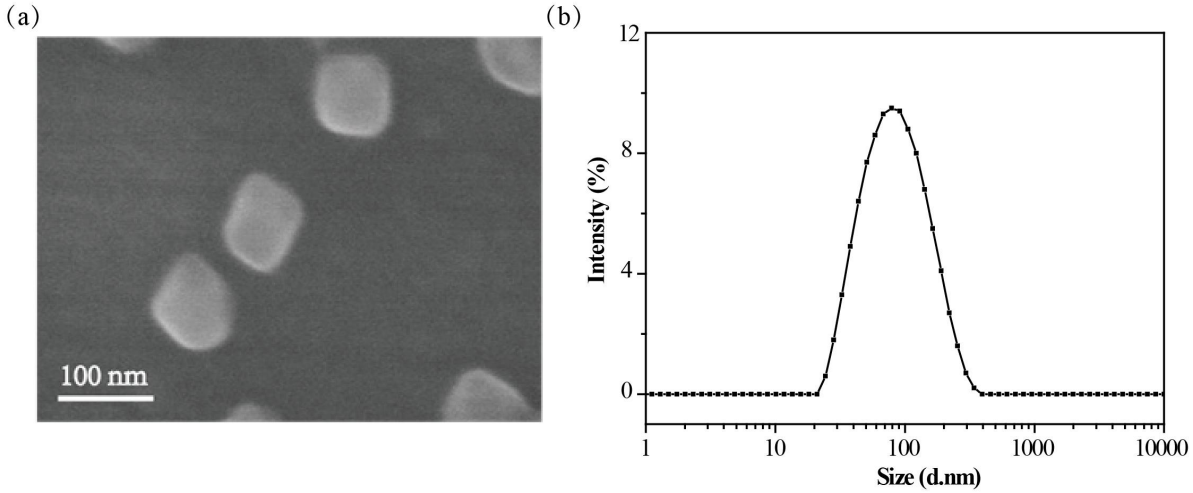


Fig. S8 (a) SEM image and (b) DLS distribution of PM@mPEG.

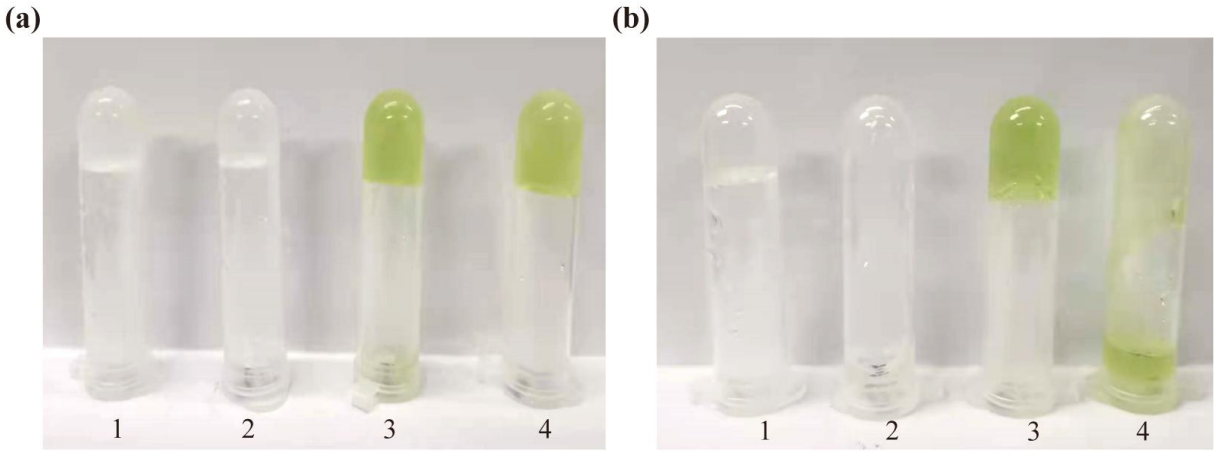


Fig. S9 Gelatin test experimental images (a) at the beginning of addition of materials and (b) 4 h later, respectively. (1) gelatin + HA; (2) gelatin + HA + HAase; (3) gelatin + HA + PM@mPEG; (4) gelatin + HA + PM@HAase-mPEG.

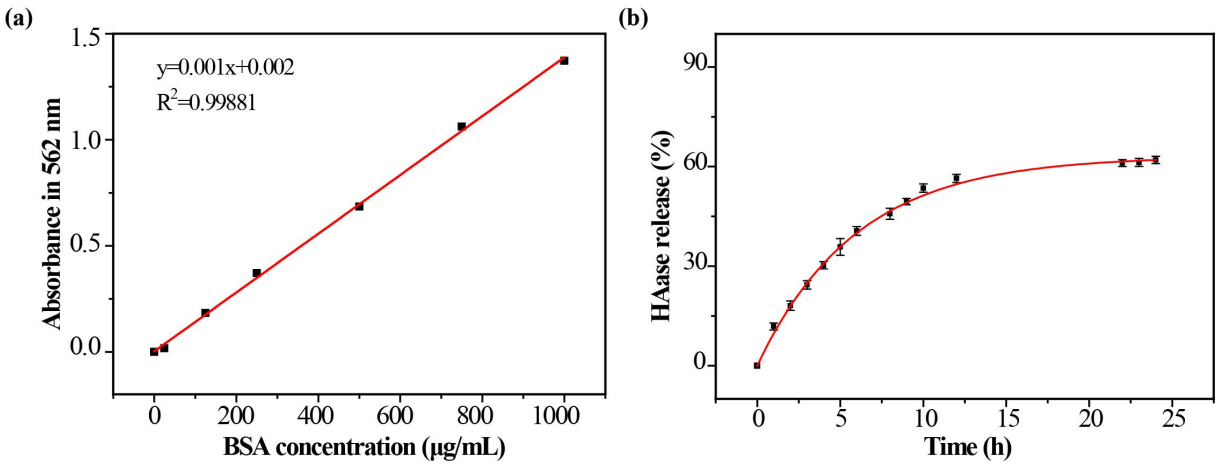


Fig. S10 The release of HAase from PM@HAase-mPEG by BCA protein assay (a) standard curve and (b) HAase release profile at 37 °C for different time. Data are presented as means ± S.D (n=3).

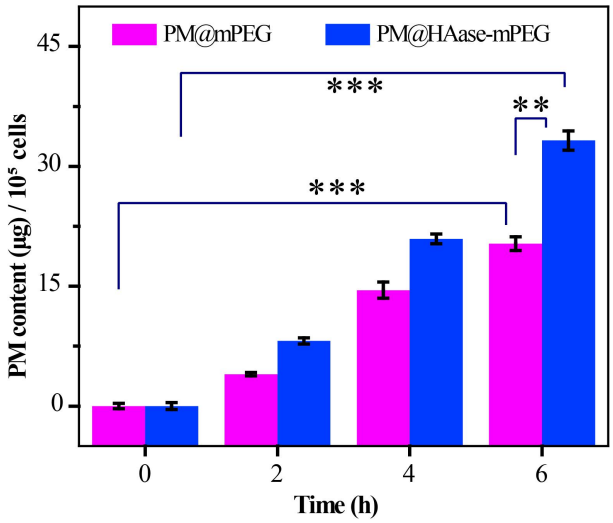


Fig. S11 Cellular uptake of PM@mPEG and PM@HAase-mPEG after 2, 4 or 6 hours of incubation. The concentrations were determined by measuring Zn content inside cells by ICP. Data are presented as means ± SD (n = 3). **p < 0.01, ***p < 0.001.

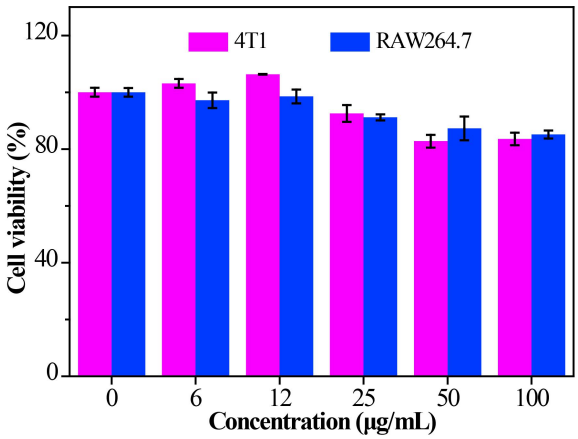


Fig. S12 Cell viabilities of 4T1 and RAW 264.7 cells incubated with different concentration of PM@mPEG in dark for 24 h. Data are presented as means ± SD (n=3).

	C_p	PDT efficiency
PM@PEG	42 μM	55%
PM@PEG	84 μM	67%
Zn-TAPP	84 μM	31%
PCN-224	68 μM	22%
Hf-TCPP NMOF	51 μM	50%
NPMOF	75 μM	25%

Table S1 PDT efficiencies for TAPP ligand and MOFs reported in the literature. C_P represents the porphyrin concentration.

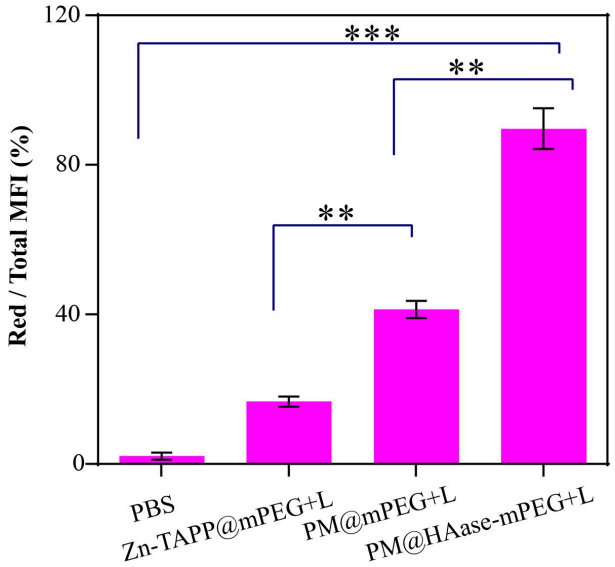


Fig. S13 Quantitative analysis of red fluorescence intensity ratio in calcein-AM /PI staining of each group. Data are presented as means ± SD (n = 3). ***p < 0.001.

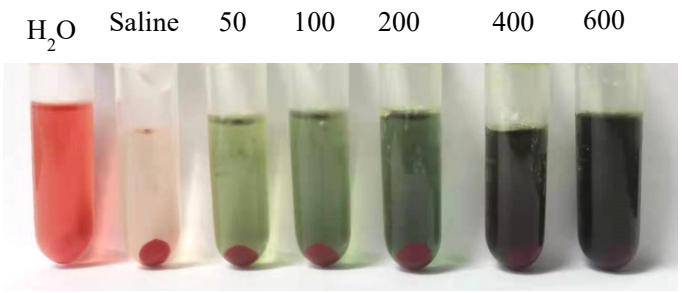


Fig. S14 Red blood cells were incubated with various concentrations of PM@HAase-mPEG for 2 h. PBS and pure water were employed as the negative and positive control, respectively.

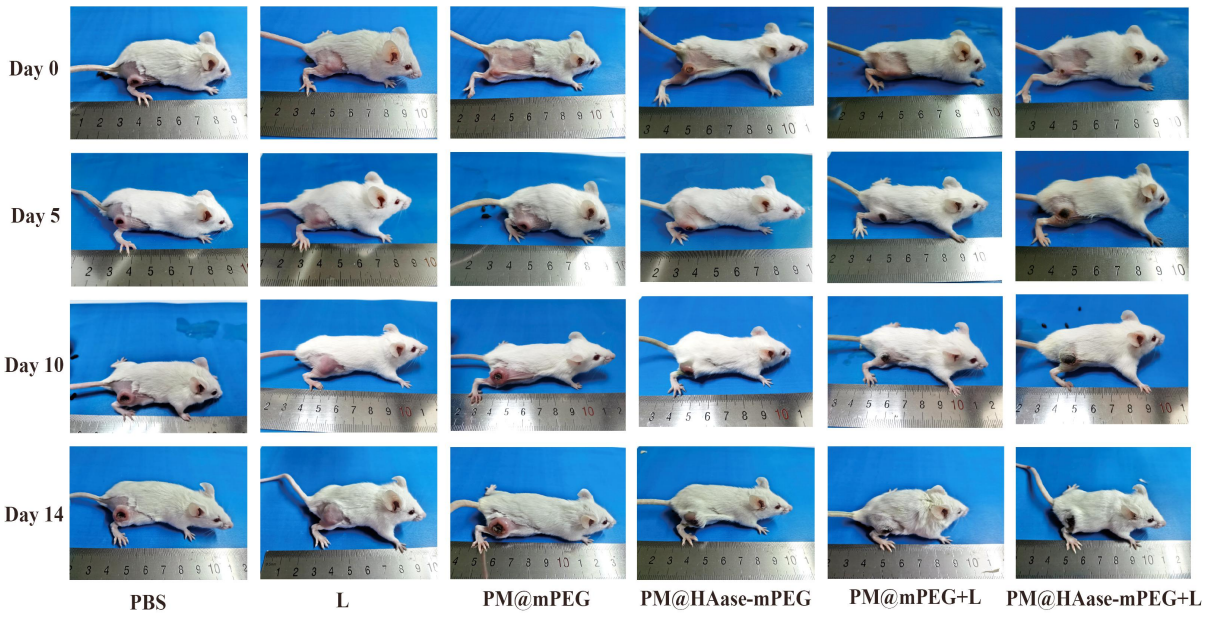


Fig. S15 Photographs of tumor-bearing mice on days 0, 5, 10 and 14 days of during follow-up treatment.

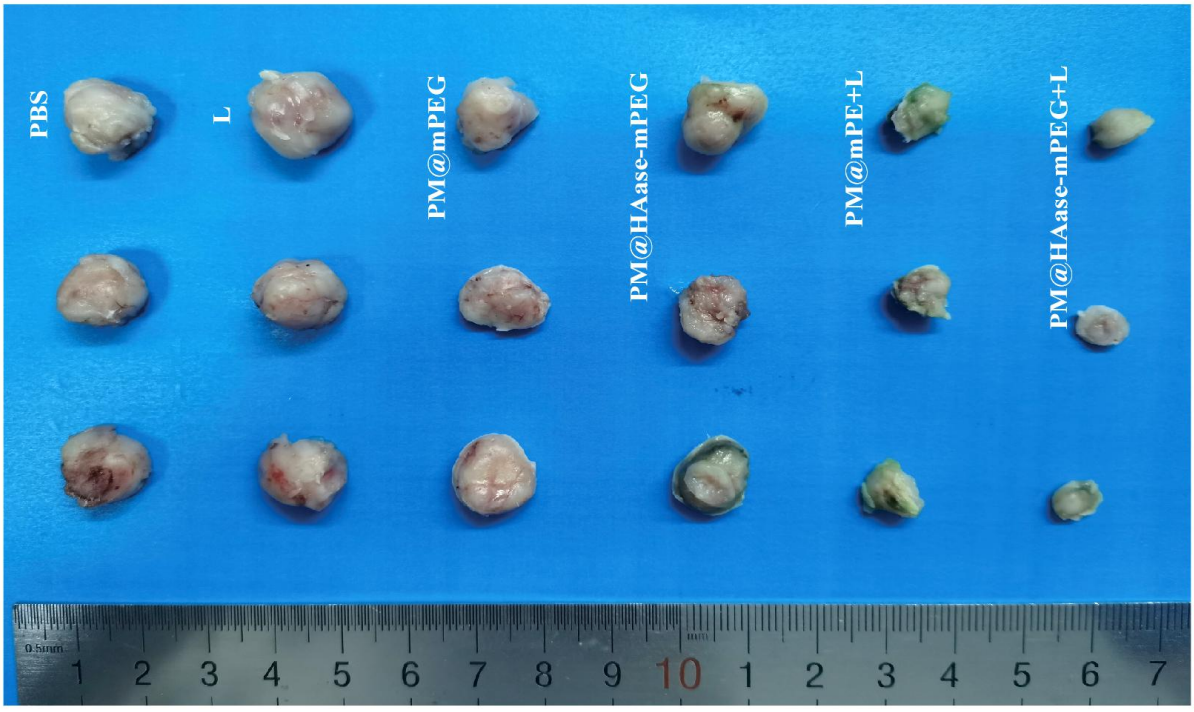


Fig. S16 Photographs of the tumors after treatment for 14 days.

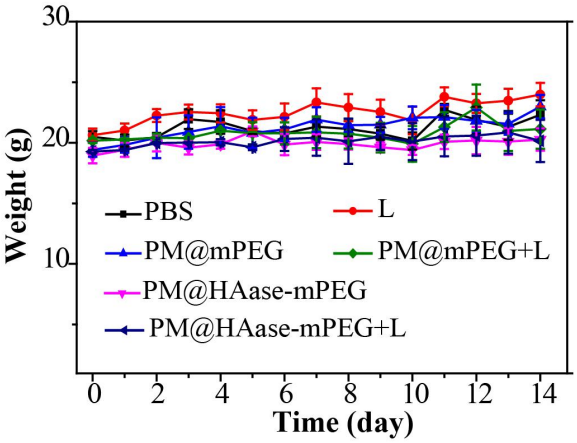


Fig. S17 Body weight of 4T1 tumor-bearing mice during various treatment for 14 days. Data are presented as means±S.D (n=3).

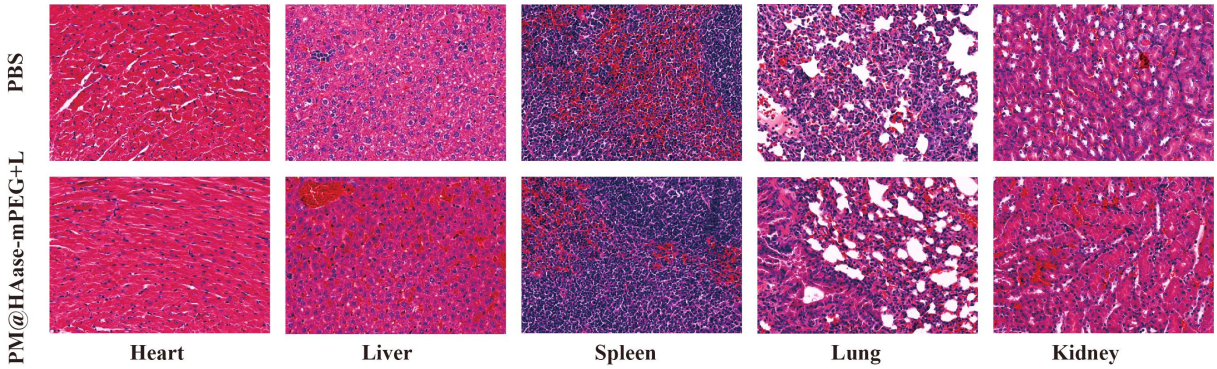


Fig. S18 H&E staining of mice heart, liver, spleen, lung and kidney after treatment by PM@HAase-mPEG with irradiation.