Supplementary Material

Pyropheophorbide a (PPa, Mw=534.65) was purchased from Shanghai Xianhui Pharmaceutical Technology Co. Ltd. L_{TPA} (Mw=2320) was synthesized in the previous research and kindly provided by the co-author Dr. Xiaohe Tian. 2-(Diisopropylamino)ethyl methacrylate (DPA) was purchased from Scientific Polymer Products and purified using a DHR-4 column supplied by the manufacturer before storing in a refrigerator prior to use. 2-Hydroxypropyl methacrylate (HPMA; an isomeric mixture of 75% HPMA and 25% 2-hydroxyisopropyl methacrylate), 4-(2-Hydroxyethyl) morpholine (99%), 2-bromoisobutyryl bromide (98%), 2,2'-bipyridine (99%), succinic anhydride (SA), DAPI, Syto9, 2',7'-dichlorofluorescin diacetate (DCFH-DA) Chlorpromazine, Chloroquine, Nocodazole, 2-Deoxy-D-glucose, Colchicine and NH_4Cl were obtained from Sigma-Aldrich. Regenerated cellulose dialysis membrane (Spectra/Por 6, molecular weight cut-off = 1,000 Da) was supplied by Fisher. Silica gel 60 (0.063-0.2 mm diameter) was supplied by Merck (Darmstadt, Germany). 9,10-Anthracene-dipropionic acid disodium salt (ADPA) was purchased from Santa Cruz Biotechnology.

Synthesis PSPMA-PDPA diblock copolymer via atom transfer radical polymerization (ATRP)

The PSPMA-PDPA diblock copolymer was prepared as described in literature.[13] Briefly, ATRP initiator (99 mg, 1.00 mmol) and HPMA monomer (10.22 g, 70 mmol, Dp=70) were dissolved with 2-propanol/water (95:5 w/w) in a 100 mL two-neck round-bottomed flask to obtain a 50% w/w aqueous solution of HPMA. The atmosphere of the mixture was protected by using a stream of nitrogen for 1 h in advance. Cu(I)Cl catalyst and 2,2'-bipyridine (relative molar ratio ME-Br: Cu(I)Cl: Bpy 1:1:2) were then added quickly under a nitrogen blanket to start the polymerization. The color of mixture turned into dark brown and became viscous as the reaction progressed. After 6 hours, the polymerization was completed (more than 98% conversion) as judged by ^1H NMR. A separately degassed 50% w/w solution of DPA in 2-propanol/water was added to the

reaction solution with a syringe. The polymerization was quenched by exposing the reaction mixture to air, followed by dilution with THF (80 mL). The remaining ATRP catalyst was removed by flushing the green solution through a silica gel (0.063-0.2 nm particle diameter; Merck, Germany) column. Most THF was removed under vacuum, and the crude block copolymer was precipitated with 500 mL deionized water. The recovered off-white copolymer was redissolved in THF and precipitated with water and filtered to remove unreacted DPA monomer. White solid copolymer was obtained after drying under vacuum. In a 100 mL round-bottomed flask, 2.0 g of PSHPMA-PDPA diblock copolymer precursor obtained was dissolved in 25 mL of anhydrous THF. An excess amount of SA and TEA was then added and the esterification was proceed at 20^{o}C for at least 48 h. THF was then removed under vacuum, and the crude zwitterionic diblock copolymer was dissolved in saturated NaCl solution. The completely esterified copolymer solutions were purified by dialysis in pure water with excess $NaHCO_3$ for at least 2 days to ensure the complete removal of the small impurities followed by 3 days of dialysis against pure water. All the samples were then freeze-dried from water overnight. The final esterified diblock copolymers were obtained as white solids and were denoted ‘PSPMA-PDPA’.

Molecular weights and molecular weight distributions of the homopolymer and the diblock copolymer precursors were determined using a GPC setup comprising Polymer Laboratories PL gel 5 μm MIXED-C columns. The GPC eluent was HPLC grade THF containing 2.0% (v/v) TEA and the flow rate was 1.0 mL min^{-1}. The BHT stabilizer was used as an internal standard, and the column temperature was set at 30^{o}C. Calibration was carried out using a series of near-monodisperse poly(methyl methacrylate) (PMMA) standards. Data were analyzed using PL Cirrus GPC software (version 2.0) supplied by Polymer Laboratories.

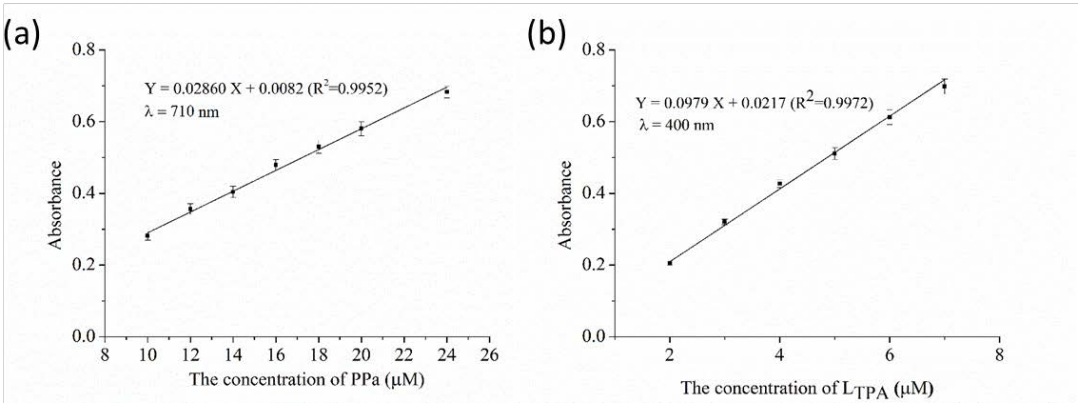

Figure S1 The calibration curves of (a) PPa and (b) L_{TPA}

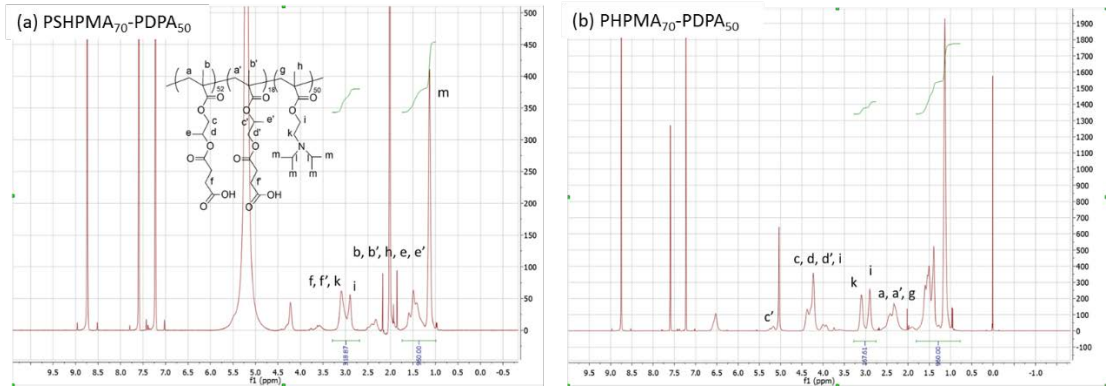

Figure S2. ^{1}H NMR spectra recorded for (a) succinic anhydride esterified $PSHPMA_{70}$-$PDPA_{50}$ and (b) $PHPMA_{70}$-$PDPA_{50}$ diblock copolymer in d5-pyridine.

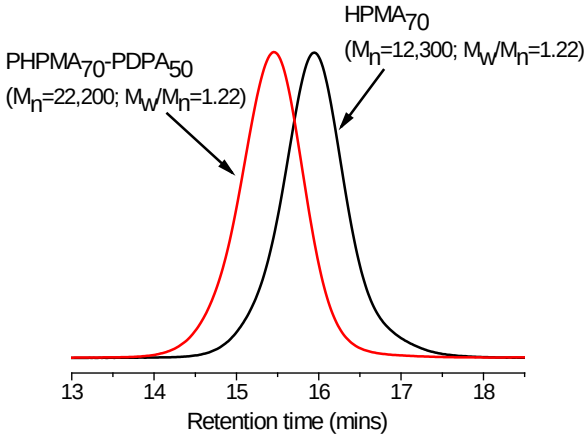

Figure S3. The GPC measurements of $PHPMA_{70}$-$PDPA_{50}$ (red line) and $HPMA_{70}$ homopolymer (black line) in THF.

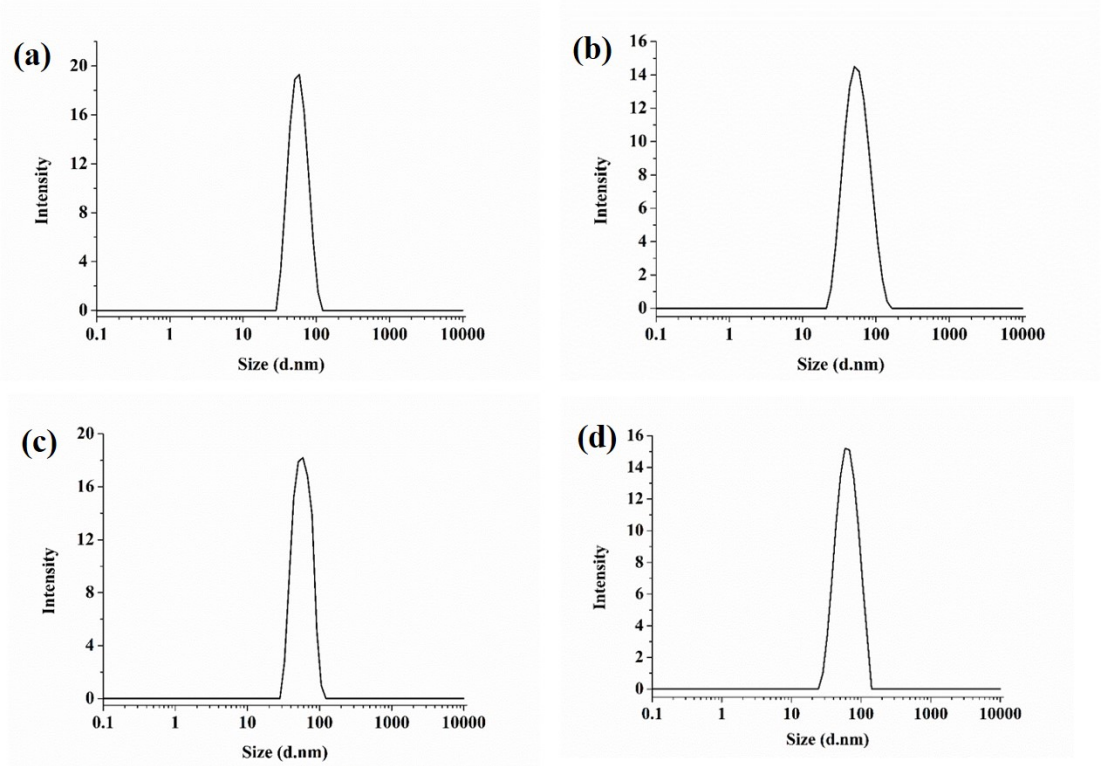

Figure S4. Size distribution determined by DLS at pH 7.4: (a) BM (empty micelles**), (b) $L_{TPA}M$ (**L_{TPA}-encapsulated micelles**), (c) PM (**PPa-encapsulated micelles**) and (d) $PL_{TPA}M$ (**PPa/L_{TPA} co-encapsulated copolymer micelles**).**

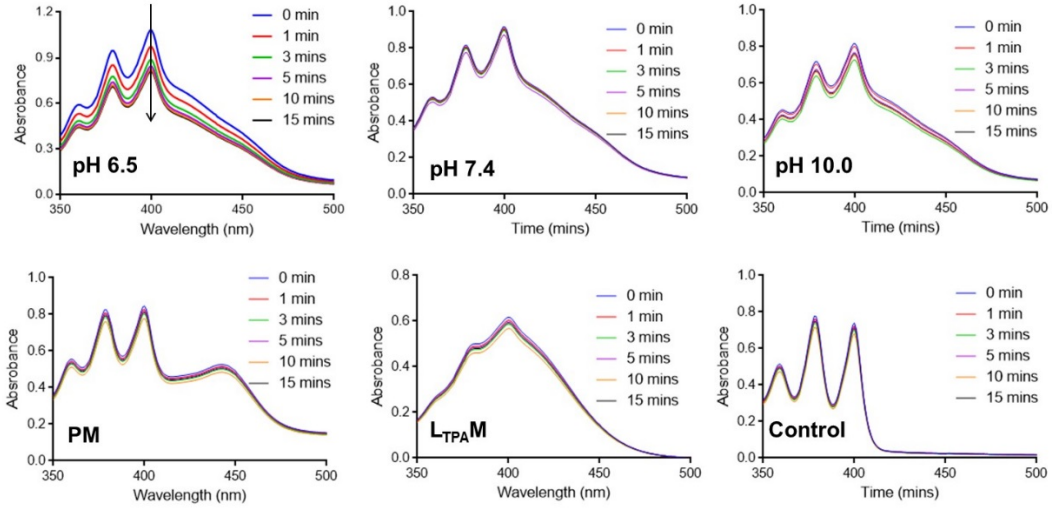

Figure S5. Supporting the results in Figure 3b. Samples were irradiated with 808 nm laser except PM which was irradiated with 660nm laser.

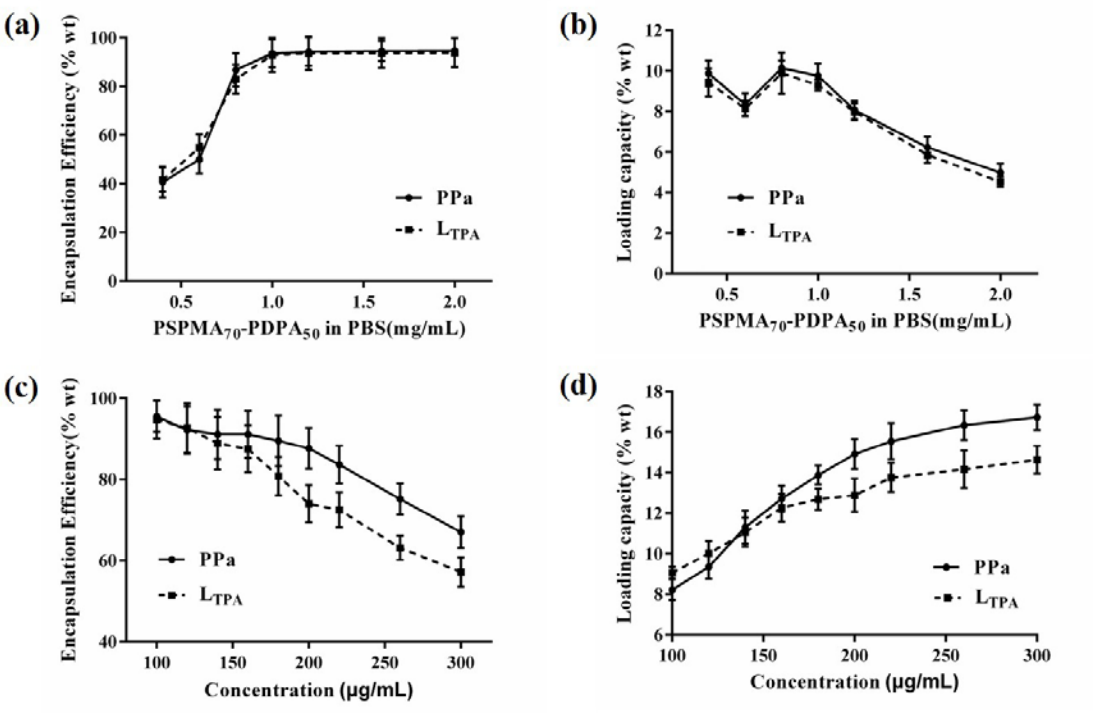

Figure S6. Determination of (a) the encapsulation efficiency and (b) loading capacity of PPa/L_{TPA} in $PL_{TPA}M$ at different $PSPMA_{70}$-$PDPA_{50}$ concentrations. Determination of (c) encapsulation efficiency and (d) loading capacity of PPa/L_{TPA} in $PL_{TPA}M$ at different PPa/L_{TPA} concentrations. Data were reported as two independent experiments ± SD (n=3).

The encapsulation efficiency (EE, %wt) and drug loading capacity (LC, %wt) were obtained according to the following formulas:

$$\text{EE \%wt} = \frac{\text{Weight of drug in micelles}}{\text{Weight of drug fed initially}} \times 100$$

$$\text{LC \%wt} = \frac{\text{Weight of drug in micelles}}{\text{Weight of drug in micelles and polymer}} \times 100$$

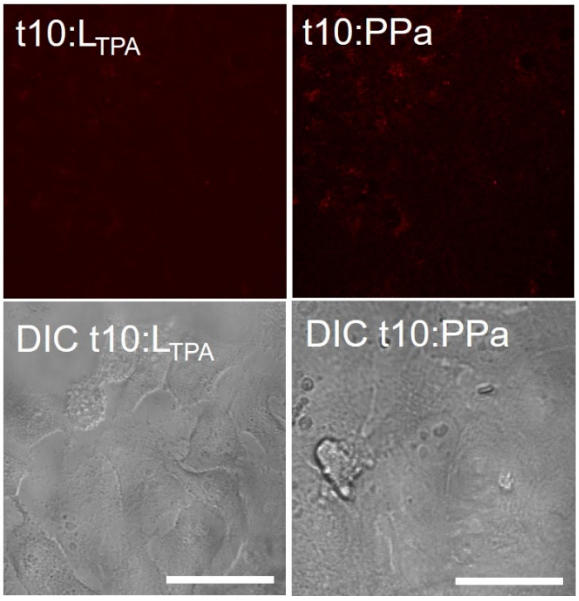

Figure S7. Cellular uptake of free PPa (5.1 μM) and free L_{TPA} (1.3 μM) treating for 30 minutes (t10).

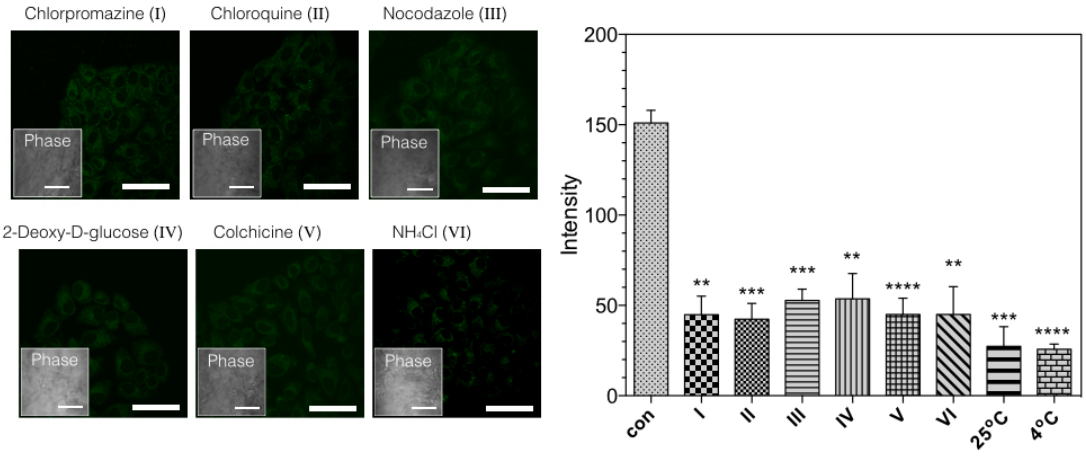

Figure S8. Effects of endocytic inhibitors (Chlorpromazine, Chloroquine, Nocodazole, 2-Deoxy-D-glucose, Colchicine, and NH_4Cl) and temperatures (4°C, 25°C, and 37°C) on the cellular uptake of $PL_{TPA}M$ on 4T1 murine breast cancer cells. One-way ANOVA was used for statistical analysis with data point＝30, $p < 0.005$. Error bars: SEM.

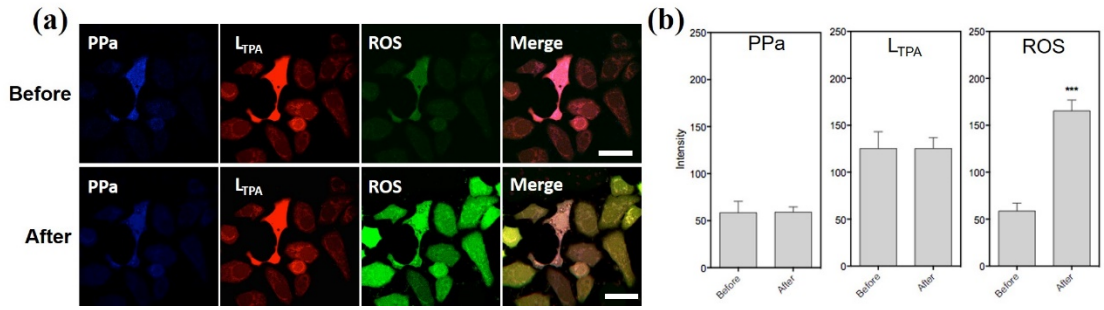

Figure S9. ROS generated in cells treated with $PL_{TPA}M$ and then irradiated with an 808 nm laser (0.3 W/cm^2, 30 scans of 10 seconds each). (a) Two photon confocal microscopy images. Green fluorescence indicated positive staining for specific fluorescence marker of ROS (Ex=502 nm, Em=523 nm). (b) Average fluorescence intensity of PPa, L_{TPA}, and ROS marker channels. ***p<0.001 compared to the ROS generation before irradiation using a one-way ANOVA.

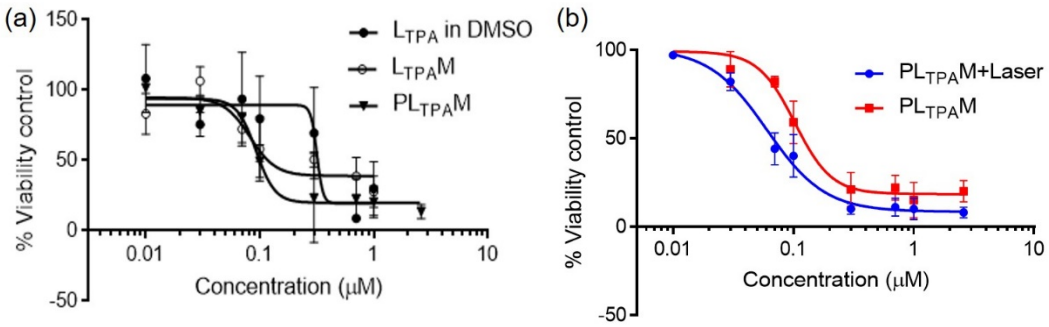

Figure S10. (a) Supporting the results in Table 1. The IC_{50}s of L_{TPA} in DMSO, $L_{TPA}M$ and $PL_{TPA}M$ treated murine breast cancer 4T1 cells. (b) The IC_{50}s of $PL_{TPA}M$ treated murine breast cancer 4T1 cells in the dark or exposed with laser. (90 J/cm^{-2}) Shown values are average values ±SD (n=3).

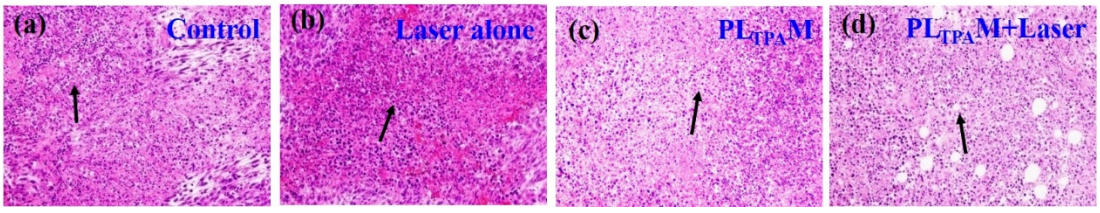

Figure S11. Images of hematoxylin and eosin (H&E) stained tumor tissues harvested from mice after initial treatments for 20 days. Corresponding groups: (a) PBS control, (e) Laser alone, (f) $PL_{TPA}M$. (g) $PL_{TPA}M$+laser. Necrotic cells were observed in tumor tissues (black arrows).

Electronic supplementary video. Real time cell morphology collapsing induced by two-photon-induced PDT.