Supporting Information

Redox-triggered activation of nanocarriers for mitochondria-targeting cancer chemotherapy

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Materials and methods

Polyethylene glycol monostearate (C_{18}-PEG_{2000}-OH, CPO)，polyethylene glycol monomethyl ether 4000 ($mPEG_{4000}$), 3,3'-dithiodipropionic acid ($C_6H_{10}O_4S_2$) and suberic acid ($C_8H_{14}O_4$) were purchased from Tokyo Chemical Industry Co., Ltd. Coumarin, dicyclohexylcarbodiimide (DCC), 4-(dimethylamino)pyridine (DMAP) and triethylamine (TEA) were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). 1,2-Dilauroyl-sn-glycero-3-phosphoethanolamine (DLPE) was obtained from J&K Chemical (Guangzhou, China). Paclitaxel (PTX), 5-bromovaleric acid (Br-C_4H_8-COOH), triphenylphosphine (TPP), poly(lactic-co-glycolic acid) (PLGA 65:35, Mw 40000-75000 Da),3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and Hoechst 33258 were purchased from Sigma–Aldrich Chemicals (St. Louis, MO, USA). Mitotracker® Red CMXRos were purchased from Invitrogen (Eugene, OR, USA). Trypsin-EDTA, fetal bovine serum (FBS, Gibco®), trypsin (Gibco®), Dulbecco's Modified Eagle Medium (DMEM, HYCLONE®), penicillin-streptomycin solution (Hyclone®), BCA Protein Assay Kit (BD biosciences), Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI)

apoptosis detection kit (BD Biosciences),Streptavidin/Peroxidase immunohistochemical kit (Zymed), anti-cytochrome c (Beyotime), diaminobenzidine (DAB) kit, colorimetric assay kit (Beyotime) and XF24 extracellular flux analyzer kit (Seahorse Bioscience) were provided by domestic suppliers. Other chemicals and reagents are of analytical grade and used without further purification.

FTIR spectra were measured by FTS 6000 spectrum instrument (Bio-Rad Company, Hercules, USA). ^{1}H NMR was detected by Mercury VX-300 type NMR spectrometer at 300 MHz, $CDCl_3$ as a solvent, tetramethylsilane (TMS) as the internal standard. Gel permeation chromatography (GPC) measurements were carried out by using a Waters 2690D HPLC equipped with Styragel HR3 column. Samples were detected with a Waters 2410 differential refractive index detector. DMF was used as the mobile phase at a flow rate of 0.3 mL min^{-1}. The column temperature was 40 °C. Poly(methyl methacrylate) was used as the standard.

Synthesis of 4-carboxbutyltriphenylphosphonium bromide (1)

To a solution of 5-bromovaleric acid (1 g, 5.5 mmol) in acetonitrile (15 mL), TPP (1.6 g, 6.1 mmol) was added and refluxed for 24h. The reaction solution was evaporated under vacuum to remove acetonitrile. The residue was dissolved in dichloromethane. The solution was added dropwise into diethyl ether. The white solid was collected by filtration, washed with benzene, hexane and diethyl ether successively, and then dried to give 2.4 g product (yield 98%).

Synthesis of C_{18}-PEG_{2000}-TPP (CPT, 2)

4-Carboxbutyl triphenylphosphonium bromide (443.3 mg, 1 mmol), C_{18}-PEG_{2000}-

OH (2.48 g, 1 mmol) and 4-(dimethylamino)pyridine (DMAP, 0.018 g, 0.15 mmol) were dissolved in anhydrous DMF (20 mL) under an nitrogen atmosphere. Subsequently, N,N′-dicyclohexylcarbodiimide (DCC) (247.6 mg, 1.2 mmol) in 3 mL anhydrous DMF was added dropwise into the above solution at 0 °C with stirring. The mixture was stirred for 24 h at room temperature. After filtration, the solution was dialyzed against pure water for 48 h using a dialysis tube (MWCO 1000 Da). During dialysis, water was exchanged each six hours. The solution was filtered and freeze-dried to obtain white product (2.00g, yield 73%).^{1}H NMR (300 MHz, $CDCl_3$: δ (ppm) 0.86 (s, 3H, CH_3), 1.23 (s, 30H, CH_2CH_2), 2.75 –2.77(t, 4H, CH_2COO), 3.55–3.71 (m, CH_2CH_2O),7.60 –7.92 (d, 15H, C_6H_5).

Synthesis of mPEG$_{4000}$-S-S-COOH (3) and mPEG$_{4000}$-C-C-COOH (4)

mPEG$_{4000}$ (4.0 g, 1 mmol), DMAP (0.018 g, 0.15 mmol) and TEA (150 μL, 1.1 mmol) were added to an ice cooled solution of3,3'-dithiodipropionic acid (0.252 g, 1.2 mmol) in 10mL anhydrous DMF. Then, a solution of DCC (0.248 g, 1.2 mmol) in anhydrous DMF (3 mL) was added dropwise into the mixture and stirred for 24 h at room temperature. After filtration, the solution was added dropwise into cold diethyl ether. The white solid was collected and dried overnight in vacuum. The crude product was purified by dissolution–precipitation in dichloromethane/diethyl ether twice and dried in vacuum to obtain the compound **3** (2.20g, yield 54%).

The synthesis of mPEG$_{4000}$-C-C-COOH (**4**) was similar to that of compound **3** except suberic acid (0.172 g, 1 mmol) was used instead of 3,3'-dithiodipropionic acid.

Synthesis of DLPE-S-S-mPEG$_{4000}$ (DSSP, 5) and DLPE-C-C-mPEG$_{4000}$ (DCCP, 6)

$mPEG_{4000}$-S-S-COOH (**3**) (4.250 g, 1 mmol), DLPE (0.580 g，1 mmol), DMAP (0.018 g, 0.15 mmol) and TEA (150μL 1.1mmol) were dissolved in anhydrous chloroform (20 mL) at 0 °C under nitrogen atmosphere. Subsequently, DCC (0.248 g, 1.2mmol) in 3 mL anhydrous chloroform was added dropwise into the solution at 0 °C. The mixture was stirred overnight at room temperature. After filtration, the solution was added dropwise into cold diethyl ether to give white solid. The white solid was purified by dissolution–precipitation in dichloromethane/diethyl ether twice. The product was obtained after drying in vacuum (3.40g, yield 72%).^{1}H NMR (300 MHz, $CDCl_3$: δ (ppm) 0.86 (s, 3H, CH_3), 1.26 (s, 72H, CH_2CH_2), 2.45–2.50(t, 4H, CH_2COO), 2.79–2.80 (t, 2H, CH_2COO), 2.90–2.92 (t, 4H,CH_2SS), 3.41 (s, 3H, OCH_3), 3.55–3.71 (m, CH_2CH_2O),4.24–4.26 (t, 2H, $COOCH_2$).

The synthesis of DLPE-C-C-$mPEG_{4000}$ (DCCP, **6**) was similar to that of DLPE-S-S-$mPEG_{4000}$ (**5**) except $mPEG_{4000}$-C-C-COOH (**4**) (4.150 g, 1 mmol) was used instead of $mPEG_{4000}$-S-S-COOH (**3**).^{1}H NMR (300 MHz, $CDCl_3$: δ (ppm) 0.86 (s, 3H, CH_3), 1.26 (s, 40H, CH_2CH_2), 2.75 –2.77(t, 2H, CH_2COO), 2.79 –2.80 (t, 2H, CH_2COO), 3.41 (s, 3H, OCH_3), 3.55–3.71 (m, CH_2CH_2O),4.24 –4.26 (t, 2H, $COOCH_2$).

Preparation of blank LPNPs

C_{18}-PEG_{2000}-TPP (CPT, 3mg) and C_{18}-PEG_{2000}-OH (CPO, 2 mg) were dispersed in 7 mL ultrapure water with stirring at 45 °C for 30 min. The solution of 50 mg PLGA in 3mL DMF was added to the aqueous phase and whirled violently for 30 min. Subsequently, the suspension was stirred at room temperature for 2 h and dialyzed

against ultrapure water using a dialysis tube (MWCO 7000) for 48 h. Ultrapure water was exchanged each 6 hr during dialysis.The solution of LPNPs (PLGA-CPT) was extruded repeatedly through a syringe filter (0.22 μM) and was stored at 4 °C before usage.

The preparation of other LPNPs, PLGA-DCCP, PLGA-DSSP, PLGA-CPT-DCCP and PLGA-CPT-DSSP, was similar to PLGA-CPT. The composition of LPNPs was summarized in Table S2.

Characterization of LPNPs

200 μL of LPNPs solution was diluted in 3 mL of PBS buffer.The average particle sizesand zeta potentials of LPNPs were measured using a ZETA-SIZER Nano Series ZEN3600 (Malvern Instruments Ltd, UK). The diluted LPNPs solutionwas dropped on a copper grid (300 mesh) with formvar film and then stained with a drop of 1% phosphotungstic acid for 2 min. The morphologies of LPNPs were observedusing Jeol JEM-100CXII transmission electron microscopy (TEM, Tokyo, Japan) at an acceleration voltage of 100 kV.

Effect of GSH treatment on sizes and zeta potentials of LPNPs

200 μL of prepared LPNPs solution was mixed with 2.8 mL of PBS containing 0.03 μmol or 0.03 mmol GSH in a cell. The final concentration of GSH is 10 μM or 10 mM. The cell was placed in a shaking bed at 37 °C. The sizes and zeta potentials of LPNPs were measured at predetermined time intervals. The sizes and zeta potentials of LPNPs incubated in PBS without GSH were measured similarly.

Protein adsorption of LPNPs

Fetal bovine serum (FBS) was used to measure protein adsorption of LPNPs. 0.5 mL of LPNPs solution in PBS at different concentrations (1, 2, 3, 4 and 5 mg/mL) was separately added into 0.5 mL mixture of FBS and PBS (1:4, v:v), and then incubated under agitation in shaking bed at 37 °C for 2 h. Subsequently, the mixture was centrifuged at 5000 rpm for 3 min and the supernatant was harvested and analyzed using BCA Protein Assay Kit. 25 μL of supernatant was added into 96-well plates, mixed with 200 μL of BCA working reagent and incubated at 37°C for 30 min. Absorbance was measured at 570 nm with a microplate reader (Thermo Scientific, USA). The final mass of FBS (M_f) was obtained from a calibration curve, which was linear over the concentration of standard protein in the kit from 2 mg/mL to 0.025 mg/mL. Initial mass of FBS (M_i) was measured after similar treatment of FBS and PBS mixture using 0.5 mL PBS instead of LPNPs solution. The mass of adsorbed FBS (M_a) was calculated as $M_a=M_i-M_f$.

Variation of sizes and charges in the presence of FBS

200 μL of prepared PTX-LPNPs solution was mixed with 2.8 mL of fresh DMEM medium in a cell. The final concentration of FBS is 10% (V/V). The cell was placed in a shaking bed at 37 °C for 2h. After 2 h, the LPNPs were harvested by centrifuging (12,000 rpm) and resuspended in 3 mL PBS to measure the sizes and zeta potentials.

Preparation of PTX-LPNPs

The preparation of PTX-LPNPs was similar to that of blank LPNPs except 6 mg PTX was added into 3mL DMF together with 50 mg PLGA. Drug loading content (DLC) of PTX-LPNPs was measured using HPLC analysis. PTX-LPNPs nanoparticles were obtained by freeze-drying of PTX-LPNPs solution. 1 mg PTX-LPNPs nanoparticles

were dissolved in 1 mL acetonitrile.10 μLsolution was analyzed using SHIMADZU SPD-15C high performance liquid chromatograph spectrophotometer (HPLC, Tokyo, Japan) equipped with a reverse-phase Angel C18 column (250×4.6 mm^2, 5 mm). The samples were detected with UV detector at a wavelength of 227 nm. Acetonitrile/water (60:40, v:v) was used as the mobile phase at a flow rate of 0.5 mL/min. The column temperature was 25°C. The mass of PTX was calculated from a standard curve that was linear over the concentration of PTX from 0.01 mg/mL to 0.3 mg/mL.

Release of PTX from PTX-loaded LPNPs

In vitro release of PTX from LPNPs in PBS with or without GSH was carried out using the dialysis method. 0.5mL PTX-LPNPs solution in dialysis tube (MWCO 14 000) was immersed into a tube containing 10 mL buffer (PBS+0.1 M Tween-80) or buffer (PBS+10 mM GSH+0.1 M Tween-80) and kept at 37 °C in a shaking water bath. 2 mL sample was withdrawn at predetermined time intervals (0 h, 0.5 h, 1 h, 2 h, 4 h, 6 h, 8 h, 12 h, 24 h, 36 h, and 48 h) and harvested by freeze-drying. Meanwhile, 2 mL fresh buffer (PBS+0.1 M Tween-80) or buffer (PBS +10 mM GSH+0.1 M Tween-80) was replenished into the tube. Subsequently, freeze-drying powder was dissolved in 2 mL acetonitrile and the amount of PTX released was measured by HPLC. The determination of coumarin release from LPNPs was similar to PTX release from LPNPs.

Cells culture

Michigan Cancer Foundation-7 (MCF-7) cells were purchased from the China Center

for Type Culture Collection (Wuhan University, Wuhan, People's Republic of China) and cultured in DMEM medium which was supplemented with 10% FBS and 1% streptomycin/penicillin. Cells were kept at 37 °C in a humidified incubator containing 5% CO_2.

Co-localization of coumarin-LPNPs and Mitotracker® Red in the mitochondria of MCF-7 cells

Co-localization of coumarin-LPNPs and mitochondria of MCF-7 cells was monitored using a CLSM (Nikon, TE2000, EZ-C1, Japan). Coumarin as a fluorescence indicator was used. Coumarin-LPNPs were prepared similar to PTX-LPNPs except PTX was replaced with coumarin. The DLC of all coumarin-LPNPs ranged from 8.0% to 8.2%. 3×10^4 MCF-7 cells were seeded in confocal dishes and incubated at 37 °C for 24 h. The media were then replaced with 1mL of media with LPNPs containing 10 μg coumarin and incubated for further 24 h in the dark at 37°C. After removing the culture medium and washing twice with PBS, the cells were stained using 1mL of 100 nM Mitotracker® Red CMXRos (Invitrogen, USA) for 15 min in the dark at 37 ℃. Subsequently, the cells were washed with PBS three times and kept in PBS for CLSM observation. The green fluorescence signals of coumarin and red fluorescence signals of Mitotracker® Red were detected using 408/520 and 543/590nm excitation/emission filters respectively. The Pearson's correlation coefficient of green fluorescence from coumarin and red fluorescence from Mitotracker® Red was calculated using Imaging J software (National Institute of Health, USA).

***In vitro* cytotoxicity assay**

The cytotoxicity offree PTX and PTX-LPNPs against MCF-7 cells was evaluated by the MTT assay. Cells were seeded at a density of 7000 cells per well in 96-well plates and cultured at 37 ℃ for 24 h. Then the medium was replaced by 100 μL DMEM medium containing free PTX or PTX-LPNPs at various concentrations of PTX from 5.0 to 0.04 μg/mL. 100 μL complete DMEM was used as a control. After 48 h incubation under 5% CO_2 at 37 °C, the medium was replaced by 100 μL of MTT solution (0.5 mg/ mL). After 4h additional incubation, the medium was removed and 150 μL DMSO was added into each well to dissolve the formazan crystals. The absorbance was measured on a microplate reader (Thermo Scientific, USA) at 570 nm. Cell viability was calculated according to the following equation: Cell viability (%)=$[(A_{sample}–A_0)/(A_{control}-A_0)]\times100$, where $A_{sample}$and $A_{control}$are the absorbance values for the treated cells and the untreated control cells. A_0 is the absorbance for the untreated control cells without addition of MTT. Data are presented as average ± SD (n=4).

The cytotoxicity of LPNPs against MCF-7 and cytotoxicity of free PTX and PTX-LPNPs against COS 7 cells were similarly measured.

Release of cytochrome c from mitochondria

A Streptavidin/Peroxidase immunohistochemical kit was used to detect cytochrome c released from the mitochondria of MCF-7 cells to the cytosol according to the manufacturer's instructions. MCF-7 cells were seeded in 6-well plates (3×10^5 cells/well) and incubated for 24 h. The medium was separately replaced by 1 mL complete medium containing free PTX or PTX-LPNPs at 3 μg/mL of PTX. The cells were incubated for 24 h, and fixed with 4% paraformaldehyde for 15 min, followed by

treated with 3% H_2O_2 (v/v). After that, blocking buffer, primary antibody anti-cytochrome c, enhanced secondary antibody and enhanced streptavidin HRP in the kit (Shanghai Beyotime Biotechnology,Co, Ltd, China) were used to treat cells, respectively. After treatment with freshly prepared diaminobenzidine-tetrahydrochloride (DAB) in the kit, an optical microscope(Nikon, XSP-8CZ, Japan) was used to observe cytochrome c release in the cells.

Caspase-9 and caspase -3 activity

Colorimetric assay kits (Shanghai Beyotime Biotechnology, Co, Ltd, China) were used to measure caspase-9 and caspase-3 activity ratios of MCF-7 cells. MCF-7 cells were seeded in 6-well plates (3×10^5 cells/well) and incubated for 24 h. The medium was separately replaced by 1 mL complete medium containing free PTX or PTX-LPNPs at 3 μg/mL of PTX). After further 24 h incubation, the cells were harvested and washed twice with PBS, then treated with lysis buffer provided in the kit for 1 h in the ice bath. Subsequently, the solution was centrifuged at 10,000 rpm for 2 min at 4 °C and the supernatants were collected. Caspase-9 or caspase-3 substrates in the kit were separately incubated with the supernatants at 37 °C for 4 h in the dark. A microplate reader (Thermo Scientific, USA) was used to measure caspase-9 and caspase-3 activity and the value was calculated according to the manufacture's instruction. The absorbance for the cells untreated with PTX-containing sample was used a control. Data are presented as average ± SD (n=3).

Cell apoptosis assay

Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) apoptosis

detection kit (BD Biosciences, OR, USA) was used to determine apoptotic rate of MCF-7 cells.MCF-7 cells were seeded in 6-well plates at 4×10^4 cells/well and incubated for 24 h. The medium was separately replaced by 1 mL complete medium containing free PTX or PTX LPNPs at 3 μg/mL of PTX. After additional 24 h incubation, the cells were harvested and washed twice with PBS. Thereafter, the cells were centrifuged and resuspended in 300 μL of 1×binding buffer in the kit. 5 μL of annexin V-FITC and 10 μL of PI were added to each cell suspension. The cell suspension was incubated at room temperature for 15min in dark, followed by adding 200 μL of 1× binding buffer in the kit. The fluorescence intensity of cells was measured using a flow cytometer (CyAN-ADP, Beckman). The cells untreated with PTX-containing sample were used as a control.

Cell OCR analysis

O_2 consumption rate (OCR) was investigated in real time using XF24 extracellular flux analyzer instrument (Seahorse Bioscience, USA) and measuring kits were provided by Seahorse Bioscience. MCF-7 cells were seeded on XF24-well cell plates at 5×10^4 cells/well in 150 μL DMEM medium and then incubated for 24 h at 37 °C in a humidified incubator containing 5% CO_2. After 24 h, cells were treated with 100 μL medium containing free PTX or PTX-LPNPs at 3 μg/mL of PTX for 15 h at 37 °C in 5% CO_2 atmosphere. Then, all but 50 μL of the culture medium was removed from each well and cells were washed two times with 1 mL of XF stress test medium(provided in the kit) pre-warmed to 37 °C.Finally 450 μL of test medium was added to each well and the plate was placed at 37 °C without CO_2 for 1 h prior to assay. XF sensor catridges

(provided in the kit)used in the processshould be hydrated prior to assay. 1 mL of Seahorse calibrant (provided in the kit) was added into the each wellof an XF utility plate (provided in the kit), on the top of which XF sensor cartridges were placed, and then catridges together with XF utility plate were kept at 37 °C incubator without CO_2 for 24 h. According to the manufacturer's instructions, OCR was measured in basal condition or after addition of Electron transport inhibitors oligomycin (1.0 μM), FCCP (0.25μM), a mixture of antimycin-A (0.5 μM) and rotenone (0.5 μM) sequentially. Test samples on each well had three replicates.

HPLC measurement of PTX content in whole cells and the mitochondria of MCF-7 cells

Cells were seeded at a density of 5 x 10^4 cells per well in 6-well plates and cultured at 37 ℃ for 24 h. Then the medium was replaced by 1 mL complete medium containing free PTX or PTX-LPNPs at 3 μg/mL of PTX. After 24 h incubation under 5% CO_2 at 37 °C, cells were harvested and washed twice with PBS. Cells were resuspendedin 1mL PBS and divided into two parts; one was lyased and analyzed using BCA Protein Assay Kit (purchased from BD biosciences), the other one was used for mitochondrial isolation.To obtain mitochondria of cancer cells, mitochondria isolation kit (purchased from Beyotime) was used. Cells were harvested and washed with cold PBS, followed by resuspended in 2 mL mitochondria isolated solution (provided in the kit) and then kept in the ice bath for 15 min. After that, cells solution were transferred into the homogenizer and homogenized 10 times, followed by centrifuged at 600 rpm for 10 min at 4 °C. The supernate was transfered into another tube and centrifuged at 11,000

rpm for 10 min at 4 °C. The precipitate was harvested and mitochondria of cancer cells were obtained. In order to measure PTX content in the mitochondria, the precipitate was resuspended in 200 μL mitochondrial lysate (provided in kit) and kept in the ice bath for 15 min. The mitonchondria-lysated solution was divided into two parts. 100μL of solution was used to mearsure the amount of total protein using BCA Protein Assay Kit. Another 100μL of solution was added into 400 μL chloroform. The mixture was whirled violently for 5 min and centrifuged at 12,000 rpm for 15 min. The organic phase was separated to dryness at 60 °C. The residue was redissolved in 100 μL acetonitrile and whirled violently for 5 min. After centrifugation at 12,000 rpm for 5 min, 10 μL of supernatant was analyzed by HPLC. The mass of PTX was calculated from a standard curve that was linear over the concentration of PTX from 0.01 μg/mL to 5 μg/mL.

In vivo therapeutic study

Four weeks old female BALB/c nude mice were purchased from Beijing Hua Fu Kang Biological Technology Co, Ltd of China. All animals received care in compliance with the guidelines outlined in the Guide for the Care and Use of Laboratory Animals and the procedures were approved by the Wuhan University of China Animal Care and Use Committee. To develop the tumor xenograft model, MCF-7 cells (5×10^6 cells) suspended in 200 μL PBS were subcutaneously injected into the right back of the mice. A caliper was used to measure the length (L) and width (W) of tumor. Tumor volume (mm^3) was calculated as $V=L\times W^2/2$. Once the tumor volumes reached around 90 mm^3 in the12^{th} day post-tumor inoculation, the mice were randomly divided into seven groups (n=5 per group). The mice bearing MCF-7 xenograft tumors were separately

treated with 200 μL of free PTX, PTX-PLGA/CPT, PTX-PLGA/DCCP, PTX-PLGA/DSSP, PTX-PLGA/CPT/DCCP and PTX-PLGA/CPT/DSSP *via* intravenous injection of an equivalent dose of 7.5 mg PTX per kg of mouse body weight.The treatment with 200 μL of PBS was used as a control. The tumor volumes and the body weights of mice were monitored each two days. The tumor inhibitory rate (TIR) was calculated as $TIR=[1-(V_{tf}-V_{ti})/(V_{pf}-V_{pi})]\times100\%$. V_{tf} and V_{ti} represent the final and initial tumor volume of the treatment group, while the V_{pf} and V_{pi} represent the final and initial tumor volume of the PBS group, respectively.

The mice were sacrificed on the 18^{th} day post-drug injection. The tumors were removed and washed with PBS, followed by fixed in 4% formaldehyde for histological and immunohistochemical analysis.

Biodistribution of PTX

Twenty-four mice bearing MCF-7 tumor (90 mm^3) were randomly divided into six groups (*n*=4 per group), and separately treated with 200 μL of free PTX, PTX-PLGA/CPT, PTX-PLGA/DCCP, PTX-PLGA/DSSP, PTX-PLGA/CPT/DCCP and PTX-PLGA/CPT/DSSP *via* intravenous injection of an equivalent dose of 7.5 mg PTX per kg of mouse body weight. After 24 h, the mice were sacrificed. The main tissues, including heart, liver, spleen, lung, kidney and tumors, were collected, washed with cold PBS, dried on filter paper, weighted and cut into small pieces. The samples were individually ground to a homogenate in $KH_2PO_4$with a tissue pulverizer (IKA, T 25 digital S 25, Germany) for subsequent operation. 100 μL of the whole tissue homogenate was added into 400 μL chloroform. The mixture was whirled violently for

5 min and centrifuged at 12,000 rpm for 15 min. The organic phase was separated to dryness at 60 °C. The residue was redissolved in 100 μL acetonitrile and whirled violently for 5 min. After centrifugation at 12,000 rpm for 5 min, 10 μL of supernatant was analyzed by HPLC. The percentage of PTX in the different tissue was calculated by following formula: % PTX/g organ = 100 * $Q_{PTX\ in\ tissue}$ /(Q_{Organ} * $Q_{PTX\ injected}$), where $Q_{PTX\ in\ tissue}$ and Q_{Organ} were the total amount of PTX in the tissue and weight of the tissue, respectively.

Measurement of PTX content in whole cancer cells and the mitochondria of cancer cells after *i. v.* injection

Eighteen mice bearing MCF-7 tumor (100 mm^3) were randomly divided into six groups (n=3 per group), and separately treated with 200 μL of free PTX, PTX-PLGA/CPT, PTX-PLGA/DCCP, PTX-PLGA/DSSP, PTX-PLGA/CPT/DCCP and PTX-PLGA/CPT/DSSP *via* intravenous injection of an equivalent dose of 7.5 mg PTX per kg of mouse body weight. After 24 h, the mice were sacrificed, meanwhile, tumors were collected, washed with hank's solution two times. Following, tumors were cut into small fragment (1-2 mm^3) and ground to 5mL DF12 medium containing type I and type IV collagenases (2 mg/mL, Sigma, St. Louis, MO, USA) to collagen digested for 2 h at 37°C. During digestion times, the mixture was dispersed by pipetting each 30 min. After 2 h digestion, the mixture was filtered by filtration sieve cells (40 mesh) and cells were harvested for subsequent operation.Cancer cells were isolated by differential adhesion method[1]. Cells medium were transferred into culture bottle (marked as bottle A) and 10 mL fresh DMEM was added into bottle A. After cultured for 20 min at 37°C,

bottle A was swayed gently, and the medium was then transferred into culture bottle B. The process was performed three times repeatedly, following medium were harvested and centrifuged at 1000 rpm for 10 min to obtain cancer cells. The cancer cells were divided into two parts. One part of cells were used to measure the PTX content in whole cells. Another part of cells were used to measure the PTX content in the mitochondria of cancer cells. The separation and analysis methods were same as described above. PTX content was measured by HPLC analysis.

Statistical analysis

Statistical analyses were performed using Student's *t*-test. $P<0.05$ was considered significant. Data were expressed as mean ± SD.

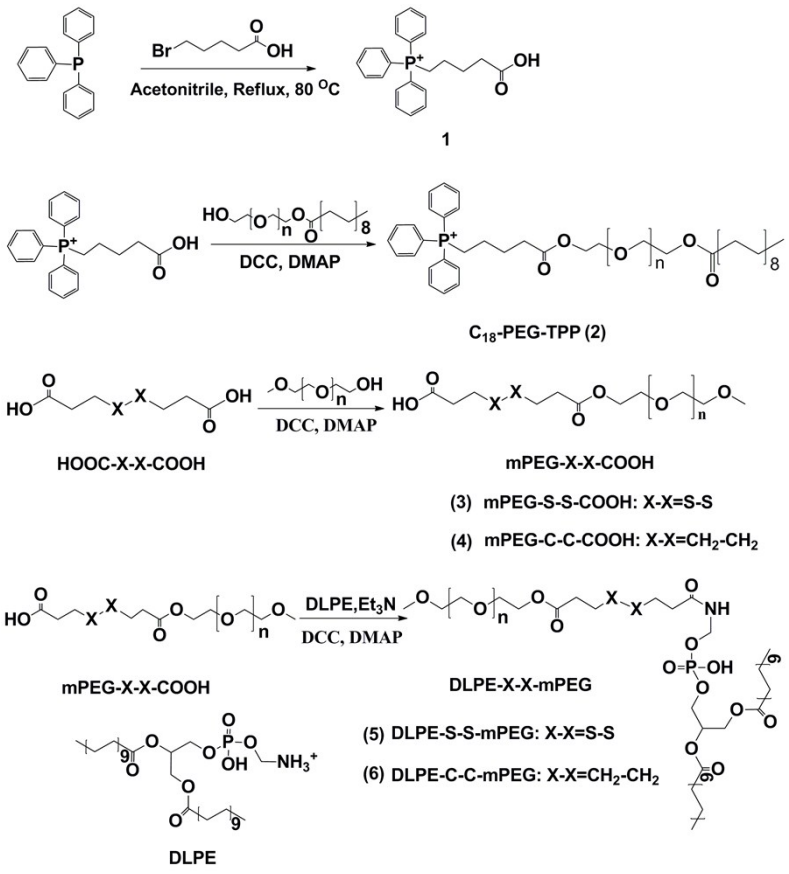


Figure S1. Syntheses of C_{18}-PEG_{2000}-TPP (CPT, **2**), DLPE-S-S-$mPEG_{4000}$ (DSSP, **5**)

and DLPE-C-C-mPEG$_{4000}$ (DCCP, **6**).

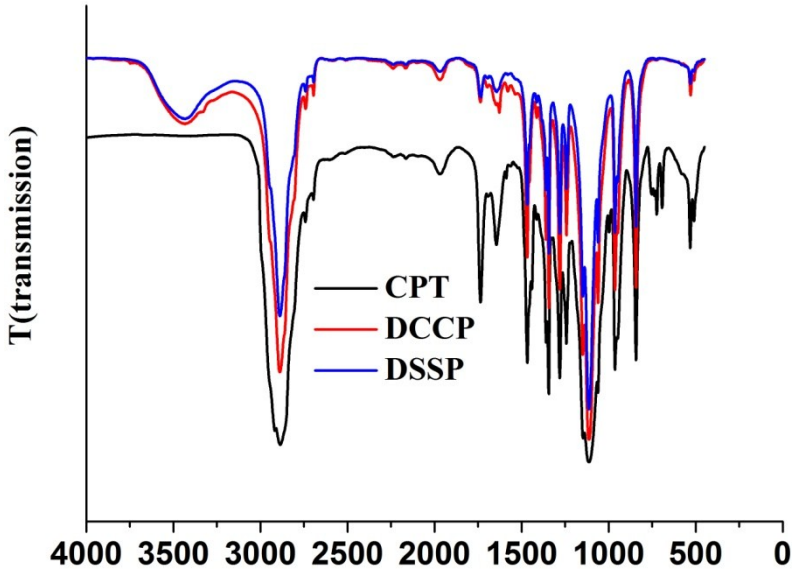


Figure S2. FT-IR spectra of C_{18}-PEG$_{2000}$-TPP (CPT, **2**), DLPE-S-S-mPEG$_{4000}$ (DSSP,**5**) and DLPE-C-C-mPEG$_{4000}$ (DCCP, **6**).

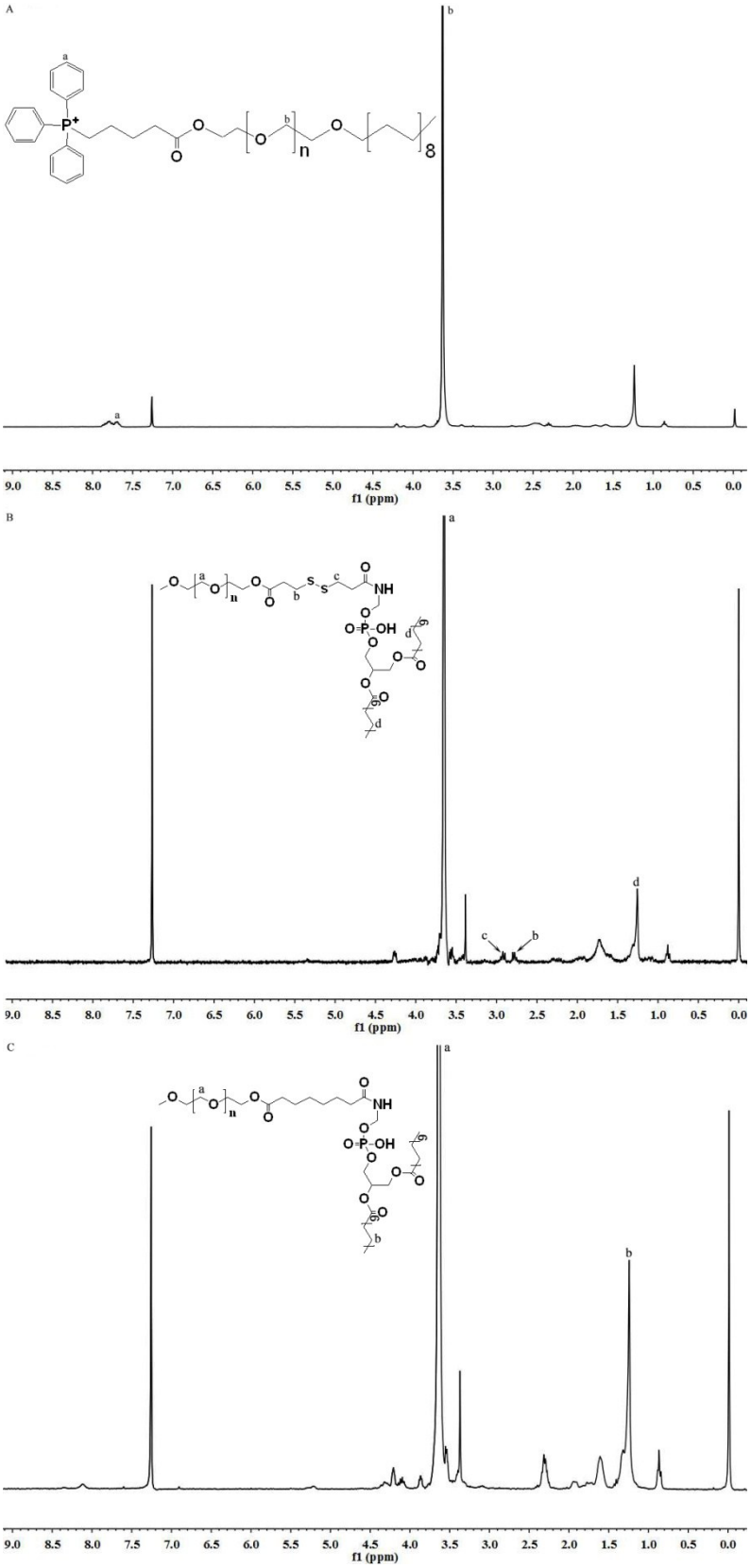


Figure S3. ^{1}H NMR spectra of C_{18}-PEG_{2000}-TPP (CPT, **2**), DLPE-S-S-$mPEG_{4000}$ (DSSP, **5**) and DLPE-C-C-$mPEG_{4000}$ (DCCP, **6**).

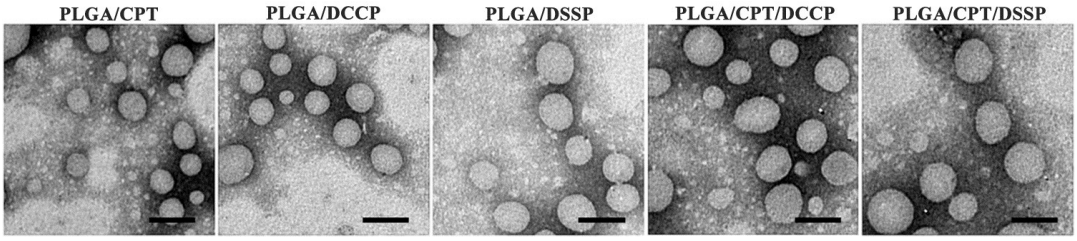


Figure S4. Transmission electron microscope (TEM) images of LPNPs.

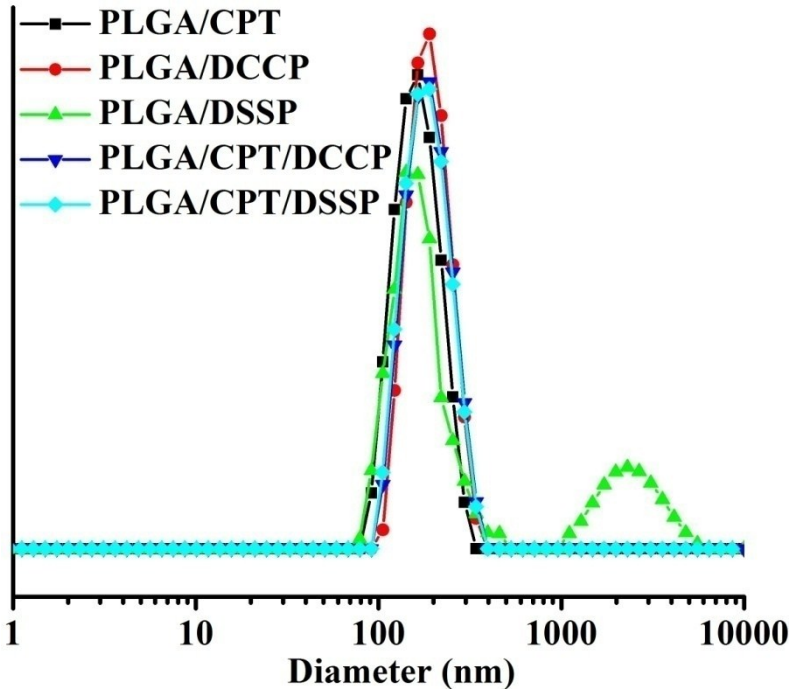


Figure S5. The size and distribution of LPNPs treated with 10 mM GSH in PBS measured by Dynamic light scattering (DLS).

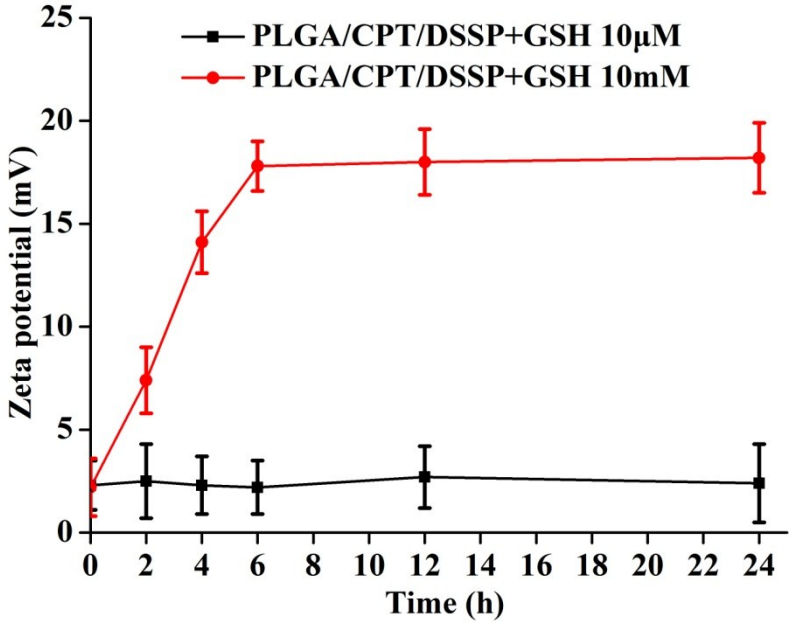


Figure S6. Zeta potential variation of PLGA/CPT/DSSP treated with 10 μM or10 mM GSH in PBS.

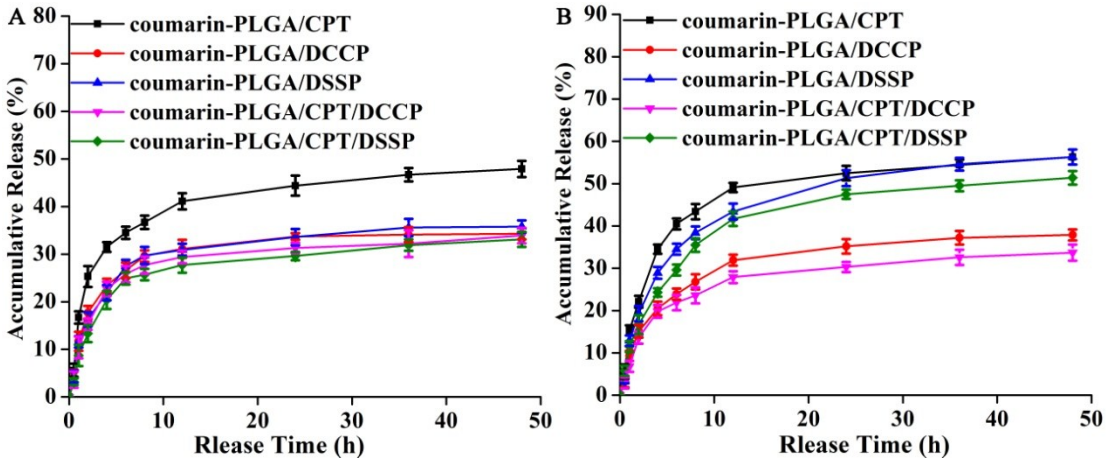
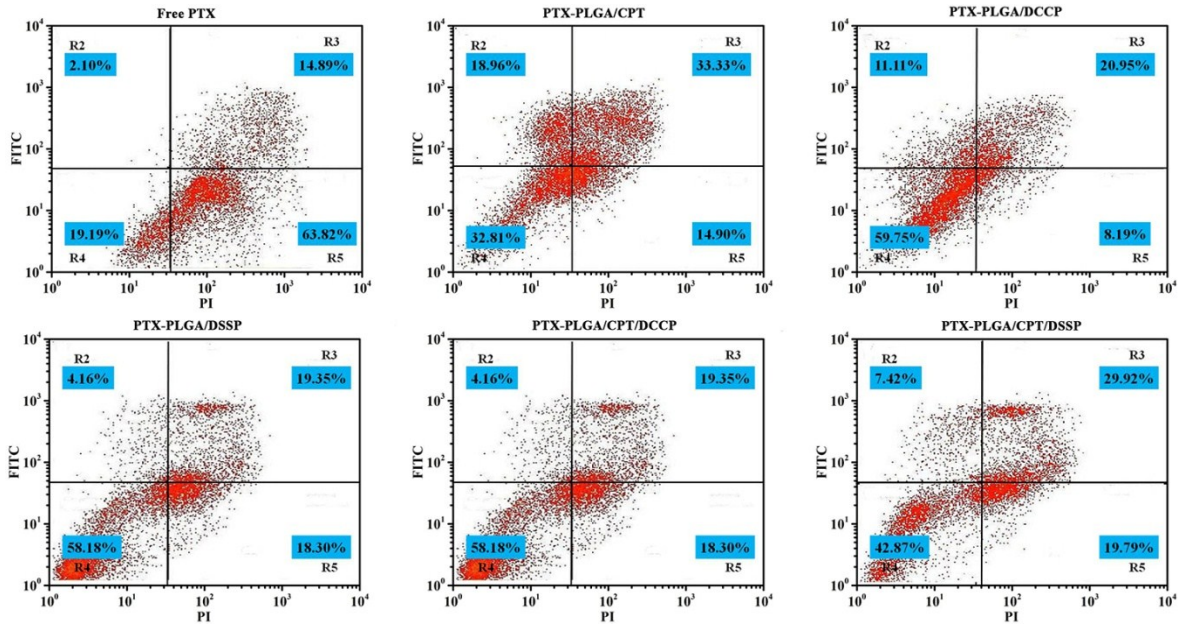


Fig S7. Coumarin release from LPNPs in PBS buffer (A) and PBS buffer containing 10 mM GSH (B). Data is presented as mean ± SD (n=3).



Figuer S8. Typical flow cytometry data of MCF-7 cells after incubation with different PTX formulations at a concentration of 3 μg/mL PTX for 24 h.

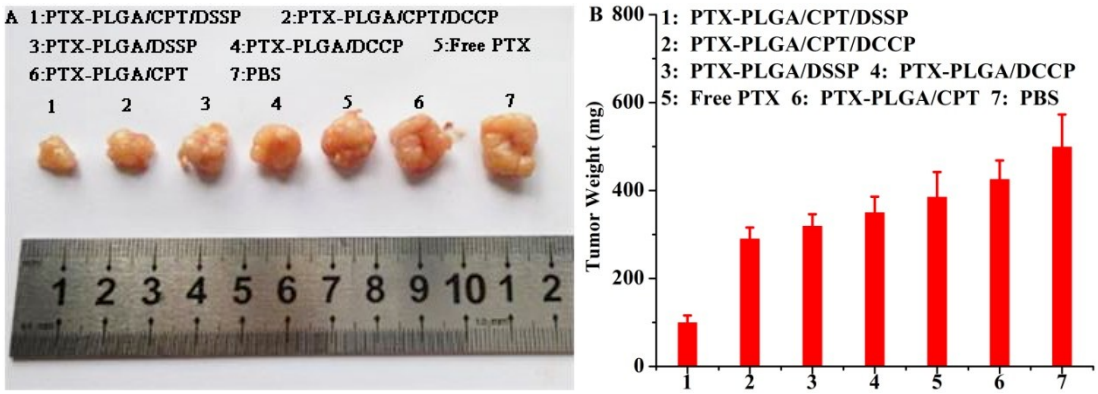


Fig S9. (A) Representative photos of excised tumors 18 d after treatments. (B) Weight of excised tumors 18 d after treatments.

Table S1. Molecular weights of CPT, DCCP and DSSP

Sample name	Mn(10^3)	Mw(10^3)	Polydispersity (Mw/Mn)
CPT	3.19	3.74	1.185
DCCP	6.70	6.96	1.028
DSSP	8.11	8.39	1.032

Table S2. Compositions of LPNPs

Sample	PLGA (mg)	CPT (mg)	CPO (mg)	DCCP (mg)	DSSP (mg)
PLGA/CPT	50	3	2	/	/
PLGA/DCCP	50	/	/	5	/
PLGA/DSSP	50	/	/	/	5
PLGA/CPT/DCCP	50	3	/	2	/
PLGA/CPT/DSSP	50	3	/	/	2

Table S3. Sizes and zeta potentials of LPNPs in PBS

Sample	LPNPs in PBS		
	Size[a]	PDI[a]	Zeta Potential[b]
	nm		mv
PLGA/CPT	149.9±2.2	0.157±0.003	21.8±1.2
PLGA/DCCP	156.2±2.1	0.229±0.005	-2.5±0.6
PLGA/DSSP	153.4±1.3	0.203±0.002	-2.7±0.3
PLGA/CPT/DCCP	168.4±1.5	0.171±0.006	2.7±0.4
PLGA/CPT/DSSP	178.6±1.2	0.153±0.003	2.4±0.8

a The average sizes were measured by DLS.

b Zeta Potentials were measured by ZETA-SIZER Nano Series.

Table S4. Sizes and zeta potentials of PTX-LPNPs in PBS and DMEM cell culture medium containing 10% FBS

Sample	PTX-LPNPsin PBS			PTX-LPNPs in DMEM			
	Size[a]	PDI[a]	Zeta potential[b]	Size[a]	PDI[a]	DLC[c]	Zeta potential[b]
	nm		mv	nm		%	mv
PLGA/CPT	164.5±3.6	0.143±0.002	21.9±0.4	248.3±5.7	0.323±0.003	7.38±0.04	7.6±1.5
PLGA/DCCP	168.8±4.1	0.155±0.003	-2.0±0.3	176.7±3.3	0.147±0.003	7.47±0.07	-3.5±0.8
PLGA/DSSP	177.8±1.8	0.143±0.002	-3.2±0.3	188.1±2.7	0.149±0.001	7.55±0.06	-3.9±0.7
PLGA/CPT/DCCP	184.4±2.2	0.166±0.006	1.9±0.6	192.9±2.6	0.171±0.004	8.34±0.02	1.5±0.9
PLGA/CPT/DSSP	193.2±2.4	0.183±0.004	1.1±0.5	198.2±2.2	0.197±0.002	8.29±0.06	0.7±0.4

a The average sizes were measured by DLS.

b Zeta potentials were measured by ZETA-SIZER Nano Series.

c Feed ratio of polymer to PTX at 10:1(w/w).

Table S5. Sizes and zeta potentials of coumarin-LPNPs in PBS

Sample	coumarin-LPNPs in PBS			
	Size[a]	PDI[a]	DLC[c]	Zeta potential[b]
	nm		%	mv
PLGA/CPT	173.7±2.8	0.126±0.004	8.01±0.03	22.1±0.3

PLGA/DCCP	168.4±2.4	0.150±0.003	8.12±0.05	-2.6±0.2
PLGA/DSSP	181.4±3.2	0.138±0.008	8.11±0.03	-3.5±0.3
PLGA/CPT/DCCP	184.4±2.2	0.147±0.005	8.17±0.04	1.7±0.4
PLGA/CPT/DSSP	186.8±2.6	0.172±0.004	8.20±0.02	1.4±0.2

a The average sizes were measured by DLS.

b Zeta potentials were measured by ZETA-SIZER Nano Series.

c Feed ratio of polymer to coumarin at 10:1(w/w).

Table S6. IC_{50} of free PTX and PTX-LPNPs against MCF-7 and COS 7 cells

Sample	MCF-7	COS 7
	IC_{50}	IC_{50}
	(μg/mL)	(μg/mL)
Free PTX	0.78 ± 0.08	1.34 ± 0.08
PTX-PLGA/CPT	1.65 ± 0.04	5.67 ± 016
PTX-PLGA/DCCP	5.22 ± 0.13	10.07 ± 0.20
PTX-PLGA/DSSP	2.74 ± 0.10	10.03 ± 0.18
PTX-PLGA/CPT/DCCP	4.62 ± 0.13	7.76 ± 0.16
PTX-PLGA/CPT/DSSP	1.79 ± 0.07	7.81 ± 0.22

1. Biernaskie, J. A.; McKenzie, I. A.; Toma, J. G.; Miller, F. D. *Nature Protocols* **2006,** 1, 2803-2812.