Supplementary Materials

Supramolecular Nanoparticles Self-Assembled from Reduction-Responsive Cabazitaxel Prodrugs for Effective Cancer Therapy

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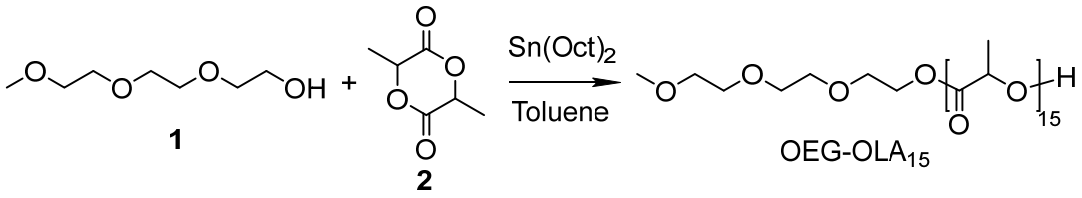
Materials and instruments

Cabazitaxel (CTX) was purchased from Knowshine Pharmachemicals Inc. (Shanghai, China). All reagents were obtained from commercial suppliers (Sigma-Aldrich, Tokyo Chemical Industry (TCI), or J&K Chemical) and used without further purification.

Analytical reverse phase high-performance liquid chromatography (RP-HPLC) was carried out on a Hitachi Chromaster 5000 system equipped with a YMC-Pack C_8 ODS reverse-phase column (5 μm, 250 × 4.6 mm, YMC Co., Ltd., Kyoto, Japan) at a flow rate of 1.0 mL/min. A gradient of 30%-100% acetonitrile in water containing 0.1% TFA within 20 min was employed as the mobile phase. UV detection was performed at 220 nm. TEM images of **OEG-OLA$_n$-CTX SNPs** were obtained using TECNAL 10 (Philips) at an acceleration voltage of 80 kV. The hydrodynamic diameters (D_H) were analyzed on a Malvern Nano-ZS90 instrument (Malvern Instruments, Malvern, UK) at 25°C.

Methods

Synthesis of OEG-OLA$_{15}$



OEG-OLA$_{15}$ was synthesized by ring-opening polymerization. Briefly, $_{D,L}$-lactide (60.3 mmol, 8.68 g) and trirthylene glycol monomethyl ether (8.04 mmol, 1.32 g) dissolved in 50 mL toluene were added into a round-bottom flask, followed by an addition of stannous octoate ($Sn(Oct)_2$). After stirring for 12 h at 140°C, the solvent was removed by evaporation under vacuum. The residue was dissolved in dichloromethane (DCM) and then precipitated in excess cold diethyl ether and filtered. The molecular weight of pLA$_{15}$ was determined by ^{1}H NMR.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.26-5.13 (m, 15H), 4.38-4.25 (m, 2H), 3.70-3.68 (m, 2H), 3.64-3.63 (m, 6H), 3.56-3.54 (m, 2H), 3.38 (s, 3H), 1.61-1.52 (m, 45H).

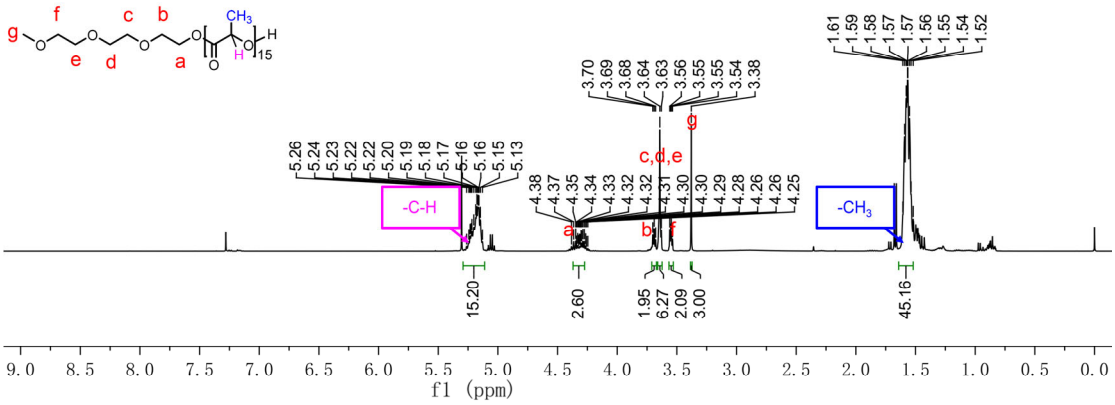
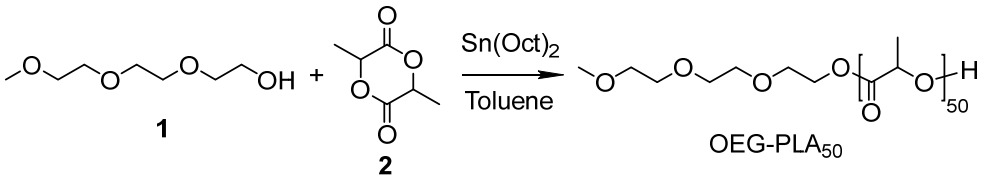


Figure S1. ^{1}H NMR spectrum of OEG-OLA$_{15}$ measured in $CDCl_3$.

Synthesis of OEG-OLA$_{50}$



OEG-OLA$_{50}$ was also synthesized by ring-opening polymerization. Briefly, $_{D,L}$-lactide (66.39 mmol, 9.56 g) and trirthylene glycol monomethyl ether (2.66 mmol, 0.44 g) dissolved in 50 mL toluene were added into a round-bottom flask, followed by an addition of stannous octoate ($Sn(Oct)_2$). After stirring for 12 h at 140°C, the solvent was removed by evaporation under vacuum. The residue was dissolved in DCM and then precipitated in excess cold diethyl ether and filtered. The molecular weight of pLA$_{50}$ was determined by ^{1}H NMR.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.25-5.13 (m, 50H), 4.38-4.24 (m, 2H), 3.70-3.68 (m, 2H), 3.65-3.63 (m, 6H), 3.56-3.53 (m, 2H), 3.38 (s, 3H), 1.59-1.55 (m, 150H).

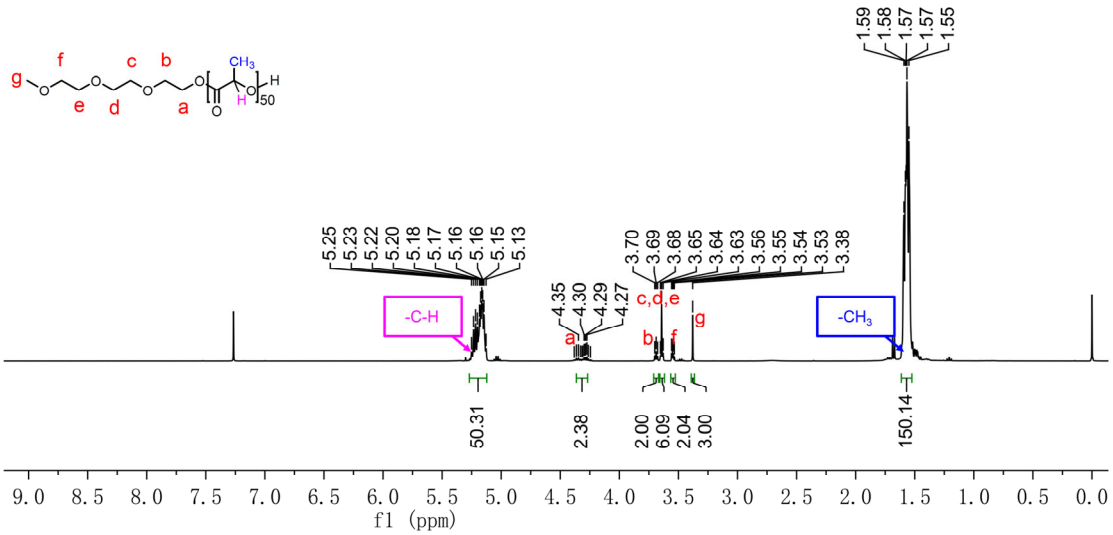
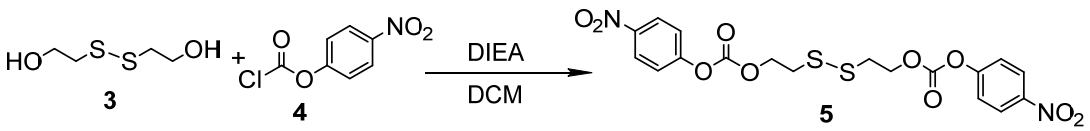


Figure S2. ^{1}H NMR spectrum of OEG-OLA$_{50}$ measured in $CDCl_3$.

Synthesis of carbonate 5



Carbonate **5** was synthesized *via* a base-catalyzed alcoholysis reaction of *p*-nitrophenyl chloroformate and bis(2-hydroxyethyl) disulfide. *p*-Nitrophenyl chloroformate (15.38 mmol, 3.10 g) and bis(2-hydroxyethyl) disulfide (7.00 mmol, 1.08 g) dissolved in 6 mL DCM were added into a round-bottom flask, followed by an addition of DIEA (2.10 mmol, 0.27 g). After stirring for 4 h at 45°C, the solvent was removed by evaporation under vacuum and then was washed by 5% citric acid, saturated $NaHCO_3$, and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated under vacuum. The crude residue was purified by flash column chromatography on silica gel (hexane/ethyl acetate=2:1) to afford the conjugate **1** (light yellow solid, 1.3 g, 38.4%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.29-8.27 (d, 4H, *J* =8), 7.40-7.38 (d, 4H, *J* =8), 4.59-4.56 (t, 4H, *J* =6), 3.10-3.07 (t, 4H, *J* = 6).

^{13}C NMR (100 MHz, $CDCl_3$): δ 155.3, 152.3, 145.5, 125.4, 121.8, 66.8, 36.7.

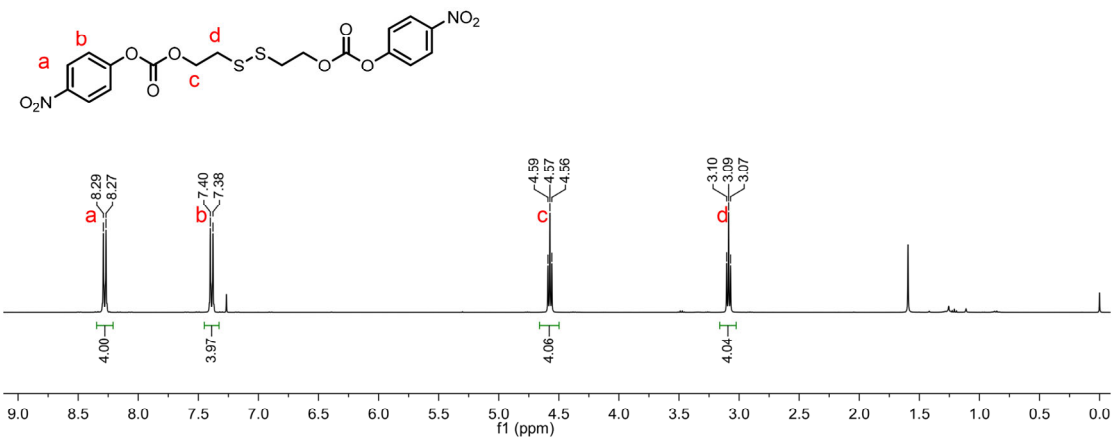


Figure S3. ^{1}H NMR spectrum of carbonate **5** measured in $CDCl_3$.

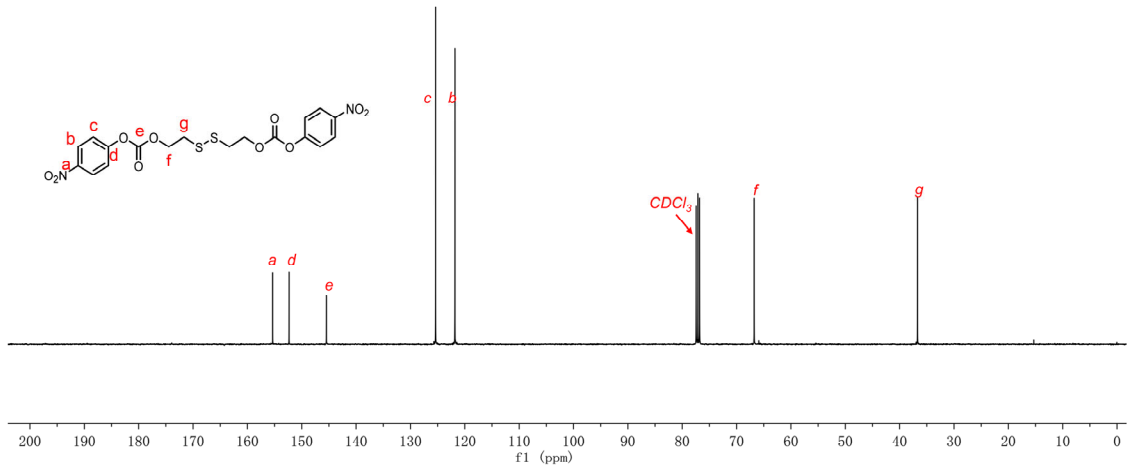
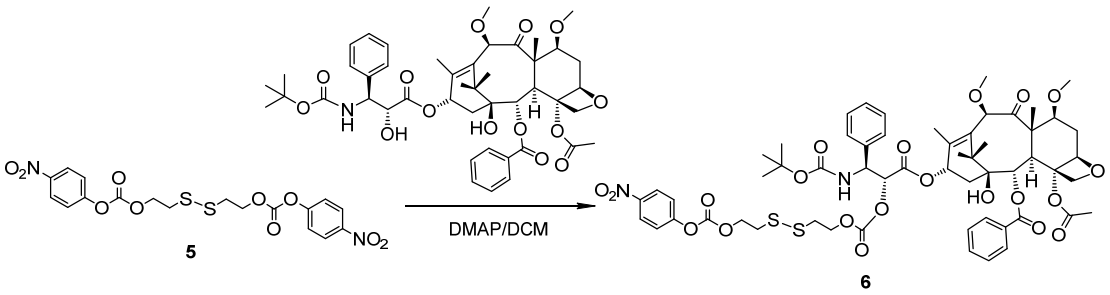


Figure S4. ^{13}C NMR spectrum of carbonate **5** measured in $CDCl_3$.

Synthesis of intermediate compound 6



The carbonate **5** (0.51 mmol, 243.95 mg) and 4-dimethylaminopyridine (DMAP, 0.53 mmol, 64.5 mg) were dissolved in 4 mL of DCM. Under stirring at 45°C, a solution of cabazitaxel (0.48 mmol, 400 mg) in 2 mL of DCM was added dropwise into the mixture.

After stirring for 30 min, the residue was immediately purified by flash column chromatography on silica gel (hexane/ethyl acetate=1:1) to afford intermediate compound **6** (white powder, 202.3 mg, 35.7%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.30-8.28 (m, 2H), 8.12-8.10 (d, 2H, *J* = 8), 7.63-7.59 (m, 1H), 7.52-7.48 (m 2H), 7.43-7.38 (m, 4H), 7.35-7.31 (m, 3H), 6.31-6.27 (br, 1H), 5.66-5.65 (d, 1H, *J* = 4), 5.52-5.46 (m, 2H), 5.27 (s, 1H), 5.01-4.99 (d, 1H, *J* = 8), 4.83 (s, 1H), 4.57-4.47 (m, 2H), 4.44-4.31 (m, 3H), 4.19-4.16 (m, 1H), 3.92-3.84 (m, 2H), 3.44 (s, 3H), 3.30 (s, 3H), 3.03-2.91 (m, 4H), 2.75-2.67 (m, 1H), 2.44 (s, 3H), 2.33-2.20 (m, 2H), 2.01 (s, 3H), 1.82-1.77 (m, 1H), 1.72 (s, 3H), 1.34 (s, 9H), 1.25 (s, 1H), 1.22-1.21 (m, 6H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 205.0, 169.8, 168.1, 167.0, 155.4, 155.2, 154.0, 152.3, 145.5, 139.4, 137.0, 135.1, 133.6, 130.2, 129.2, 129.2, 129.0, 128.7, 128.7, 128.3, 128.3, 126.5, 126.5, 125.4, 125.4, 121.8, 121.8, 84.2, 82.5, 81.6, 80.7, 80.5, 78.8, 76.4, 74.7, 74.7, 72.4, 66.8, 66.6, 57.2, 57.1, 56.8, 47.3, 43.4, 36.7, 36.7, 35.0, 32.0, 29.7, 29.7, 29.7, 28.1, 26.7, 22.8, 21.0, 14.5, 10.4.

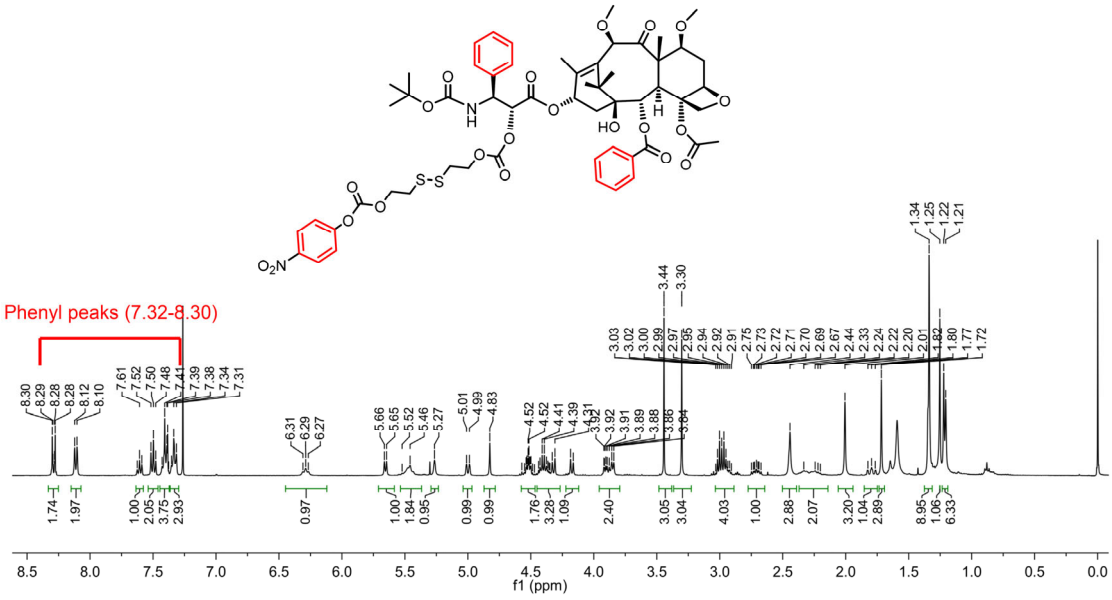


Figure S5. ^{1}H NMR spectrum of the intermediate compound **6** measured in $CDCl_3$.

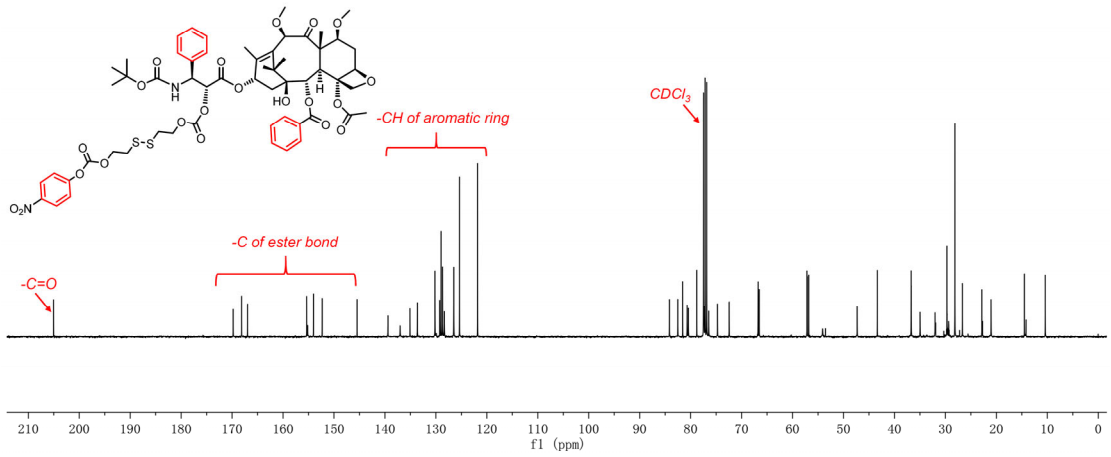
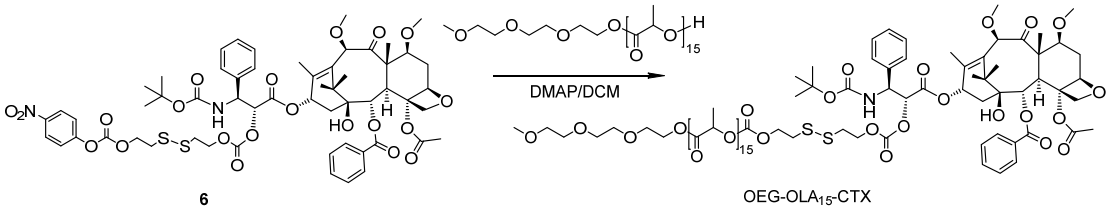


Figure S6. ^{13}C NMR spectrum of the intermediate compound **6** measured in $CDCl_3$.

Synthesis of OEG-OLA$_{15}$-CTX



Intermediate compound **6** (0.07 mmol, 80 mg), and OEG-OLA$_{15}$ (0.06 mmol, 69.85 mg) were dissolved in 2 mL of DCM and mixed with DMAP (0.07 mmol, 8.55 mg). The reaction mixture was stirred at 45°C for 4 h. Following washing with 5% citric acid, saturated $NaHCO_3$, and brine, the organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated under vacuum. The residue was purified by flash column chromatography on silica gel (hexane/ethyl acetate=1:2) to afford the OEG-OLA$_{15}$-CTX conjugate (white powder, 65.3 mg, 49.0%).

^{1}H NMR (400 MHz, $CDCl_3$): δ8.13-8.11 (d, 2H, *J* = 8.0), 7.63-7.59 (m, 1H), 7.52-7.48 (m, 2H), 7.44-7.40 (m, 2H), 7.36-7.32 (m, 3H), 6.31-6.26 (br, 1H), 5.67-5.65 (d, 1H, *J* = 8.0), 5.48 (s, 1H), 5.26-5.17 (m, 15H), 5.13 (s, 1H), 5.02-4.99 (d, 1H, *J* = 12.0), 4.83 (s, 1H), 4.38-4.37 (m, 3H), 4.33-4.25 (m, 3H), 4.19-4.16 (m, 1H), 3.92-3.88 (m, 1H), 3.86-3.85 (m, 1H), 3.70-3.68 (t, 2H, *J* = 4.0), 3.64-3.63 (m, 6H), 3.56-3.54 (m, 2H), 3.45 (s, 3H), 3.38 (s, 3H), 3.31 (s, 3H), 2.92 (s, 3H), 2.75-2.68 (m, 1H), 2.46 (s, 3H), 2.34-2.22 (m, 2H), 2.01 (s, 3H),

1.82-1.76 (m, 1H), 1.72 (s, 3H), 1.60-1.52 (m, 45H), 1.35 (s, 9H), 1.25 (s, 1H), 1.22-1.21 (m, 6H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 205.0, 170.1-169.1, 168.1, 167.0, 155.2, 154.2, 154.0, 139.4, 137.1, 135.1, 133.6, 130.2, 129.3, 129.3, 129.0, 128.7, 128.7, 128.3, 128.3, 126.5, 126.5, 84.2, 82.5, 81.6, 80.7, 80.4, 78.9, 76.5, 74.8, 74.8, 72.4, 71.9, 71.5, 70.6, 70.6, 69.2-69.0, 68.8, 66.6, 66.0, 64.4, 59.0, 57.2, 57.1, 56.8, 47.4, 43.4, 36.8, 36.8, 36.7, 35.0, 32.1, 29.7, 29.7, 29.7, 28.1, 26.7, 22.8, 21.0, 16.8-16.7, 14.5, 10.4.

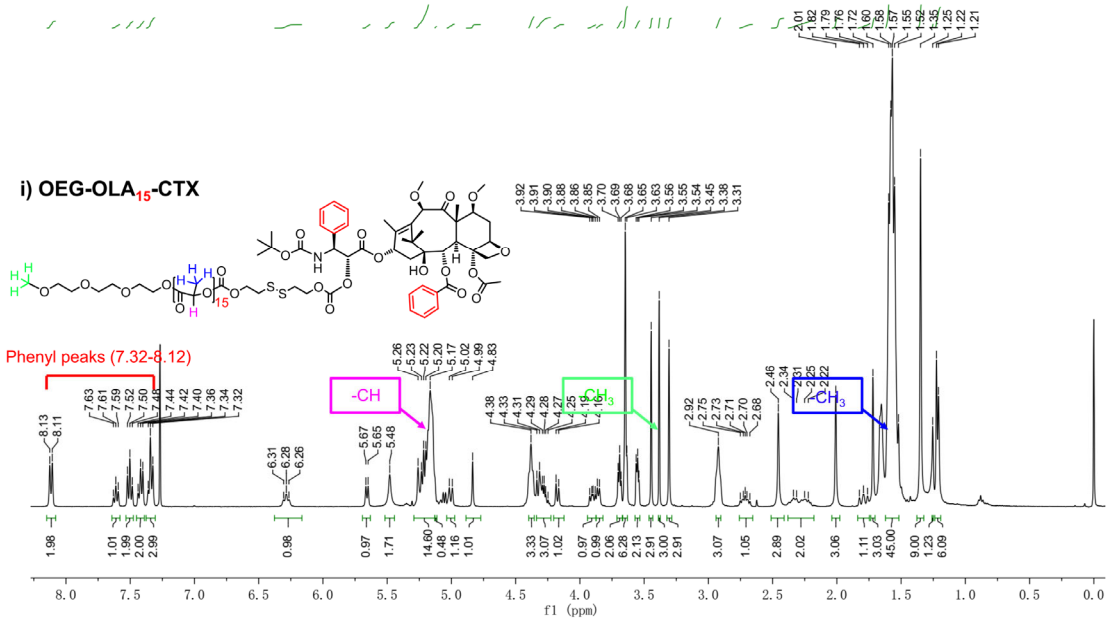


Figure S7. ^{1}H NMR spectrum of the OEG-OLA$_{15}$-CTX conjugate measured in $CDCl_3$.

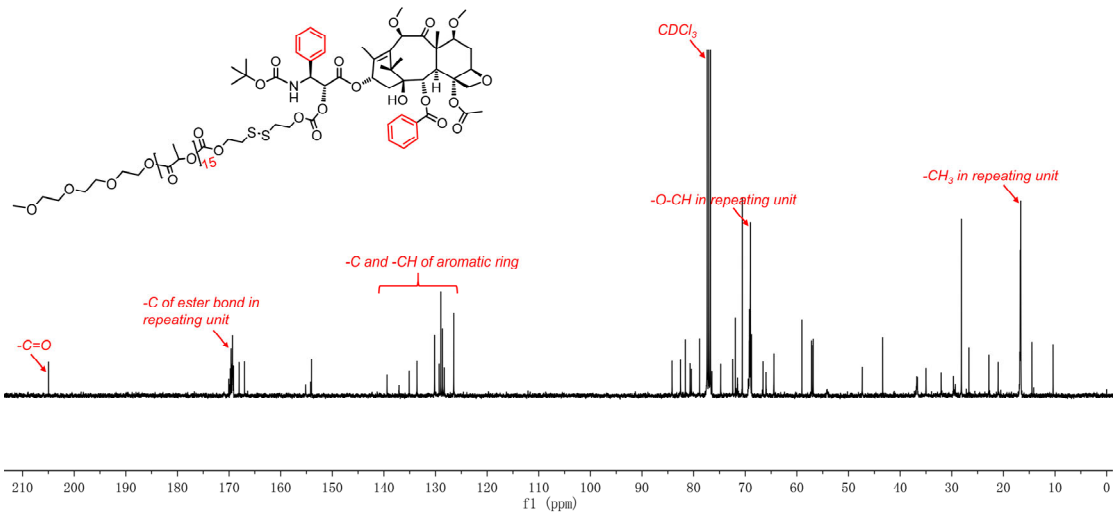
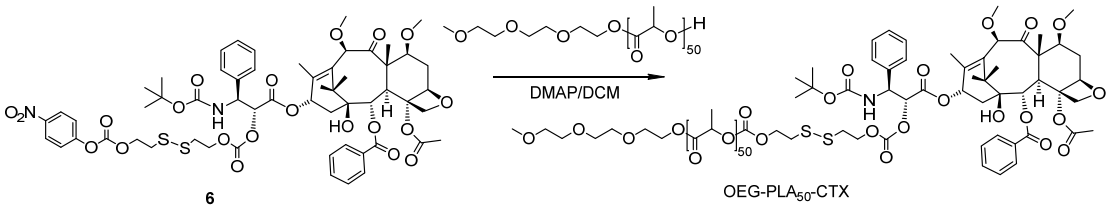


Figure S8. ^{13}C NMR spectrum of the OEG-OLA$_{15}$-CTX conjugate measured in $CDCl_3$.

Synthesis of OEG-OLA$_{50}$-CTX



Intermediate compound **6** (0.08 mmol, 96 mg), and OEG-OLA$_{50}$ (0.07 mmol, 254.91 mg) were dissolved in 4 mL of DCM and mixed with DMAP (0.08 mmol, 9.77 mg). The reaction mixture was stirred at 45°C for 4 h. Following washing with 5% citric acid, saturated $NaHCO_3$, and brine, the organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated under vacuum. The residue was purified by flash column chromatography on silica gel (hexane/ethyl acetate=1:2) to afford OEG-OLA$_{50}$-CTX compound (white powder, 182.1 mg, 53.9%).

^{1}H NMR (400 MHz, $CDCl_3$): δ8.12-8.10 (d, 2H, *J* = 8.0), 7.62-7.59 (m, 1H), 7.52-7.48 (m, 2H), 7.43-7.40 (m, 2H), 7.35-7.32 (m, 3H), 6.31-6.26 (br, 1H), 5.66-5.65 (d, 1H, *J* = 4.0), 5.47 (s, 1H), 5.25-5.15 (m, 50H), 5.13 (s, 1H), 5.01-4.99 (d, 1H, *J* = 8.0), 4.83 (s, 1H), 4.39-4.36 (m, 3H), 4.33-4.23 (m, 3H), 4.19-4.16 (m, 1H), 3.92-3.88 (m, 1H), 3.86-3.85 (m, 1H), 3.70-3.68 (t, 2H, *J* = 4.0), 3.64-3.63 (m, 6H), 3.56-3.54 (m, 2H), 3.44 (s, 3H), 3.38 (s, 3H), 3.30 (s, 3H), 2.94-2.92 (m, 3H), 2.75-2.67 (m, 1H), 2.45 (s, 3H), 2.36-2.20 (m, 2H), 2.01 (s, 3H), 1.82-1.76 (m, 1H), 1.72 (s, 3H), 1.59-1.53 (m, 150H), 1.35 (s, 9H), 1.25 (s, 1H), 1.22-1.21 (m, 6H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 205.0, 170.1-169.1, 168.1, 167.0, 155.2, 154.2, 154.0, 139.4, 137.0, 135.0, 133.6, 130.2, 129.3, 129.3, 129.0, 128.7, 128.7, 128.3, 128.3, 126.5, 126.5, 84.1, 82.5, 81.6, 80.7, 80.4, 78.8, 76.4, 74.7, 74.7, 72.4, 71.9, 71.5, 70.6, 70.6, 69.4-69.0, 68.8, 66.5, 66.0, 64.4, 59.0, 57.1, 57.1, 56.8, 47.3, 43.3, 36.7, 36.7, 36.6, 34.9, 32.0, 29.7, 29.7, 29.7, 28.1, 26.6, 22.8, 21.0, 16.7-16.7, 14.5, 10.4.

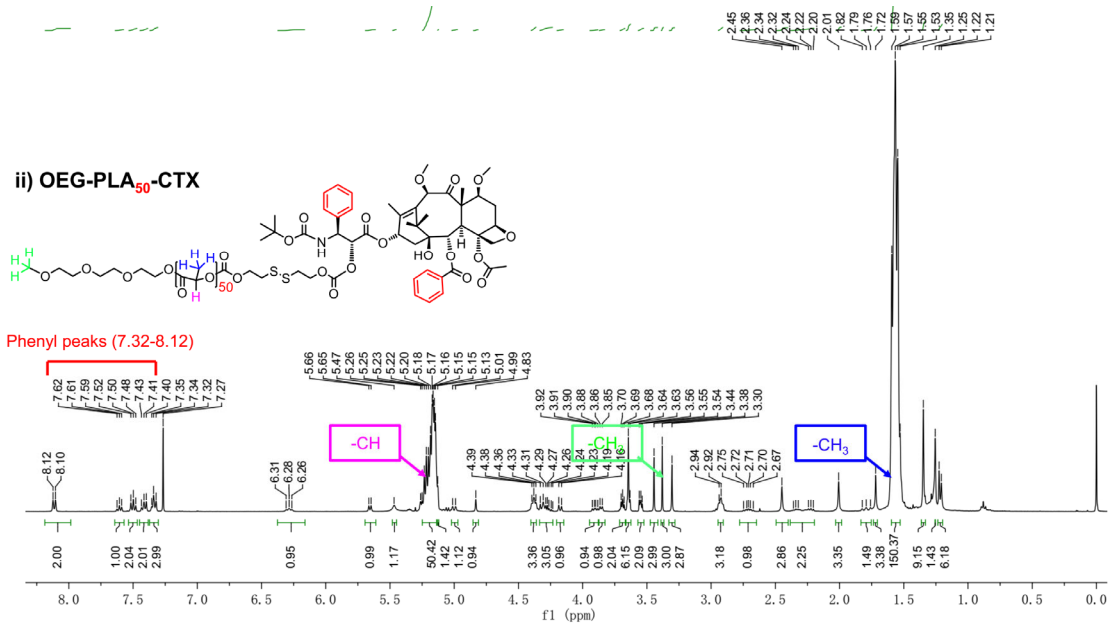


Figure S9. ^{1}H NMR spectrum of the OEG-OLA$_{50}$-CTX conjugate measured in $CDCl_3$.

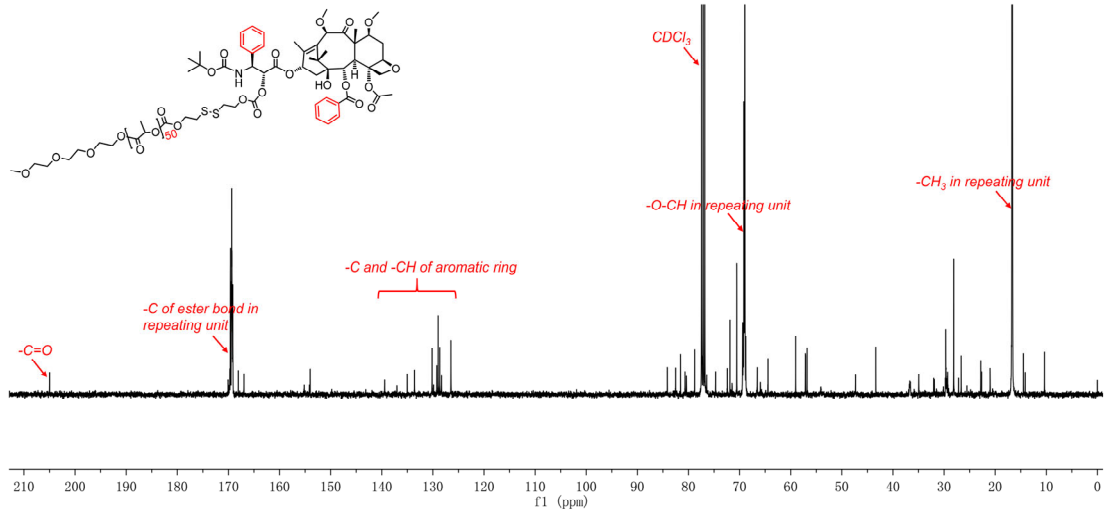


Figure S10. ^{13}C NMR spectrum of the OEG-OLA$_{50}$-CTX conjugate measured in $CDCl_3$.

Analysis of molecular weight distribution

The molecular weight distribution of the OEG-OLA$_n$-CTX conjugates (n = 15 or 50) was analyzed by gel permeation chromatography (GPC) measurement (Waters 1525/2414, USA) at 35°C.

Preparation of OEG-OLA$_n$-CTX self-assembled nanoparticles (SNPs)

OEG-OLA$_n$-CTX SNPs were prepared by a reprecipitation method. Briefly, the OEG-OLA$_n$-CTX conjugate dissolved in 100 μL of DMSO was rapidly injected into 900 μL of deionized (DI) water under ultrasound. The drug concentration of **OEG-OLA$_n$-CTX SNPs** was determined with RP-HPLC.

Characterization of OEG-OLA$_n$-CTX SNPs

Transmission electron microscopy (TEM) and Scanning electron microscopy (SEM) analysis

For TEM observation, **OEG-OLA$_n$-CTX SNPs** (at 0.3 mg/mL CTX equivalence) were dipped onto a 300-mesh copper grid. The surface solution was removed with filter paper after 2-min deposition. Then, 2 wt% aqueous uranyl acetate solution was used to positively stain samples for about 1 min. All samples were observed on a TECNAL 10 (Philips) operating at an acceleration voltage of 80 kV. For SEM observation, samples were directly observed with Nova Nano 450 without positive staining.

Dynamic light scattering (DLS)

The hydrodynamic size and distribution of **OEG-OLA$_n$-CTX SNPs** were determined with Malvern Nano-ZS90 instrument (Malvern, UK) at 25°C.

Stability of OEG-OLA$_{15}$-CTX SNPs *in vitro*

OEG-OLA$_{15}$-CTX SNPs were incubated in DI water and DI water supplemented with 10% or 50% FBS (v/v) at 37°C. At predetermined time intervals, the hydrodynamic size (D_H) and zeta potential were monitored over several days.

Redox-responsive drug release from OEG-OLA$_{15}$-CTX SNPs

Drug release profiles using **OEG-OLA$_{15}$-CTX SNPs** was investigated by a dialysis method. Briefly, **OEG-OLA$_{15}$-CTX SNPs** at a CTX-equivalent concentration of 0.1 mg/mL (3 mL) were dialyzed against PBS buffer with 10 mM DTT (20 mL, pH 7.4, 0.2% polysorbate 80). **OEG-OLA$_{15}$-CTX SNPs** dialyzed against PBS without DTT was included as a reference. The dialysis assay was performed at 37°C with shaking. At predetermined time intervals, 1 mL of sample was collected while supplementing the same volume of PBS buffer or PBS buffer with 10 mM DTT. All samples were hydrolyzed with sodium hydroxide (0.1 mol/L)

for 2 h at 37°C as previously reported.[1] The release of **OEG-OLA$_{15}$-CTX SNPs** was analyzed *via* RP-HPLC equipped with a C8 reversed-phase column. A gradient of 30% to 100% acetonitrile in water within 20 min at a flow rate of 1.0 mL/minute was applied.

GSH-responsive hydrolysis of the OEG-OLA$_{15}$-CTX conjugate

The *in vitro* hydrolysis of the OEG-OLA$_{15}$-CTX conjugate in the presence or absence of 10 mM GSH was analyzed by RP-HPLC. The conjugate in DMSO (0.9 mL) was mixed with PBS (0.1 mL, 10 mM, 7.4) and further was incubated with 10 mM GSH. The solutions without GSH was also included as a control. All solutions were incubated at 37°C with shaking. At predetermined time intervals, samples were collected and analyzed on RP-HPLC equipped with a C8 reversed-phase column at a flow rate of 1.0 mL/min. A gradient of 30% to 100% acetonitrile in water within 20 min was applied.

***In vitro* cytotoxicity evaluated by the CCK8 assay**

The cytotoxicity of **OEG-OLA$_{15}$-CTX SNPs** was determined by the CCK8 assay against melanoma cell lines and noncancerous cells, including B16-F10, A375 melanoma cells and murine macrophage cells (RAW 264.7). Cells were seeded in 96-well plates (1000-2000 cells per well) and incubated at 37°C overnight. Then, cells were exposed to serial dilutions of free CTX or **OEG-OLA$_{15}$-CTX SNPs** for another 72 h. Subsequently, the medium for each well was replaced with fresh medium containing 10% CCK8 (v/v). After 2-h incubation, the absorbance at a wavelength of 450 nm was measured with a microplate reader (Multiskan FC, Thermo Scientific). The IC_{50} values for drug formulations were extrapolated from the dose-response curves. Utilizing GraphPad Prism® software (GraphPad Software Inc., San Diego, CA), the data were fit by non-linear regression to the following formula: $y = 100/(1+(x/IC_{50})^{p})$, where the initial values for IC_{50} and p are 1.0 and 0.05, respectively. The goodness-of-fit coefficients R^2 are >0.95.

Colocalization study using confocal laser fluorescence microscopy

B16-F10 cells (8×10^4 cells) were seeded into a glass-bottom dish and cultured at 37°C overnight. Following different time incubation with a fluorescent dye DiI-labeled **OEG-OLA$_{15}$-CTX SNPs**, the cell culture medium was discarded and cells were washed with PBS for three times. Subsequently, cells were stained with LysoTracker green and

Hoechst 33342 for 0.5 h. After three-time PBS wash, cells were observed under confocal laser fluorescence microscopy (Olympus IX81-FV3000, Japan).

Endocytosis pathway study for OEG-OLA$_{15}$-CTX SNPs

B16-F10 cells were seeded into 6-well plates at a density of 2.5×10^5 cells per well and cultured at 37°C overnight. To determine endocytosis pathway for **OEG-OLA$_{15}$-CTX SNPs**, cells were preincubated with specific endocytosis inhibitors including chlorpromazine (10 μg/mL), cytochalasin D (40 μM), and filipin III (5 μg/mL) at 37°C for 0.5 h. Untreated cells were included as reference. Subsequently, cells were incubated with DiI-labeled **OEG-OLA$_{15}$-CTX SNPs** for another 4 h. Following brief wash with PBS, cells were harvested and analyzed with flow cytometry.

Hemolysis assay

Hemolysis assay was conducted to assess the hemocompatibility of **OEG-OLA$_{15}$-CTX SNPs**, free CTX formulated in polysorbate 80 was included as reference. Briefly, red blood cells (RBCs) were isolated from fresh rat blood mixed with EDTA by centrifugation at 1500 rpm for 10 min and washed three times with saline. Subsequently, the RBCs suspension at a final concentration of 2% (v/v) was prepared by a dilution with saline. RBCs suspension (300 μL) was mixed with same volume of **OEG-OLA$_{15}$-CTX SNPs** and free CTX formulated in polysorbate 80 at serial concentrations (0.5, 1, 1.5, 2, and 3 mg/mL). RBCs suspensions incubated with saline and 1% Triton X-100 were identified as negative group and positive group, respectively. After 1h incubation at 37°C, samples were centrifuged at 1500 rpm for 10 min and photographed. 100 μL of supernatant was transferred into 96-well plates, the absorbance was measured at 540 nm on a microplate reader (Multiskan FC, Thermo Scientific). The hemolysis was calculated as follows:

$$Hemolysis\ (\%) = 100\% \times (A_{Sample} - A_{Saline}) / (A_{Triton} - A_{Saline})$$

Assessment of apoptosis-inducing ability using acridine orange/ethidium bromide (AO/EB) staining assay

B16-F10 and A375 cells were seeded into 48-well plates at a density of 8000 cell/well and cultured for 24 h, respectively. Then, cells were treated with free CTX and **OEG-**

OLA_{15}-CTX SNPs for 48 h (0.5 nM CTX equivalence for A375, 8 nM CTX equivalence for B16-F10). AO and EB solutions were premixed (1:1, v/v) and diluted with PBS buffer. Medium for each well was replaced with AO/EB solutions (AO: 100 ug/mL, EB: 100 ug/mL). Following three washes with PBS, cells were imaged on a fluorescence microscopy. The apoptosis index was identified as the ratio of orange cells to green cells.

Inhibition on the proliferation induced by OEG-OLA_{15}-CTX SNPs

A Click-iT® EdU Alexa Fluor® 488 Assay Kit (Invitrogen) was used to determine the inhibition on cell proliferation induced by **OEG-OLA_{15}-CTX SNPs** and free CTX. Cells were plated in 48-well plates (8000 cells per well) and incubated for 24 h at 37°C. Subsequently, cells were treated with free CTX and **OEG-OLA_{15}-CTX SNPs** for 48 h (0.5 nM CTX equivalence for A375, 8 nM CTX equivalence for B16-F10). Cells not treated with drug formulations were identified as a control. After removing the medium, fresh medium containing 10 μM EdU was added into each well and incubated for 2 h to label the proliferating cells. Upon brief washes, cells were fixed with 4% paraformaldehyd for 30 min at room temperature. To ensure the permeability of cytomembrane, cells were further permeabilized with 0.5% Triton X-100 for 20 min. Then, Alexa Fluor 488 azide solutions were added to stain the cells labeled by EdU for 30 min in the dark. Following staining with Hoechst 33342, cells were observed on a fluorescence microscope (Olympus, IX71).

Animal studies

Mice (5-6 weeks old) for animal studies were purchased from Shanghai Experimental Animal Centre, Chinese Academy of Science. All animal experiments were performed in compliance with the guidelines of the Zhejiang University Committee for Animal Use and Care. The studies involving animals were approved by the Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine. They were housed under aseptic conditions and given an autoclaved rodent diet and sterile water.

Evaluation of toxicity *in vivo*

We evaluated the *in vivo* toxicity of **OEG-OLA_{15}-CTX SNPs** and free CTX in healthy ICR mice (4-5 weeks old). Mice were randomly divided into six groups. **OEG-OLA_{15}-CTX**

SNPs (at 5, 10, 15, 20 mg/kg CTX equivalence) were intravenously injected on days 0, 3, 6, while free CTX formulated in polysorbate 80/ethanol (1:1, v/v) at a dose of 5 or 10 mg/kg was injected as a control. The body weight of mice treated with drug formulations was recorded every 3 days for the evaluation of toxicity. At the end of investigation, mice were scarified and the main organs (heart, liver, spleen, lung and kidney) were collected for histological analysis.

The hematotoxicity of **OEG-OLA$_{15}$-CTX SNPs** was compared with free CTX in healthy ICR mice. Mice were randomly divided into five groups. **OEG-OLA$_{15}$-CTX SNPs** (at 10 or 20 mg/kg CTX equivalence) were intravenously injected on days 0, 3, and 6, while free CTX formulated in polysorbate 80/ethanol (1:1, v/v) at a dose of 10 or 20 mg/kg was injected as control. Blood samples were collected and analyzed on days 3, 6, and 10 after drug administration.

Establishment of a melanoma PDX tumor model and in vivo antitumor study

The melanoma PDX tumor model was established as follows. Fresh tumor tissue was surgically resected from patients and then subcutaneously implanted into Balb/c nude mice with ~1 mm^3 pieces. A fifth passage of melanoma was used in this research. When the average tumor volume reached ~100 mm^3, mice were randomly divided into four groups (n=6 mice in each group). The mice were intravenously injected with free CTX (formulated in polysorbate 80/ethanol, 1:1, v/v, 6 mg/kg), **OEG-OLA$_{15}$-CTX SNPs** (6, 12 mg/kg) three time every three days, respectively. Mice treated with saline were included as a reference. Tumor volume and body weight were monitored and the volume of tumor was calculated as the following formula: $V = (L \times W^2)/2$, W is shorter than L. On day 21, mice were sacrificed and the tumors were excised for photograph.

***In vivo* antitumor activity assessment in a mouse model bearing B16F10 cell-derived xenograft (CDX)**

C57BL/6 mice bearing B16F10 xenograft were established by subcutaneous injection of B16F10 cells (5×10^5 cells mL^{-1}, 100 μL per mouse). When the average tumor volume reached ~100 mm^3, mice were randomly divided into three groups. The mice were intravenously injected with **OEG-OLA$_{15}$** and **OEG-OLA$_{15}$-CTX SNPs** (12 mg/kg) three

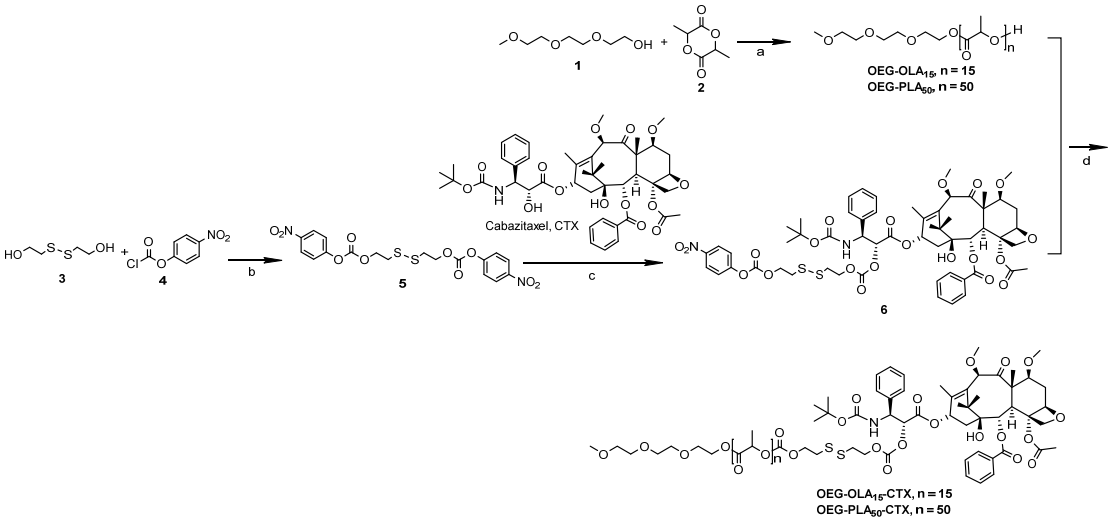
times every three days. Mice treated with saline were included as references. Tumor volume and body weight were monitored, and the volume of tumors was calculated as the following formula: $V = (L \times W^2)/2$, W is shorter than L.

Histological analysis

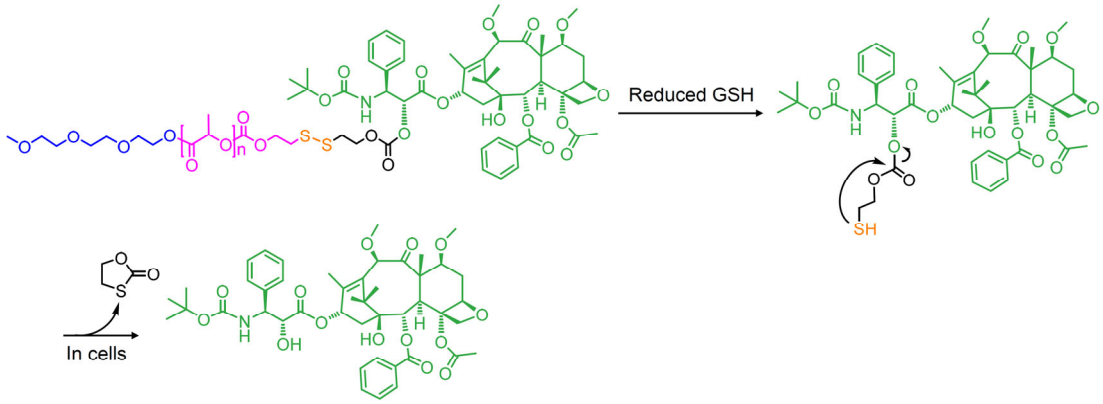
The excised tumors on day 12 and organs were fixed in 4% formaldehyde, embedded in paraffin, and sectioned into 5-μm slices. Subsequently, slices were stained with hematoxylin and eosin (H&E, Sigma). For TUNEL assay, an in Situ Cell Death Detection Kit was used to stain the fixed tumor sections to detect the DNA fragmentation. To investigate the proliferation of tumor cells for each group, tumor sections were incubated with a Ki67 antibody. The nuclei were stained with 4', 6- diamino-2-phenylindole (DAPI). All stained slices were captured using a microscope (Olympus, IX71).

Reference:

(1) Wan, J.; Qiao, Y.; Chen, X.; Wu, J.; Zhou, L.; Zhang, J.; Fang, S.; Wang, H.: Structure-Guided Engineering of Cytotoxic Cabazitaxel for an Adaptive Nanoparticle Formulation: Enhancing the Drug Safety and Therapeutic Efficacy. *Advanced Functional Materials* **2018**, *28*, 1804229.



Scheme S1. Synthetic scheme of the OEG-OLA_n-CTX conjugates (n = 15 or 50). Reaction conditions: a) $Sn(Oct)_2$, toluene, 140 °C; b) DIEA, DCM, reflux; c) DMAP, DCM, reflux; d) DMAP, DCM, reflux.



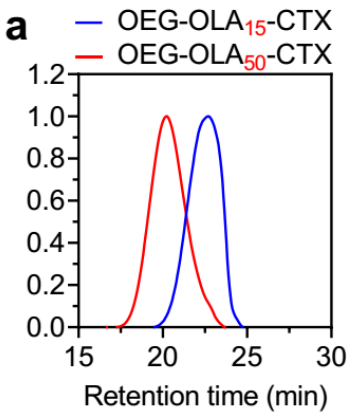
Scheme S2. Schematic illustration of active CTX release in response to intracellular reduced glutathione (GSH).

Table S1. The number of mouse survival after treatment with various drug formulations at different doses for ICR mice.

Drug formulations	Dose[a]	Survival at endpoints[b]
Free CTX	5 mg/kg (q036d)	3/3
	10 mg/kg (q036d)	1/3
pLA_{15}-SS-CTX NPs	5 mg/kg (q036d)	3/3
	10 mg/kg (q036d)	3/3
	15 mg/kg (q036d)	3/3
	20 mg/kg (q036d)	3/3

[a] Drug formulations were intravenously injected very three days *via* the tail vein.

[b] The mice were defined to be dead when the body weight loss exceeded 15%.



b

	M_n (g·mol^{-1})	$Đ$
OEG-OLA$_{15}$-CTX	2200	1.25
OEG-OLA$_{50}$-CTX	6200	1.32

Figure S11. Characterization of the OEG-OLA$_n$-CTX conjugates (n = 15 or 50) with gel permeation chromatography (GPC) measured in tetrahydrofuran (THF). M_n, number-average molar mass; $Đ$, polydispersity.

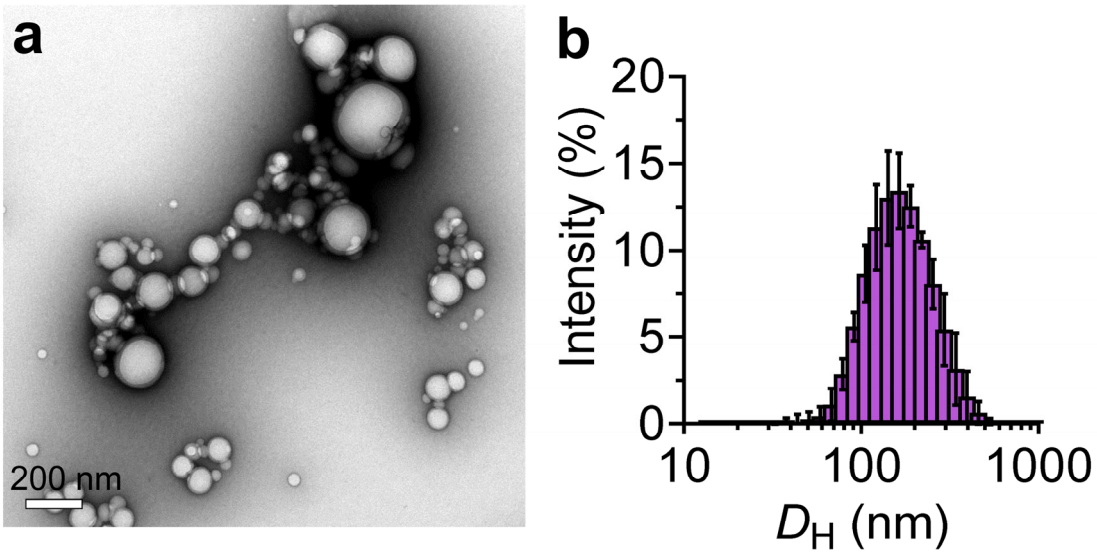


Figure S12. Characterization of **OEG-OLA$_{50}$-CTX SNPs**. Transmission electron microscopy (TEM) image (a) and size distribution determined by dynamic light scattering (DLS) (b).

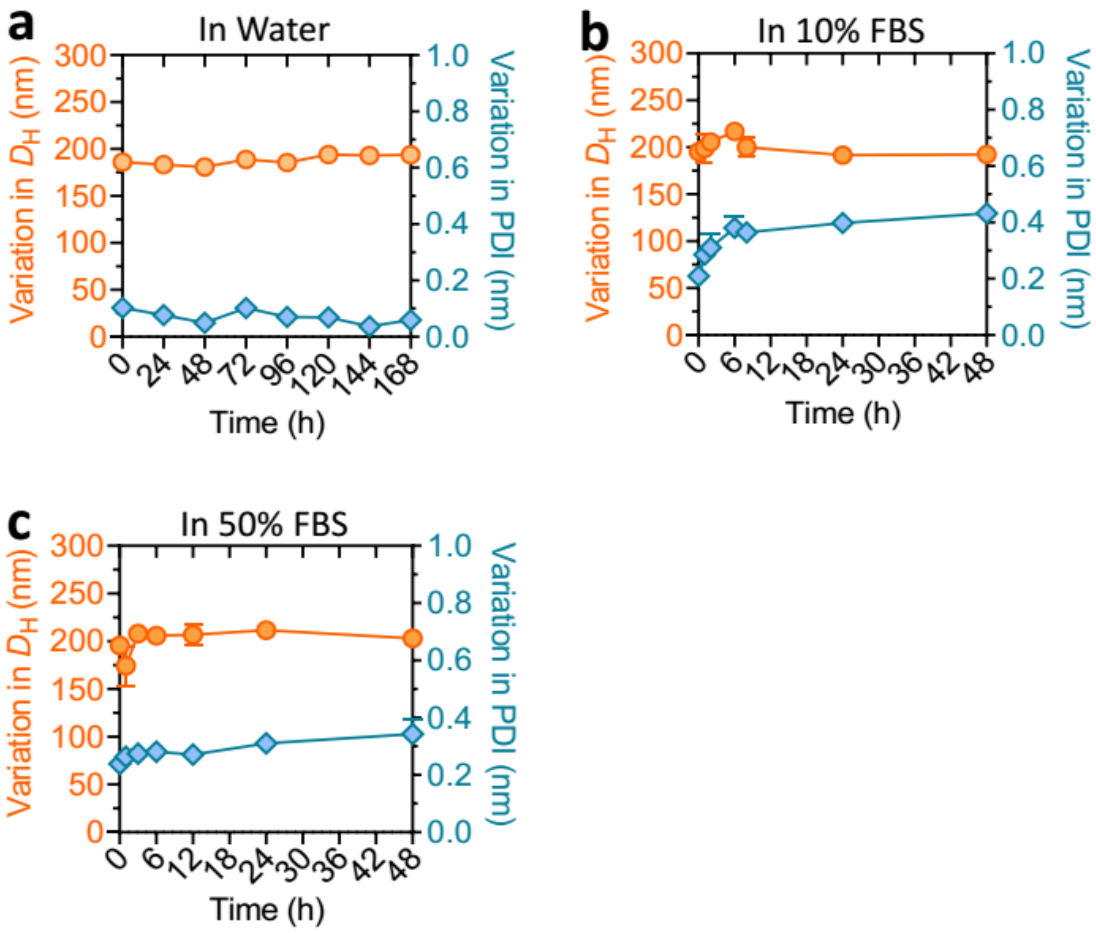


Figure S13. Stability of **OEG-OLA$_{15}$-CTX NPs** in **a**) DI water, **b**) DI water containing 10% FBS and **c**) DI water containing 50% FBS. The particle sizes and PDI were evaluated by DLS.

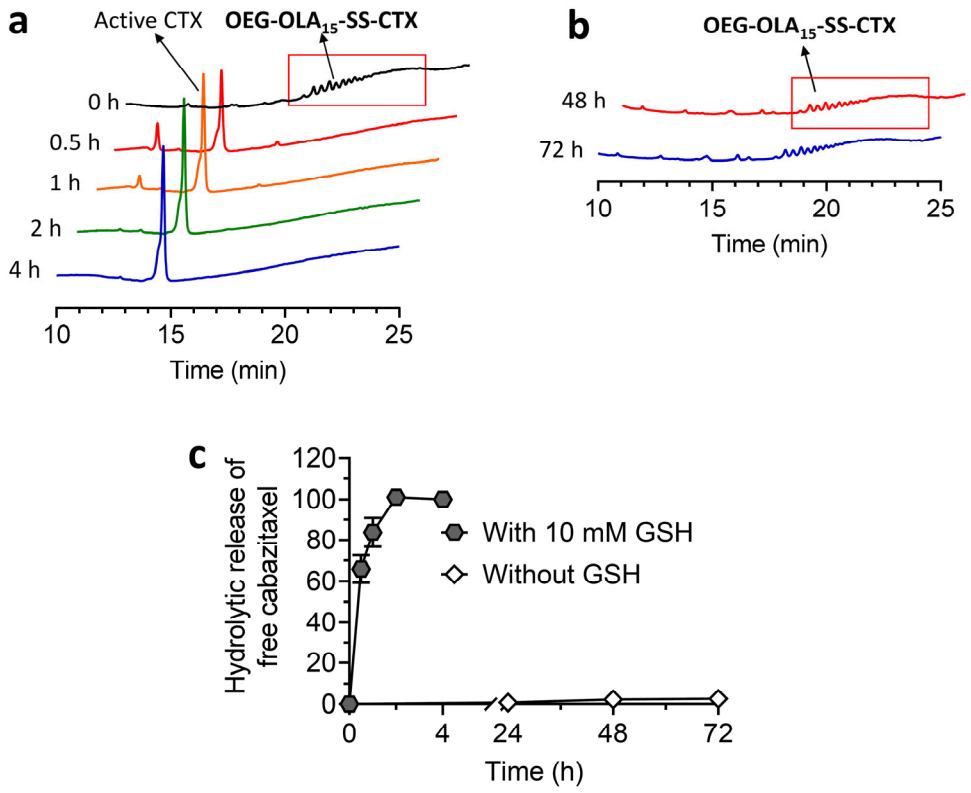


Figure S14. Representative HPLC chromatograms of the OEG-OLA_{15}-CTX conjugate in the presence (**a**) or in the absence (**b**) of 10 mM GSH. **c**) The release profiles of active CTX from the conjugate.

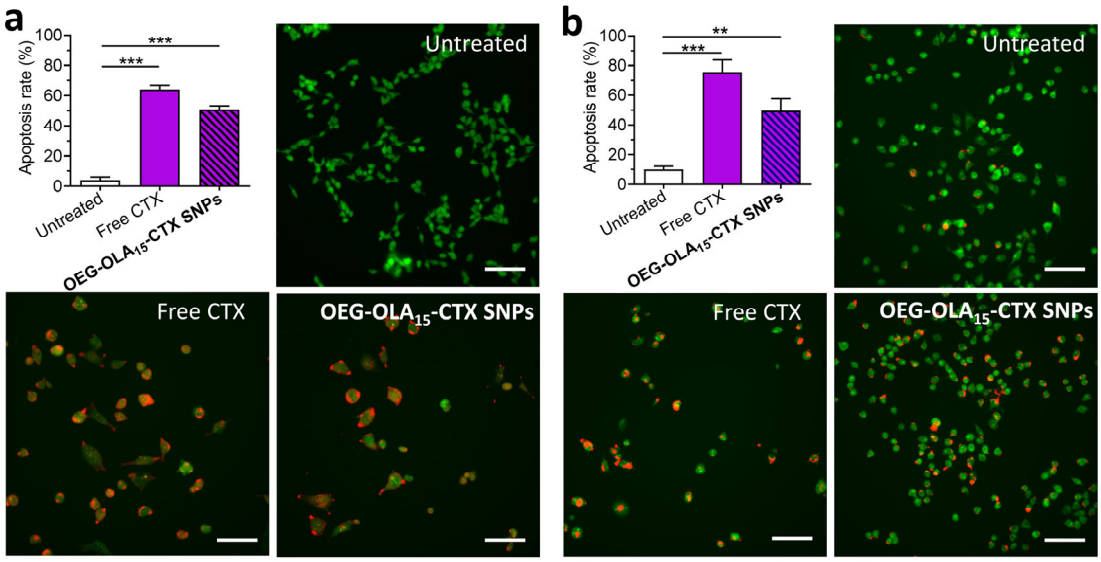


Figure S15. An AO/EB staining assay was performed to examine the activity of **OEG-OLA$_{15}$-CTX SNPs** in B16F10 (**a**) and A375 (**b**) cells. Scale bars: 100 μm. Data are presented as means ± SD. $^{**}p < 0.01$; $^{***}p < 0.001$.

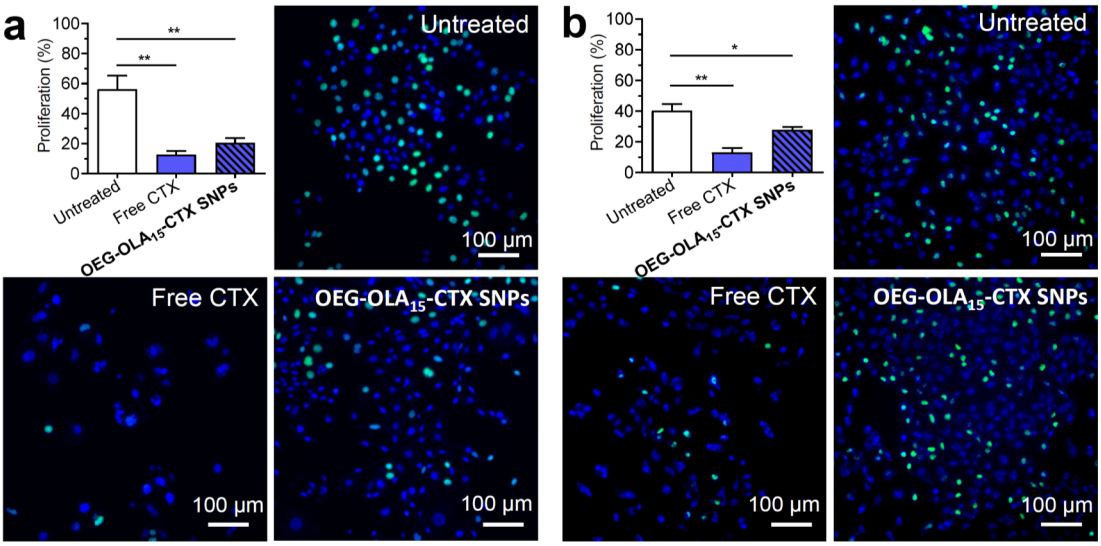


Figure S16. A Click-iT EdU assay was performed to evaluate the inhibition on proliferation of a) B16-F10 and b) A375 cells. Cells were treated with free CTX and **OEG-OLA$_{15}$-CTX SNPs** (CTX-equivalent concentration: 0.5 nM for A375 cells and 8 nM for B16-F10 cells) for 48 h. Data are presented as means ± SD. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

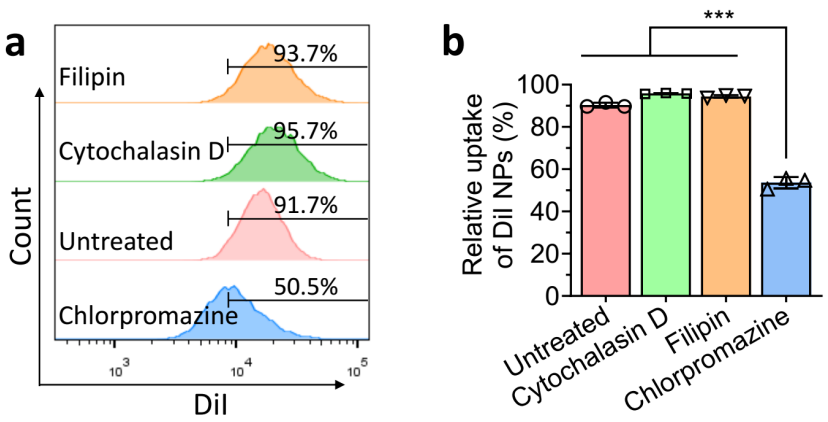


Figure S17. Cellular uptake of DiI-labeled **OEG-OLA$_{15}$-CTX SNPs** using flow cytometry analysis in the presence or absence of endocytosis inhibitors. Data are presented as means ± SD. ***, $p < 0.001$.

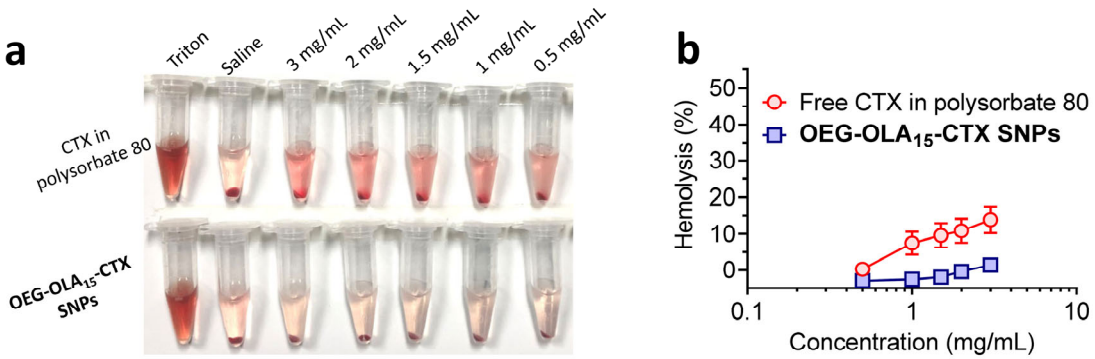


Figure S18. Hemolysis of free CTX and **OEG-OLA$_{15}$-CTX SNPs** at a range of concentration from 0.5 mg/mL to 3 mg/mL. Saline and 1% Triton X-100 were included as negative and positive controls, respectively. Data are presented as means ± SD.

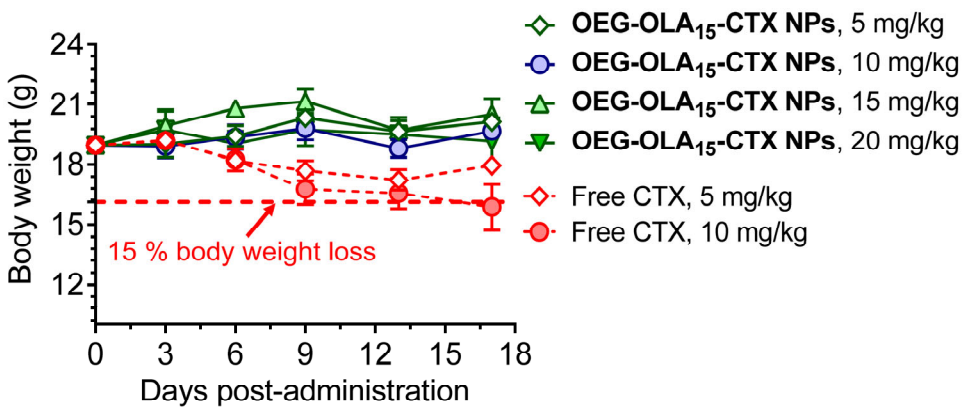


Figure S19. Variation in body weights following three injections of free CTX (5 and 10 mg/kg) and **OEG-OLA$_{15}$-CTX SNPs** (5, 10, 15 or 20 mg/kg, at CTX-equivalent doses) on days 0, 3, and 6. Data are presented as means ± SD.

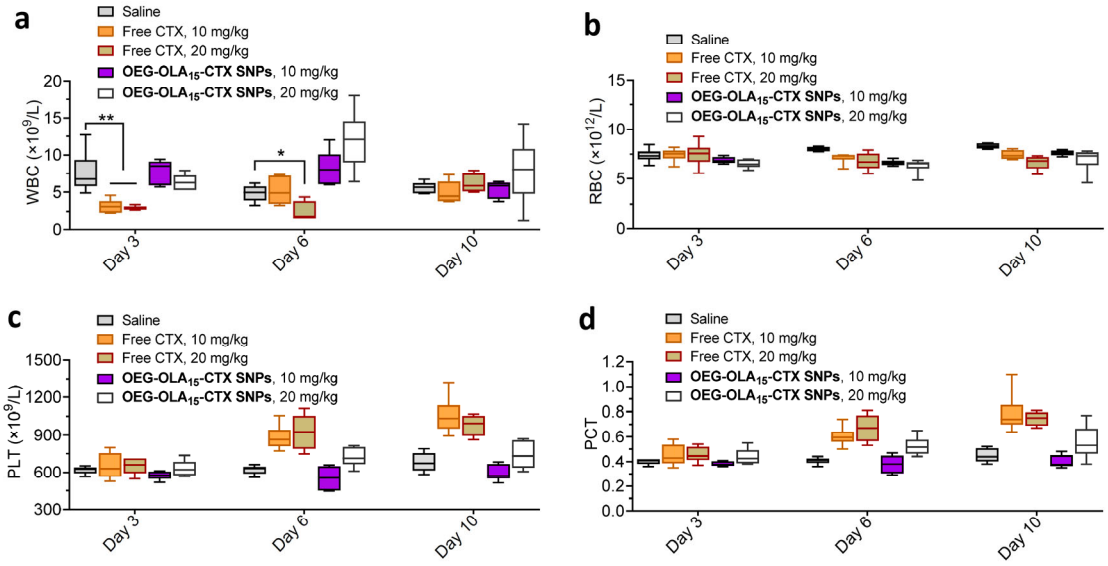


Figure S20. Variations in **a**) WBC, **b**) RBC, **c**) PLT counts and **d**) PCT following three injections of free CTX (10 and 20 mg/kg) and **OEG-OLA$_{15}$-CTX SNPs** (10 and 20 mg/kg, at CTX-equivalent doses) on days 0, 3, and 6. Data are presented as means ± SD. * p < 0.05; ** p < 0.01. WBC, white blood cell; RBC, red blood cell; PLT, platelet; PCT, plateletcrit.

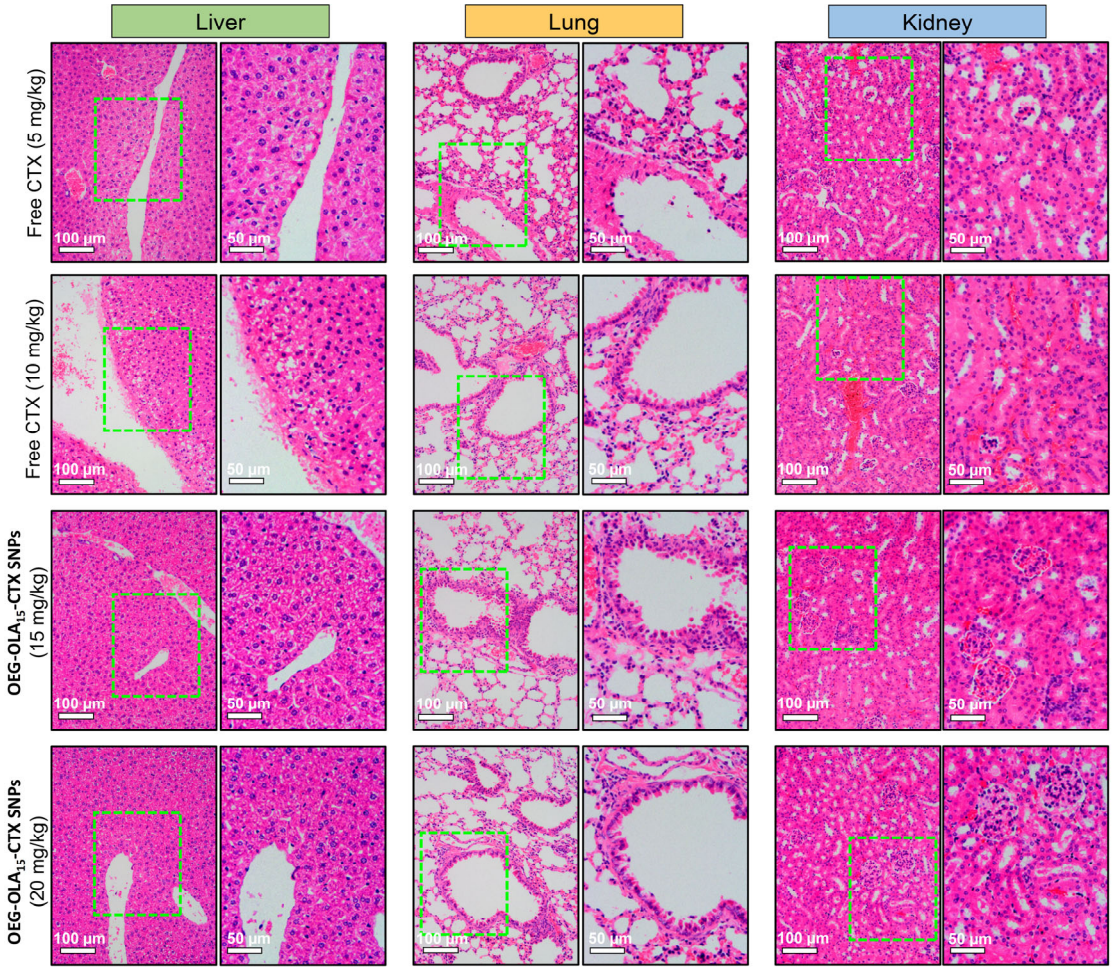


Figure S21. Representative H&E staining of major organs (liver, lung, and kidney) excised from the mice treated with free CTX (5 or 10 mg/kg) and **OEG-OLA$_{15}$-CTX SNPs** (15 or 20 mg/kg, at a CTX-equivalent dose).

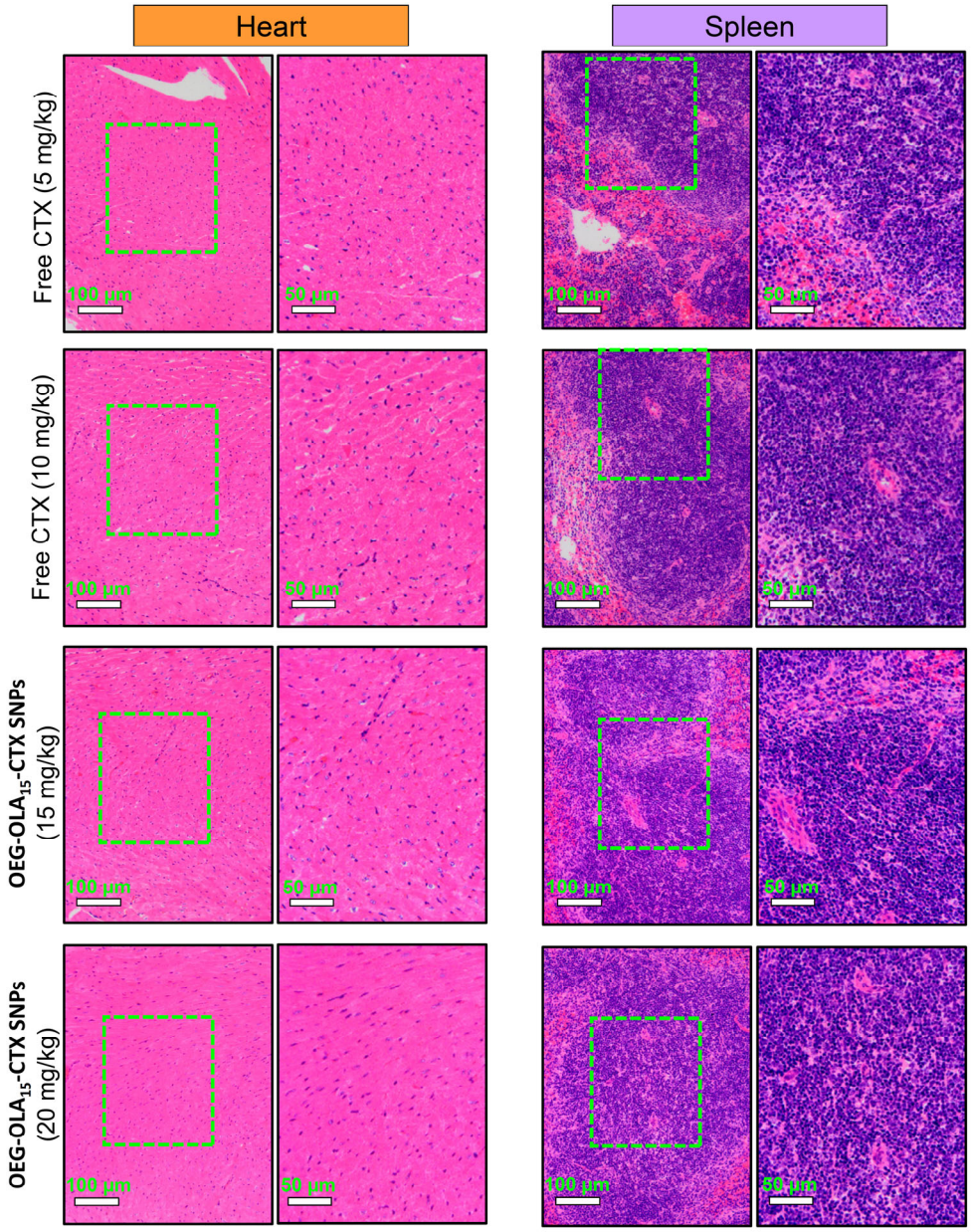


Figure S22. Representative H&E staining of major organs (heart and spleen) excised from the mice treated with free CTX (5 or 10 mg/kg) and **OEG-OLA$_{15}$-CTX SNPs** (15 or 20 mg/kg, at a CTX-equivalent dose).

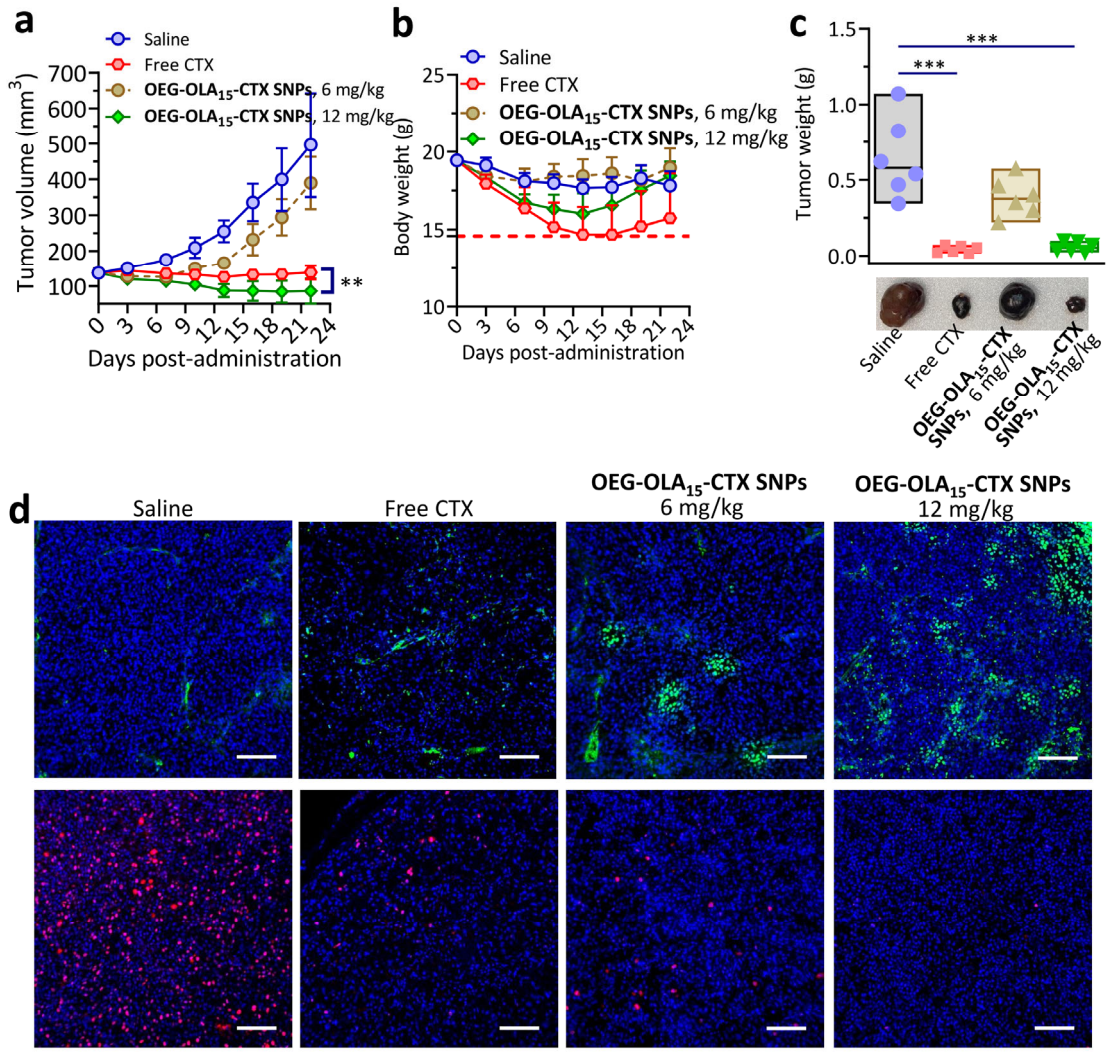


Figure S23. *In vivo* efficacy of the nanotherapy was evaluated in a melanoma PDX model. (a) Tumor growth curve following three intravenous injections of **OEG-OLA$_{15}$-CTX SNPs** and free CTX. (b) Variation in body weights after drug administration. (c) Tumor weights and photographs of the tumors in each group excised on day 21. (d) Histological analysis of the tumor sections after treatment. Scale bars: 100 μm. The data are presented as the means ± SD (n = 6). *p < 0.05; **p < 0.01; ***p < 0.001.

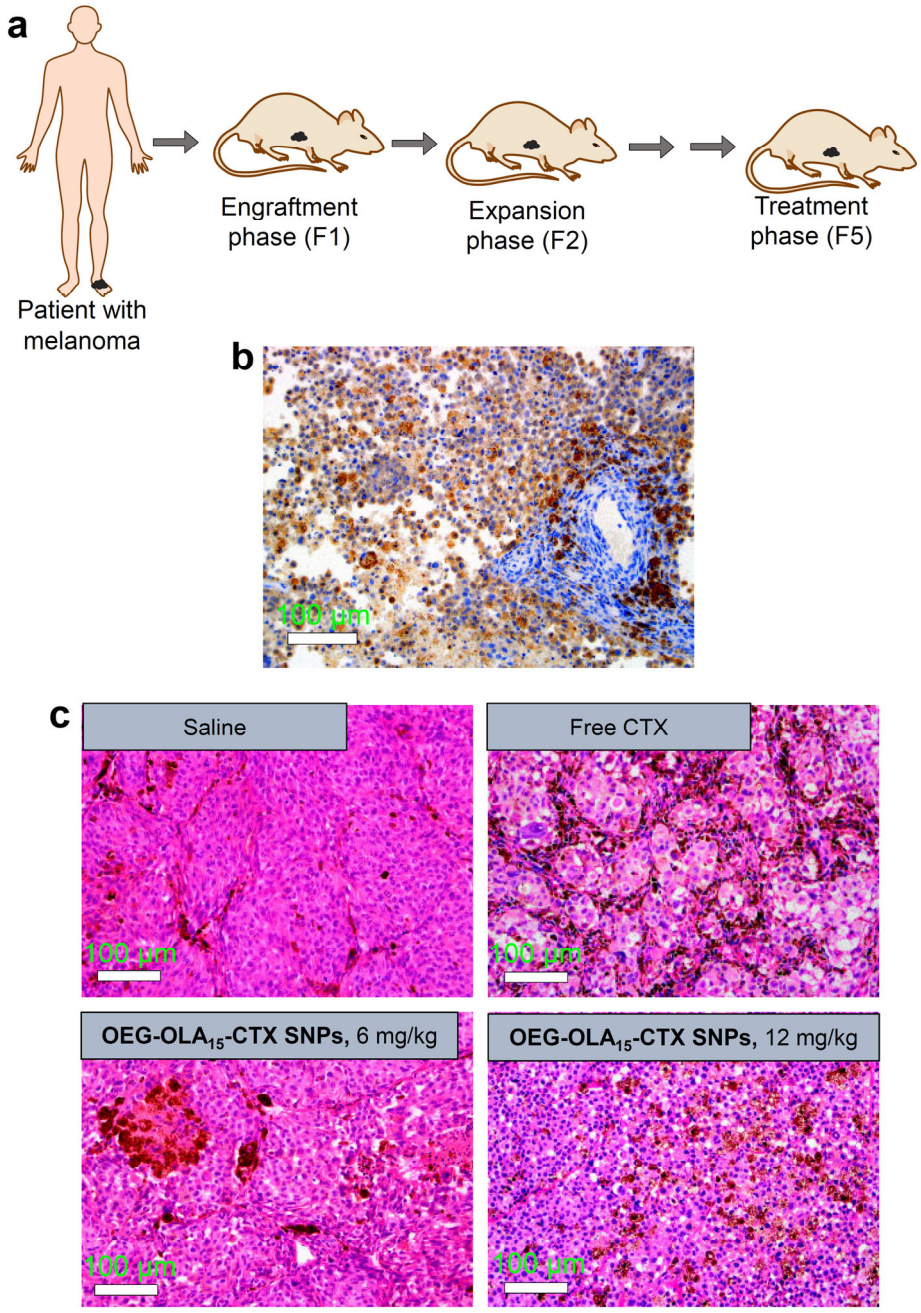


Figure S24. *In vivo* antitumor efficacy against an orthotopic melanoma PDX model in Balb/c nude mice. a) Schematic illustration for the establishment of an orthotopic melanoma PDX mouse model. b) Immunohistochemistry (IHC) staining (Melan-A) of the melanoma PDX. c) H&E staining of the tumors excised from the mice administered with free CTX (6 mg/kg) and **OEG-OLA$_{15}$-CTX SNPs** (6 or 12 mg/kg, at a CTX-equivalent dose).

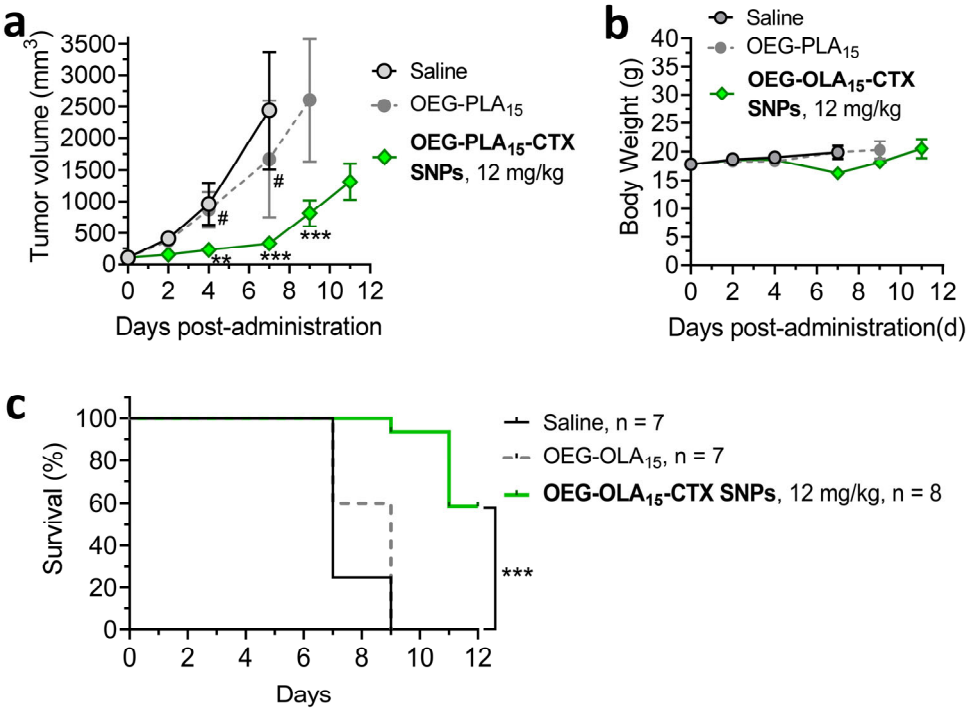


Figure S25. *In vivo* efficacy of the nanotherapy was evaluated in the mouse model of melanoma B16F10 xenograft. **a**) Tumor growth curve following three intravenous injections of saline, OEG-OLA_{15}, and **OEG-OLA_{15}-CTX SNPs**. **b**) Variation in body weights after drug administration. **c**) Survival percentage of the mice in each treatment group. We defined the mouse death when the tumor volume exceeded 1500 mm^3. Data are presented as means ± SD. #$p > 0.05$, OEG-OLA_{15} *versus* saline; **$p < 0.01$ and *** $p < 0.001$, **OEG-OLA_{15}-CTX SNPs** *versus* OEG-OLA_{15}.