Supporting Information for:

Design and development of multifunctional polyphosphoester-based nanoparticles for ultrahigh paclitaxel dual loading

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Materials. Paclitaxel was purchased from BIOTANG, Inc. (Lexington, MA). 2-Chloro-2-oxo-1,3,2-dioxaphospholane (95%) was used as received from Thermo Fisher Scientific, Inc. (Pittsburgh, PA). Chelex® 100 resin was used as received from Bio-Rad Laboratories (Hercules, CA). Tetrahydrofuran (THF) and dichloromethane (DCM) were dried through solvent purification system (J. C. Meyer Solvent Systems, Inc., Laguna Beach, CA). Poly(ethylene glycol) methyl ether ($M_n = \sim 2$ kDa, $M_w/M_n = 1.05$) was purchased from Sigma-Aldrich Co. (St. Louis, MO) and was used as received. Slide-A-Lyzer dialysis cassettes (10 kDa molecular weight cut-off, MWCO) were purchased from Pierce Biotech. (Rockford, IL). The Spectra/Por dialysis membranes (MWCO 12-14 kDa) were purchased from Spectrum Laboratories, Inc. (Rancho Dominguez, CA). Nanopure water (18 MΩ•cm) was acquired by means of a Milli-Q water filtration system, Millipore Co. (Bedford, MA). RAW 264.7 and OVCAR-3 cell lines, as well as, RPMI and DMEM media, were obtained from the American Type Culture Collection (Manassas, VA). Fetal bovine serum and penicillin/streptomycin were obtained from Sigma-Aldrich Co. (St. Louis, MO). Cell culture 96-well rounded bottom plates were purchased from Corning Costar Co. (Corning, NY). The Cell-Titer 96 non-radioactive cell proliferation assay was obtained from Promega Co. (Madison, WI).
**Instrumentation.** $^1$H NMR and $^{13}$C NMR spectra were recorded on a Varian Inova 300 or Varian Inova 500 spectrometer interfaced to a UNIX computer using VnmrJ software. Chemical shifts were referenced to the solvent residual signals. FTIR spectra were recorded on an IR Prestige 21 system using a diamond ATR lens (Shimadzu Corp., Japan) and analyzed using IRsolution v. 1.40 software. The DMF gel permeation chromatography (GPC) was conducted on a Waters Chromatography, Inc. (Milford, MA) system equipped with an isocratic pump model 1515, a differential refractometer model 2414, and a three-column set of Styragel HR 4.5 μm DMF (300 × 7.5 mm), Styragel HR 4E 5 μm DMF (300 × 7.5 mm), and Styragel HR 2.5 μm DMF (300 × 7.5 mm). The system was equilibrated at 70 °C in pre-filtered DMF containing 0.05 M LiBr, which served as polymer solvent and eluent (flow rate set to 1.00 mL/min). Polymer solutions were prepared at a concentration of *ca.* 3 mg/mL and an injection volume of 200 μL was used. Data collection and analysis were performed with Empower 2 v. 6.10.01.00 software (Waters, Inc.). The system was calibrated with polystyrene standards (Polymer Laboratories, Amherst, MA) ranging from 615 to 442,800 Da.

Glass transition temperatures ($T_g$) were measured by differential scanning calorimetry on a Mettler-Toledo DSC822® (Mettler-Toledo, Inc., Columbus, OH), with a heating rate of 10 °C/min. Measurements were analyzed using Mettler-Toledo Star® v. 7.01 software. The $T_g$ was taken as the midpoint of the inflection tangent, upon the third heating scan. Thermogravimetric analysis was performed under N$_2$ atmosphere using a Mettler-Toledo model TGA/SDTA851e, with a heating rate of 10 °C/min. Measurements were analyzed by using Mettler-Toledo Star® v. 7.01 software.

Dynamic light scattering (DLS) measurements were conducted using a Delsa Nano C (Beckman Coulter, Inc., Fullerton, CA) instrument equipped with a laser diode operating at 658 nm. Size measurements were made in nanopure water ($n = 1.3329$, $\eta = 0.890$ cP at 25 ± 1 °C). Scattered light was detected at a 165° angle and analyzed using a log correlator over 70 accumulations for a 3.0 mL sample in a glass sizing cell (4.0 mL capacity). The photomultiplier aperture and the attenuator were automatically adjusted to obtain a photon counting rate of *ca.* 8 kcps. Calculations of the particle size distribution and distribution averages were performed using CONTIN particle size distribution analysis routines. The peak averages of
histograms from number distributions out of 70 accumulations were reported as the average diameters of the particles. The particle ζ-potential values were determined by a Delsa Nano C particle analyzer (Beckman Coulter, Fullerton, CA) equipped with a 30 mW dual laser diode (658 nm). The ζ-potential of the particles in suspension was obtained by measuring the electrophoretic movement of charged particles under an applied electric field. Scattered light was detected at a 15° angle at 25 °C. The ζ-potential was measured at five regions in the flow cell, and a weighted mean was calculated. These five measurements were used to correct for electro-osmotic flow that was induced in the cell due to the surface charge of the cell wall. All determinations were repeated six times.

Transmission electron microscopy (TEM) images were collected on a JEOL 1200EX operating at 100 kV and micrographs were recorded at calibrated magnifications using a SIA-15C CCD camera. The samples as aqueous solutions (4 μL) were deposited onto carbon-coated copper grids, and after 10 min, the excess of the solution was quickly wicked away by a piece of filter paper. The grids were allowed to dry in air overnight. High-resolution scanning transmission electron microscopy (STEM) and elemental mapping were conducted on a FEI Tecnai G2 F20 FE-TEM coupled with energy-dispersive X-ray (EDX), operating at a voltage of 200 kV with a Gatan CCD camera.

The atomic force microscopy (AFM) imaging was performed on a MFP-3D system (Asylum Research) in tapping mode using standard silicon tips (Aspire probes, resonance constant: 28 kHz, tip radius: <10 nm, spring constant: 0.1 N/m). For AFM sample preparation, nanoparticles were dissolved in nanopure water at 0.05 mg/mL, and 10 μL of the sample was spin-coated onto a mica surface and allowed to dry in air overnight.

1. **Synthesis of PEBP-b-PBYP-g-PEG copolymer.**

The hydrophobic functional block terpolymer, poly(2-ethylbutoxy phospholane)-block-poly(2-butynyl phospholane) (PEBP-b-PBYP, \( M_n^{NMR} = 19300 \) Da, \( D = 1.25 \)), and the amphiphilic block terpolymer, poly(2-ethylbutoxy phospholane)-block-poly(2-butynyl phospholane)-graft-poly(ethylene glycol) (PEBP-b-PBYP-g-PEG, \( M_n^{NMR} = 27300 \) Da, \( D = 1.24 \)), were synthesized and reported previously.\(^1,2\)
2. **Synthesis of 6-azidohexanoic acid.**

In a 250 mL round-bottom flask equipped with a magnetic stir bar, 6-bromohexanoic (5.01 g, 25.7 mmol) and sodium azide (3.34 g, 51.4 mmol) were added and dissolved in DMF (50 mL). After stirring under heating at 45 °C for 7 d, the reaction mixture was added into 75 mL of dichloromethane (DCM) and then extracted with water (40 mL), brine (40 mL) and saturated NaHCO₃ aqueous solution (40 mL), respectively. The combined organic layers were dried over Na₂SO₄ and filtered. The filtrate was concentrated in vacuo and the resulting mixture was purified by column chromatography on silica gel using hexane/EtOAc gradient as eluent, which gave 6-azidohexanoic acid as a pale yellow liquid (1.46 g, yield: 36.0 %). ¹H NMR (300MHz, CDCl₃, ppm): δ 1.44 (m, 2H, N₃CH₂CH₂CH₂CH₂CH₂), 1.65 (m, 4H, N₃CH₂CH₂CH₂CH₂CH₂), 2.38 (t, 2H, J = 7 Hz, CH₂CH₂COOH), 3.29 (t, 2H, J = 7 Hz, N₃CH₂CH₂CH₂CH₂CH₂), 11.55 (br, 1H, COOH). ¹³C NMR (75 MHz, CDCl₃, ppm): δ 24.2, 26.2, 28.5, 33.9, 51.2, 180.1.

3. **Synthesis of azido-PTX.**

In a 25 mL round-bottom flask equipped with a magnetic stir bar, 6-azidohexanoic acid (44.4 mg, 0.281 mmol) and PTX (201.2 mg, 0.2356 mmol) were added and dissolved in dichloromethane (5 mL). After stirring at r.t. for 1 h, DCC (58.4 mg, 0.283 mmol) and DMAP (5.7 mg, 0.047 mmol) were added. The mixture was heated to reflux for 5 d, filtered, concentrated and then purified by flash chromatography using silica gel with hexane and ethyl acetate as eluent in gradient (hexane/ethyl acetate = 64/36, v/v) to give the product as a pale yellow solid (210.0 mg, yield: 90.2 %). ¹H NMR (300 MHz, CDCl₃, ppm): δ 1.15 (s, 3H, (C-16)-C₃H₃), 1.22-1.47 (m, 5H, (C-17)-C₃H₃ and N₃CH₂CH₂CH₂CH₂CH₂), 1.49-1.78 (m, 7H, (C-19)-CH₃ and N₃CH₂CH₂CH₂CH₂CH₂), 1.84-2.00 (m, 5H, (C-6)-CH, 1-OH and (C-18)-CH₃), 2.16 (m, 1H, (C-14)-CH), 2.24 (s, 3H, 10-0Ac), 2.28-2.70 (m, 8H, 4-OAc, (C-6)-CH, (C-4)-CH, 7-OH and CH₂CH₂COO(PTX)), 3.22 (t, 2H, J = 7 Hz, N₃CH₂CH₂CH₂), 3.83 (d, 1H, J = 7 Hz, (C-3)-CH), 4.21 (d, 1H, J = 8 Hz, (C-20)-CH), 4.33 (d, 1H, J = 8 Hz, (C-20)-CH), 4.46 (m, 1H, (C-7)-CH), 5.00 (dd, 1H, J = 9 Hz, 2 Hz, (C-5)-CH), 5.52 (d, 1H, J = 3 Hz, (C-2')-CH), 5.70 (d, 1H, J = 7 Hz, (C-2)-CH), 5.98 (dd, 1H, J = 9 Hz and 3 Hz, (C-3')-CH), 6.22-6.33 (m, 2H, (C-10)-CH), 6.88 (d, 1H, J = 9 Hz, 3'-NH), 7.32-7.66 (m, 11H, PhH), 7.75 (d, 2H, J = 8 Hz, PhH), 8.15 (d,
2H, \( J = 8 \text{ Hz}, \text{PhH} \). \(^{13}\text{C} \) NMR (75 MHz, CDCl\(_3\), ppm): \( \delta \) 9.6, 14.9, 20.9, 22.2, 22.7, 24.2, 25.0, 26.0, 26.9, 28.4, 31.0, 33.5, 35.6, 43.2, 45.6, 51.1, 52.8, 58.5, 71.8, 72.2, 73.9, 75.1, 75.6, 76.5, 79.2, 81.1, 84.5, 126.5, 127.1, 128.5, 128.8, 129.1, 129.2, 130.3, 132.1, 132.8, 133.7, 137.0, 142.8, 165.1, 167.1, 167.2, 168.1, 169.8, 171.3, 172.5, 203.9.

4. **Synthesis of PEBP-b-PBYP-g-PTX/PEG.**

In a 10-mL Schlenk flask equipped with a magnetic stir bar, PEBP-b-(PBYP-g-PEG) (300.0 mg, 0.0110 mmol), azido-PTX (109.1 mg, 0.110 mmol), CuBr (7.9 mg, 0.055 mmol) and PMDETA (19.0 mg, 0.0110 mmol) were added and dissolved in DMF (6 mL). The reaction mixture was deoxygenated by freeze-pump-thaw (3×) and then placed in a preheated water bath at 37 °C and stirred overnight. The resulting mixture was precipitated from acetone into ethyl ether (3×) to remove unreacted azido-PTX. The crude product was collected and dissolved in nanopure water (5 mL) and purified by Sephadex G-25 column to remove copper. The solution was transferred to dialysis tubing (MWCO 12-14 kDa) and dialyzed against nanopure water for 2 d. The micelle solution was lyophilized to give a white powder (271.3 mg, 66.3 %). \(^{1}H \) NMR (500 MHz, CDCl\(_3\), ppm): \( \delta \) 0.89 (t, \( J = 7.4 \text{ Hz}, \text{POCH}_2\text{CH(}\text{CH}_2\text{CH}_3\text{)}_2\)), 1.08-1.31 (broad multiple peaks, protons from PTX), 1.37 (m, POCH\(_2\text{CH(}\text{CH}_2\text{CH}_3\text{)}_2\)), 1.52 (m, POCH\(_2\text{CH}_2\)), 1.56-2.80 (broad multiple peaks, protons from PTX, POCH\(_2\text{CH}_2\text{C=CH)}\)), POCH\(_2\text{CH}_2\text{C=CH)}\)), 3.00-3.18 (brs, NCH\(_2\text{CH}_2\)), 3.38 (s, OCH\(_3\)), 3.64 (s, OCH\(_2\text{CH}_2\) from PEG), 3.78 (m, CH from PTX(C-3)-CH), 3.96-4.02 (t, \( J = 5.5 \text{ Hz}, \text{POCH}_2\text{CH}_2\)), 4.02-4.56 (br, POCH\(_2\text{CH}_2\), POCH\(_2\text{CH}_2\text{OP)}\), and protons from PTX), 4.90-5.00 (br, CH from PTX(C-5)-CH), 5.07 (d, \( J = 8.0 \text{ Hz}, \text{OCH}_2\text{Ar})\)), 5.48 (br, CH from PTX(C-2')-CH), 5.65 (br, CH from PTX(C-2')-CH), 5.90 (br, CH from PTX(C-3')-CH), 6.15 (br, CH from PTX(C-13)-CH), 6.30 (br, CH from PTX(C-10)-CH), 7.28-7.70 (broad multiple peaks, PhH from PTX, CH\(_2\text{CH}_2\text{CH=CHNCH}_3\)), 7.75 (br, PhH from PTX), 8.11 (br, PhH from PTX). \(^{31}\text{P} \) NMR (121 MHz, CDCl\(_3\), ppm): \( \delta \) -0.98 and -1.70. \(^{13}\text{C} \) NMR (125 MHz, CDCl\(_3\), ppm): \( \delta \) 9.8, 11.0, 14.9, 20.6-20.8, 21.0, 21.4-21.8, 22.1, 22.8, 24.1, 25.8, 26.9, 27.1, 29.8-30.0, 33.5, 35.5, 35.9, 41.6, 43.3, 45.1, 45.7-46.2, 49.9, 50.2, 58.5, 65.9, 66.2-67.3, 70.2, 70.7,7.5-72.2, 74.4, 75.2, 75.7, 76.4-76.6, 79.1, 81.2, 84.6, 127.1, 127.4, 128.5, 128.6, 128.9, 129.1, 130.1, 132.0, 133.0,
5. **Labeling of PEBP-b-PBYP-g-PTX/PEG with Alexa Fluor 488.**

In a typical experiment, a vial containing a magnetic stir bar was charged with PEBP-b-PBYP-g-PTX/PEG (10 mg, 0.27 μmol, 1 eq.), Alexa Fluor 488 azide (0.116 mg, 0.134 μmol, 0.5 eq.), copper(II) sulfate pentahydrate (0.067 mg, 0.27 μmol, 1 eq.), and 5.0 mL nanopure water. The reaction mixture was degassed for 10 min via nitrogen bubbling before addition of sodium ascorbate (0.532 mg, 2.70 μmol, 10 eq.), and then was wrapped with aluminum foil and stirred for another 24 h at 37 °C. The solution was subsequently passed through a Sephadex G-25 desalting column and dialyzed against Chelex® 100 resin in nanopure water in presoaked dialysis tubing (MWCO 12–14 kDa) for 2 d, to remove copper ions and unreacted dye. The purified product was lyophilized and stored at –20 °C for further use. Conjugation efficiency, as measured by UV-vis: 75%; Yield: 92%.

6. **Self-assembly of PEBP-b-PBYP-g-PTX/PEG.**

The polymer was directly dissolved in water and sonicated for 5 min and micelles were formed spontaneously. The solubility could be as high as 110 mg/mL, with a PTX concentration of 25.3 mg/mL. Hydrodynamic diameters of micelles in nanopure water, as measured by DLS: $D_h$(intensity) = 22 ± 12 nm, $D_h$(volume) = 11 ± 5 nm, $D_h$(number) = 8 ± 2 nm; ζ-potential in nanopure water: -43 ± 3 mV.

7. **PTX loading into PEBP-b-PBYP-g-PTX/PEG.**

In a typical experiment, to a 20 mL vial containing 50.0 mg of polymer in ethanol (50.0 mg/mL), a solution of PTX (5.56 mg, 2.0 mg/mL in ethanol) was added. The flask was shaken vigorously to mix the solution, and then ethanol was removed in vacuo. Subsequently, 1.0 mL of nanopure water was added to the vial to resuspend the polymer and PTX mixture. After sonication for 5 min, a well-dispersed nanoparticle suspension was obtained. PTX loading: 10 wt% was physically loaded and 21 wt% was chemically conjugated. DLS and TEM were used to characterize these PTX-loaded nanoparticles, and HPLC was used to confirm the actual loading amount of PTX. Hydrodynamic diameters of micelles in nanopure water, as measured by DLS: $D_h$(intensity) = 26 ± 15 nm, $D_h$(volume) = 12 ± 6 nm, $D_h$(number) = 9 ± 3 nm; ζ-potential in nanopure water: -40 ± 4 mV.
8. PTX loading into PEBP-\textit{b}-PBYP-\textit{g}-PEG.

In a typical experiment, to a 20 mL vial containing 50.0 mg of polymer in ethanol (50.0 mg/mL), a solution of PTX (5.56 mg, 2.0 mg/mL in ethanol) was added. The flask was shaken vigorously to mix the solution, and then ethanol was removed \textit{in vacuo}. Subsequently, 1.0 mL of nanopure water was added to the vial to resuspend the polymer and PTX mixture. After sonication for 5 min, a well-dispersed nanoparticle suspension was obtained. DLS and TEM were used to characterize these PTX-loaded nanoparticles, and HPLC was used to confirm the actual loading amount of PTX. Hydrodynamic diameters of micelles in nanopure water, as measured by DLS: $D_h$ (intensity) = 43 ± 23 nm, $D_h$ (volume) = 21 ± 10 nm, $D_h$ (number) = 15 ± 4 nm; $\zeta$-potential in nanopure water: -37 ± 5 mV.

9. Cytotoxicity assays.

Human ovarian adenocarcinoma cells (OVCAR-3) ($5 \times 10^3$ cells/well) and osteosarcoma cells (SJSA) ($5 \times 10^3$ cells/well) were plated in 96-well plates in RPMI-1640 medium (OVCAR-3) or Dulbecco’s Modified Eagle Medium (DMEM) (1% penicillin/streptomycin, and 20% or 10% fetal bovine serum (FBS), for OVCAR-3 and SJSA, respectively). Cells were incubated at 37 °C in a humidified atmosphere containing 5% CO$_2$. The medium was replaced with fresh medium 24 h after seeding. Drug formulations were prepared at a range of different concentrations. For each well, 20 μL of each formulation was added to 100 μL of the medium. Negative controls were prepared by addition of 20 μL of PBS to wells containing cells and 100 μL of the medium. The cells were incubated for 72 h, and after this period, the medium was replaced with 100 μL of fresh medium. Then, 20 μL of the MTS combined reagent was added to each well (Cell Titer 96$^\text{TM}$ Aqueous Non-Radioactive Cell Proliferation Assay, Promega Co., Madison, WI). The cells were incubated with the reagent for 2 h at 37 °C in a humidified atmosphere containing 5% CO$_2$, and protected from light. Absorbance was measured at 490 nm using SpectraMax M5 (Molecular Devices Co., Sunnyvale, CA). The cell viability was calculated based on the relative absorbance to the control untreated cells. The calculation of the IC$_{50}$ values and the statistical analysis were performed using GraphPad Prism (GraphPad Software, Inc.,
La Jolla, CA). The 0% and 100% cell viabilities are for media control (no cells) and cells with no treatment, respectively.

10. Cellular uptake.

_In vitro_ cellular uptake was investigated using confocal laser scanning microscopy on RAW 264.7 cells and OVCAR-3 cells. To a glass bottom 6-well plate (MatTek Co., Ashland, MA), 0.5 mL of the cell solution was added to each well by placing 0.1 mL spots in a star pattern around the glass surface of the well. DMEM (RAW 264.7) or RPMI-1640 (OVCAR-3) supplemented with FBS was added to each well to achieve a final volume of 2.0 mL. Plates were incubated for 24 h in a humidified atmosphere with 5% CO₂. Then, Alexa 488 labeled formulations were added to each well after removing 500 μL of the media, and mixing 400 μL of media with 100 μL of the prepared formulations. Formulations were incubated for 2 h prior to imaging. After 1.5 h, DRAQ 5 (Excitation/emission: 647/681, Life Technologies, Carlsbad, CA) was added to each well and incubated for 15 min to stain the nucleus. The wells were washed twice with the medium and imaged live on a laser scanning confocal microscope (FV-1000, Olympus Co., Tokyo, Japan).

11. _In vitro_ tumor spheroids inhibition and imaging.

SJSA cells were seeded in a rounded-bottom, ultra-low attachment spheroid microplates (Corning Inc., Corning, NY) at a density of 500 cells per well in 100 μL DMEM/F-12 media (Life Technologies, Carlsbad, CA) supplemented with 10% FBS, 100 U/mL penicillin, 200 μg/mL streptomycin, and 0.25 μg/mL Fungizone (Lonza, Basel, Switzerland). Spheroids were formed after incubating at 37 °C, 5% CO₂, overnight. Afterwards, an additional 50 μL of PTX, Abraxane®, or chemically loaded, physically loaded, or dual-loaded PTX micelles, prepared in the same feeding media, was added to the wells to a final concentration of 1 μM PTX. In order to prepare the PTX in media, 0.25% v:v DMSO (final concentration) was used to solubilize PTX. Control consisted of 0.25% DMSO in media. The spheroids were imaged on day 3 and day 7 following addition of the treatments using trans-illumination on a confocal microscope in order to measure the cross-sectional area. After 7 days, the spheroids were stained with LIVE/DEAD® stain (calcein-AM with ethidium homodimer-1, Life Technologies) _per_
To measure the tumor-killing efficacy of the treatments, the tumor spheroids were imaged at 200× magnification on a laser scanning confocal microscope (FV-1000, Olympus Co.) with a laser wavelength of 635 nm using a transmitted light detection unit at a resolution of 256 x 256 pixels. Images were analyzed in ImageJ (NIH, v. 1.50i) using the Bio-Formats package (v. 5.5.0) by drawing a region of interest around the spheroid and using the measure function to determine the cross-sectional area. To image the LIVE/DEAD® stained spheroids, laser wavelengths of 488 nm (calcein-AM “live” stain), 543 nm (ethidium homodimer-1 “dead” stain), and 635 nm (transmitted light) were used along in depth scanning mode (step size = 10 μm). Maximum intensity z-stack projections of the fluorescent images were then overlain on their corresponding transmitted light images.
Scheme S1: Synthesis polyphosphoester PEBP-\textit{b-}\textit{PBYP-g-PEG}.
Scheme S2: Synthesis of PTX conjugated polyphosphoester PEBP-b-PBYP-g-PTX/PEG.

Br-CO-OH + NaN₃ $\xrightarrow{\text{DMF, } 45^\circ C, \text{ 7 d}}$ N₃-CO-OH $\xrightarrow{\text{DCC, DMAP, DCM, reflux, 5 d}}$ Azido-PTX

PEBP-b-PBYP-g-PEG + 10 Azido-PTX $\xrightarrow{\text{CuBr, PMDETA, DMF, 37 }^\circ C, \text{ overnight}}$ PEBP-b-PBYP-g-PTX/PEG

PTX
Scheme S3: PEBP-b-PBYP-g-PTX/PEG labeling of Alexa 488 dye.
Figure S1. $^1$H NMR and $^{31}$P NMR of PEBP-$b$-PBYP-$g$-PEG in CDCl$_3$. 
Figure S2. $^1$H NMR of azido-PTX in CDCl$_3$. 

Figure S3. $^1$H NMR and $^{31}$P NMR of PEBP-$b$-PBYP-$g$-PTX/PEG in CDCl$_3$. 
Figure S4: Zeta-potential of nanoparticles: (A) PTX chemically conjugated NP, (B) PTX physically loaded NP, and (C) PTX dual loaded NP.

Figure S5. PTX release from physically loaded NP (black) and dual loaded NP (red).
Figure S6. Confocal laser scanning microscopy analysis of the cellular uptake of Alexa Fluor 488 labelled PTX chemically conjugated NP (A-C), and PTX dual loaded NP (D-F) into OVCAR cancer cells. Blue: DRAQ-5 stained nucleus; Green: Alexa Fluor 488 labelled polymer.

Figure S7. In vitro cytotoxicity against OVCAR-3 cancer cells. Numbers in parentheses are calculated IC$_{50}$ values. For the dual loaded NP, only physically loaded PTX were used to calculate IC$_{50}$ values. Data are represented as mean ± SD.
Author contributions

F. Z. and K. L. W. conceived and designed the project; F. Z., S. K., R. L., J. A. S and A. A. J. performed the experiments; F. Z., S. K., R. L., J. A. S., S. Z., G. Z., L. S., M. E., X. C. and K. L. W. analyzed data and co-wrote the paper; K. L. W. supervised all studies. All the authors have approved the final version.