Supplementary Information

Experimental Section:

Materials. All solvents and reagents were purchased from Sigma-Aldrich and used as received unless otherwise noted. 5-methyl-5-benzyloxycarbonxyl-1,3-trimethylene carbonate (TMCC-Bn) was synthesized as previously reported. [1] 3,6-dimethyl-1,4-dioxane-2,5-dione) and 1-[3,5-bis-(trifluromethyl)phenyl]—3-[(1*R*,2*R*)-(-)-2(dimethylamino)cyclohexyl] thiourea (Strem Chemicals, Newburyport, MA) and NH_2-PEG-OMe (10 kg/mol, Rapp Polymere, Tubingen, Germany) were used as received. Docetaxel was purchased from LC Laboratories (Woburn, MA). Peptide synthesis reagents, including Fmoc-Amino Acids, were purchased from AnaSpec (Fremont, CA).

Instruments. ^{1}H NMR spectra were recorded at 500 MHz at room temperature using an Agilent DD2 spectrometer. Molecular weights and polydispersity of P(LA- co-TMCC) were measured by gel permeation chromatography (GPC) in THF (containing 0.25% tetrabutyl ammonium bromide) relative to polystyrene standards at room temperature on a Waters 515 HPLC pump with a RI detector (VE3580) and a UV detector (KNAUER 2500) at a flow rate of 0.6 mL/min. Chemicals shifts (δ) are in ppm. Fluorescence and absorbance measurements were performed with the Tecan Infinite M200 pro fluorescent plate reader. Docetaxel was quantified using an Agilent 1100 HPLC equipped with an AB Sciex API 4000 triple quadropole mass spectrometer with electrospray ionization source detector. Serum stability studies were performed on a GE AKTA purifier 10 Fast Protein Liquid Chromatography System equipped with a UV900 monitor.

Synthesis of P(LA-co-TMCC)-g-PEG. P(LA-*co*-TMCC) was synthesized as previously described. [2] Briefly, 3,6-dimethyl-1,4-dioxane-2,5-dione (10.4 mmol) and 5-methyl-5-benzyloxycarbonyl-1,3-trimethylene carbonate (2.3 mmol) were co-polymerized for 7 days in dry, distilled dichloromethane (10 mL) under argon by a ring opening polymerization initiated with 1-pyrenebutanol (0.0625 mmol) and catalyzed by 1-[3,5-bis-(trifluromethyl)phenyl]—3-[(1*R*,2*R*)-(-)-2(dimethylamino)cyclohexyl] thiourea (0.35 mmol). GPC analysis revealed a backbone M_n of 12,869 and a PDI of 1.09. Following purification by silica column to remove the catalyst, the copolymer was deprotected for 7 days using Pd/C catalyst (20 wt%) in 50:50, v/v THF:EtOAc under hydrogen gas (91% yield). NH_2-PEG-OMe was grafted to the backbone at 3 PEG/backbone, as previously described. [3] P(LA-co-TMCC) (100 mg) was dissolved in DMF (5 mL) to which N,N'-diisopropylcarbodiimide (DIC, 100 uL) and hydroxybenzotriazole (HOBt, 16.88 mg) were added, and the solution was stirred for 30 min at room temperature. NH_2-PEG-OMe (PEG, 10 kg/mol, 416 mg) was dissolved in DMF (5 mL) and added to the copolymer solution under argon. The reaction was stirred at room temperature for 24 h, after which 500 uL of borate buffer (pH 9, 500 mM) was added and the solution was dialyzed against distilled water (2 kg/mol MWCO). Unreacted PEG was removed using a Sepharose CL-4B column equilibrated with distilled water. Collected fractions with polymer were combined and lyophilized to give a white solid (~60% yield). ^{1}H NMR ($CDCl_3$): δ1.23 (m, CH_3 from TMCC), 1.57 (m, CH_3 from LA), 3.64 (bs, CH_2 from PEG), 4.34 (m, CH_2 from TMCC), and 5.16 (m, CH from LA). Average PEG conjugation was calculated by end group analysis comparing area under the curve for PEG (3.64 ppm) vs. LA (5.17 ppm) as previously reported. [3]

Synthesis of P(LA-co-TMCC-co-TMCC-Bn)-g-PEG (P_{Bn}). P(LA-*co*-TMCC-Bn) was synthesized as described above with controlled deprotection of the benzyl group. Deprotection by palladium-catalyzed hydrogenolysis was monitored by ^{1}H NMR in order to control the number of pendant benzyl groups. Copolymers were synthesized with 2 or 5% benzylated monomers (20 or 50% Bn-TMCC). 3 PEGs were grafted onto the benzylated copolymer as described above, and purified by Sepharose CL4B column to remove free PEG. The final product, P_{Bn}, was lyophilized and characterized by ^{1}H NMR (72% yield). ^{1}H NMR (DMSO-d_6): δ1.28 (m,

CH_3 from TMCC), 1.47 (m, CH_3 from LA), 3.51 (bs, CH_2 from PEG), 4.29 (m, CH_2 from TMCC), 5.22 (m, CH from LA), 7.36 (m, aromatic protons from Bn-TMCC).

Synthesis of P(LA-co-TMCC)-g-PEG,DTX (P_{DTX}). P(LA-*co*-TMCC) was synthesized as previously described. A Steglich esterification was performed to graft docetaxel onto the backbone as previously reported. [4] Briefly, the copolymer (100 mg) was dissolved in distilled DCM (5 mL) and pre-activated with N,N'-dicyclohexylcarbodiimide (DCC, 17.2 mg) and 4-dimethylaminopyridine (DMAP, 10.2 mg) for 30 min with stirring under Ar. Docetaxel was dissolved in DCM (5 mL) and added dropwise to the polymer solution over ice. The solution was sealed under argon and stirred for 2 h over ice followed by 22 h at room temperature. The solution was filtered to remove dicyclohexylurea, and then extracted against water (3 times) and 0.5 M ammonium chloride (1 time). The organic fraction was collected and dried with magnesium sulfate and filtered. The dichloromethane was removed by rotary evaporation, the polymer precipitated into cold hexane and dried under vacuum (with any remaining docetaxel removed in the subsequent step of PEG grafting). PEG was grafted as reported above (3 PEG/polymer backbone), and dialyzed against water, prior to purification by Sepharose CL4B column to remove free PEG. The final product, P_{DTX}, was lyophilized and characterized by ^{1}H NMR (81% yield, 2.5 DTX/polymer backbone). ^{1}H NMR ($CDCl_3$): δ1.25 (m, CH_3 from TMCC), 1.58 (m, CH_3 from LA), 3.64 (bs, CH_2 from PEG), 4.26 (m, CH_2 from TMCC), 5.17 (m, CH from LA), 8.12 (br, ortho-aromatic Hs on DTX).

Synthesis of P(LA-co-TMCC)-g-PEG, TBP (P_{TBP}) or P(LA-co-TMCC)-g-PEG, Scrambled (P_{SCR}). P(LA-*co*-TMCC) was synthesized as previously described. The copolymer (200 mg, 12,000 g/mol) was dissolved in distilled DCM (5 mL) and pre-activated for 30 min with DIC (15.5 uL) and HOBt (13.5 mg). 3,3'-dithiobis(propionic hydrazide) (23.8 mg) [5] was pre-dissolved in 5 mL of distilled DCM with a few drops of DMF to increase solubility, before being added dropwise to the pre-activated polymer. The solution was sealed under argon and stirred. After 24 h, DCM was removed by rotary evaporation and the product was precipitated into hexane and dried under vacuum. PEG was grafted as described above (3 PEG/polymer backbone). After Sepharose CL4B purification to remove unreacted PEG, the polymer was reduced using DTT (50 eq) in PBS (1x, pH 7.5) for 6 h. The product was dialyzed against dilute HCl (pH 4.5) to ensure free thiols remain stable and to remove byproducts. An Ellman's assay was used to quantify free thiols against an L-Cysteine standard curve (1.1 thiols/backbone). 3-maleimidopropinoic-taxol binding peptide (Mal-PGFAPLTSRGSQQYAAG) and 3-maleimidopropinoic-scrambled peptide (Mal-PRSAYAIFGGSQPQTLG) were synthesized by conventional solid-phase microwave peptide synthesis techniques (CEM Liberty 1). The peptide was dissolved in phosphate buffer (0.1 M, pH 8) with a small amount of acetonitrile to increase solubility. The polymer-SH solution was added dropwise to the peptide, and the pH of the final solution was increased to 7 using NaOH. The polymer was allowed to react with the peptide for 48 h, followed by quenching of the unreacted thiols with N-(2-hydroxyethyl) maleimide (20 eq) for 24 h. The final solution was dialyzed for 2 days against 0.1 M Arginine and 0.1 M NaCl to remove unreacted peptide (20 kg/mol MWCO) followed by dialysis against water for 24 h to remove salts. The final products, P_{TBP} or $P_{Scr,}$ were lyophilized and characterized by amino acid analysis (84% and 62% yield respectively, 0.8 peptide/polymer backbone).

Nanomicelle formation by dialysis. Polymer (4 mg) and DTX (2.4 mg) were dissolved in DMF (1 mL) to which 50 uL of borate buffer (pH 9, 500 mM) was added and the solution left at room temperature for 15 min. The borate buffer is added to reduce nanomicelle size as described in detail previously. [2] 0.5 mL of distilled water was added dropwise at a rate of ~1 drop every 3 s. The solution was dialyzed against distilled water for 24 h, changing the water six times (MWCO 2 kg/mol). The solution was centrifuged to remove free DTX aggregates prior to use and characterization. DTX concentration was quantified by HPLC-MS/MS by diluting micelle solutions into 80:20 v/v acetonitrile: water and comparing to a DTX standard curve (3.125 ng/mL-

200 ng/mL) using paclitaxel as an internal standard (100 ng/mL). Loading was normalized between groups by calculating based on the molecular weight of the modified-hydrophobic backbone (P(LA-co-TMCC)) in order to avoid measuring differences due to a shift in the hydrophobic-hydrophillic balance.

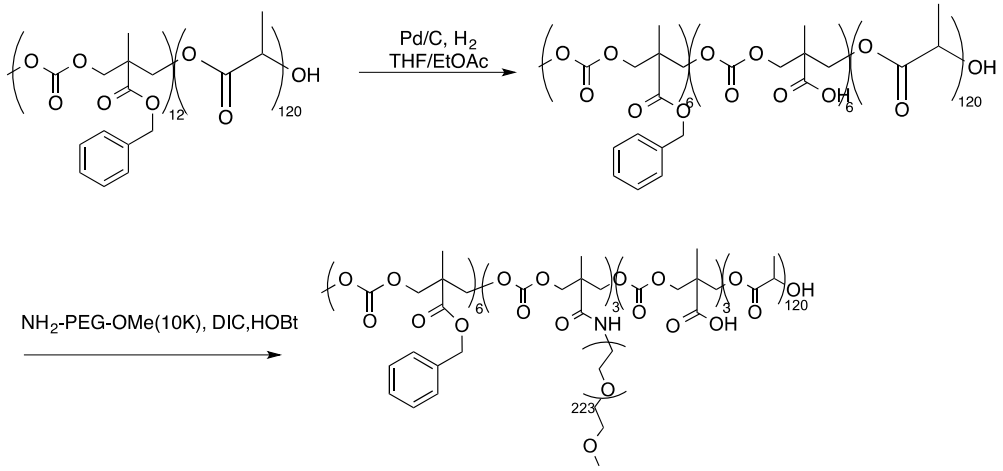
Dynamic light scattering (DLS), Zeta Potential and Transmission electron microscopy (TEM). The hydrodynamic diameter was quantified at 25°C using a Malvern Zetasizer Nano ZS, equipped with a 4 mW, 633 nm laser. All samples were prepared at a concentration of 1 mg/mL and centrifuged to remove aggregates prior to use. Hydrodynamic diameters (d_h) were measured in polystyrene cuvettes (Kuvetten, Germany) and calculated using the Strokes-Einstein equation $d_h = k_BT/3\pi\eta D$, where k_B is the Boltzmann constant, T is the absolute temperature, η is the solvent viscosity and D is the diffusion coefficient. The autocorrelation functions of the scattered intensity were analyzed by means of the cumulant method to yield the effective diffusion coefficient (D) as a function of the scattered angle. Micelles prepared from P_{TBP} were concentrated to ~13 mg/mL polymer using a centrifugal filter device (Millipore, MWCO 30 kg/mol). Zeta potential was measured using folded capillary cells (Malvern, DTS 1060). The average of three individual samples, prepared under the same conditions with 36 runs each is reported. For TEM, a sample (5 µL) was deposited onto a freshly glow-discharged 400 mesh carbon coated copper TEM grid (Ted Pella, Inc.) and allowed to adhere for 4 min. Excess liquid was removed with filter paper and particles then stained with 2% uranyl acetate (w/v, 5 µL, pH 4.3) for 15 seconds. The stain was removed and samples imaged using a Hitachi H-7000 microscope operating at 75 kV. Images were captured using an Advanced Microscopy Techniques (AMT) XR-60 CCD camera with typical magnifications between 50,000 – 100,000x. Particles (360 over 14 images) were analyzed using ImageJ 64 software.

In vitro serum stability. The stability of docetaxel-loaded micelles was determined using fast protein liquid chromatography by previously established method. [3,6] Docetaxel micelles at a polymer concentration of 1 mg/mL in 1x PBS (pH 7.4) were incubated with 20% fetal bovine serum (FBS, Charcoal Stripped) at 37 °C. At 0, 24 and 48 h, 500 µL aliquots were removed and injected onto a Superdex 200 gel filtration column. Samples were run with a flow rate of 1.5 mL/min and 1x PBS as the mobile phase. 1 mL fractions from the middle of the micelle peak (11 mL elution volume) were collected and DTX concentration was analyzed by HPLC-MS/MS. The drug loading was quantified as described above.

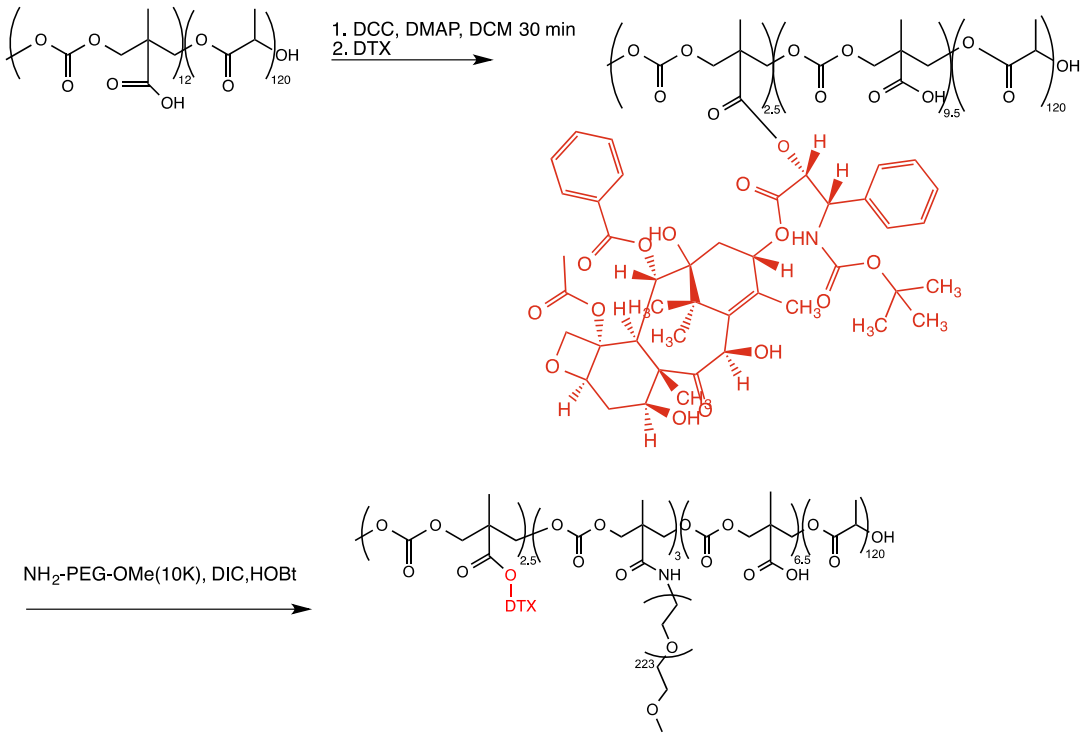
Cell culture cytotoxicity assay. SKBR-3 cells were cultured in McCoy's 5A media supplemented with 10% FBS and 1% penicillin-streptomycin in a humidified 37 °C incubator at 5% CO_2. Cells were seeded into 96-well flat-bottomed tissue culture plates at a density of 5,000 cells per well, and incubated for 24 h before use. Control and P_{TBP} micelles were synthesized as described, and dose-matched with free docetaxel to a concentration of 10 ng/mL (determined to be the IC_{50} of docetaxel in SKBR-3 cells). Dilutions of micelles were done with blank polymeric micelle solutions in McCoy's media (100 ug/mL polymer). Treatment with DTX was left on for 5 h, followed by media replacement and incubation for 48 h. At 48 h, the cell culture medium was replaced with 110 uL of Presto Blue solution (Life Technologies).[7,8] The plate was incubated for 2 h at 37°C, allowing viable cells to reduce the resazurin dye to the highly red fluorescent resorufin derivative. The fluorescence of individual wells was measured with an excitation at 560 nm and an emission at 590 nm by a microplate reader. Each measurement is an average of 6 repeat wells/plate, with 3 plates of separate passages of SKBR-3 cells.

Statistics. All statistical analyses were performed using Graph Pad Prism version 5.00 for Macintosh (Graph Pad Software, San Diego, California, www.graphpad.com). Differences among groups were assessed by one-way ANOVA with Bonferroni *post hoc* correction to identify statistical differences among three or more treatments. Alpha levels were set at 0.05 and a p-value <0.05 was set as the criteria for statistical

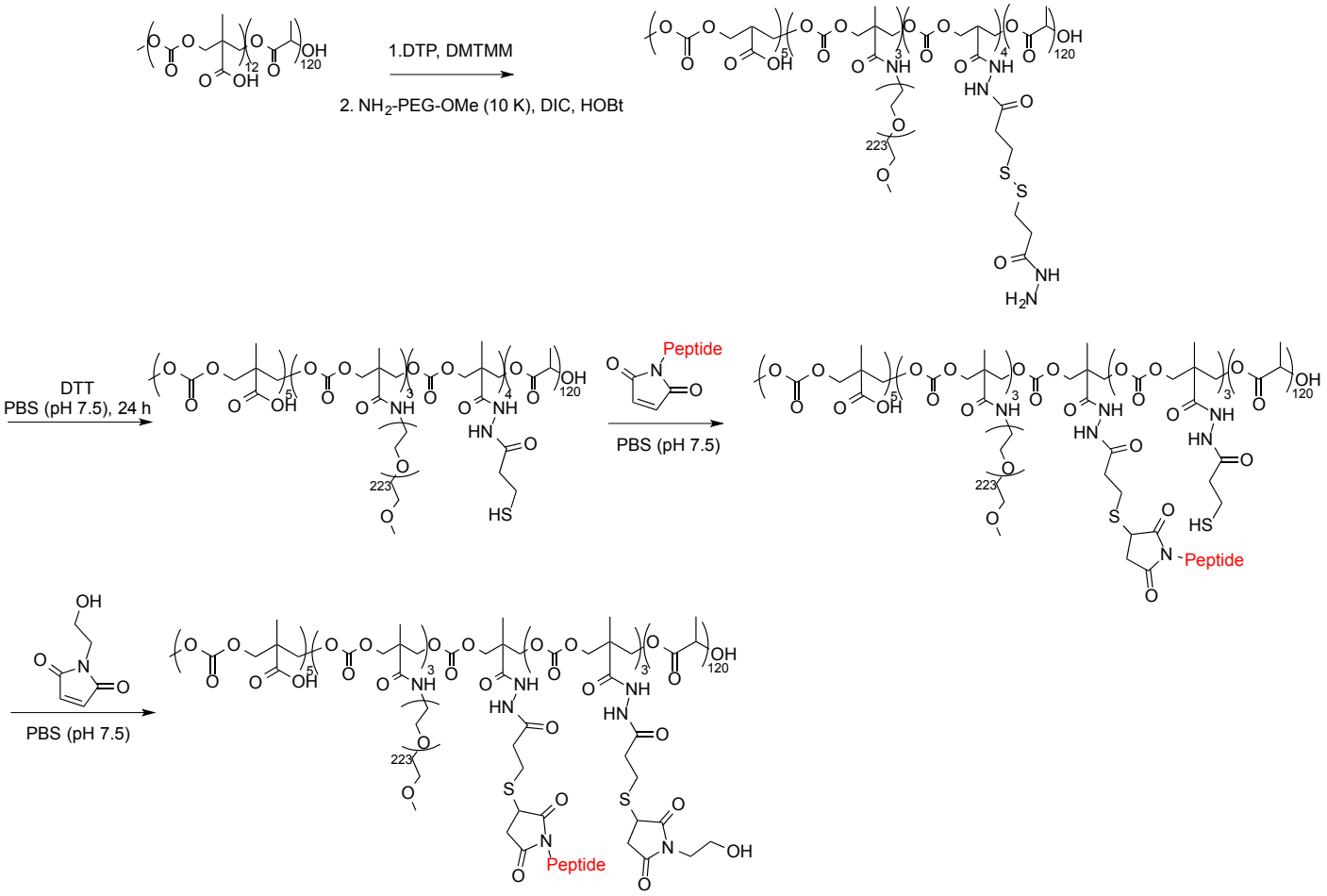
significance. Graphs are annotated where p-values are represented as *p<0.05, **p<0.01 or ***p<0.001. All data are presented as mean + standard deviation.



Scheme S1: By controlling the palladium catalyzed deprotection of benzyl groups, we achieved P(LA-*co*-TMCC-Bn) with as many as 6 benzyl groups per polymer backbone (50% of the TMCC groups or 5% of the polymer backbone)



Scheme S2: Steglich esterification was used to conjugate docetaxel (DTX) to P(LA-*co*-TMCC) backbone carboxylic acid functional groups, after which NH_2-PEG-OMe was coupled.



Scheme S3: Synthesis of P_{TBP} and the scrambled analog, P_{SCR}, were achieved by first modifying P(LA-co-TMCC) backbone carboxylic acid groups with DTP and NH_2-PEG-OMe followed by reduction of the DTP with DTT to expose thiol groups for Michael addition of maleimide-modified peptides: either (a) taxol binding peptide (TBP) – PGFAPLTSRGSQQYAA; or (b) scrambled peptide control (SCR) - PRSAYAIFGGSQPQTLG.

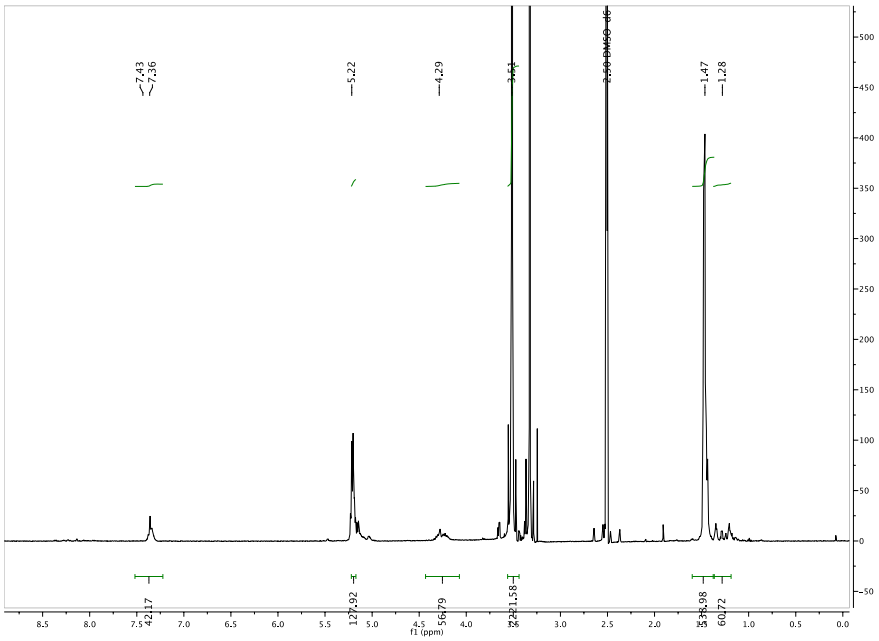


Figure S1: ^{1}H NMR of P_{Bn} (DMSO-d_6): δ1.28 (m, CH_3 from TMCC), 1.47 (m, CH_3 from LA), 3.51 (bs, CH_2 from PEG), 4.29 (m, CH_2 from TMCC), 5.22 (m, CH from LA). 7.36 (m, aromatic protons from Bn-TMCC).

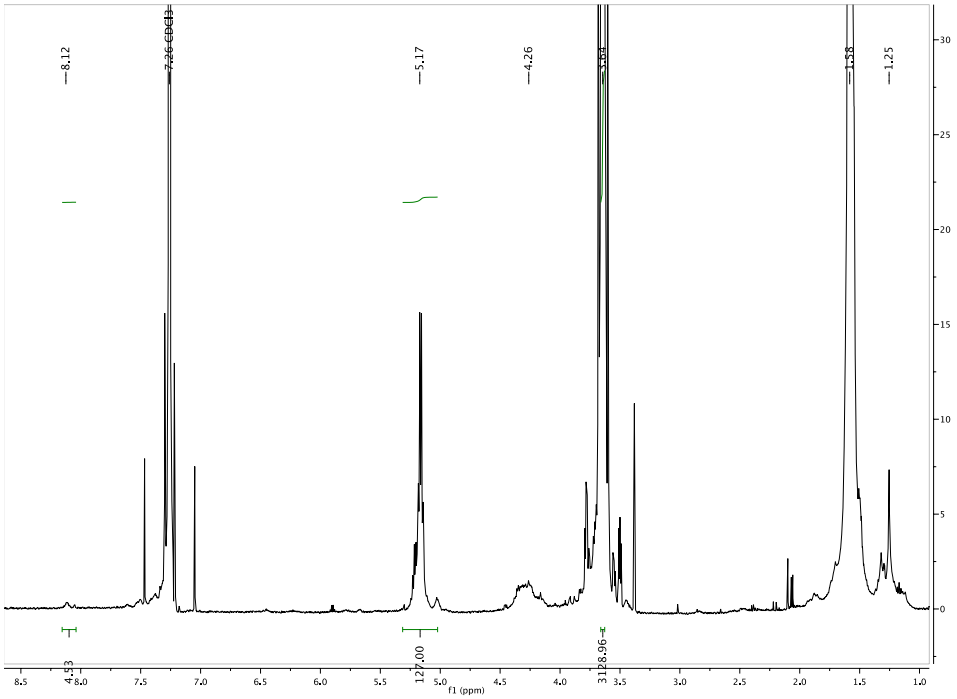


Figure S2: ^{1}H NMR of P_{DTX} ($CDCl_3$): δ1.25 (m, CH_3 from TMCC), 1.58 (m, CH_3 from LA), 3.64 (bs, CH_2 from PEG), 4.26 (m, CH_2 from TMCC), 5.17 (m, CH from LA). 8.12 (b, o-Bz on DTX).

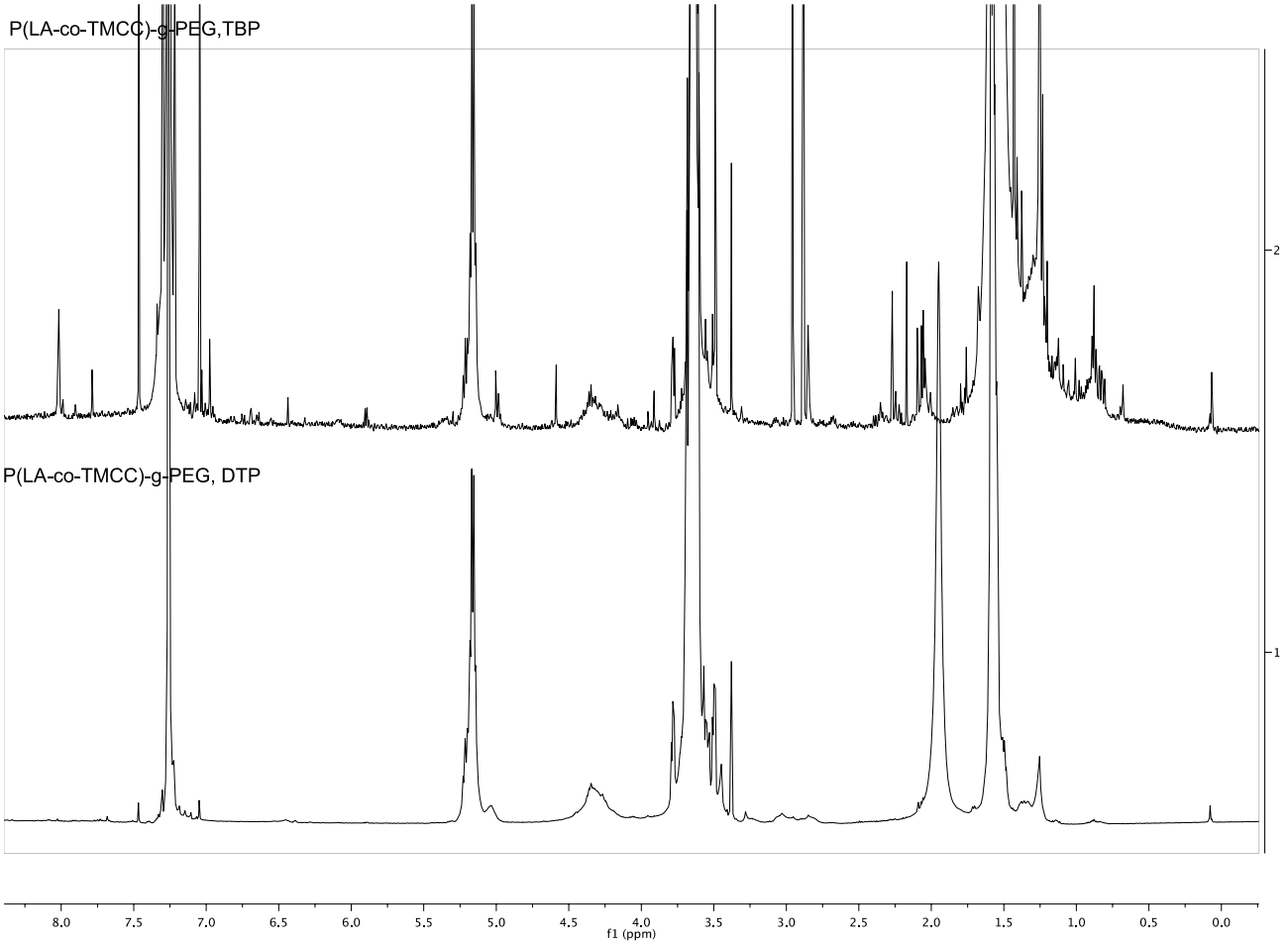


Figure S3: ^{1}H NMR of P(LA-*co*-TMCC)-g-PEG, DTP (bottom) and P(LA-co-TMCC)-g-PEG,TBP (top) showing successful conjugation of the PEG and peptide ($CDCl_3$): δ1.25 (m, CH_3 from TMCC), 1.58 (m, CH_3 from LA), 3.64 (bs, CH_2 from PEG), 4.26 (m, CH_2 from TMCC), 5.17 (m, CH from LA). Peptide conjugation was determined by amino acid analysis to be 0.8 peptides/backbone.

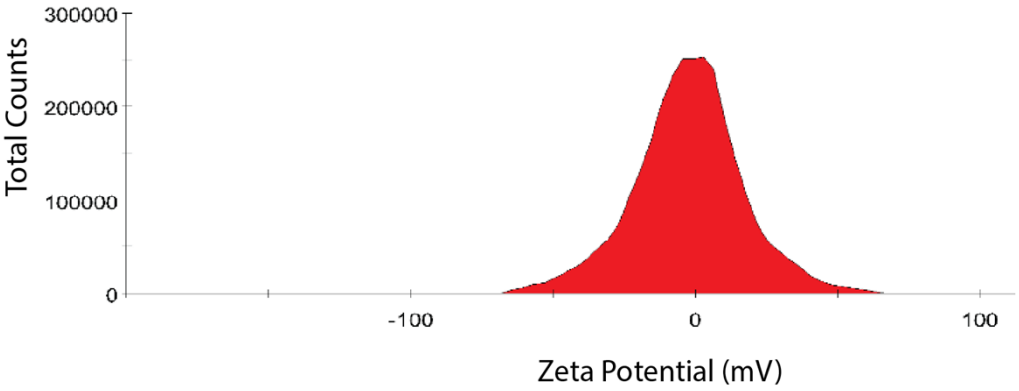


Figure S4: Zeta potential characterization of P_{TBP} micelles showing neutral zeta potential.

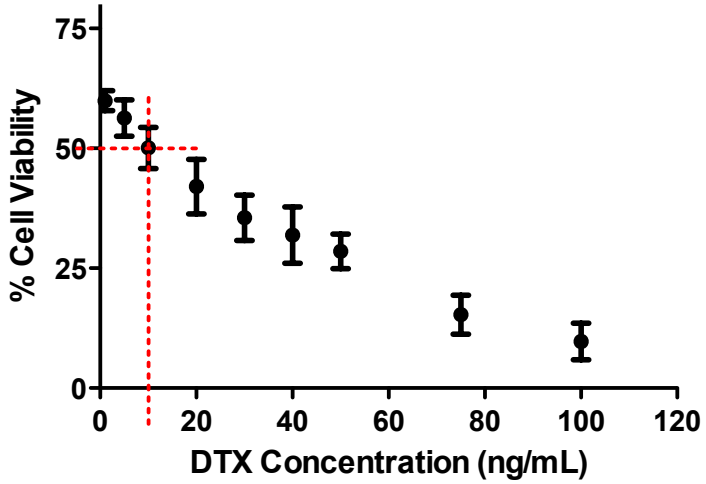


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Table S1: Average molar mass of polymers (measured by ^{1}H NMR end group analysis) and the corresponding diameters of self-assembled polymeric nanomicelles as measured by dynamic light scattering.

Polymeric Micelle	M_n (^{1}H NMR)*	Size (nm, mean ± SD)
Unmodified P(LA-*co*-TMCC)-*g*-PEG	40800	94.2 ± 3.5
P_{Bn}	37500	99.0 ± 7.6
P_{DTX}	41000	148.0 ± 0.4
P_{TBP}	39700	108.4 ± 15.9

*calculated by end group analysis comparing area under the curve for PEG (3.64) vs. LA (5.17) with backbone M_n calculated as previously reported[3]

References:

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