Electronic Supplementary Material (ESI) for Soft Matter.

Effect of side-chain length on solute encapsulation by amphiphilic heterografted brush copolymers

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Supporting Information

Materials, characterization methods and additional methods

All reagents were commercially available and used as received unless otherwise noted. Glycidyl methacrylate (GMA, Aldrich 97%) was passed through a short basic alumina column to remove the inhibitor. 2,2′-Azobis(isobutyronitrile) (AIBN) and D,L-lactide were recrystallized from methanol and anhydrous ethyl acetate, respectively. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) was kept over molecular sieves (3 Å) overnight. Deionized water was purified in a Barnstead Nanopure system to a final resistance of 18.2 mΩ; it will be referred to as Nanopure water.

Gel permeation chromatography (GPC) was performed on a Waters 1515 Isocratic HPLC equipped with two Styragel® columns (HR4 and HR3, 300 mm x 7.8 mm) connected in series, a differential refractive index detector (Waters 2414) and a UV-visible detector (Waters 2489). HPLC grade THF was used as the eluent, at a flow rate of 1 mL/min. Molecular weights are reported referenced to polystyrene standards (Shodex SL-105). 1H NMR spectra of polymers were recorded on a Bruker AV 400 MHz spectrometer in either CDCl3 or DMSO-d_6.

^{1}H NMR spectra of micelles were recorded in either D_2O or a mixture of D_2O and acetone-d_6. Spectra were referenced to $CDCl_3$ (7.26 ppm) or DMSO-d_6 (2.50 ppm).

Dynamic light scattering was conducted on a Malvern Instruments Nano-ZS ZetaSizer equipped with a 4 mW He-Ne laser operating at 633 nm. All measurements were performed at 25 °C at a scattering angle of 173°.

Fluorescence spectroscopy was carried out on a Fluorolog-3 system (HORIBA Jobin Yvon Inc., NJ).

Bright-field TEM imaging was performed on a FEI Technai 12 Twin transmission electron microscope operated at an acceleration voltage of 100 kV. All TEM images were recorded by a SIS Megaview III wide-angle CCD camera. TEM grids (carbon-coated copper or formvar-carbon grid, Electron Microscopy, Hatfield, PA) were ionized under plasma before preparation. Grids were placed on top of a single drop of sample suspension (20 μL) for 5 min, washed with 5 drops of doubly distilled water, and placed on a drop of 2 wt% aqueous uranyl acetate solution for 30 s. Excess solution was blotted off with filter paper and samples were allowed to dry at room temperature prior to imaging.

Refractive index measurements. The change in refractive index with concentration (dn/dc) was measured by an Optilab-rEX refractive index detector (Wyatt Technology) using a laser light wavelength of 658 nm. The temperature was set at 25 °C throughout the measurement. Samples were passed through 0.45 μm PVDF syringe filters (Thermo Scientific) and injected via a syringe pump (New Era Pump System, NE-1000) at a fixed velocity of 0.2 mL/min. Ten concentrations were surveyed for the polymer in dimethylformamide (anhydrous), ranging from 0.05 to 2 mg/ mL. Twelve samples were surveyed for nanoparticle suspensions in Nanopure water, ranging from 0.02 to 0.2 mg/mL. (dn/ dc) values were analyzed by the Astra 6.1 software. Azidolyzed polymer stock solution was prepared in dimethyformamide at 2.5 mg/mL and diluted to six samples with concentrations of 0.05, 0.1, 0.2, 0.35, 0.5 and 0.75 mg/mL.

Static light scattering measurements were performed with a DAWN HELEOS II (Wyatt Technology) using a 120 mW GaAs linearly polarized laser operated at 658 nm. Filtered samples

(described above for refractive index measurements) were injected at a fixed velocity of 0.2 mL/ min. Polystyrene (20 kDa, Fluka; 5 mg/mL solution in DMF) and dextran (9 - 11 kDa, Aldrich, 5 mg/mL solution in Nanopure water) were used as standards to normalize measurements for polymer and micelles respectively. (dn/dc) values were used to determine absolute molecular weights, and the weight average molecular weight (Mw) was extracted from Zimm plots using Debye and Zimm models (for the polymer and micelles, respectively) using Astra 6.1.

Polymer self-assembly. The polymer was dissolved in THF at a concentration of 10 mg/mL. Rapid self- assembly was carried out in a four-inlet vortex mixer described elsewhere.85 Nanopure water was charged into three 50 mL syringes (Hamilton, NJ) and the organic solution into a 10 mL syringe, and mounted on two separate syringe drivers (PHD Ultra, Harvard Apparatus). Flow rates of water and organic streams were 108 mL/min (water) and 12 mL/min (THF) to achieve a 10% (v/ v) THF concentration. Samples were collected and dialyzed (6-8 kDa MWCO, Fisherbrand) against Nanopure water for 24 h at 20 °C. Water was replenished every 4 h throughout the dialysis process. Samples were stored in clean scintillation vials, and unless otherwise noted, kept under refrigeration at 4 °C.

Flash nanoprecipitation. The protocol described above was used to prepare solute loaded nanoparticles, with the exception that both the polymer and the solutes (rose bengal lactone or probucol) were dissolved together in tetrahydrofuran. Dialyzed samples were collected and filtered through 0.45 μm PVDF syringe filters (Thermo Scientific) and stored in clean centrifuge tubes (Falcon® Tube). Drug loading capacity (DLC) and drug loading efficiency (DLE) of solutes in nanoparticles (NP) are estimated as shown below:

$$DLC(\%) = \frac{mass\ of\ drug\ in\ nanoparticles}{mass\ of\ polymer} \times 100 \quad ; \quad DLE(\%) = \frac{mass\ of\ drug\ in\ nanoparticles}{mass\ of\ drug\ used} \times 100$$

Drug loading capacity and efficiency were determined with a Varian Cary 50 UV/Vis spectrophotometer (Agilent Technologies, Santa Clara, CA). Loaded micelles were lyophilized and dissolved in DMF. Solute concentrations were calculated from calibration curves in DMF, with reference to their absorption at 565 nm (for rose bengal lactone) or 271 nm (for probucol).

Cryogenic transmission electron microscopy. Cryo-TEM imaging was performed on the FEI Tecnai 12 TWIN Transmission Electron Microscope, operating at 80 kV. 5 μL of sample solution were placed on a lacy carbon film, supported on a TEM copper grid (Electron Microscopy Services, Hatfield, PA). All the TEM grids used for cryo-TEM imaging were treated with plasma in air to render the lacy carbon film hydrophilic. A thin film of the sample solution was produced using the Vitrobot with a controlled humidity chamber (FEI). After loading of the sample solution, the lacy carbon grid was blotted using preset parameters and plunged instantly into a liquid ethane reservoir precooled by liquid nitrogen. Vitrified samples were then transferred to a cryo-holder and cryo-transfer stage, which was cooled by liquid nitrogen. To prevent sublimation of vitreous water, the cryo-holder temperature was maintained below -170 °C during the imaging process. All images were recorded by a SIS Megaview III wide-angle CCD camera.

Small-angle Neutron Scattering (SANS). Polymers were dissolved in DMSO-d6, THF-d7 or DMF-d8 at a concentration of 10 mg/mL. Micelles (starting polymer concentration of 1 wt %) were dialyzed against deuterium oxide overnight, and the dialysis mediums were collected as

solvent baselines. SANS experiments were performed on the NG-7 30 m SANS instrument at the National Institute of Standards and Technology, Center for Neutron Research. An incident wavelength of 6.0 Å was used with sample-detector distances of 1, 4 and 13 m to cover a q-range from 0.003 to 0.55 Å-1. All measurements were performed at ambient temperature. Raw data were reduced and analyzed by the IGOR Pro (WaveMetrics), using the SANS reduction and analysis packages provided by NIST27. The raw data were corrected for the background and empty cell scattering, sample and empty cell transmission, detector sensitivity and cell thickness. Data were rescaled to an absolute intensity by the beam flux method. Scattering from the solvent was subtracted in proportion to its volume fraction.

Synthesis Protocols

The synthesis of the PGMA backbone and its azidolysis were previously reported by our group. We therefore limit the discussion of synthesis methods to the last two steps of the reaction, as described below.

PLA "grafting from" ($PGMA_{721}$-g-PLA_{11}). $PGMA_{721}$-*g*-N_3 (150 mg, 0.81 mmol –OH group) and D,L-lactide (1.1685 g, 8.11 mmol) were loaded into a round bottom flask, and placed under high vacuum at 36 °C for ~ 5 hours. After backfilling with argon, anhydrous DMF (~ 35 mL) was added to dissolve the reagents. DBU (45.6 μL, 0.31 mmol) was then injected and the reaction was allowed to proceed for 1.5 h under argon at room temperature. Polymerization was quenched by addition of benzoic acid (93.4 mg, 0.76 mmol). DMF was removed under vacuum and the polymer was re-dissolved in THF, followed by precipitation into a mixture of Nanopure water and methanol (1:1, vol). Solids were lyophilized to remove water. Yield: 86.5%. ^{1}H NMR of the resulting grafted polymer is shown in **Figure S1**. The degree of polymerization of PLA side chains was calculated based on signals e and e'.

Synthesis of $PGMA_{721}$-g-PEG_{113}/PLA_{11}. Alkynl-PEG_{113} was grafted onto $PGMA_{721}$-*g*-PLA_{11} via 'click' chemistry, catalyzed by $CuSO_4$•$5H_2O$/ascorbic acid. Molar ratio of -N_3, alkynyl groups, $CuSO_4$•$5H_2O$ and ascorbic acid was kept at 1:1.05:0.2:1. $PGMA_{721}$-*g*-PLA_{11} (200 mg, 0.11 mmol) and alkynyl-PEG_{113} (617.1 mg, 0.12 mmol) were placed in a round bottomed flask and dissolved in DMF (16 mL). Ascorbic acid (19.9 mg, 0.11 mmol) was added and the solution was bubbled with argon for 30 min. Finally, $CuSO_4$•$5H_2O$ (5.5 mg, 0.02 mmol) was added under argon and the reaction was allowed to take place at room temperature for 12 h under argon. The product was purified by precipitation in diethyl ether and dried in vacuum oven. Yield: 96.1%. ^{1}H NMR spectrum of the polymer is shown in **Figure S2**.

Synthesis of PEG_{113}-b-PLA_{125}. mPEG_{113} (750 mg, 0.15 mmol) was loaded into a round bottom flask, and placed under high vacuum at 90 °C for ~1 h. After cooling and backfilling with argon, D,L- lactide (750 mg, 5.2 mmol) was added to the flask and placed under high vacuum at 36 °C for ~3 h. After backfilling with argon, 8 mL anhydrous dichloromethane was added to dissolve all reagents. DBU (8 μL, 0.05 ml) was then injected and the reaction was allowed to proceed for 1 h under argon at room temperature. Polymerization was quenched by addition of benzoic acid (15 mg, 0.12 mmol), followed by precipitation into 2-propanol. The ^{1}H NMR spectrum of the sample is provided in **Figure S3**.

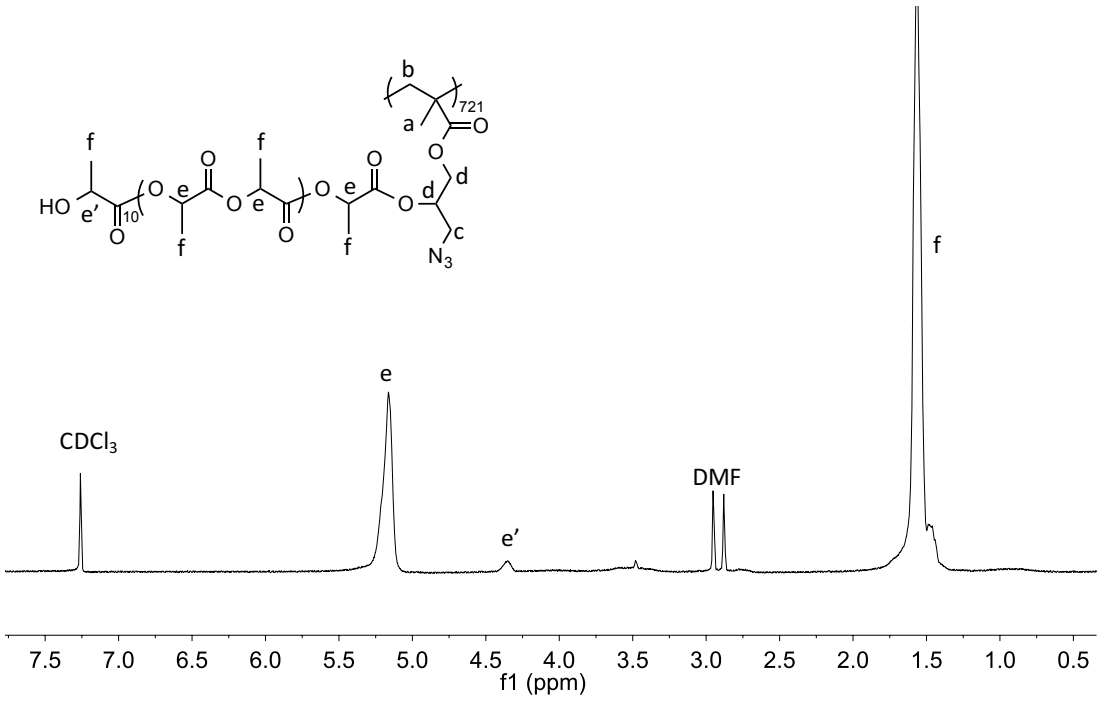


Figure S1. ^{1}H NMR spectrum and peak assignments of $PGMA_{721}$-*g*-PLA_{11}.

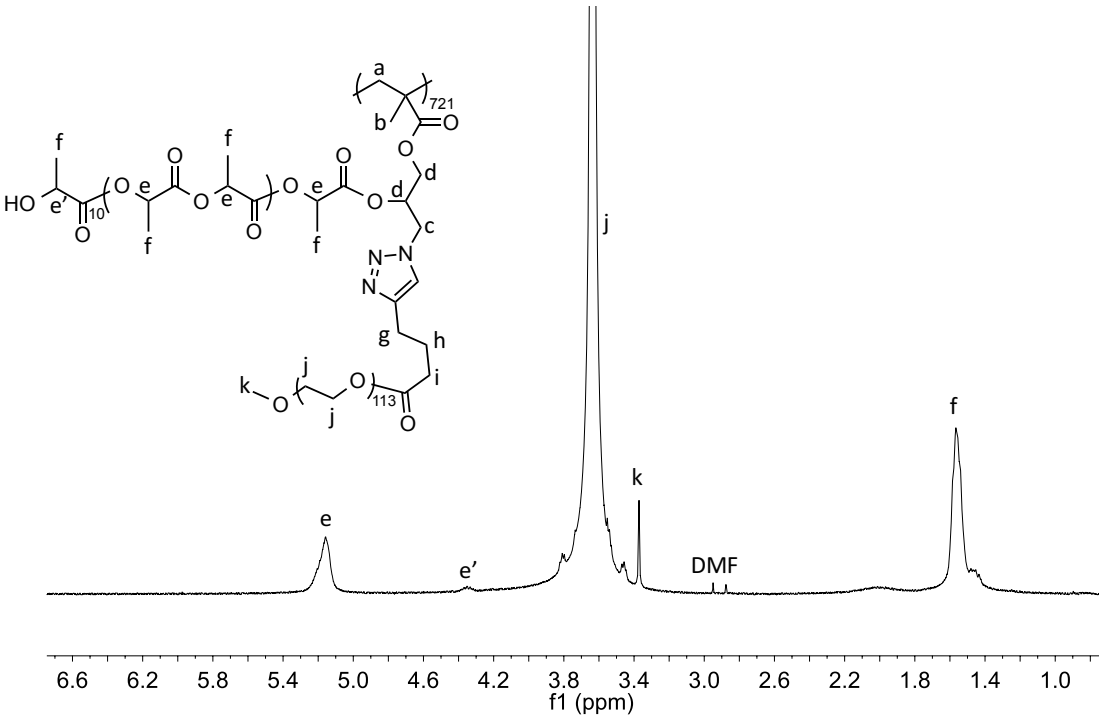


Figure S2. ^{1}H NMR spectrum and peak assignments of $PGMA_{721}$-*g*-PLA_{11}/PEG_{113}.

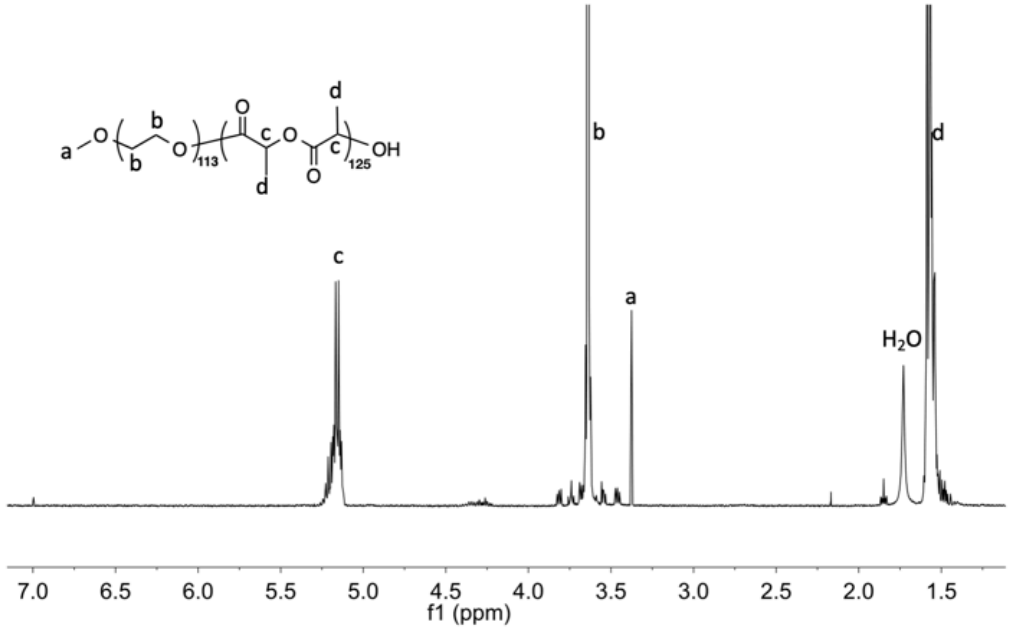


Figure S3. ^{1}H NMR spectrum and peak assignments of PEG_{113}-*b*-PLA_{125}.

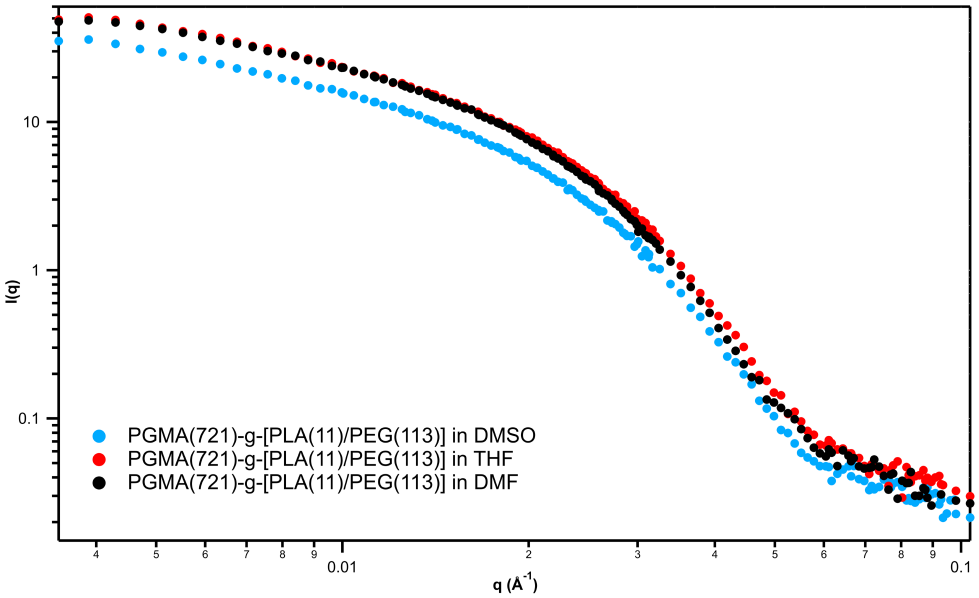


Figure S4. Small-angle neutron scattering profiles of **B2** double-bottlebrushes in three different good solvents, showing no variation among samples.

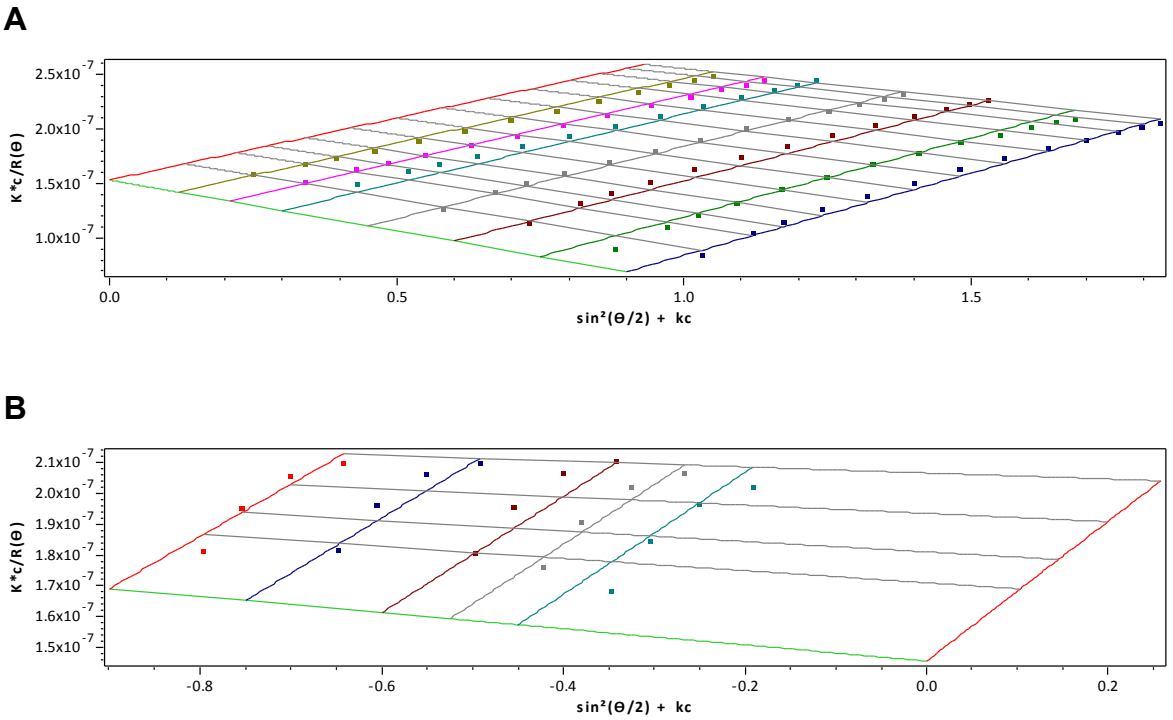
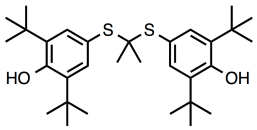
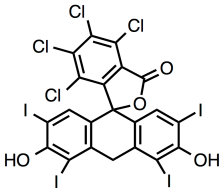


Figure S5. Static light scattering measurements of weight average molecular weights of **B2** in DMF (**A**, 6.5 × 10^6 Da) and water (**B**, 6.9 × 10^6 Da).

Table S1. Physicochemical properties of model drugs

Solute (model drug)	Probucol	Rose bengal lactone
Structure		
Molar mass (g/mol)	516.84	973.67
logP[a]	9.46	9.31
logP[b]	8.92	5.85
logS[b]	-7.1	-6.27
Solubility (mg/mL)[c]	4.11×10^{-5}	5.23×10^{-4}

[a] source: molinspiration.com; [b] source: ALOGPS; [c] calculated from logS.

Table S2. Summary table of Guinier-Porod fits of **B2** in water and RBL-loaded particles.

Solute feed (w/w_p, %)	Solvent	Dimension parameter, s	Radius of gyration (cross-sectional), $R_{g,cs}$ (nm)	Radius, R (nm)
0	DMF-d_7	0.7	6.7	9.5
0	D_2O	1.0	6.8	9.6
5	D_2O	0.9	7.2	10.2
15	D_2O	0.9	7.1	10.0

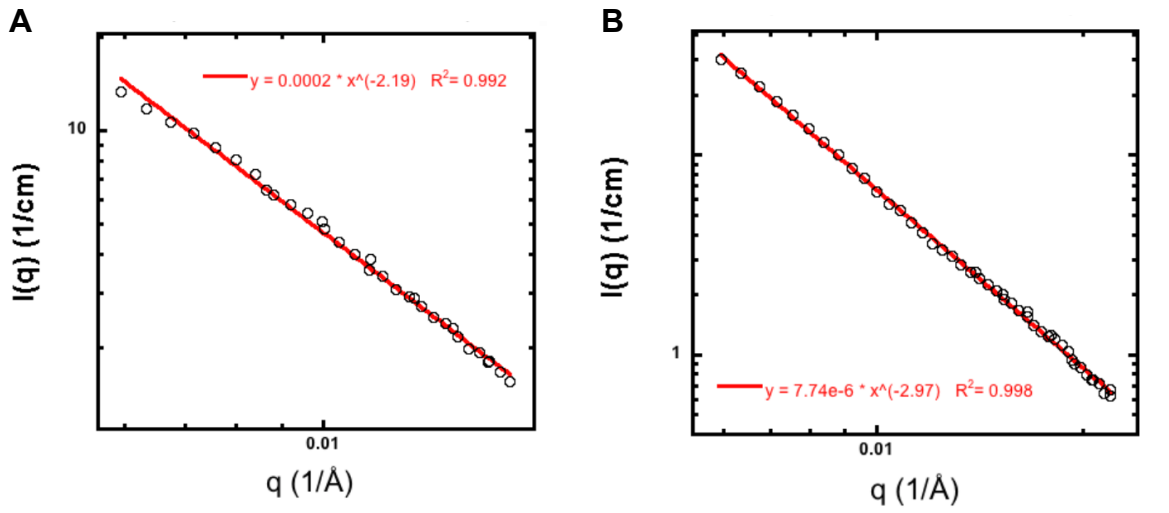


Figure S6. Results from power-law fits of SANS data for probucol-loaded **B2** at 5% w/w_p (**A**) and 15% w/w_p (**B**) solute feeds.

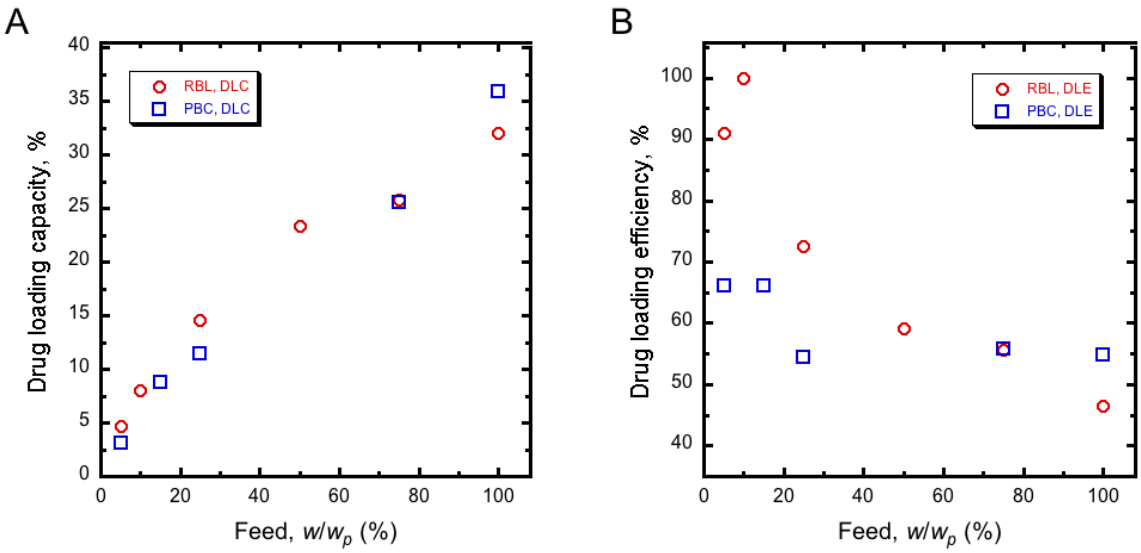


Figure S7. Drug loading capacity and efficiency of RBL and PBC nanoparticles stabilized by a linear diblock copolymer (PEG_{113}-*b*-PLA_{125}). DLC = mass drug in NP/mass polymer in NP; DLE = mass of drug in NP/mass of drug fed.