

Investigating the Self-Assembly and Shape Transformation of poly(ethylene glycol)-b-poly(D,L-lactide) (PEG-PDLLA) Polymersomes by Tailoring Solvent-Polymer Interactions

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Supporting Information

S1. Experimental Materials and Methods

Materials

All chemicals were used as received unless stated otherwise. For the synthesis of poly(ethylene glycol)-*b*-poly(D,L-lactide) (PEG-PDLLA), poly(ethylene glycol) 1K was purchased from JenKem technology and freeze-dried twice before use. D,L-lactide was purchased from Acros and used as supplied. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) was purchased from Sigma Aldrich and used as supplied. All other chemicals were supplied by Sigma-Aldrich and were used without any purification. Ultra pure MilliQ water, obtained from a Merck Millipore Q-Pod system (18.2 M Ω) with a 0.22 μ m Millipore® Express 40 filter, was used for the polymersome self-assembly and dialysis. Dialysis membranes MWCO 12-14000 g mol⁻¹ Spectra/Por® were used for dialysis during the tubes formation. Sodium chloride was purchased from Merck.

Methods

Nuclear Magnetic Resonance (NMR): Proton nuclear magnetic resonance measurements were performed on a Bruker 400 Ultrashield™ spectrometer equipped with a Bruker SampleCase autosampler, using CDCl₃ as a solvent and TMS as internal standard.

Gel permeation chromatography (GPC): GPC was conducted using a Shimadzu Prominence-*i* GPC system with a PL gel 5 μ m mixed D and mixed C column (Polymer Laboratories) with PS standard and equipped with a Shimadzu RID-20A differential refractive index detector and THF used as an eluent with a flow rate of 1 mL min⁻¹.

Dynamic Light Scattering (DLS): DLS measurements were performed on a Malvern instrument Zetasizer (model Nano ZSP) equipped with an autosampler. Zetasizer software was used to process and analyse the data.

Cryogenic transmission electron microscopy (cryo-TEM): Experiments were performed using a FEI Titan (300 kV electron source) equipped with a LaB₆ filament and an autoloader station. Samples for cryo-TEM were prepared by treating the grids (Lacey carbon coated, R2/2, Cu, 200 mesh, EM sciences) in a Cressington 208 carbon coater for 40 seconds. Then, 4 μ l of the polymersome solution was pipetted on the grid and blotted in a Vitrobot MARK III at 100% humidity. The grid was blotted for 3 seconds (offset -3) and directly plunged and frozen in liquid ethane.

S2.Experimental Procedures

Ring opening polymerization (ROP) of poly(ethylene glycol)-poly(D,L-lactide) PEG-PDLLA block copolymers:

PEG₂₂-PDLLA₄₅ was synthesized according to previous publication by ring opening polymerization starting from PEG as macro initiator and using DBU as a catalyst yielding PEG₂₂-PDLLA₄₅ polymer with ±80 % yield. ¹ Copolymer composition was determined by integrating the protons of PEG (3.65-3.7 ppm), terminal methyl unit (singlet at 3.37 ppm) and PDLLA CH (multiplet at 1.4-1.8 and 5.11-5.28 ppm). Đ values were calculated to be less than 1.2 using GPC with PS standards for calibration.

Preparation of spherical polymersomes:

In a 15 mL vial, PEG₂₂-PDLLA₄₅ was dissolved in 2 mL of THF and dioxane (4:1 v/v) unless stated otherwise and the vial was sealed with a rubber septum. The solution was stirred at 400 rpm for a minimum of 30 minutes prior to the addition of MilliQ (2 mL, 1 mL h⁻¹) *via* a syringe pump. The resulting cloudy suspension was transferred into a prehydrated dialysis bag (SpectraPor, MWCO: 12-14 kDa, 2 mL/cm). Dialysis was performed against MilliQ at room temperature for max. 24 hours with a water change after 1 hour.

Preparation of tubes:

Polymersomes were prepared according to the above mentioned method. Dialysis was performed against 50 mM NaCl at 5 °C for max. 24 hours with a water change after 1 hour.

Extrusion of spherical polymersomes:

In a 15 mL vial, PEG₂₂-PDLLA₄₅ was dissolved in 2 mL of THF and dioxane (4:1 v/v) unless stated otherwise and the vial was sealed with a rubber septum. 1 mL h⁻¹) *via* a syringe pump. The resulting suspension was extruded through an Avanti Mini Extruder with a 0.1 μm membrane filter in between filter supports. The solution was extruded for a minimum of 20 times. The resulting cloudy suspension was transferred into a prehydrated dialysis bag (SpectraPor, MWCO: 12-14 kDa, 2 mL/cm). Dialysis was performed against MilliQ at room temperature for max. 24 hours with a water change after 1 hour.

Shape transformation of extruded spherical polymersomes into elongated tubes:

Polymersomes were prepared according to the above mentioned extrusion method. Dialysis was performed against 100 mM NaCl at 5 °C for max. 24 hours with a water change after 1 hour.

S3. Supplementary Figures

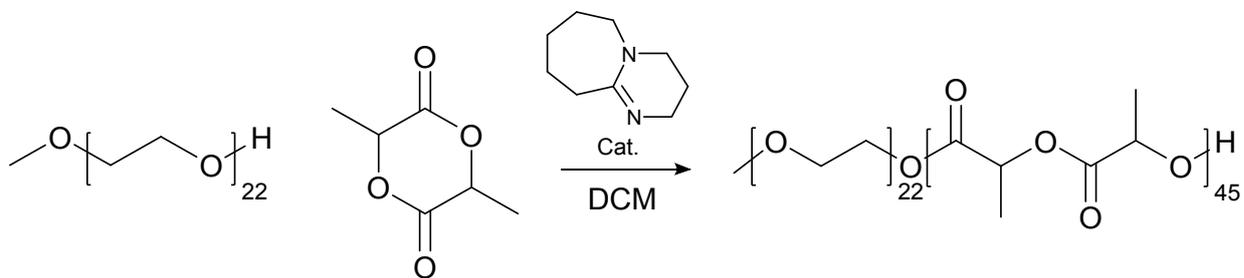


Figure S1: Organocatalyzed synthesis of PEG₂₂-PDLLA₄₅ using DBU as catalyst.

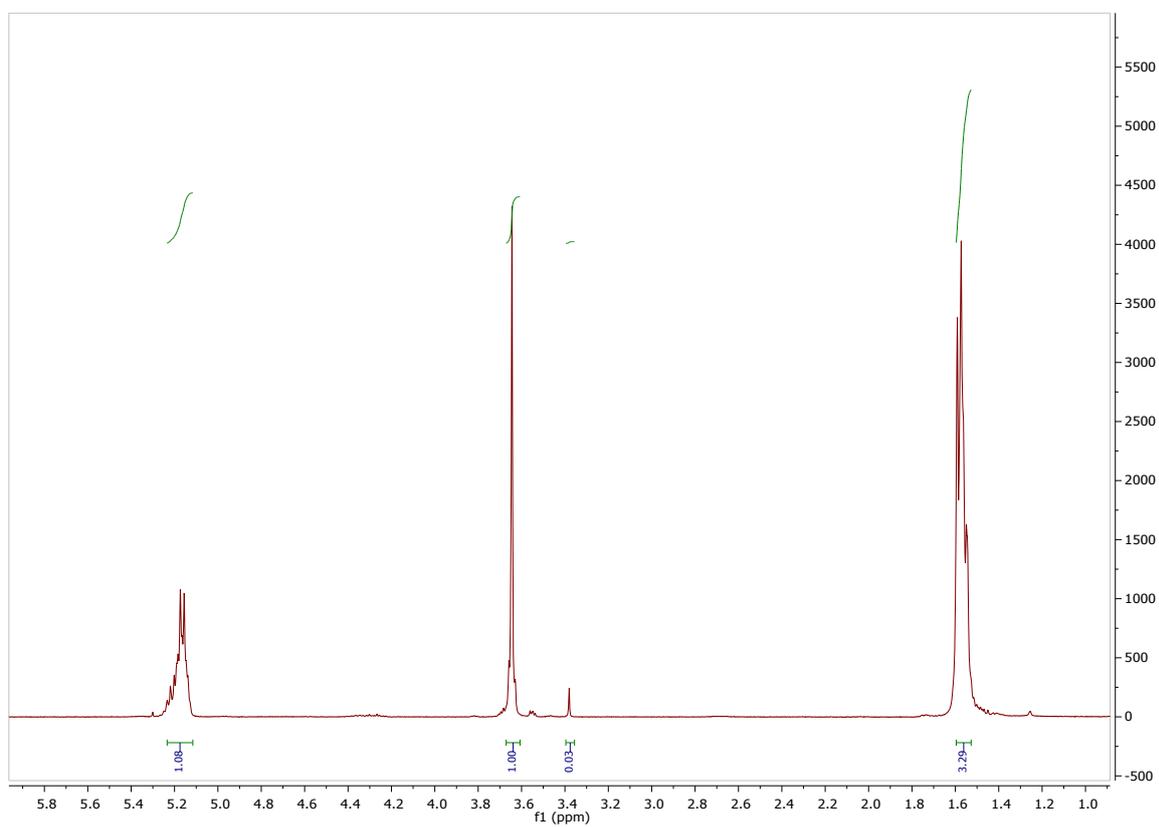


Figure S2: Typical ¹H-NMR spectrum of PEG₂₂-PDLLA₄₅

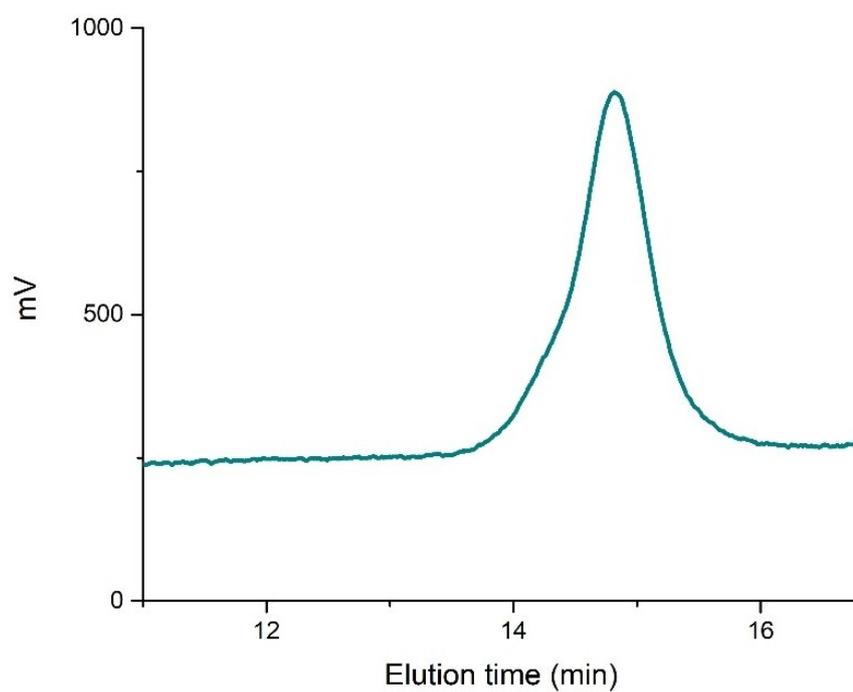


Figure S3: Typical GPC plot of PEG₂₂-PDLLA₄₅

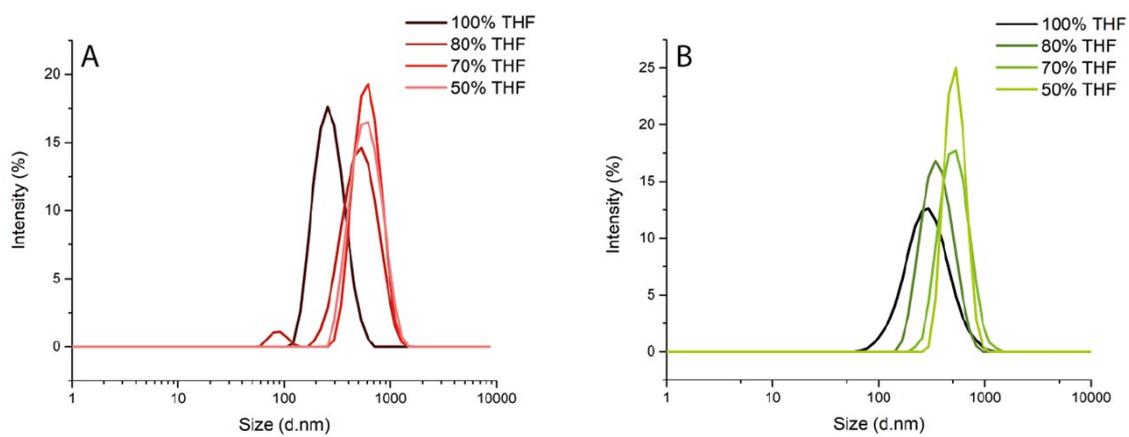


Figure S4: DLS intensity plots of spherical polymersomes (A) and tubes (B) prepared with various THF/Dioxane ratios.

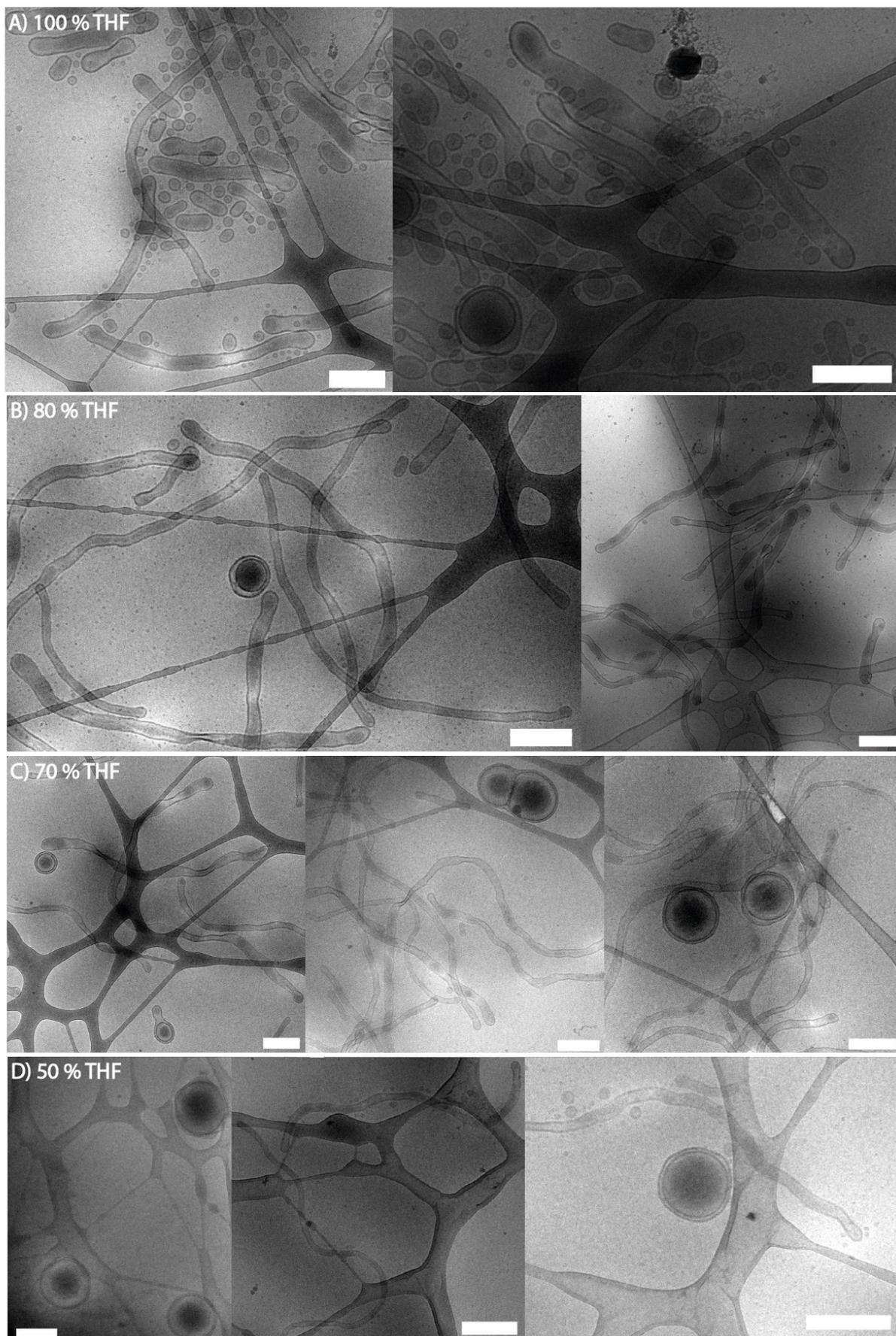


Figure S5: Cryo-TEM images of tubes prepared with THF/dioxane ratio A) 100/0, B) 80/20, C) 70/30 and D) 50/50. Scale bar is 500 nm

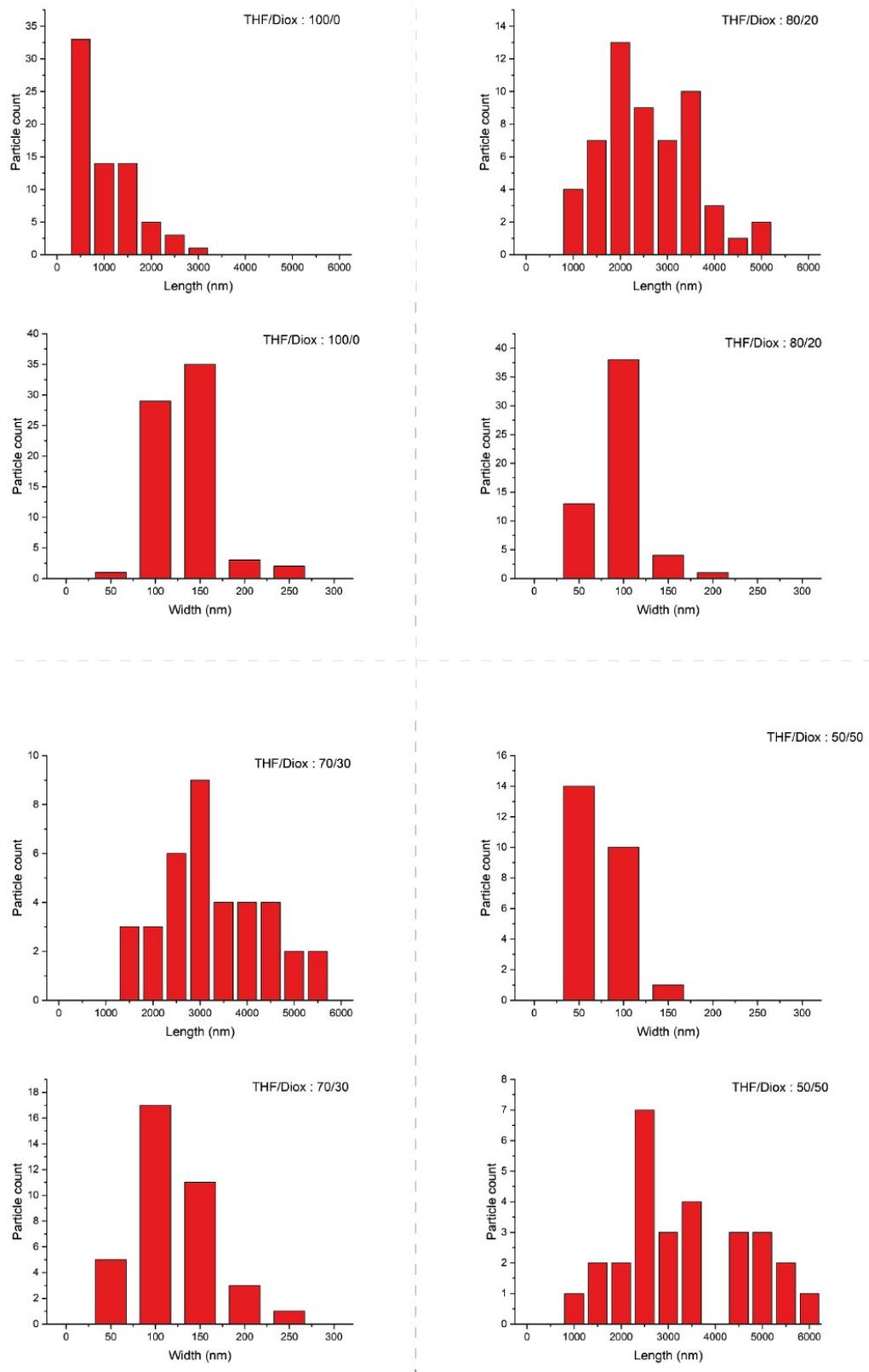


Figure S6: Bar graphs depicting the width and length of tubes measured from cryo-TEM images for each solvent ratio.

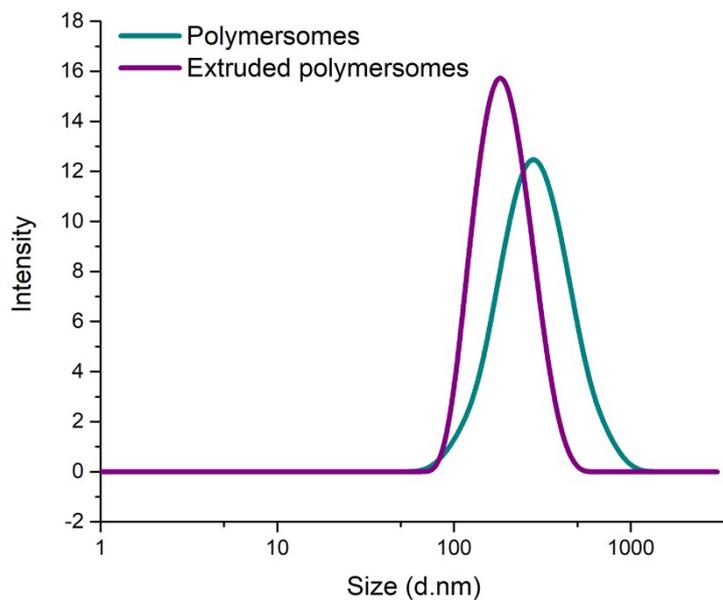


Figure S7: DLS intensity plot for polymersomes and extruded polymersomes demonstrating the size decrease after extrusion.

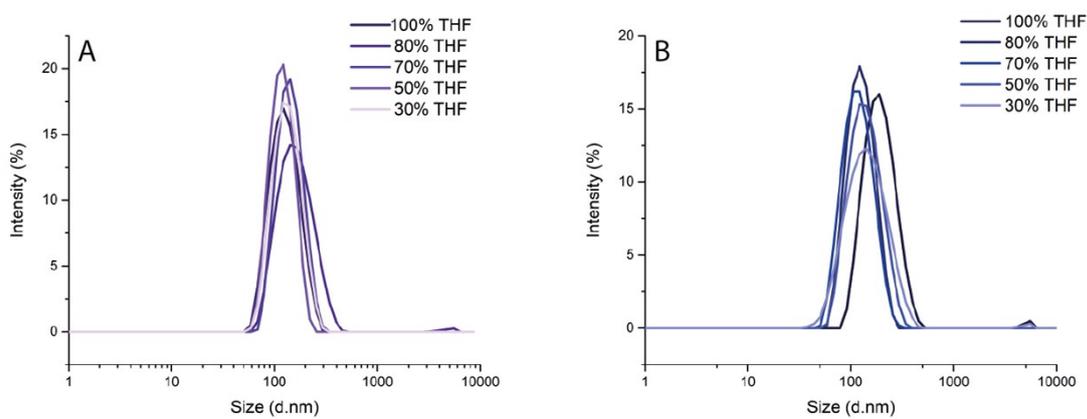


Figure S8: DLS data of extruded spherical polymersomes (A) and their counter tubes (B) prepared in various THF/dioxane ratios.

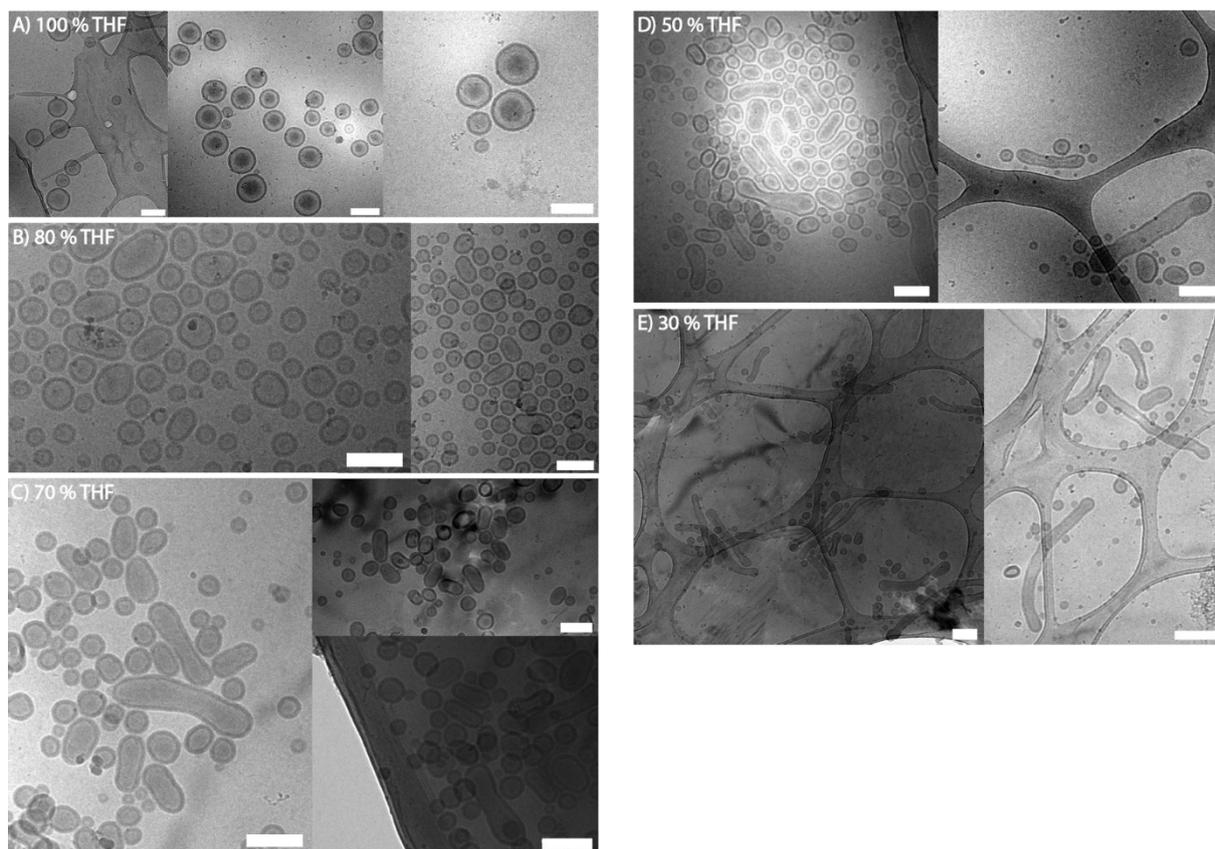


Figure S9: Cryo-TEM images of the outcome of the shape transformation process of the extruded spherical polymersomes, prepared with THF/dioxane ratio A) 100/0, B) 80/20, C) 70/30, D) 50/50 and E) 30/70. Scale bar is 200 nm.

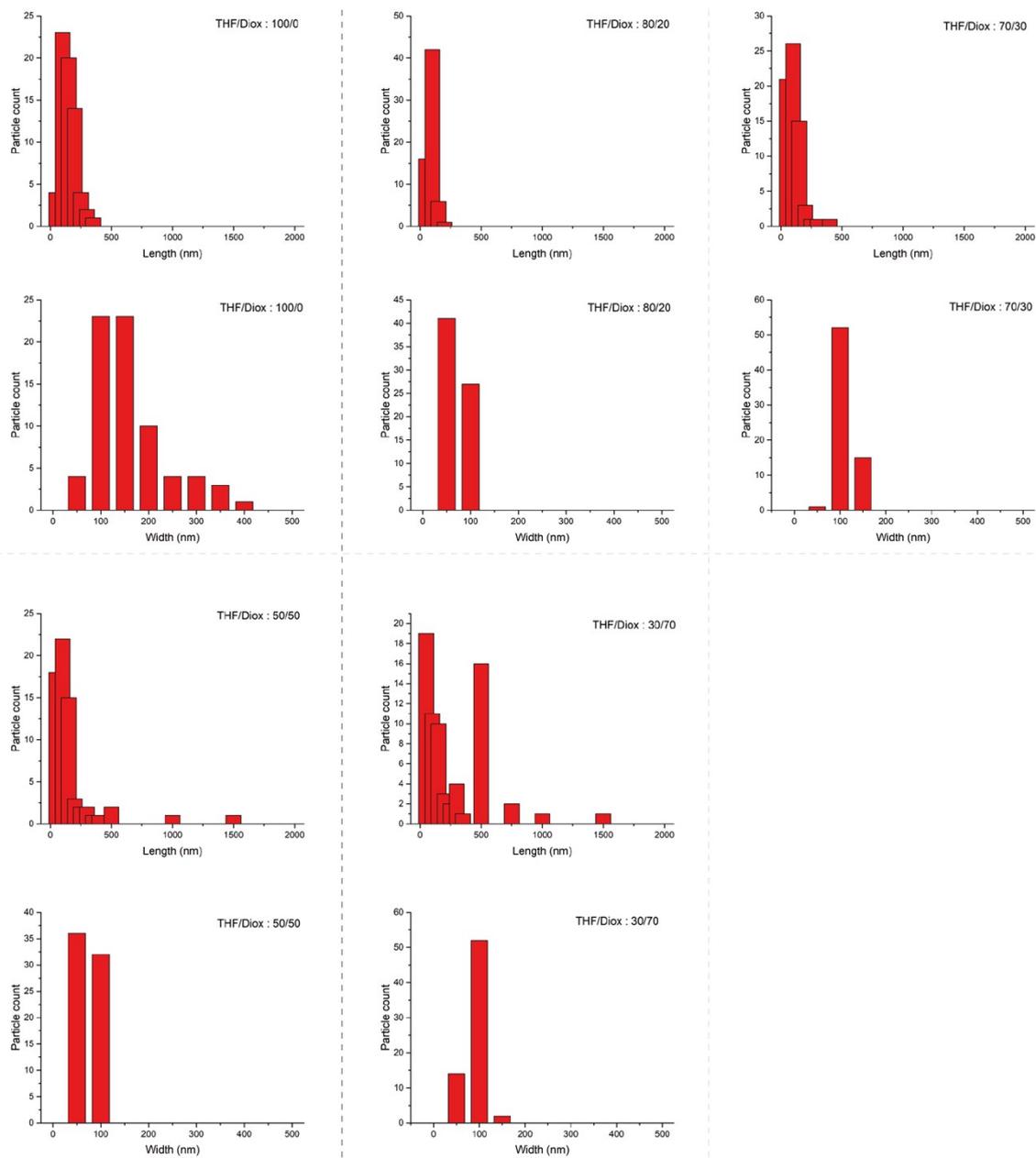


Figure S10: Bar graphs depicting the width and length of extruded tubes measured from cryo-TEM images for each solvent ratio.

References:

- 1 L. K. E. A. Abdelmohsen, D. S. Williams, J. Pille, S. G. Ozel, R. S. M. Rikken, D. A. Wilson and J. C. M. Van Hest, *J. Am. Chem. Soc.*, 2016, **138**, 9353–9356.