Membrane folding and shape transformation in biomimetic vesicles

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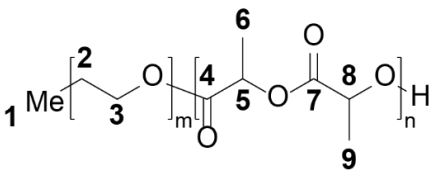
Supporting information

Materials and Methods

Nuclear Magnetic Resonance (NMR) was measured at 298 K on a Bruker 400 MHz Avance III HD nanobay spectrometer equipped with a 9.4 T Ascend magnet (400 MHz) and BBFO probe. Chemical shifts are given in parts per million (ppm) with respect to tetramethylsilane (TMS, δ 0.00 ppm) as internal standard for ^{1}H NMR. ^{1}H spectra were acquired using 48 scans and a relaxation delay of 9 seconds.



Poly(ethylene glycol)$_m$-b-poly(D,L-lactide)$_n$: **^{1}H NMR** ($CDCl_3$, 400MHz) δ 5.19 (m, 2n H, 5-C*H* + 8-C*H*), 3.64 (s, 4m H, 2-CH_2 + 3-CH_2), 3.38 (s, 3 H, 1-CH_3), 1.66-1.50 (m, 6n H, 6-CH_3 + 9-CH_3). **^{13}C NMR** ($CDCl_3$, 101MHz)**:** δ 169.58 (4-C + 7-C), 71.08 (2-C + 3-C), 68.61 (5-C + 8-C), 58.97 (1-C), 16.31 (6-C + 9-C).

Dynamic Light Scattering (DLS) measurements were performed on a Malvern Instruments Zetasizer (ZEN 1600), using Zetasizer Software (Malvern Instruments) for analysis of the data. Samples were loaded in Malvern disposable capillary cells. The average of three size measurements with 10 scans of 10 seconds was taken.

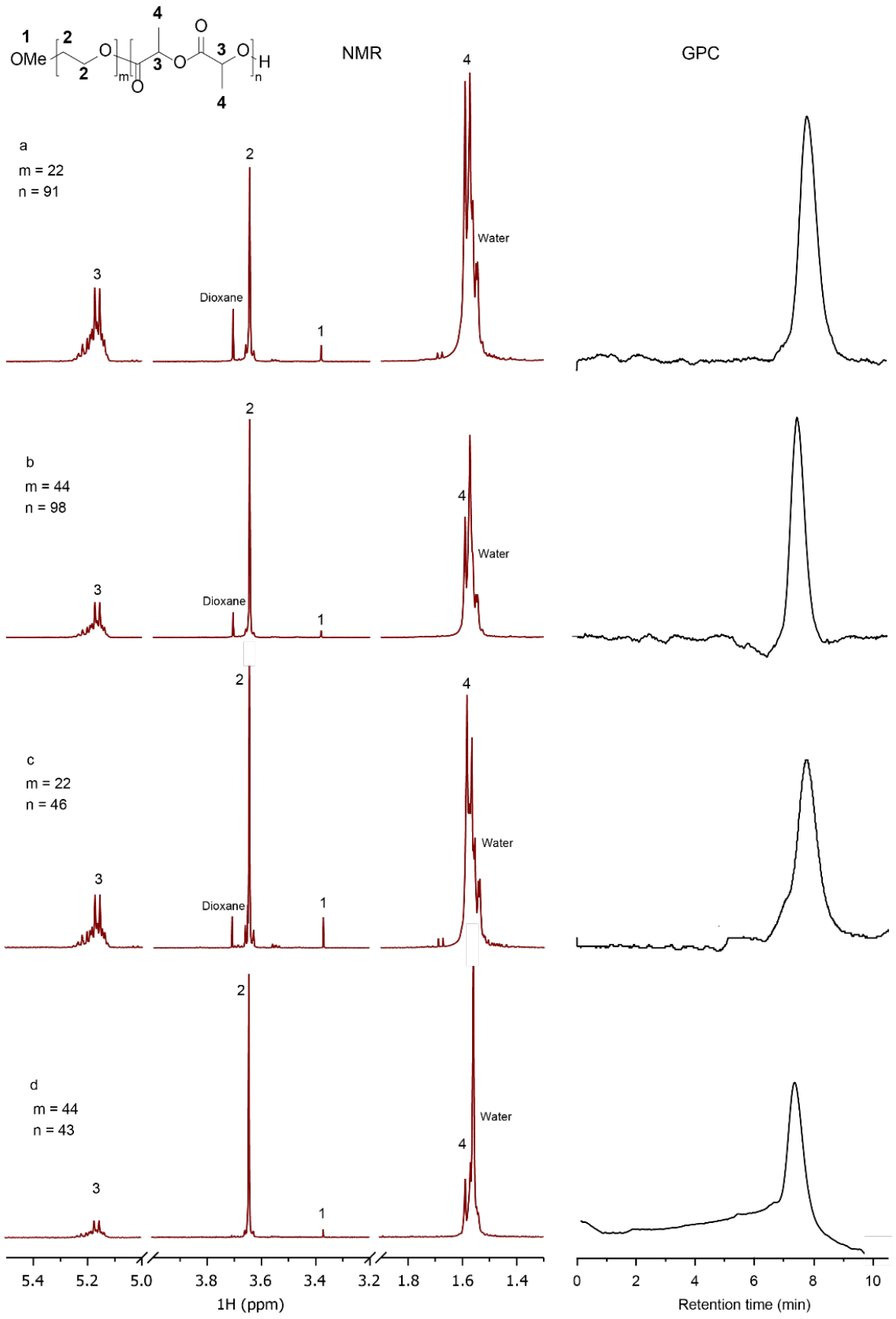
Cryogenic Transmission Electron Microscopy (cryo-TEM) pictures were taken on a JEOL TEM 2100 microscope (JEOL Japan). Analysis and processing of the data was performed using Fiji (a free program developed by NIH and available at https://fiji.sc/).

Protocol: EM Science TEM grids were glow discharged with a 208 carbon coater (Cressington). On each grid 3 µL of sample was added, blotted and immediately vitrified through freeze plunging into liquid ethane at 100% humidity using an automatic vitrification robot, FEI Vitrobot™ Mark IV (blot time 1 s, blot force 3). Samples were loaded in a 914 High tilt cryoholder (Gatan, Munich, Germany) and inserted into a JEOL Transmission Electron Microscope 2100 (Japan) at 200 kV. Images were taken with a 4096 x 4096 pixel CCD camera (Gatan). The average dimensions and membrane thickness of each sample were obtained from different regions (images) and analyzed with plot profile tools of Fiji.

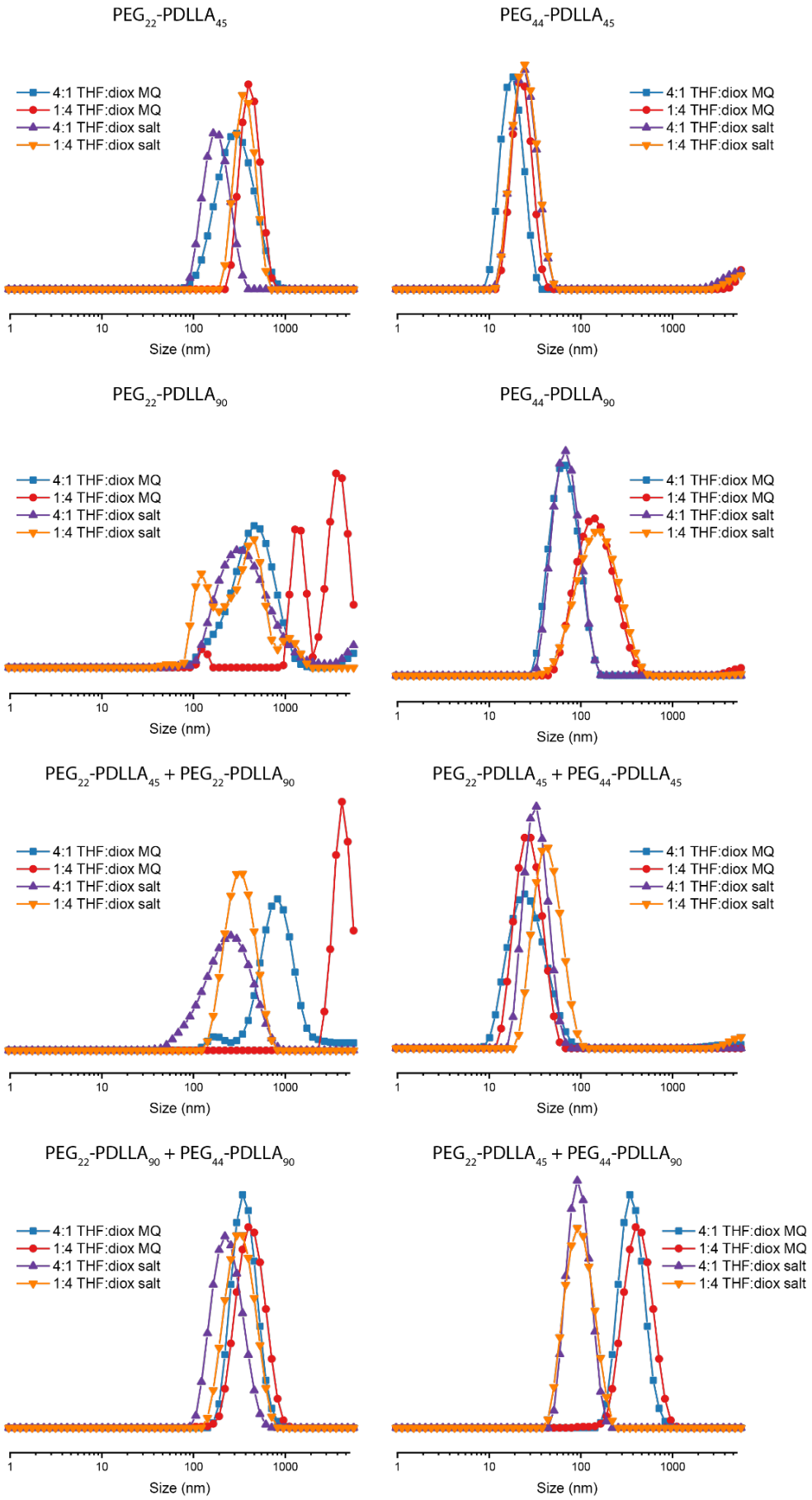
Supplementary Tables and Figures

Supplementary Table 1: *Overview of PEG-PDLLA block copolymer compositions*

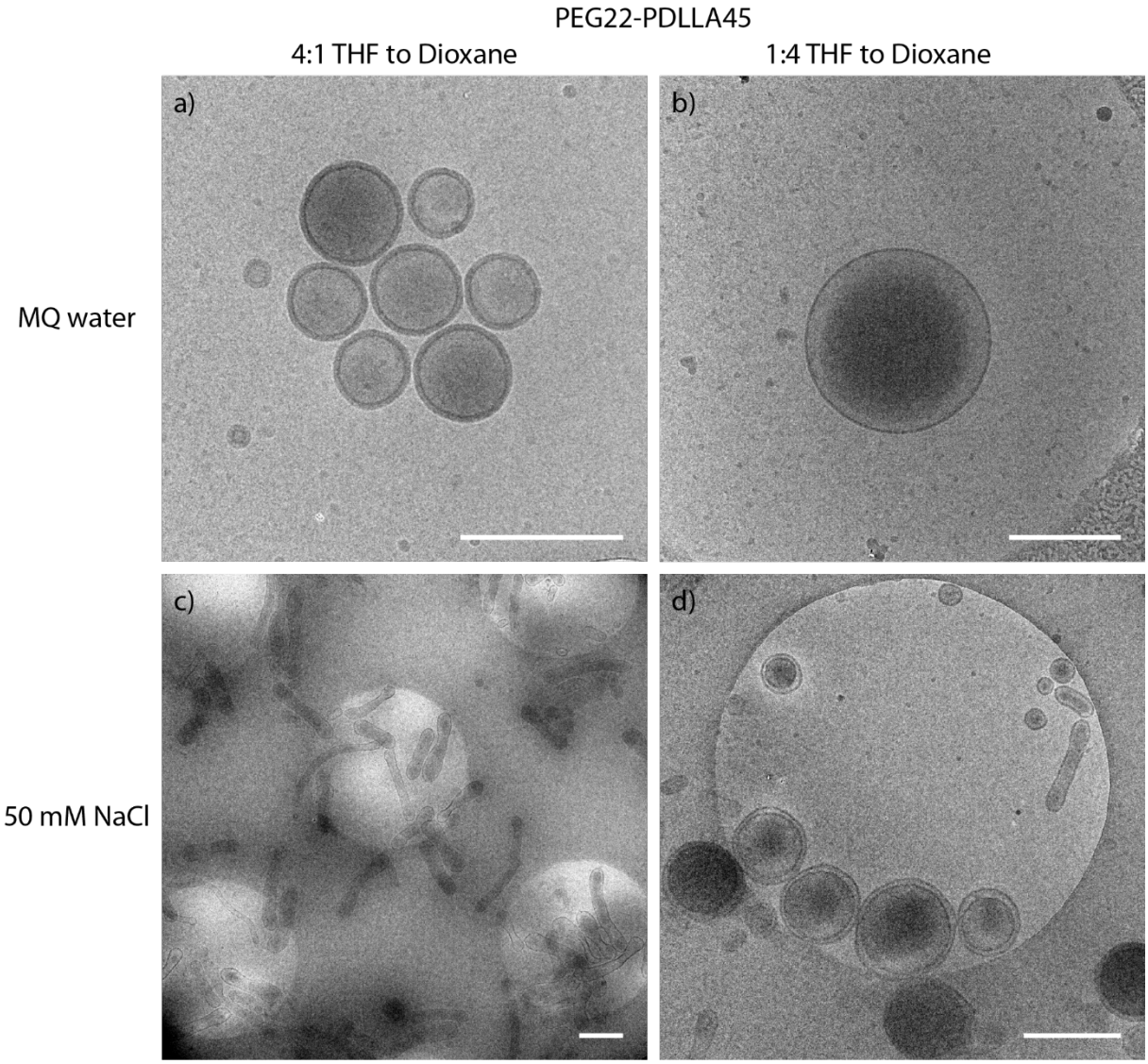
Polymer	Polymer composition (NMR)	PDI (GPC)
PEG_{22}-$PDLLA_{45}$	PEG_{22}-$PDLLA_{46}$	1.09
PEG_{44}-$PDLLA_{45}$	PEG_{44}-$PDLLA_{43}$	1.07
PEG_{22}-$PDLLA_{90}$	PEG_{22}-$PDLLA_{91}$	1.08
PEG_{44}-$PDLLA_{90}$	PEG_{44}-$PDLLA_{98}$	1.05



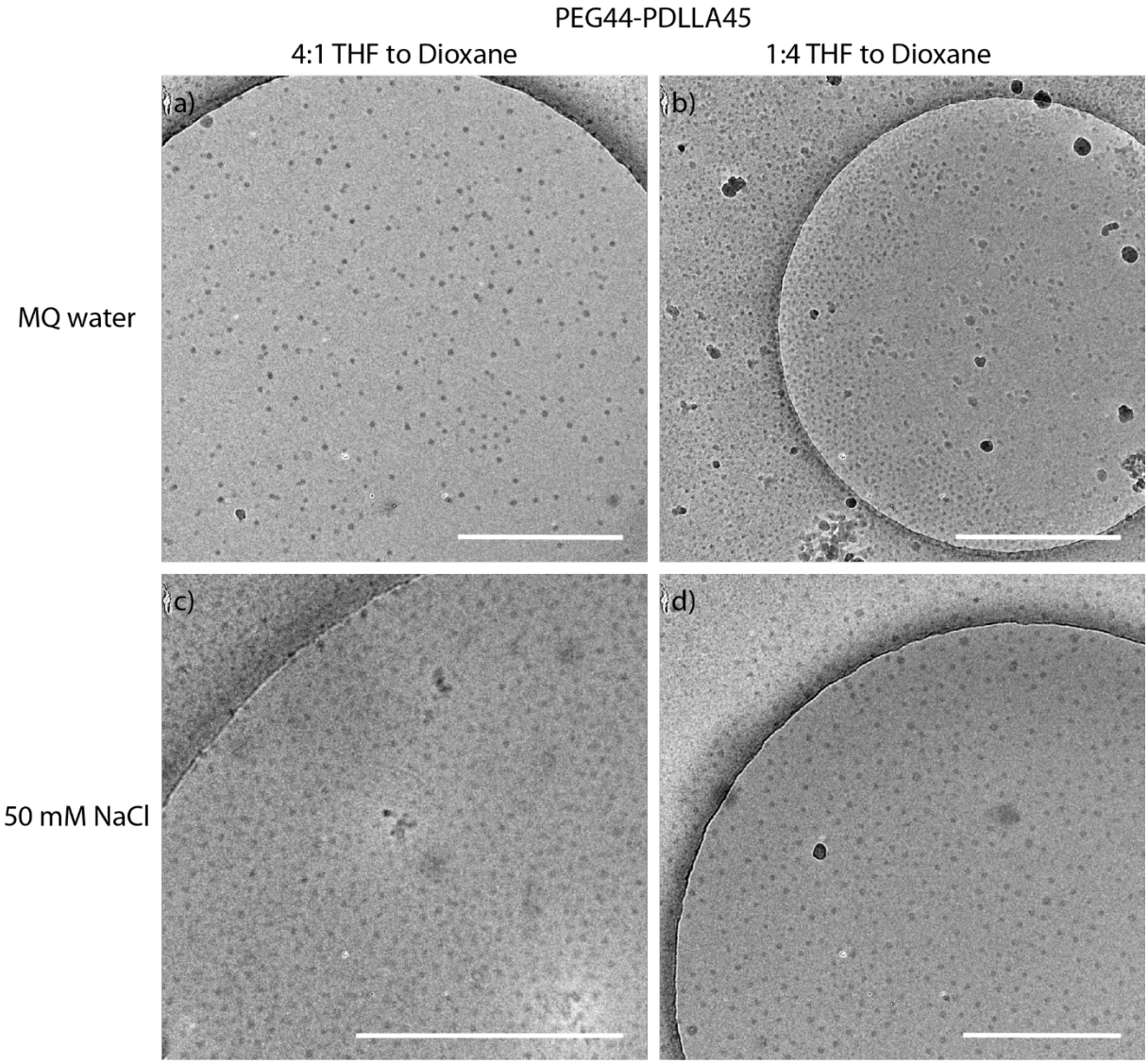
Supplementary Figure 1: Characterization of PEG-PDLLA polymers. NMR spectrum and GPC chromatogram of **a,** PEG_{22}-$PDLLA_{90}$ **b,** PEG_{44}-$PDLLA_{90}$ **c,** PEG_{22}-$PDLLA_{45}$ **d,** PEG_{44}-$PDLLA_{45}$. Compositions are calculated by integrating peaks 2 and 3 relatively to peak 1.



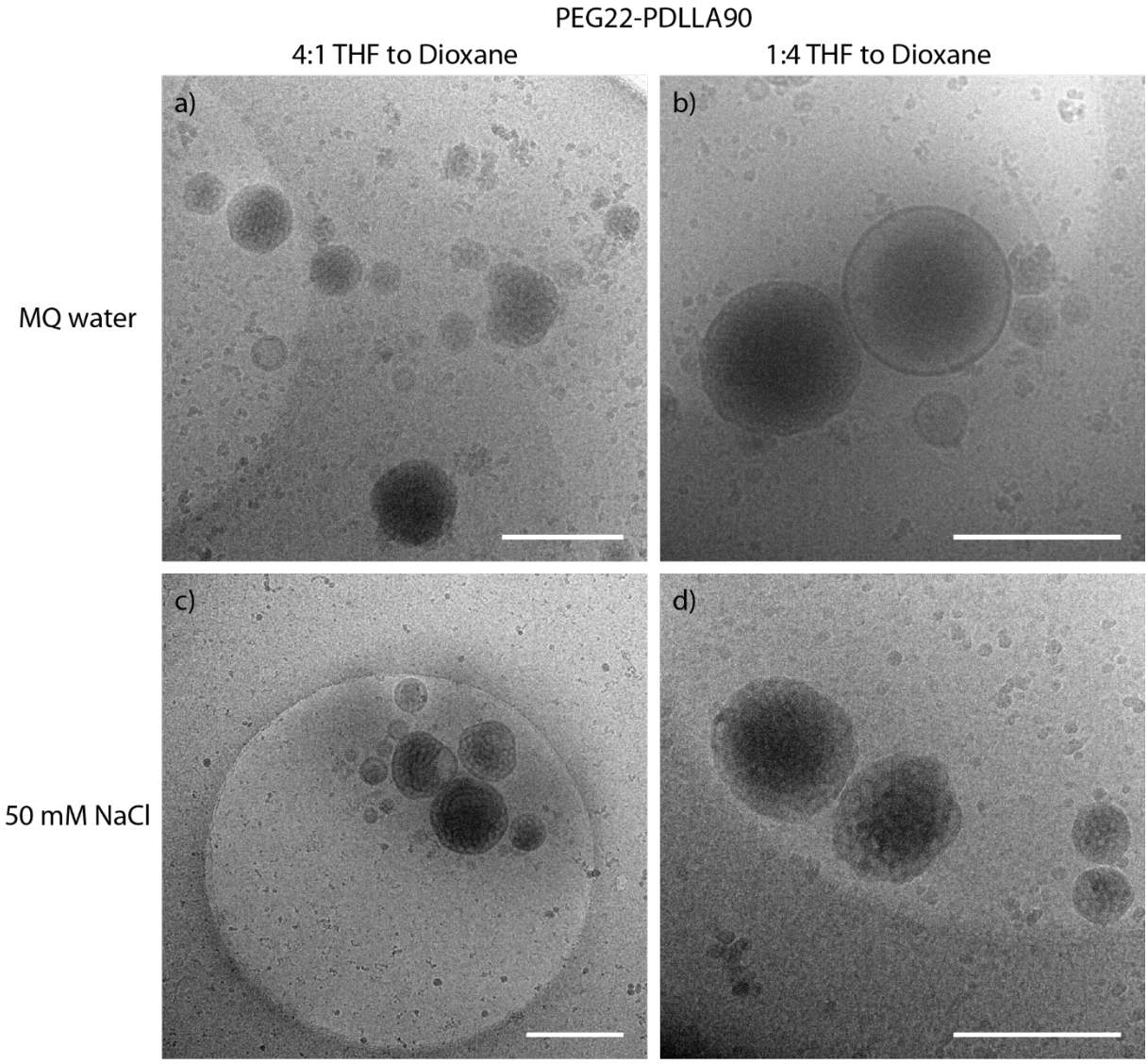
Supplementary Figure 2: DLS measurement of all polymer mixtures.



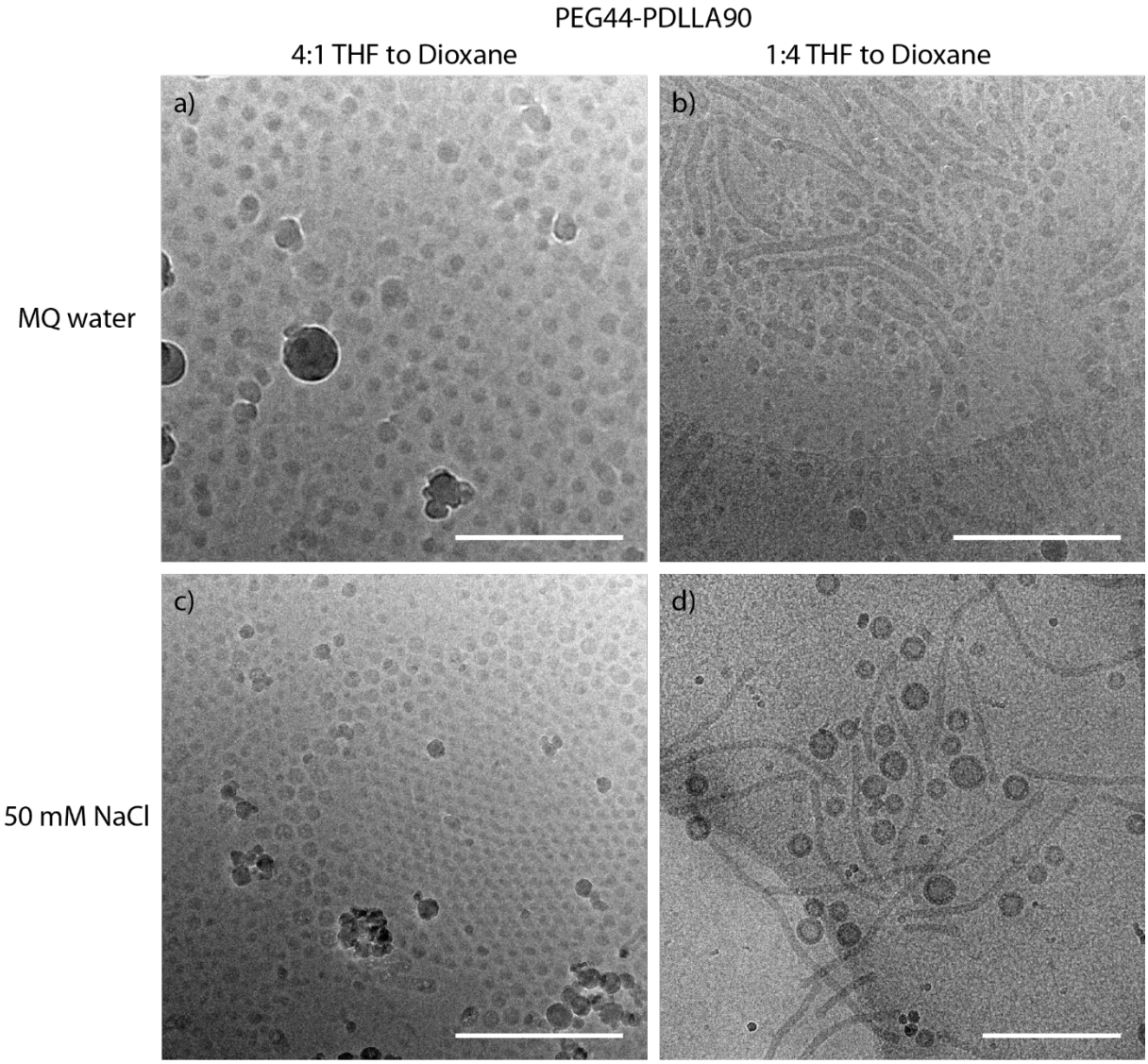
Supplementary Figure 3: Cryo-TEM images of PEG_{22}-$PDLLA_{45}$ self-assemblies. a) spherical polymersomes formed with 4:1 THF to Dioxane and dialysis against Milli-Q water. **b)** spherical polymersomes formed with 1:4 THF to Dioxane and dialysis against Milli-Q water. **c)** tubular polymersomes formed with 4:1 THF to Dioxane and dialysis against 50 mM salt. **d)** nested vesicles and tubular polymersomes formed with 1:4 THF to Dioxane and dialysis against 50 mM salt. Scale bars are 500 nm.



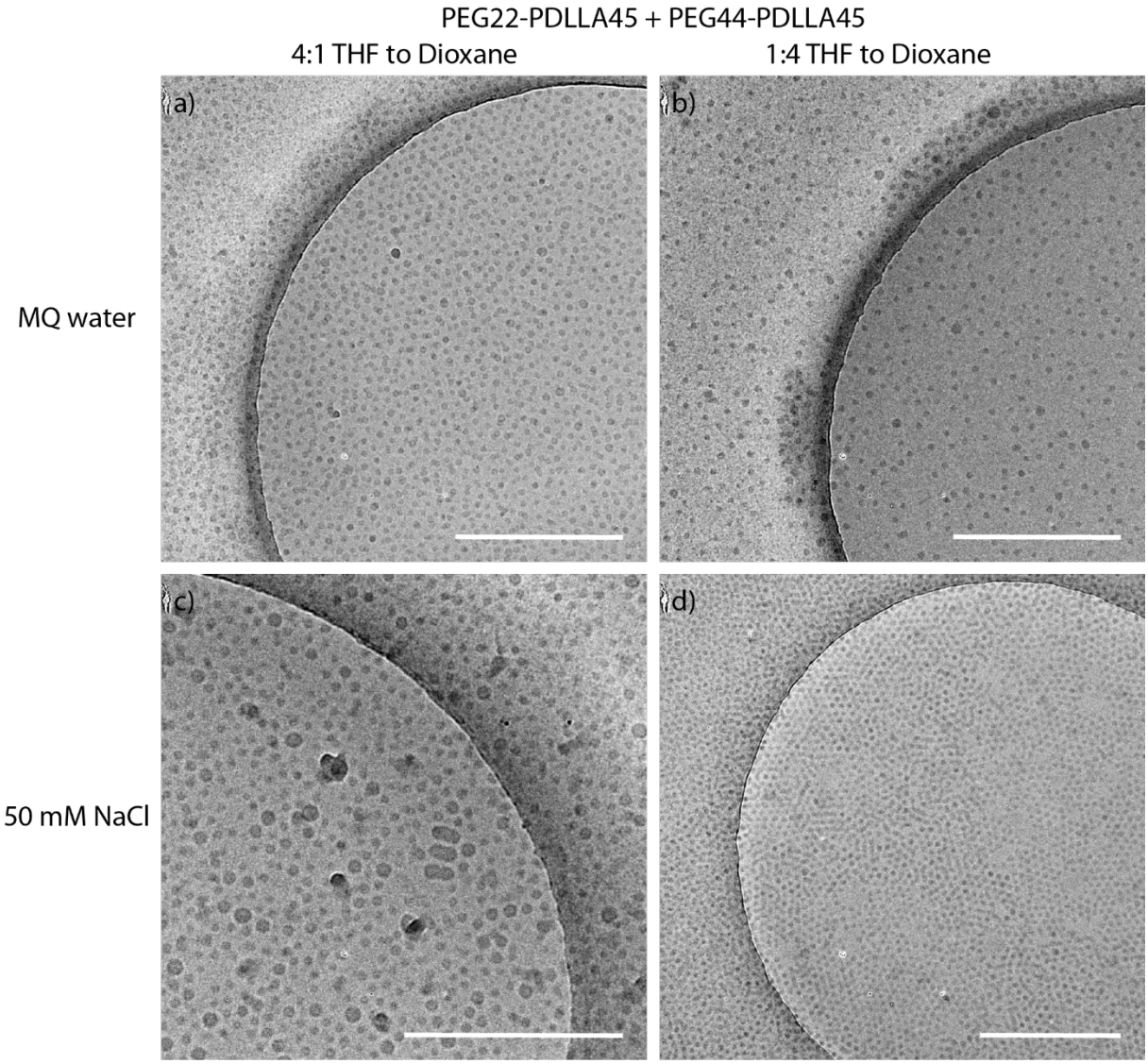
Supplementary Figure 4: Cryo-TEM images of PEG_{44}-$PDLLA_{45}$ self-assemblies. a) micelles formed with 4:1 THF to Dioxane and dialysis against Milli-Q water. **b)** micelles formed with 1:4 THF to Dioxane and dialysis against Milli-Q water. **c)** micelles formed with 4:1 THF to Dioxane and dialysis against 50 mM salt. **d)** micelles formed with 1:4 THF to Dioxane and dialysis against 50 mM salt. Scale bars are 500 nm.



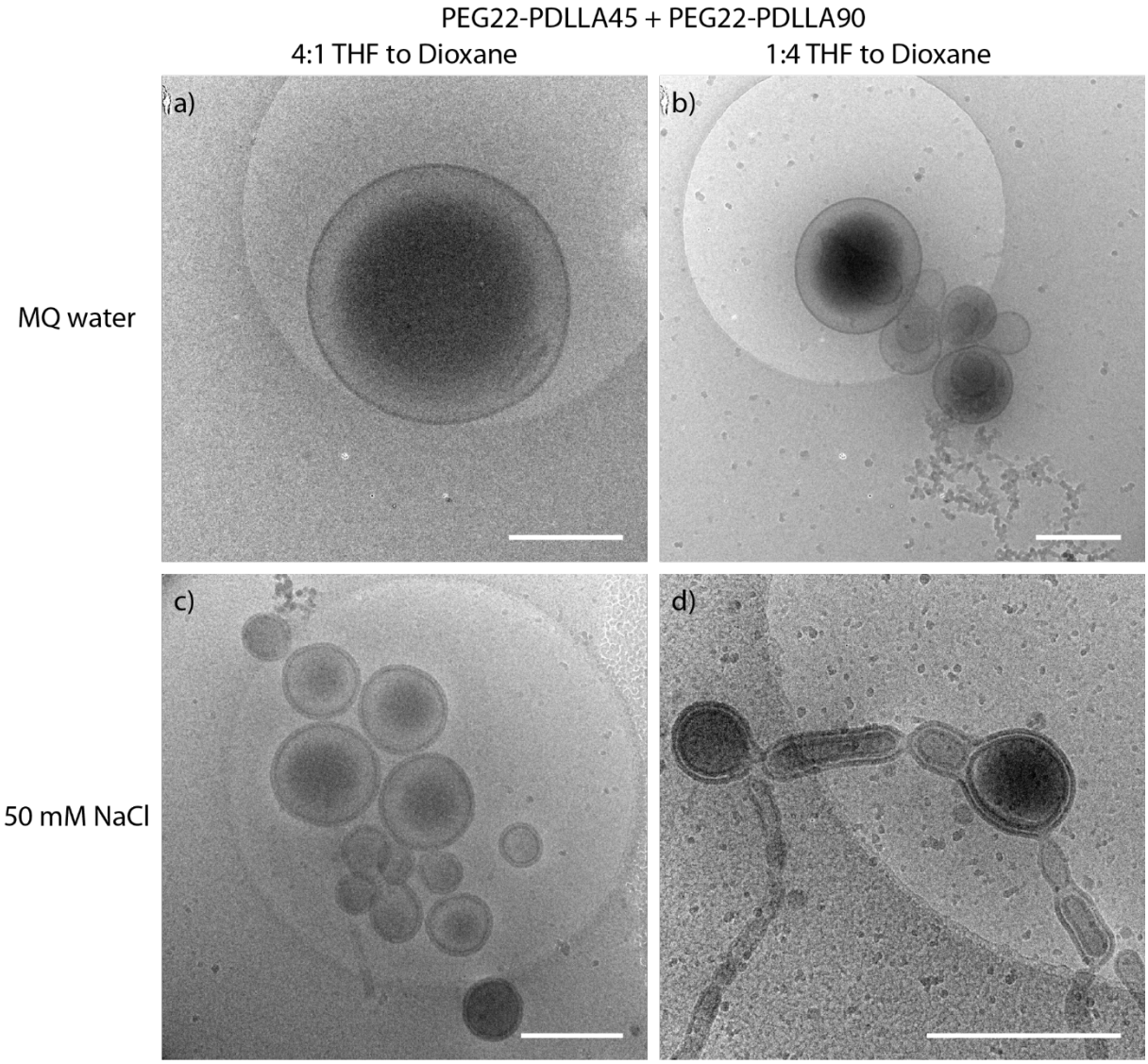
Supplementary Figure 5: Cryo-TEM images of PEG_{22}-$PDLLA_{90}$ self-assemblies. These samples contained aggregations that were too large for cryo-TEM analysis. **a)** large compound vesicles (LCVs) formed with 4:1 THF to Dioxane and dialysis against Milli-Q water. **b)** LCVs and spherical polymersomes formed with 1:4 THF to Dioxane and dialysis against Milli-Q water. **c)** LCVs formed with 4:1 THF to Dioxane and dialysis against 50 mM salt. **d)** LCVs formed with 1:4 THF to Dioxane and dialysis against 50 mM salt. Scale bars are 500 nm.



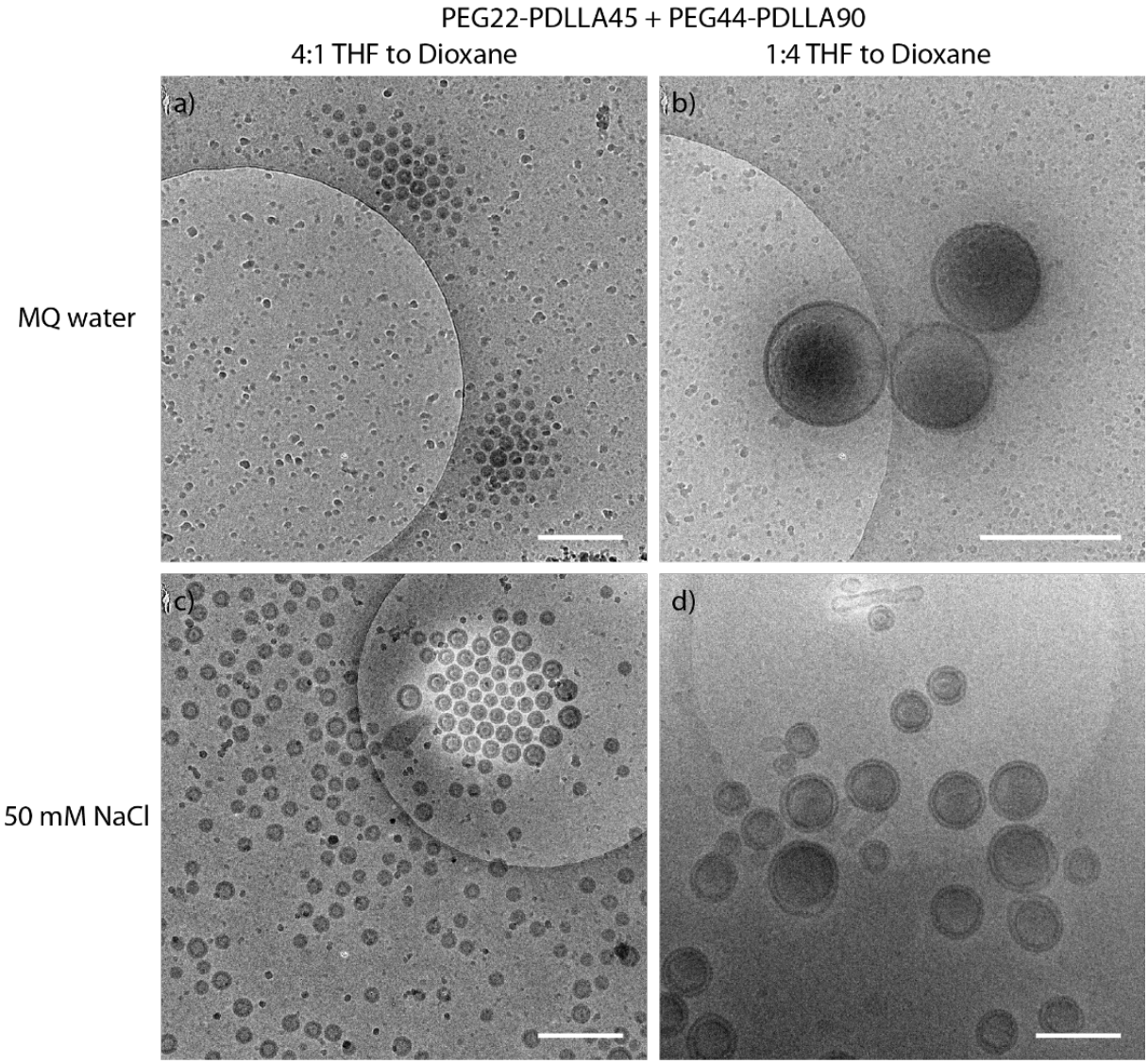
Supplementary Figure 6: Cryo-TEM images of PEG_{44}-$PDLLA_{90}$ self-assemblies. a) micelles formed with 4:1 THF to Dioxane and dialysis against Milli-Q water. **b)** micelles and worms formed with 1:4 THF to Dioxane and dialysis against Milli-Q water. **c)** micelles and worms formed with 4:1 THF to Dioxane and dialysis against 50 mM salt. **d)** worms and spherical polymersomes formed with 1:4 THF to Dioxane and dialysis against 50 mM salt. Scale bars are 500 nm.



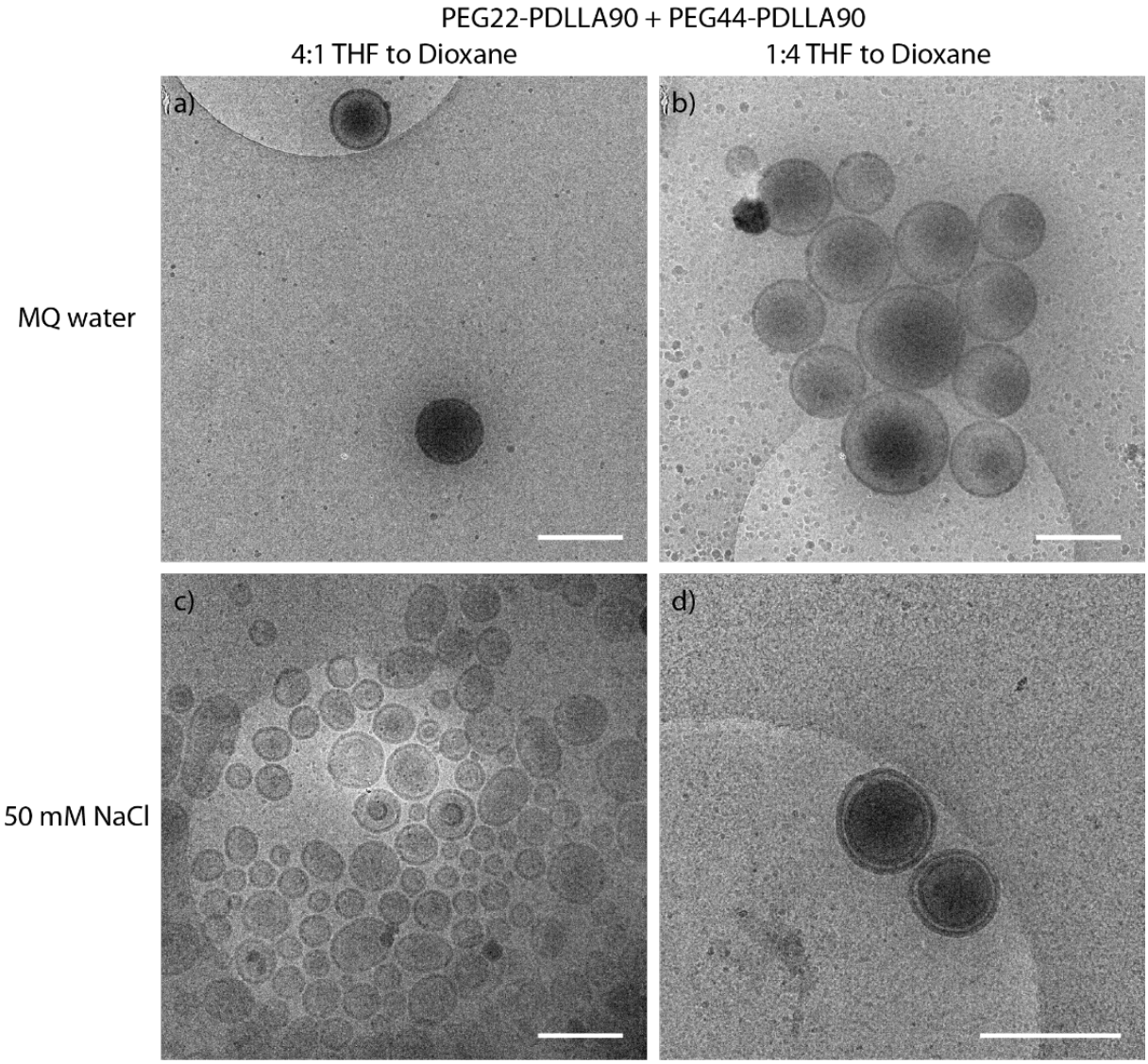
Supplementary Figure 7: Cryo-TEM images of PEG_{22}-$PDLLA_{45}$ + PEG_{44}-$PDLLA_{45}$ self-assemblies. a) micelles formed with 4:1 THF to Dioxane and dialysis against Milli-Q water. **b)** micelles formed with 1:4 THF to Dioxane and dialysis against Milli-Q water. **c)** micelles formed with 4:1 THF to Dioxane and dialysis against 50 mM salt. **d)** micelles formed with 1:4 THF to Dioxane and dialysis against 50 mM salt. Scale bars are 500 nm.



Supplementary Figure 8: Cryo-TEM images of PEG_{22}-$PDLLA_{45}$ + PEG_{22}-$PDLLA_{90}$ self-assemblies. a) spherical polymersomes formed with 4:1 THF to Dioxane and dialysis against Milli-Q water. **b)** irregular polymersomes formed with 1:4 THF to Dioxane and dialysis against Milli-Q water. **c)** nested vesicles formed with 4:1 THF to Dioxane and dialysis against 50 mM salt. **d)** irregular deflated polymersomes formed with 1:4 THF to Dioxane and dialysis against 50 mM salt. Scale bars are 500 nm.



Supplementary Figure 9: Cryo-TEM images of PEG_{22}-$PDLLA_{45}$ + PEG_{44}-$PDLLA_{90}$ self-assemblies. a) micelles and spherical polymersomes formed with 4:1 THF to Dioxane and dialysis against Milli-Q water. **b)** spherical polymersomes formed with 1:4 THF to Dioxane and dialysis against Milli-Q water. **c)** spherical polymersomes formed with 4:1 THF to Dioxane and dialysis against 50 mM salt. **d)** stomatocytes and nested vesicles formed with 1:4 THF to Dioxane and dialysis against 50 mM salt. Scale bars are 500 nm.



Supplementary Figure 10: Cryo-TEM images of PEG_{22}-$PDLLA_{90}$ + PEG_{44}-$PDLLA_{90}$ self-assemblies. a) spherical polymersomes formed with 4:1 THF to Dioxane and dialysis against Milli-Q water. **b)** spherical polymersomes formed with 1:4 THF to Dioxane and dialysis against Milli-Q water. **c)** discs formed with 4:1 THF to Dioxane and dialysis against 50 mM salt. **d)** stomatocytes formed with 1:4 THF to Dioxane and dialysis against 50 mM salt. Scale bars are 500 nm.