

Supporting Information for Non-ionic block copolymer coacervates

Aoon Rizvi¹, Urja Patel¹, Alessandro Ianaro² Paul J. Hurst¹, Jovany G. Merham¹, Joseph

P. Patterson^{1*}

¹*Department of Chemistry, University of California, Irvine, Irvine, CA 92697-2025,*

²*Adolphe Merkle institute, University of Fribourg, 1700 Fribourg, Switzerland*

Table of contents

1) Materials and instruments (pg. 2)

2) Experimental Methods (pg. 3-11)

- i. Synthesis
- ii. Preparation of organic-water mixtures for studying the phase behavior of amphiphilic block copolymers
- iii. Experiments to test the pathway dependence on the coacervation of PEO-b-PMMA block copolymers
- iv. Preparation of block copolymer nanoparticles through a coacervate intermediate
- v. Preparation of block copolymer microparticles through a coacervate intermediate
- vi. Preparation of block copolymer porous networks through a coacervate intermediate
- vii. Preparation of block copolymer fibers through a coacervate intermediate
- viii. Liquid Phase Electron Microscopy of block copolymer coacervates
- ix. Cryo-electron microscopy of block copolymer assemblies
- x. Cryo-electron microscopy of block copolymer nano-coacervates
- xi. Optical microscopy of block copolymer coacervates
- xii. Scanning Electron Microscopy of Polymer microparticles and fibers

3) Supplementary Discussion (pg. 11-14)

- i. Discussion on the relationship between block copolymer structure and pore diameter in self-assembled structures
- ii. Flory-Huggins theory for block copolymers in a solvent mixture
- iii. Calculation of the solvent switch diagrams

4) Supplementary information Tables and Figures (pg. 14-33)

5) References (pg. 33)

1) **Materials and instruments:**

All reagents were purchased from Acros Organics, Fisher Scientific, or Sigma-Aldrich, and used without further purification unless otherwise noted. Mili-Q water was used for experiments unless otherwise noted. ^1H NMR spectra were collected on a 500 MHz Bruker Avance spectrometer. All samples were taken in CDCl_3 . Chemical shifts are provided in ppm, calibrated from the residual CDCl_3 peak (7.26 ppm). All TEM experiments were performed on a JEOL 2100 equipped with a 200 keV field emission gun and a OneView camera, Irvine Materials Research Institute, University of California, Irvine. Liquid Phase imaging was performed with a DENS solutions Ocean holder using 0 nm spacer chips. SEM imaging was performed on a FEI Magellan 400 XHR SEM. Optical and fluorescence imaging were performed using a Keyence Bz-X810 all in one fluorescence microscope. Size exclusion chromatography (SEC) was performed in DMF using an Agilent 1100 chromatograph equipped with RID detector and a PL gel 5 μm 300x7.5 mm mixed column. All samples were calibrated against polystyrene standards (MW= 580, 1300, 5000, 10000, 30000, 70000, 130000 g/mol).

2) Experiential Methods

2-i) Synthesis:

Synthesis of CTA 1

To a 250 mL round bottom flask, dodecanethiol (10.1 g, 50 mmol), KOH (3.41, 60 mmol) were added and dissolved in EtOH (40 mL). After 30 min of stirring, CS₂ (3.0 mL, 39 mmol) was added via a syringe. After 4 h of stirring, Tosyl-Cl (4.65 g, 24.4 mmol) in DCM (10 mL) was added dropwise turning the reaction mixture orange. The reaction was stirred overnight. The reaction mixture was washed with brine and the organics were concentrated *in vacuo*. The solid intermediate was further dissolved in EtOAc (40 mL) and 4,4'-azobis(4-cyanovaleric acid) (5.55 g, 0.0198 mol) was added to the reaction mixture. The solution was heated at 80 °C for 12 hours. The solvent was evaporated, and the yellow product was purified using silica gel chromatography 20% ethyl acetate in hexanes. The pure product (3.7g, 20% yield) was dried in high-vac overnight to remove residual solvent. ¹H NMR (CDCl₃, 500 MHz) δ ppm: 0.95 (t, *J* = 6.9 Hz, 3H), 1.32 (m, 18H), 1.76 (m, 1H), 1.95 (s, 3H), 2.46 (m, 2H), 2.75 (t, 2H), 3.39 (t, 2H).

Synthesis of Macro PEO-CTA

To a flame dried 100 mL two-neck round bottom flask under N₂ m-PEG₄₅ (1.1 g, 0.5 mmol) was added along with DCC (212 mg, 1 mmol), anhydrous DCM was syringed in to dissolve the solids followed by the addition of **CTA-1** (417 mg, 1 mmol) dissolved in DCM (5 mL). The reaction was covered with foil under N₂ and stopped after 48 h. The dicyclohexyl urea precipitate was filtered out and the organics were evaporated *in vacuo*. The yellow solids were dissolved in minimal THF and the viscous solution was poured into cold Et₂O while vigorously stirring to precipitate the **PEO-CTA**. The precipitate also

contains unfunctionalized m-PEG₄₅ as the DCC coupling reaction was not quantitative, the macro CTA was used with the unfunctionalized m-PEG. The concentration of the functionalized PEG was determined using UV-vis analysis (Figure S19,S20). ¹H NMR (CDCl₃, 500 MHz) δ ppm: 0.88 (t, *J* = 6.9 Hz, 3H), 1.25 (m, 18H), 1.70 (m, 1H), 1.87 (s, 3H), 2.46 (m, 2H), 2.66 (, 2H), 3.32 (m, 2H), 3.38 (s, 3H), 3.64 (s, 180H).

Synthesis of PEO₄₅-b-PMMA₂₀₀

To a 10 mL Schleck tube MMA (0.62 g, 6.2 mmol), AIBN (0.5 mg, 0.62 mmol), **PEO-CTA** (70 mg, 0.0292 mmol) and toluene (300 μL) were added along with a stir bar. The mixture was vigorously stirred and sparged with N₂ for 20 min. The tube was sealed and heated to 70 °C in an oil bath. The polymerization was monitored via the consumption of MMA using ¹H NMR. After 12 hours the polymerization was “gelled”, indicating high conversion, in this case 90% conversion. The polymer was precipitated three times into cold methanol and dried in a vacuum oven at 40 °C. ¹H NMR (CDCl₃, 500 MHz) δ ppm: 0.86-1.02 (CH₂ polymer back bone), 1.81 (CH₃, PMMA), 3.38 (CH₃O- PEO End Group), 3.60 (-OCH₃, PMMA), 3.64 (-OCH₂CH₂-). All PEO₄₅-PMMA block copolymers were synthesized using this procedure, varying the monomer ratios for desired DP)

Synthesis of PDMA₇₀

To a 10 mL Schleck tube DDMAT (0.100 g, 0.274 mmol), AIBN (0.45 mg, 0.0274 mmol), DMA (1.9 g, 21.9 mmol) and toluene (300 μL) were added along with a stir bar. The mixture was vigorously stirred and sparged with N₂ for 20 min. The tube was sealed and heated to 70 °C in an oil bath. The polymerization was monitored via the consumption of DMA using ¹H NMR. After 6 hours the polymerization was “gelled”, indicating high conversion (85%). The polymer was precipitated three times into cold ether and dried in

a vacuum oven at 40 °C. ^1H NMR (CDCl_3 , 500 MHz) δ ppm: 0.88 (t, $J = 6.9$ Hz, 3H), (1.0-1.9) (CH_2 , CH polymer back bone), 2.2- 3.2 ($(\text{CH}_3)_2\text{N}$).

Synthesis of PDMA₇₀-b-PMMA₄₅₀

To a 10 mL Schleck tube PDMA₇₀ (0.200 g, 0.030mmol), AIBN (0.1mg), MMA (1.35 g, 13.5 mmol) and toluene (300 μL) were added along with a stir bar. The mixture was vigorously stirred and sparged with N_2 for 20 min. The tube was sealed and heated to 70 °C in an oil bath. The polymerization was monitored via the consumption of MMA using ^1H NMR. After 4 hours the polymerization was “gelled”, indicating high conversion (99%)The polymer was precipitated three times into cold ether and dried in a vacuum oven at 40 °C. ^1H NMR (CDCl_3 , 500 MHz) δ ppm: 0.82-0.88 (DDMAT), 1.0-1.9 (CH_2 , CH polymer back bone), 2.2- 3.2 ($(\text{CH}_3)_2\text{N}$), (CH_3 , PMMA), 3.60 ($-\text{OCH}_3$, PMMA). *SEC characterization was not carried out due to COVID-19 closure.

2-ii) Preparation of organic-water mixtures for studying the phase behavior of amphiphilic block copolymers:

The phase behavior of PEO₄₅-b-PMMA_x was mapped experimentally. Self-assembly by the solvent switch method was carried out as a function of PMMA chain length (130, 200, 300, 400) and starting polymer concentration (1, 5, 10 mg mL^{-1}). For each sample, a calculated volume of polymer solution was used, and water was subsequently added to achieve the desired ϕ_w with 1 mL being the final solution volume. The phase behavior of each sample was determined largely based on a visual inspection of the vial supplemented by optical microscopy and cryo=TEM as detailed in Figure 1. Each sample was categorized as following: clear solution (no self-assembly), milky opaque solution (coacervate formation/ LLPS), translucent blue turbid solution (nano particle formation).

This procedure was used to perform solvent switch experiments with other block copolymers as well (PDMA₇₀-*b*-PMMA₄₅₀, PEO₄₅-*b*-PCL₆) Table S1-S3 presents the details and outcomes of each sample for this study.

2-iii) Experiments to test the pathway dependence on the coacervation of PEO-*b*-PMMA block copolymers:

Coacervates of PEO₄₅-PMMA₃₀₀ were formed from different pathways to test if the formation of these droplets was path dependent. As shown earlier, increasing the water content from no water to where phase separation occurs ($\phi_W \approx 0.25$) results in the formation of coacervates. However, in (Figure S2 a) we show that if nanoparticles are first formed at higher water content, in this case $\phi_W = 0.35$, and more dioxane is added to decrease the water content to where the solution is expected to phase separate ($\phi_W \approx 0.25$), we see coacervate formation again (Figure S2 b). This suggests the coacervates form under thermodynamic control. Furthermore, addition of solid polymer to a mixture of $\phi_W = 0.25$ in dioxane formed coacervates exhibiting thermodynamic behavior. These experiments showcase the path independent behavior of coacervate formation which means this process is under thermodynamic control.

2-iv) Preparation of block copolymer nanoparticles through a coacervate intermediate

PEO₄₅-*b*-PMMA₃₀₀ nanoparticles were self-assembled by the solvent switch method. Water (500 μ L) was added using a micro-pipette within 1 second to the polymer solutions (500 μ L) of varying polymer concentrations (1, 5, 10 mg mL⁻¹). The samples were vortexed for 10 seconds. The self-assembled samples turned lightly turbid indicating the

formation of nano structures in solution. The dioxane:water solutions were dialyzed (3.5K molecular weight cutoff) extensively against DI water and analyzed by Cryo-TEM.

2-v) Preparation of block copolymer microparticles through a coacervate intermediate

PEO₄₅-*b*-PMMA₃₀₀ microparticles were self-assembled in two steps. First, water (250 μ L) was added quickly within 1 second to the polymer solutions (750 μ L, 5 mg mL⁻¹) to induce coacervation ($\phi_W = 0.25$). The coacervate solutions were vortexed for 10 seconds. Subsequently, more water (375 μ L) was added to the coacervate solution to self-assemble the micron size coacervates into micro particles. The dioxane:water solutions were dialyzed (3.5K molecular weight cutoff) extensively against DI water and analyzed by SEM.

2-vi) Preparation of block copolymer porous networks through a coacervate intermediate

PEO₄₅-*b*-PMMA₃₀₀ porous pellets were self-assembled in two steps. First, water (250 μ L) was added directly to the polymer solutions (750 μ L, 5 mg mL⁻¹) to induce coacervation ($\phi_W = 0.25$). The coacervate solutions were vortexed for 10 seconds and then centrifuged at 10,000 g for 15 minutes to separate the coacervate phase and the dilute phase. The dilute phase was decanted from the Eppendorf tubes and excess water (≈ 1000 μ L) was added on top of the coacervate phase to drive the self-assembly of mm size pellets. The solvent was discarded, and the pellet was dried using Kimwipes, the pellet was sliced using a scalpel and analyzed by SEM.

2-vii) Preparation of block copolymer fibers through a coacervate intermediate

PEO₄₅-*b*-PMMA₃₀₀ fibers were formed in two steps. First, water (250 μL) was added directly to the polymer solutions (750 μL , 5 mg mL⁻¹) to induce coacervation ($\phi_W = 0.25$). The coacervate solutions were vortexed for 10 seconds and then centrifuged at 10,000 g for 15 minutes to separate the coacervate phase and the dilute phase. The coacervate phase (≈ 10 -50 μL) was drawn from the samples and dropped onto a glass slide. Using a metal spatula, fibers were pulled from the coacervate solution on the glass slide (Supplementary Movie 3). The fibers were easily extended to lengths of 20-30 cm, depending on the volume of the coacervate solution. The fibers were dried under atmosphere and further analyzed by SEM.

2-viii) Liquid Phase Electron Microscopy of block copolymer coacervates:

The onset of coacervation of PEO₄₅-*b*-PMMA₃₀₀ was observed in real time by carrying out the solvent switch experiments inside the cell. All nano-chips used were plasma cleaned (1:1 Ar:O₂) for 4 minutes prior to the assembly of the cell. PEO₄₅-*b*-PMMA₃₀₀ solution (5 mg mL⁻¹ in dioxane) was drop casted ($\approx 0.5 \mu\text{L}$) on to the bottom chip and the cell was sealed by placing the top chip on the cell. The cell was tested for any leaks prior to inserting it in the microscope. Once in the microscope, using a 500 μL syringe, water was flowed into the tip surrounding the cell creating a diffusion gradient between the two solvents. TEM imaging was performed at an electron dose rate $\approx 10 \frac{e}{\text{nm}^2\text{s}}$ and the total dose $\approx 4 \times 10^4 \frac{e}{\text{nm}^2}$. We observed that imaging the coacervates over extended periods

results in the precipitation of polymer. Further work is underway to investigate the electron-sample interactions for the coacervates. In this paper we used image times and doses that qualitatively did not show significant beam-sample interactions. Images were recorded with an exposure of 1 second having a dead time of ≈ 3 seconds using the *TEM Recorder* script for DigitalMicrograph and collected using the Gatan Oneview. Image Stacks were manipulated in DigitalMicrograph and ImageJ. Each image was binned 4x in the x and y direction and the movie was created. Snapshots in Figure 3 are of this same processed data.

2-ix) **Cryo-electron microscopy of block copolymer assemblies**

Quantifoil Holey Carbon Films were purchased from Electron Microscopy Sciences, grids were glow discharged for 70 s to increase hydrophilicity prior to sample preparation. Vitrification was carried out by an Automatic Plunge Freezer ME GP2 (Leica Microsystems) where sample preparation onto cryoTEM grids was carried out at 95% humidity to prevent evaporation and blotted for 3 s before auto-plunging into liquid propane. Vitrified samples were studied on a JEOL-2100F TEM using a Schottky type field emission gun operating at 200 kV. Size measurements for cryoTEM images were performed using the measurement tool in ImageJ.

2-x) **Cryo-electron microscopy of block copolymer nano-coacervates**

3 μL of 5 mg mL⁻¹ PEO₄₅-PMMA₃₀₀ solution in dioxane and $\phi_W = 0.15$ was deposited onto the TEM grid inside the Leica EM GP humidity chamber. The sample was blotted using a filter paper for 1 second and plunged into liquid propane to vitrify the sample. The polymer solution crosses the phase boundaries during the sample prep as the water concentration

increases inside the humidity chamber as dioxane is a hygroscopic solvent. Figure S7 a-c show bicontinuous spheres that were self-assembled during the sample prep. These structures are expected in the self-assembled region for this system as discussed in the main text. However, for these experiments our goal was to trap the coacervates that form prior to the self-assembled structures. In other parts of the grid we observed distinct structures that have not been seen in samples that were prepared in a vial. Image d shows an overview of low contrast “field” that has a continuous structure throughout it. Figure 7 d-f capture the continuous fields at a higher magnification. We suspect the irregular shapes of such fields arise from the coalescence of the coacervate precursor that forms during the self-assembly and the flattening of these fields must be due to the thin layer of solution these structures are assembled in. Ice-layers for Cryo-TEM experiments are typically ~100-200 nm thick, forcing the liquid precursors of sizes larger than this to either be removed during blotting or to flatten out. Samples with $\phi_W = 0.25$ in dioxane formed nano-fibers due to solvent drying as shown in Figure 5c.¹

2-xi) **Optical microscopy of block copolymer coacervates**

Standard microscope slides along with 1.0 mm coverslips were used to prepare samples for bright-field imaging using the Keyence optical microscope. Coacervate samples were vortexed for 10 s and then 20 μ L of coacervate solution was sealed between the glass slide and the coverslips for imaging each sample. Images were collected with 10x and 20x objective lenses. The images were not further modified after collection.

2-xii) **Scanning Electron Microscopy of Polymer microparticles and fibers**

Microparticle solutions in pure water were deposited ($\approx 10 \mu\text{L}$) on 1x1 cm glass substrate. The samples were sputter coated with 5nm of iridium (Quorum Q150T). Polymer fibers and porous membrane cross sections were stuck on conductive tape and sputter coated with 5nm of iridium (Quorum Q150T). All samples were imaged by a FEI Magellan 400 XHR system. Secondary electron images were acquired with accelerating voltages of (5 or 10 keV), using a through lens detector operating in immersion mode. Size measurements for SEM images were performed using the measurement tool in ImageJ.

3) **Supplementary Discussion**

3-i) **Discussion on the relationship between block copolymer structure and pore diameter in self-assembled structures:**

The dimensions of block copolymer structures can be related to the total length of the polymer chains (or individual blocks) and the chain stretching factor for the block. Comparing the measured dimensions to the total extended length of a polymer (or individual block) can provide information to rule out or support a morphological assignment.² For a bicontinuous structure, the pore diameter should not be more than 2x the hydrophilic polymer chain length and the center to center distance between pores should not be more than 2x the total length of the block copolymer chain. Our model polymer, PEO₄₅-*b*-PMMA₃₀₀, has a fully extended PMMA block length of $300 \times 0.25 \text{ nm} = 87.5 \text{ nm}$ and a fully extended PEO block length of 12.75 nm .^{2,3}

3-ii) **Flory-Huggins theory for block copolymers in a solvent mixture.**

The Flory-Huggins (F-H) energy of mixing ($\bar{G}_{\text{mix}}^{\text{bcp}}$) of a block copolymer in a mixture of solvents (S1 and S2) is derived below. The solvent mixture is considered as a single solvent S, the composition of which is described by φ_{S1} and φ_{S2} , defined as:

$$\varphi_{S1} = \frac{n_{S1}}{n_S}, \quad \varphi_{S2} = \frac{n_{S2}}{n_S} = 1 - \varphi_{S1}. \quad (1)$$

Here, n_{S1} and n_{S2} are the number of molecules of S1 and S2, respectively, while $n_S = n_{S1} + n_{S2}$ is the total number of solvent molecules. The system is considered to contain n_S molecules of solvent and n_P molecules of AB; each solvent molecule is composed of $N_S = N_{S1} = N_{S2}$ segments while the blocks chain lengths are denoted as N_A and N_B , respectively. The average volume fractions of solvent, A and B segments in the system are then defined as:

$$\phi_S = \frac{n_S N_S}{n_P(N_A + N_B) + n_S N_S}, \phi_A = \frac{n_P N_A}{n_P(N_A + N_B) + n_S N_S}, \phi_B = \frac{n_P N_B}{n_P(N_A + N_B) + n_S N_S}. \quad (2)$$

The total *average* copolymer segment volume fraction in the system ϕ_P is

$$\phi_P = \frac{n_P(N_A + N_B)}{n_P(N_A + N_B) + n_S N_S} \quad (3)$$

Obviously, ϕ_A and ϕ_B are related to ϕ_P by

$$\phi_A = \phi_P \frac{N_A}{N_A + N_B}, \quad \phi_B = \phi_P \frac{N_B}{N_A + N_B} \quad (4)$$

It is assumed that the entropy of mixing block copolymers and the solvent mixture equals that of a homopolymer with total chain length $N = N_A + N_B$ in a solvent S. The block copolymer enthalpy of mixing is modelled as that of a three-component system, where

two distinct polymers are mixed in a solvent. Hence, the Gibbs energy of mixing for block copolymers can be obtained combining the Flory-Huggins mixing entropy of a two-component system with the mixing enthalpy of a three-component system⁴:

$$\bar{G}_{\text{mix}}^{\text{bcp}} = \frac{\Delta G_{\text{mix}}^{\text{bcp}}}{k_B T} = n_S \ln(\phi_S) + n_P \ln(\phi_P) + N_S n_S \phi_A \chi_{AS} + N_S n_S \phi_B \chi_{BS} + N_A n_P \phi_B \chi_{AB}. \quad (5)$$

The terms χ_{AS} , χ_{BS} and χ_{AB} are the F-H interaction parameters, which depend upon the solvent composition:

$$\chi_{AS} = \varphi_{S1} \chi_{AS1} + (1 - \varphi_{S1}) \chi_{AS2}, \quad \chi_{BS} = \varphi_{S1} \chi_{BS1} + (1 - \varphi_{S1}) \chi_{BS2}. \quad (6)$$

The parameters χ_{AS1} , χ_{AS2} , χ_{BS1} and χ_{BS2} are the Flory-Huggins interaction parameters⁵ describing the interaction of the A and B blocks with S1 and S2 respectively. The term χ_{AB} is the interaction parameter between the blocks.

The derivatives of $\bar{G}_{\text{mix}}^{\text{bcp}}$ with respect to n_P and n_S are the copolymer and solvent chemical potentials respectively, which using (3) and (4), can be written as

$$\begin{aligned} \frac{\mu_{\text{bcp}} - \mu_{\text{bcp}}^0}{k_B T} = \bar{\mu}_{\text{bcp}} &= \left(\frac{\partial \bar{G}_{\text{mix}}^{\text{bcp}}}{\partial n_P} \right)_{T,P,n_S} = \\ &= \ln(\phi_P) + \left(1 - \frac{N_A + N_B}{N_S} \right) (1 - \phi_P) + (N_A \chi_{AS} + N_B \chi_{BS}) (1 - \phi_P)^2 \\ &+ \frac{\chi_{AB} N_A N_B \phi_P}{N_A + N_B} (2 - \phi_P), \end{aligned} \quad (7)$$

$$\frac{\mu_S - \mu_S^0}{k_B T} = \bar{\mu}_S = \left(\frac{\partial \bar{G}_{\text{mix}}^{\text{bcp}}}{\partial n_S} \right)_{T,P,n_P} =$$

$$\ln(1 - \phi_P) + \left(1 - \frac{N_S}{N_A + N_B} \right) \phi_P + \frac{N_S}{N_A + N_B} \left(N_A \chi_{AS} + N_B \chi_{BS} - \frac{\chi_{AB} N_A N_B}{N_A + N_B} \right) \phi_P^2. \quad (8)$$

It is easy to show that for $N_P = N_A + N_B$, $\chi_{AB} = 0$ and $\chi_{AS} = \chi_{BS}$, the F-H chemical potentials for the homopolymer-solvent case are recovered[].

Imposing $\partial \bar{\mu}_S / \partial \phi_P = 0$ (or $\partial \bar{\mu}_{\text{bcp}} / \partial \phi_P = 0$) yields the critical conditions for phase separation. The critical polymer volume fraction ϕ_P^{crit} follows as

$$\phi_P^{\text{crit}} = \frac{\sqrt{N_A N_S + N_B N_S} - N_S}{N_A + N_B - N_S}, \quad (9)$$

and the critical demixing conditions are given by

$$N_A \chi_{AS} + N_B \chi_{BS} - \frac{\chi_{AB} N_A N_B}{N_A + N_B} = \frac{N_A + N_B}{2 N_S (1 - \phi_P^{\text{crit}})^2} \quad (10)$$

Insertion of these results into (5.6) yields the critical solvent mixture composition ϕ_{S1}^{crit}

$$\phi_{S1}^{\text{crit}} = \frac{\left[\frac{N_A + N_B + N_S + 2\sqrt{(N_A + N_B)N_S}}{2N_S} + \frac{\chi_{AB} N_A N_B}{N_A + N_B} - N_A \chi_{AS2} - N_B \chi_{BS2} \right]}{N_A (\chi_{AS1} - \chi_{AS2}) + N_B (\chi_{BS1} - \chi_{BS2})} \quad (11)$$

3-iii) Calculation of the solvent switch diagrams

The solvent switch diagrams have been calculated comparing equation (11) and equation (5) with the free energy of micelle formation from Sato and Takahashi.⁶ The values of the interaction parameters used in the calculations are reported in Table S3.

Supplementary information Tables and Figures:

Table S1: Outcomes of all solvent switch experiments of PEO₄₅-*b*-PMMA_x block copolymers for the phase diagrams presented in the main text. Outcomes (NSA = no self-assembly, LLPS = liquid liquid phase separation, NP= Nanoparticle formation).

Polymer conc (mg mL ⁻¹)	PMMA (Dp)	Dioxane (μL)	Water (μL)	ϕ_w	Outcome
1	130	900	100	0.1	NSA
1	130	750	250	0.25	NSA
1	130	725	275	0.275	NSA
1	130	700	300	0.3	NP
1	130	675	325	0.325	NP
1	130	650	350	0.35	NP
1	130	550	450	0.45	NP
1	130	500	500	0.5	NP
1	130	300	700	0.7	NP
1	200	900	100	0.1	NSA
1	200	750	250	0.25	NSA
1	200	725	275	0.275	NP
1	200	700	300	0.3	NP
1	200	675	325	0.325	NP
1	200	650	350	0.35	NP
1	200	550	450	0.45	NP
1	200	500	500	0.5	NP
1	200	300	700	0.7	NP
1	300	900	100	0.1	NSA
1	300	750	250	0.25	NSA
1	300	725	275	0.275	LLPS
1	300	700	300	0.3	NP
1	300	675	325	0.325	NP
1	300	650	350	0.35	NP
1	300	550	450	0.45	NP
1	300	500	500	0.5	NP
1	300	300	700	0.7	NP
1	400	900	100	0.1	NSA
1	400	800	200	0.2	NSA
1	400	775	225	0.225	NSA
1	400	750	250	0.25	LLPS
1	400	725	275	0.275	LLPS
1	400	700	300	0.3	LLPS
1	400	675	325	0.325	NP
1	400	650	350	0.35	NP
1	400	550	450	0.45	NP
1	400	500	500	0.5	Np
1	400	300	700	0.7	NP

5	130	900	100	0.1	NSA
5	130	750	250	0.25	NSA
5	130	725	275	0.275	NP
5	130	700	300	0.3	NP
5	130	675	325	0.325	NP
5	130	650	350	0.35	NP
5	130	550	450	0.45	NP
5	130	500	500	0.5	NP
5	130	300	700	0.7	NP
5	200	900	100	0.1	NSA
5	200	750	250	0.25	NSA
5	200	725	275	0.275	LLPS
5	200	700	300	0.3	NP
5	200	675	325	0.325	NP
5	200	650	350	0.35	NP
5	200	550	450	0.45	NP
5	200	500	500	0.5	NP
5	200	300	700	0.7	NP
5	300	900	100	0.1	NSA
5	300	800	200	0.2	NSA
5	300	775	225	0.25	NSA
5	300	750	250	0.25	LLPS
5	300	725	275	.275	LLPS
5	300	700	300	0.3	NP
5	300	675	325	0.325	NP
5	300	650	350	0.35	NP
5	300	550	450	0.45	NP
5	300	500	500	0.5	NP
5	300	300	700	0.7	NP
5	400	900	100	0.1	NSA
5	400	800	200	0.2	NSA
5	400	775	225	0.225	LLPS
5	400	750	250	0.25	LLPS
5	400	725	275	0.275	LLPS
5	400	700	300	0.3	NP
5	400	675	325	0.325	NP
5	400	650	350	0.35	NP
5	400	550	450	0.45	NP
5	400	500	500	0.5	Np
5	400	300	700	0.7	NP
10	130	900	100	0.1	NSA
10	130	750	250	0.25	NSA
10	130	725	275	0.275	NSA
10	130	700	300	0.3	NSA
10	130	675	325	0.325	LLPS

10	130	650	350	0.35	NP
10	130	550	450	0.45	NP
10	130	500	500	0.5	NP
10	130	300	700	0.7	NP
10	200	900	100	0.1	NSA
10	200	800	200	0.2	NSA
10	200	775	225	0.225	NSA
10	200	750	250	0.25	LLPS
10	200	725	275	0.275	LLPS
10	200	700	300	0.3	NP
10	200	675	325	0.325	NP
10	200	650	350	0.35	NP
10	200	550	450	0.45	NP
10	200	500	500	0.5	NP
10	200	300	700	0.7	NP
10	300	900	100	0.1	NSA
10	300	800	200	0.2	NSA
10	300	775	225	0.25	LLPS
10	300	750	250	0.25	LLPS
10	300	725	275	0.275	LLPS
10	300	700	300	0.3	NP
10	300	675	325	0.325	NP
10	300	650	350	0.35	NP
10	300	550	450	0.45	NP
10	300	500	500	0.5	NP
10	300	300	700	0.7	NP
10	400	900	100	0.1	NSA
10	400	800	200	0.2	NSA
10	400	775	225	0.225	LLPS
10	400	750	250	0.25	LLPS
10	400	725	275	0.275	LLPS
10	400	700	300	0.3	NP
10	400	675	325	0.325	NP
10	400	650	350	0.35	NP
10	400	550	450	0.45	NP
10	400	500	500	0.5	Np
10	400	300	700	0.7	Np

Table S2: Outcomes of solvent switch experiments of PEO₄₅-*b*-PMMA₃₀₀ in THF and DMF. Outcomes (NSA= no self-assembly, NP = nanoparticle formation, PPT = precipitation)

Polymer conc (mg mL ⁻¹)	Solvent	Solvent (μ L)	Water (μ L)	ϕ_w	Outcome
5	THF	900	100	0.1	NSA
5	THF	700	300	0.3	NSA
5	THF	500	500	0.5	NP
5	THF	100	900	0.9	NP
5	DMF	900	100	0.1	NSA
5	DMF	700	300	0.3	PPT
5	DMF	500	500	0.5	PPT
5	DMF	100	900	0.9	PPT

Table S3: Outcomes of solvent switch experiments of PDMA₇₀-*b*-PMMA₄₅₀ in THF, dioxane, DMF, and acetone. Outcomes (NSA= no self-assembly, NP = nanoparticle formation, PPT = precipitation)

Polymer conc (mg mL ⁻¹)	Solvent	Solvent (μ L)	Water (μ L)	ϕ_w	Outcome
5	THF	900	100	0.1	NSA
5	THF	750	250	0.25	NSA
5	THF	500	500	0.5	NP
5	THF	100	900	0.7	NP
5	DMF	300	700	0.1	NSA
5	DMF	750	250	0.25	PPT
5	DMF	500	500	0.5	PPT
5	DMF	300	700	0.7	PPT
5	Acetone	900	100	0.1	NSA
5	Acetone	720	250	0.25	LLPS
5	Acetone	500	500	0.5	NP
5	Acetone	300	700	0.7	NP

Table S4: Interaction parameters used for phase boundary calculations in Figure 2.

Interaction	χ value
χ_{AS1} (PEO : water)	0.49
χ_{AS2} (PEO : dioxane)	0.20
χ_{BS1} (PMMA : water)	2.8
χ_{BS2} (PMMA : dioxane)	0.1
χ_{AB} (PMMA : PEO)	0.005

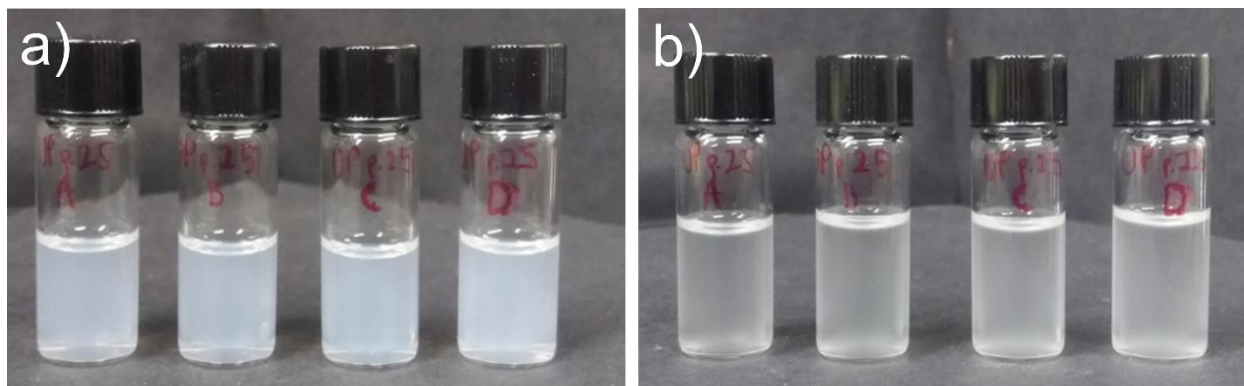


Figure S1: Pathway dependent experiments for the coacervation of PEO₄₅-*b*-PMMA₃₀₀. Nano particle solutions ($\varphi_W = 0.50$) of PEO₄₅-*b*-PMMA₃₀₀ were used to form coacervates by the addition of dioxane to reduce the water to $\varphi_W = 0.25$ (supplementary section 2-iii). a) Nanoparticle solutions ($\varphi_W = 0.50$) of PEO₄₅-*b*-PMMA₃₀₀. b) Coacervate solutions ($\varphi_W = 0.50$) of PEO₄₅-*b*-PMMA₃₀₀.

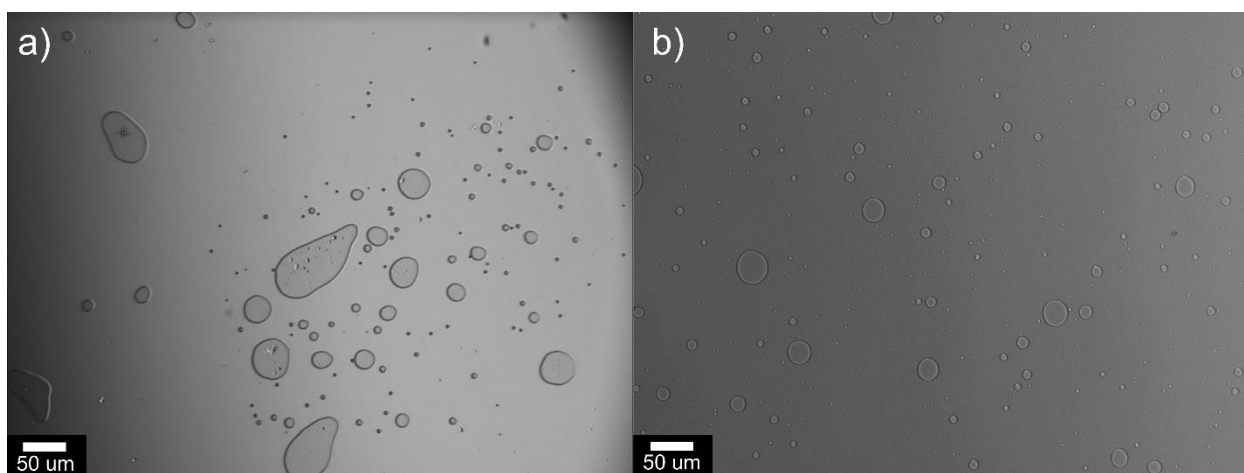


Figure S2: Bright field optical microscopy of block copolymer coacervates. a) PEO₄₅-*b*-PCL₆ coacervates $\varphi_W = 0.34$ in acetone. b) PDMA₇₀-*b*-PMMA₄₅₀ coacervates $\varphi_W = 0.25$ in THF.

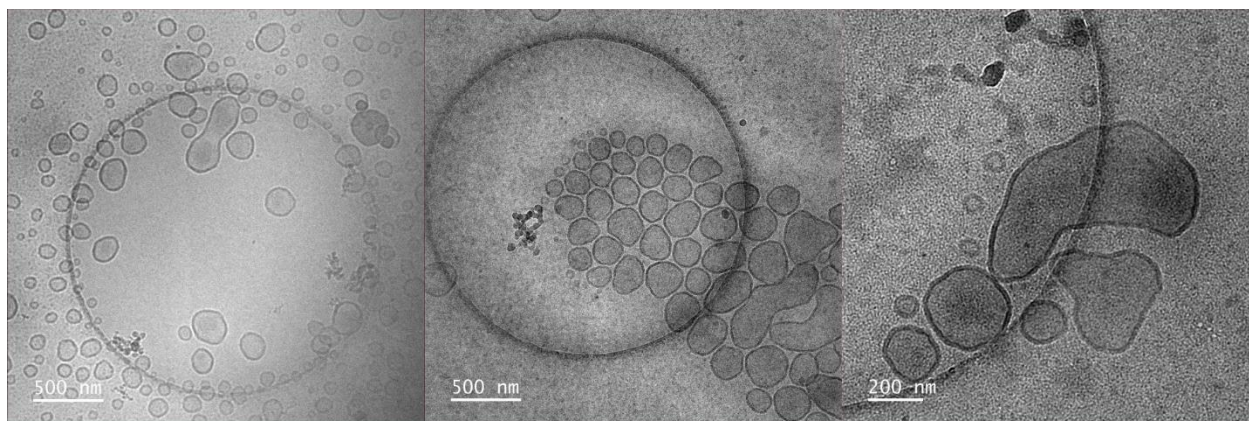


Figure S3: Cryo-TEM images of $\phi_W > 0.25$ PEO₄₅-*b*-PMMA₃₀₀ vesicles with irregular anisotropic shapes.

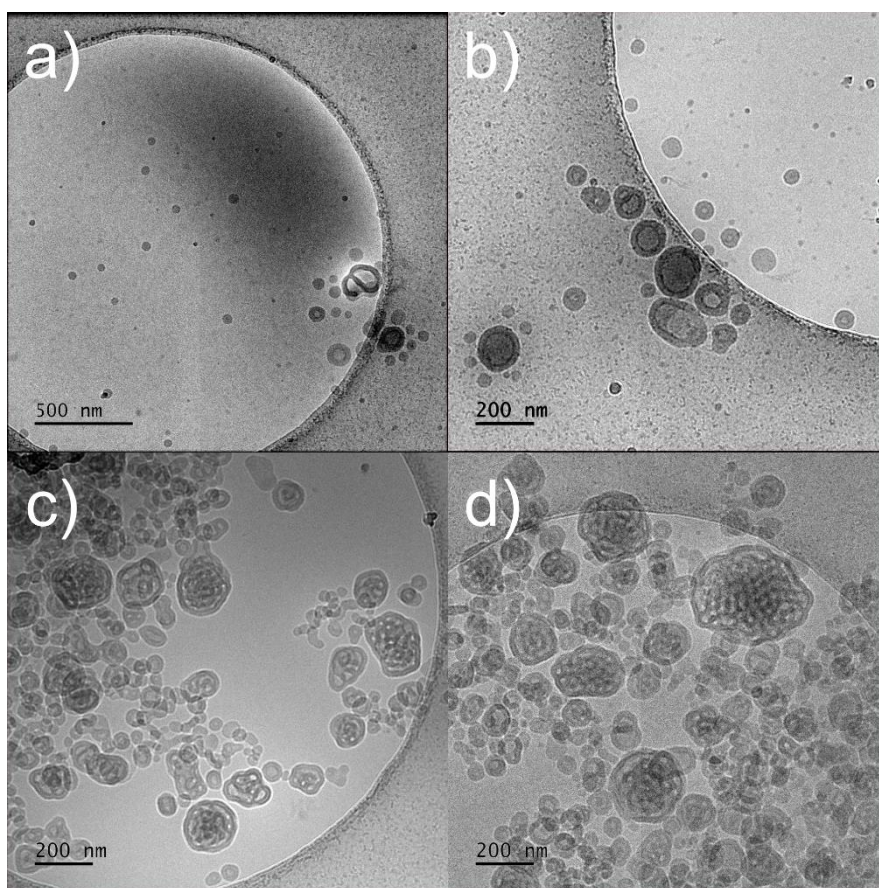


Figure S4: Cryo-TEM images of PEO₄₅-PMMA₃₀₀ assemblies after dialysis in water. a-b) Images of vesicles and spherical micelles in 1 mg mL⁻¹ PEO₄₅-*b*-PMMA₃₀₀ solution self-assembled at $\varphi_W = 0.50$ water in dioxane and further dialyzed against pure water. c-d) Images of bicontinuous spheres and vesicles 5 mg mL⁻¹ PEO₄₅-*b*-PMMA₃₀₀ solution self-assembled at $\varphi_W = 0.50$ water in dioxane and further dialyzed against pure water.

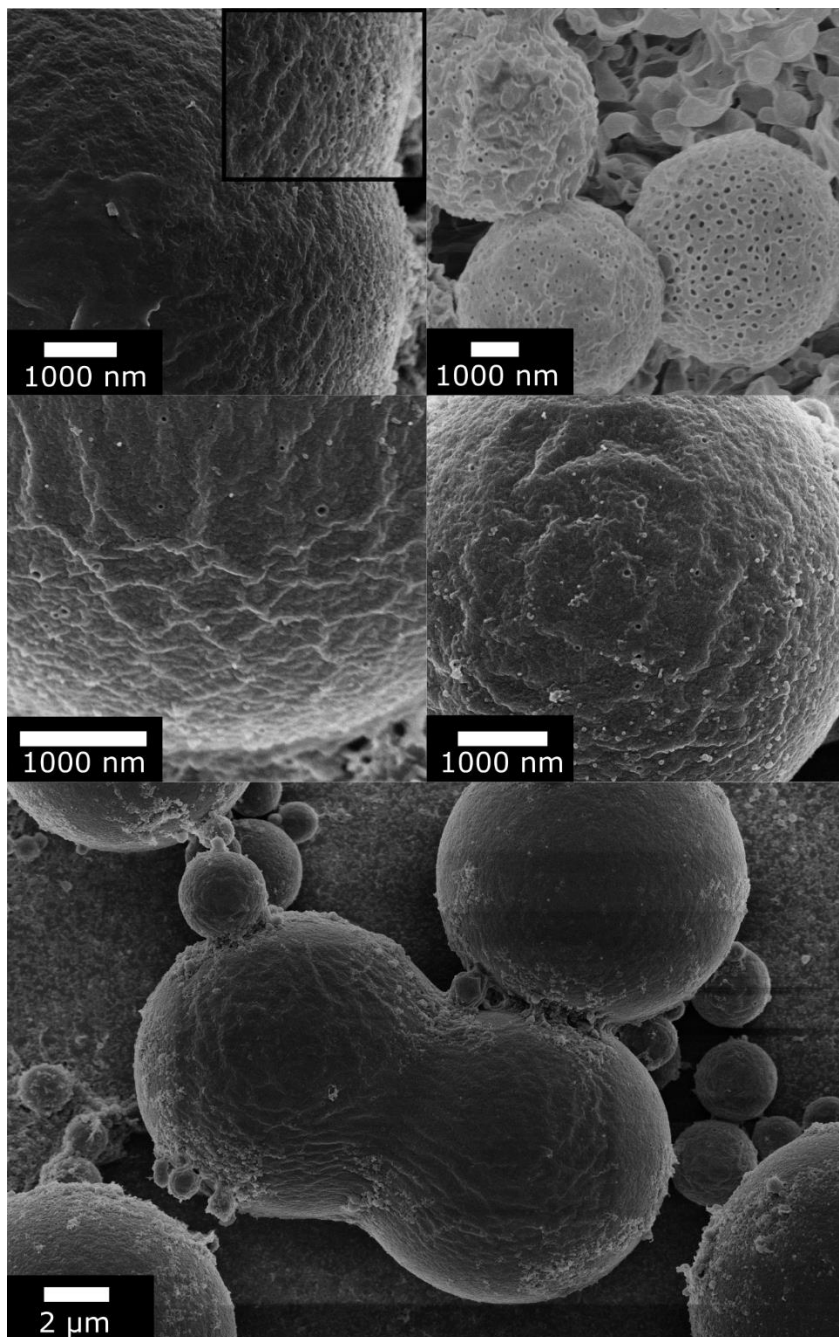


Figure S5: SEM images of PEO₄₅-*b*-PMMA₃₀₀ microparticles with pores and fused anisotropic microparticles.

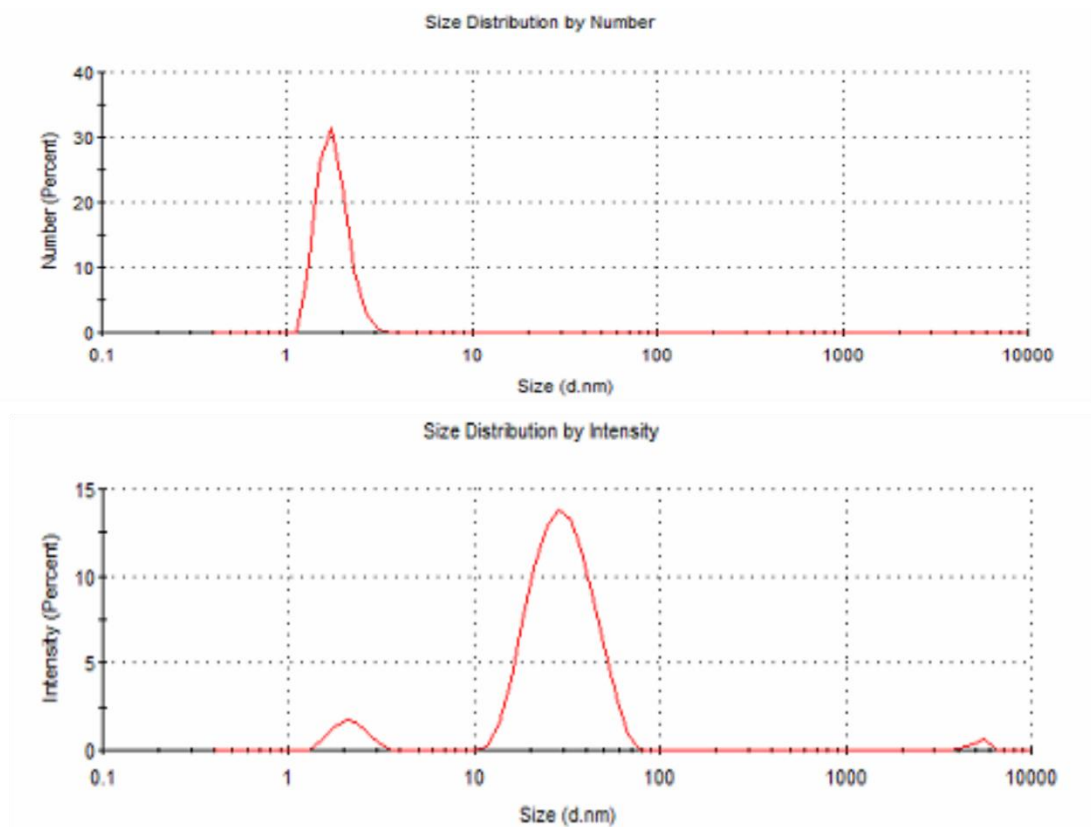


Figure S6: DLS size distribution by number and intensity of PEO₄₅-*b*-PMMA₃₀₀ homogenous sample $\varphi_W = 0.10$.

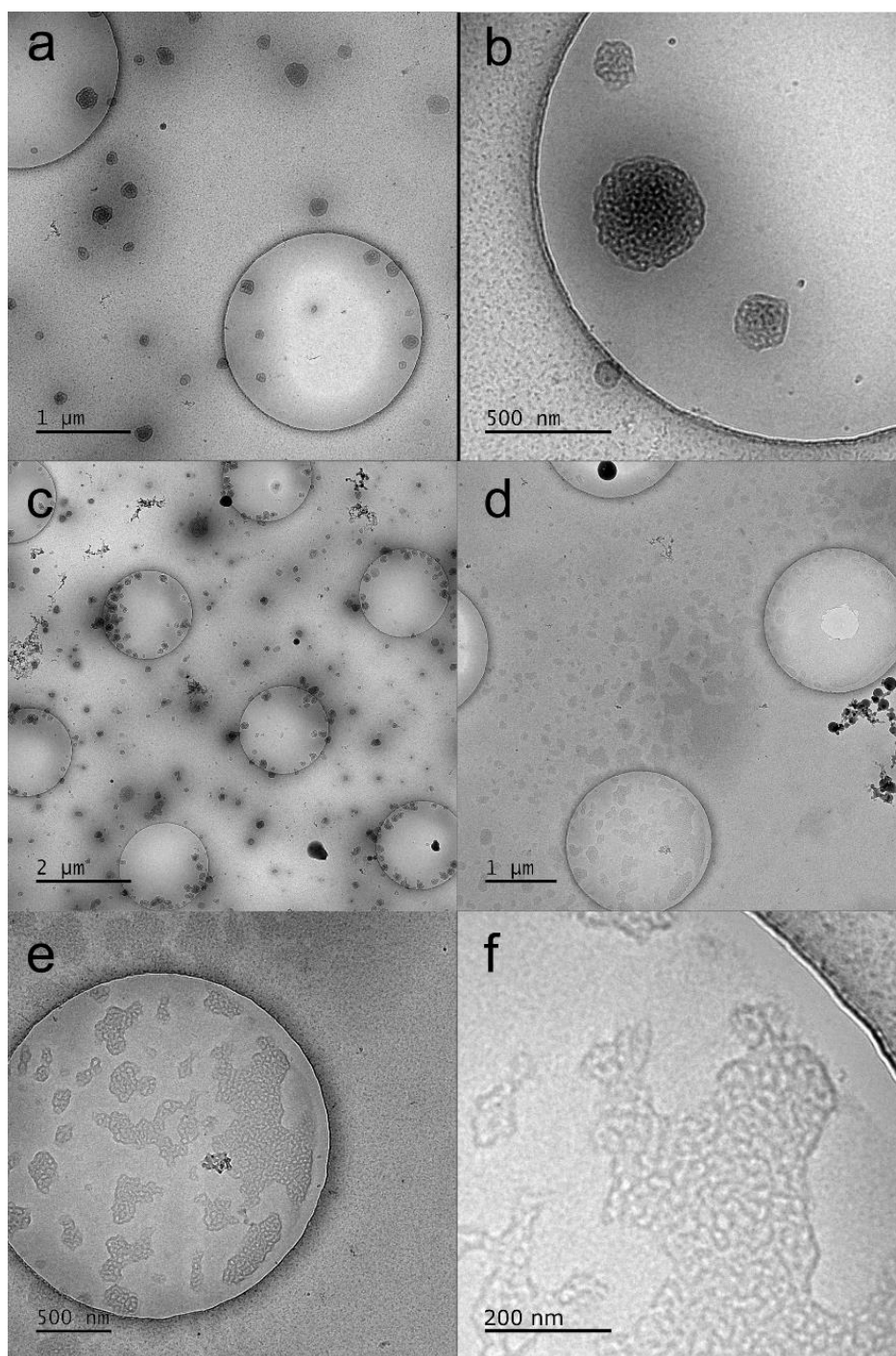


Figure S7: Cryo-TEM images of PEO₄₅-PMMA₃₀₀ assemblies formed on the TEM grid during sample prep. Initial $\phi_W = 0.15$, final $\phi_W > 0.30$ as we see nanoparticle formation around the TEM grid. (a-c) Bicontinuous nano spheres formed from nano-coacervates. (d-f) Coacervate fields that were flattened out during the sample prep with continuous structure within them.

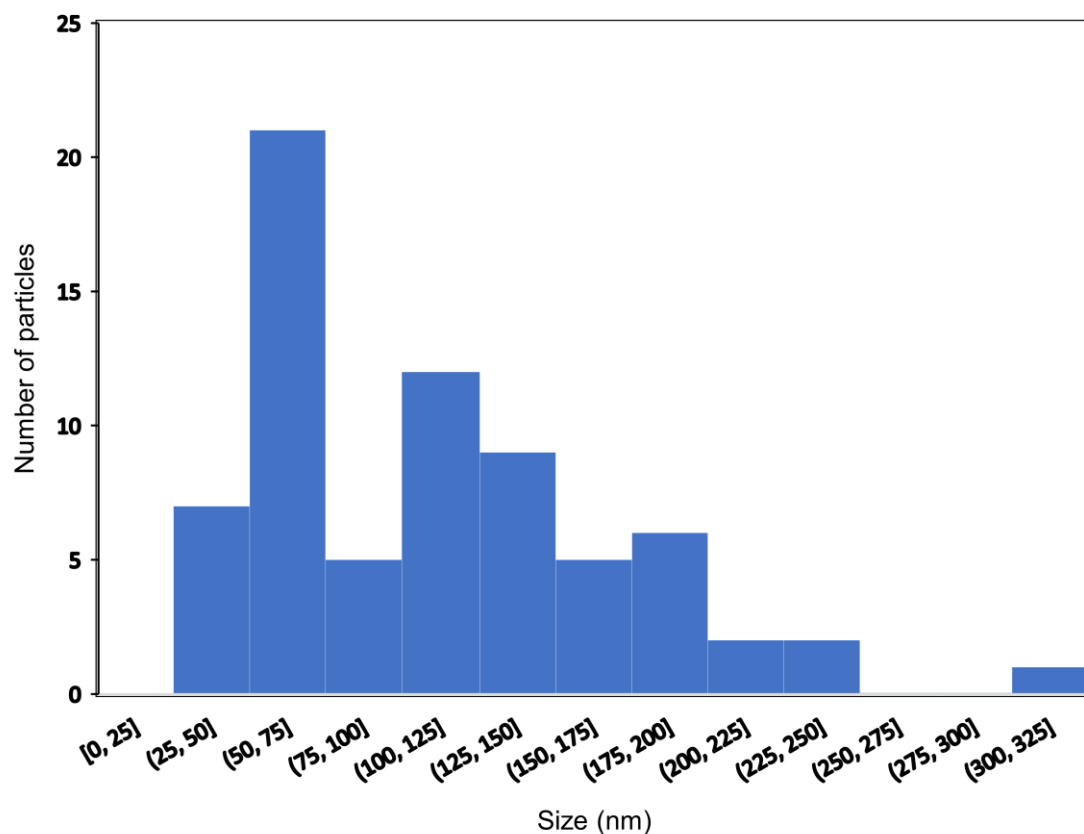


Figure S8: Size (diameter) distribution histogram of PEO₄₅-*b*-PMMA₃₀₀ bicontinuous structures from the Cryo-TEM experiments (Figure 4f).

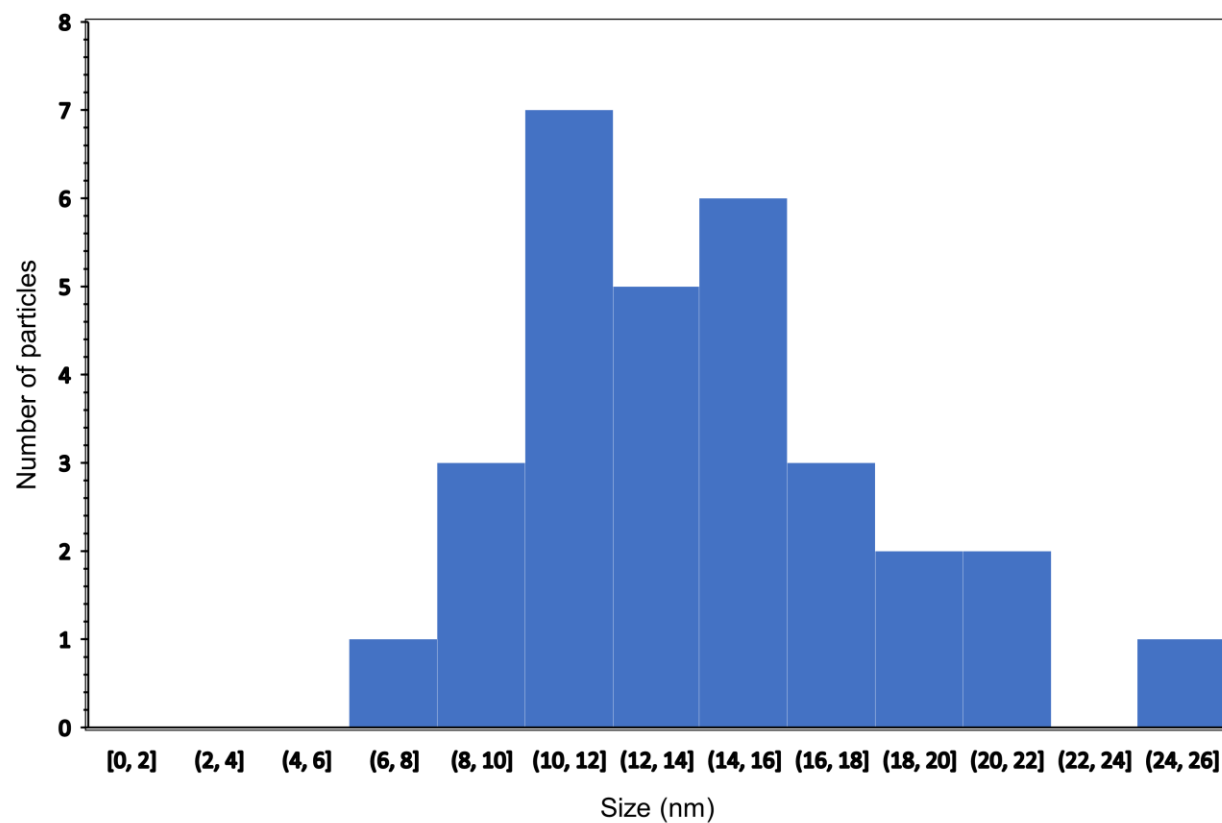


Figure S9: Size (diameter) distribution histogram of pores on the PEO₄₅-*b*-PMMA₃₀₀ bicontinuous structures from the Cryo-TEM experiments (Figure 4f).

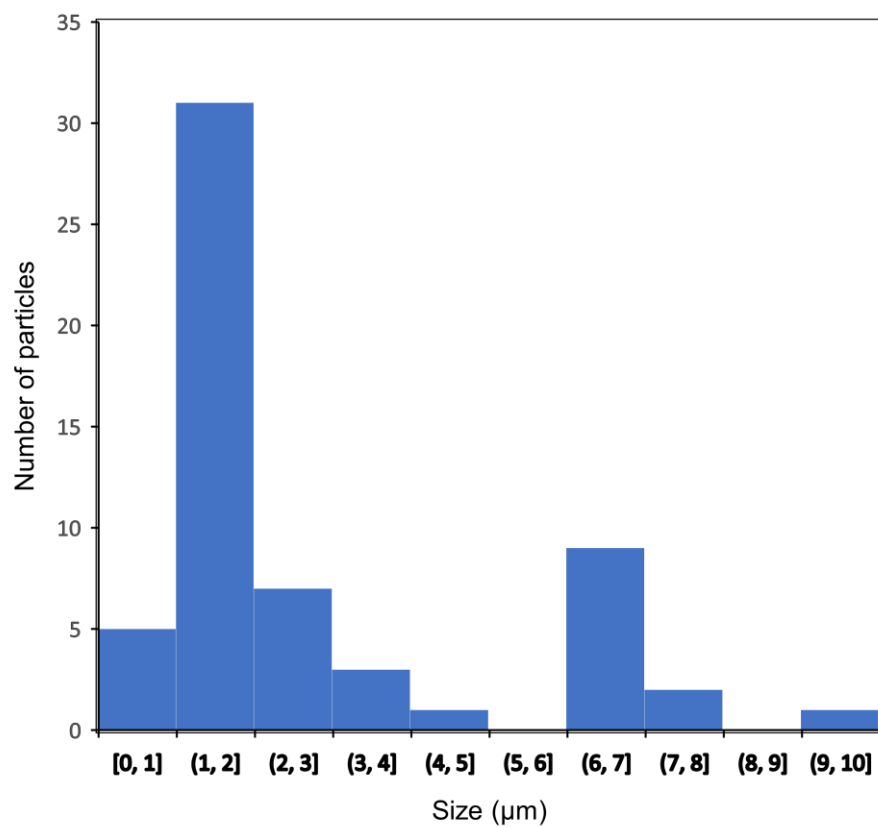


Figure S10: Size (diameter) distribution histogram of PEO₄₅-*b*-PMMA₃₀₀ microparticles from the SEM experiments (Figure 4d).

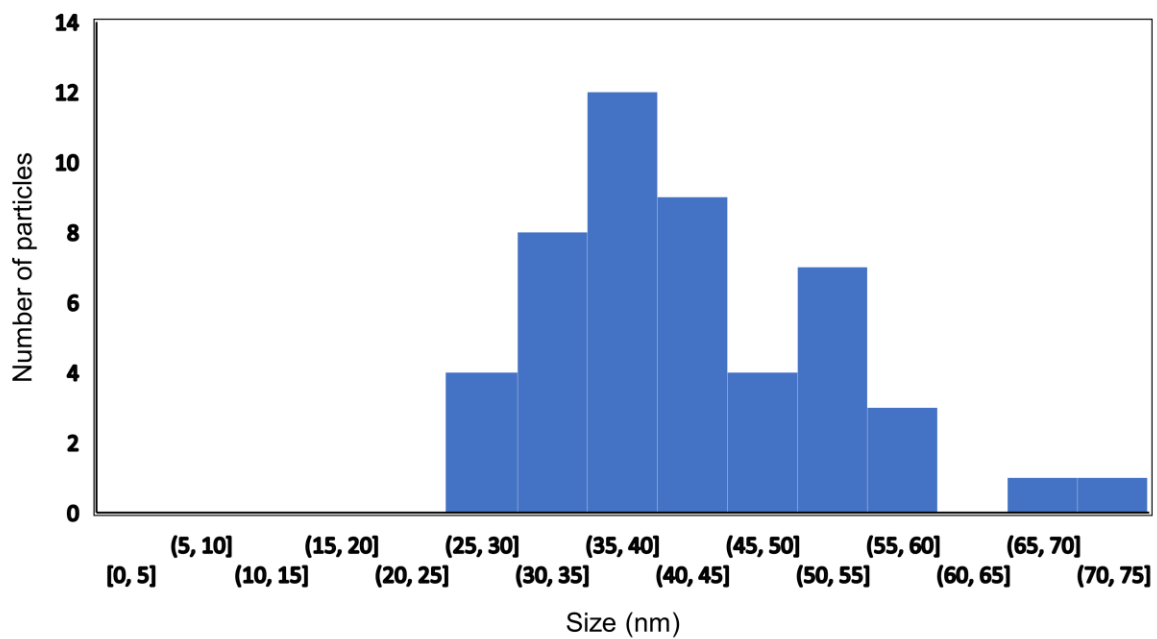


Figure S11: Size (diameter) distribution histogram of pores on the PEO₄₅-*b*-PMMA₃₀₀ microparticles from the SEM experiments (Figure 4d).

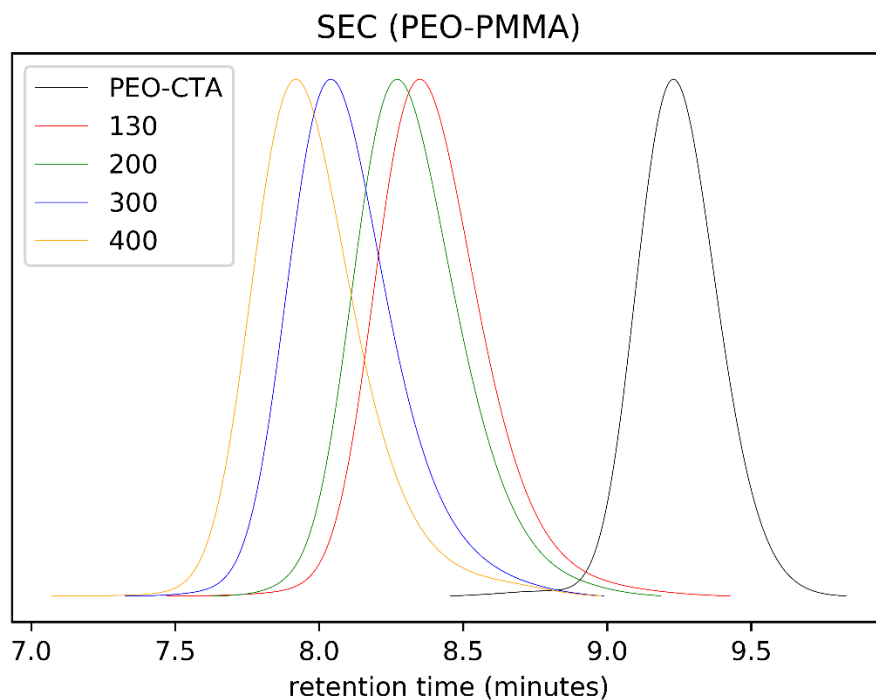


Figure S12: Chromatogram of polyethyeleneoxide MacroCTA and the series of PEO₄₅-*b*-PMMA with increasing degree of polymerization for the PMMA block.

¹H NMR:

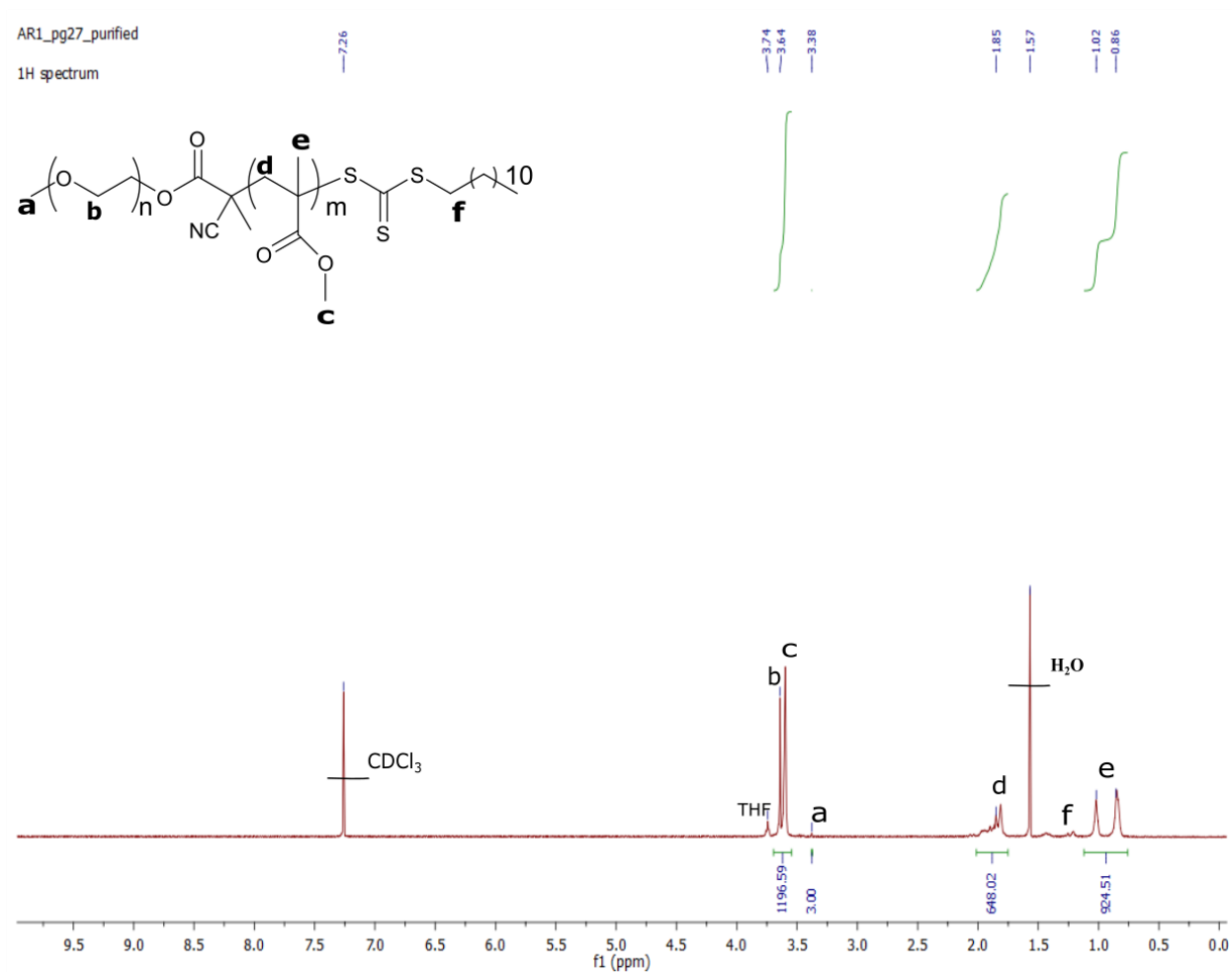


Figure S13: ¹H NMR spectrum of PEO₄₅-*b*-PMMA₃₀₀ Solvent: CDCl₃

AR1_pg89_purified2

¹H spectrum

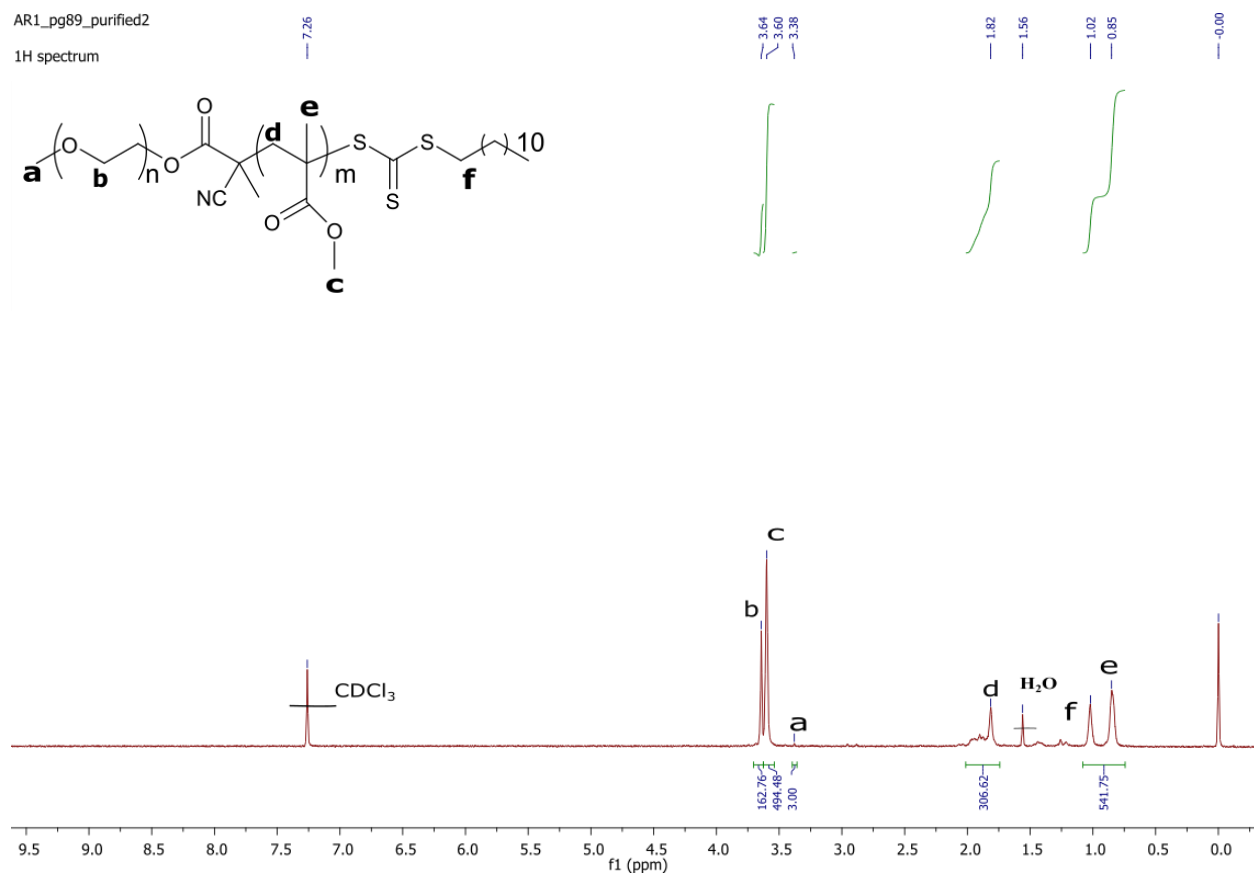


Figure S14: ¹H NMR spectrum of PEO₄₅-b-PMMA₁₃₀ Solvent: CDCl₃ (TMS)

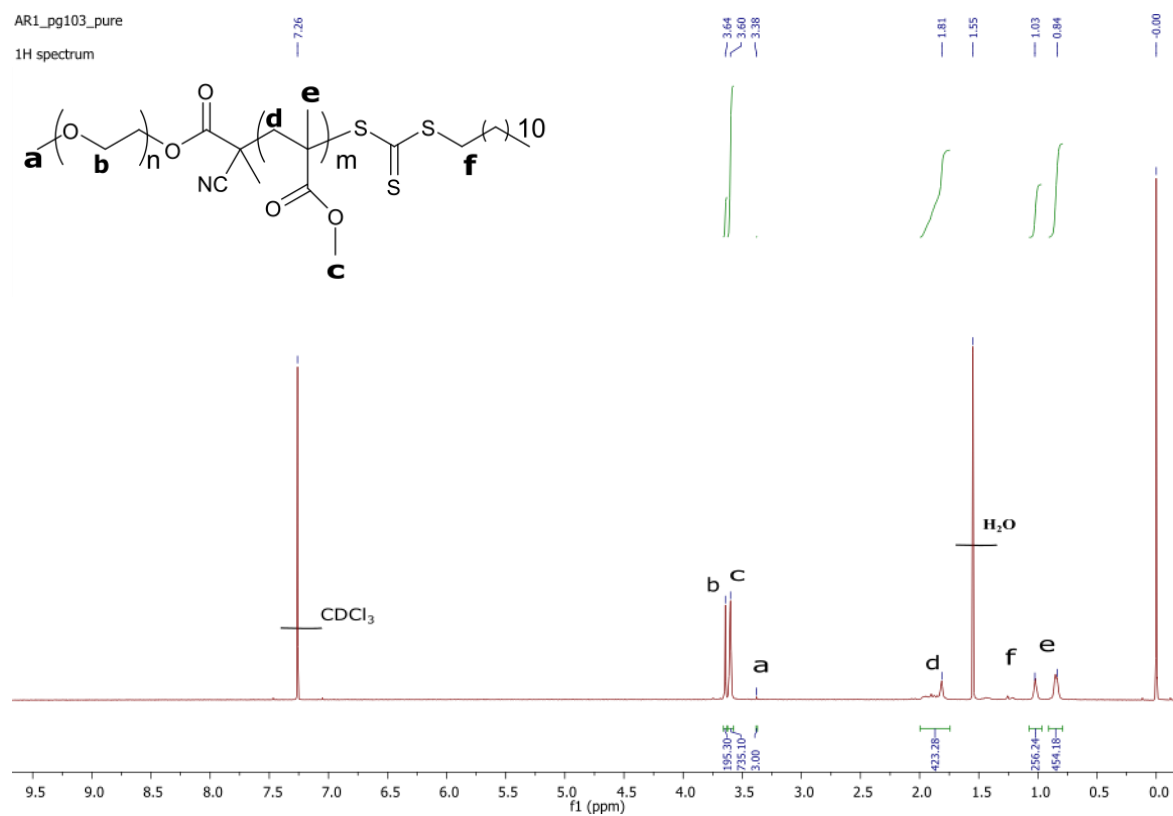


Figure S15 : ^1H NMR spectrum of $\text{PEO}_{45}\text{-}b\text{-PMMA}_{200}$ Solvent: CDCl_3 (TMS)

AR1_pg109_purified

¹H spectrum

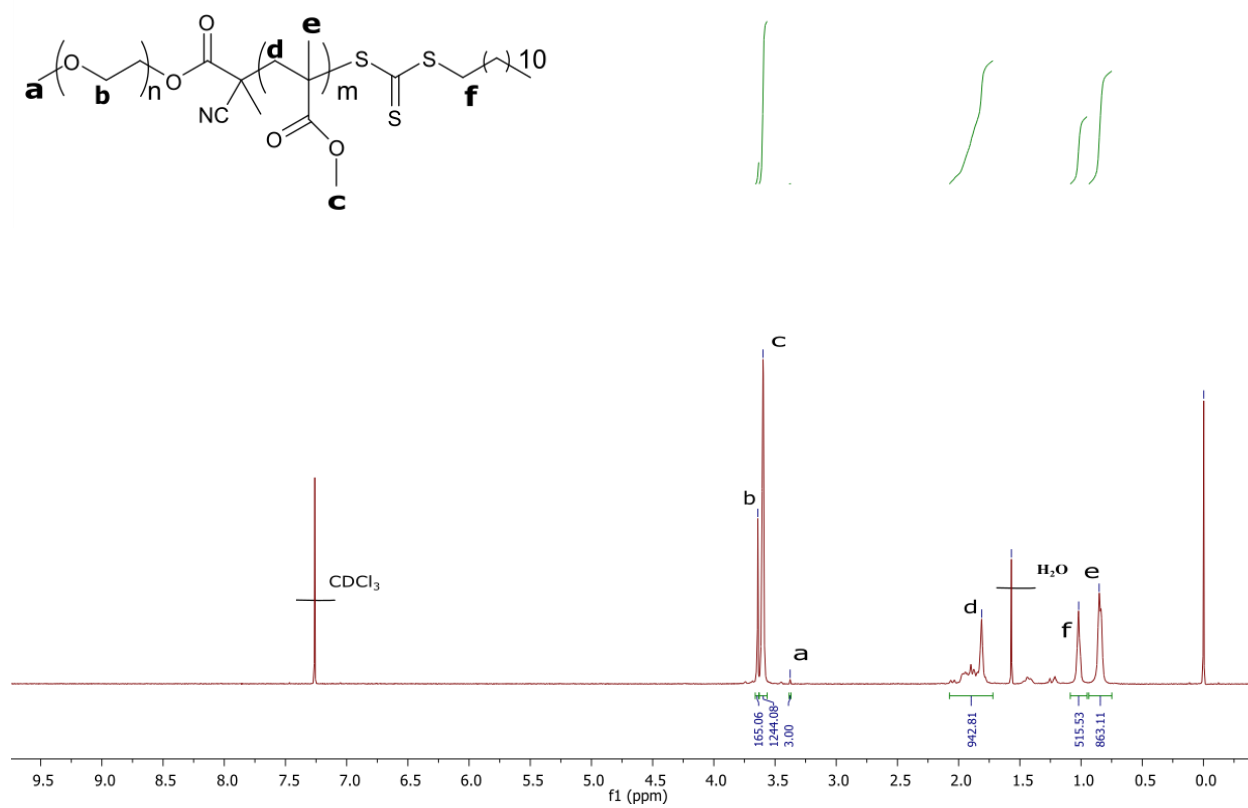


Figure S16: ¹H NMR spectrum of PEO₄₅-b-PMMA₄₀₀ Solvent: CDCl₃ (TMS)

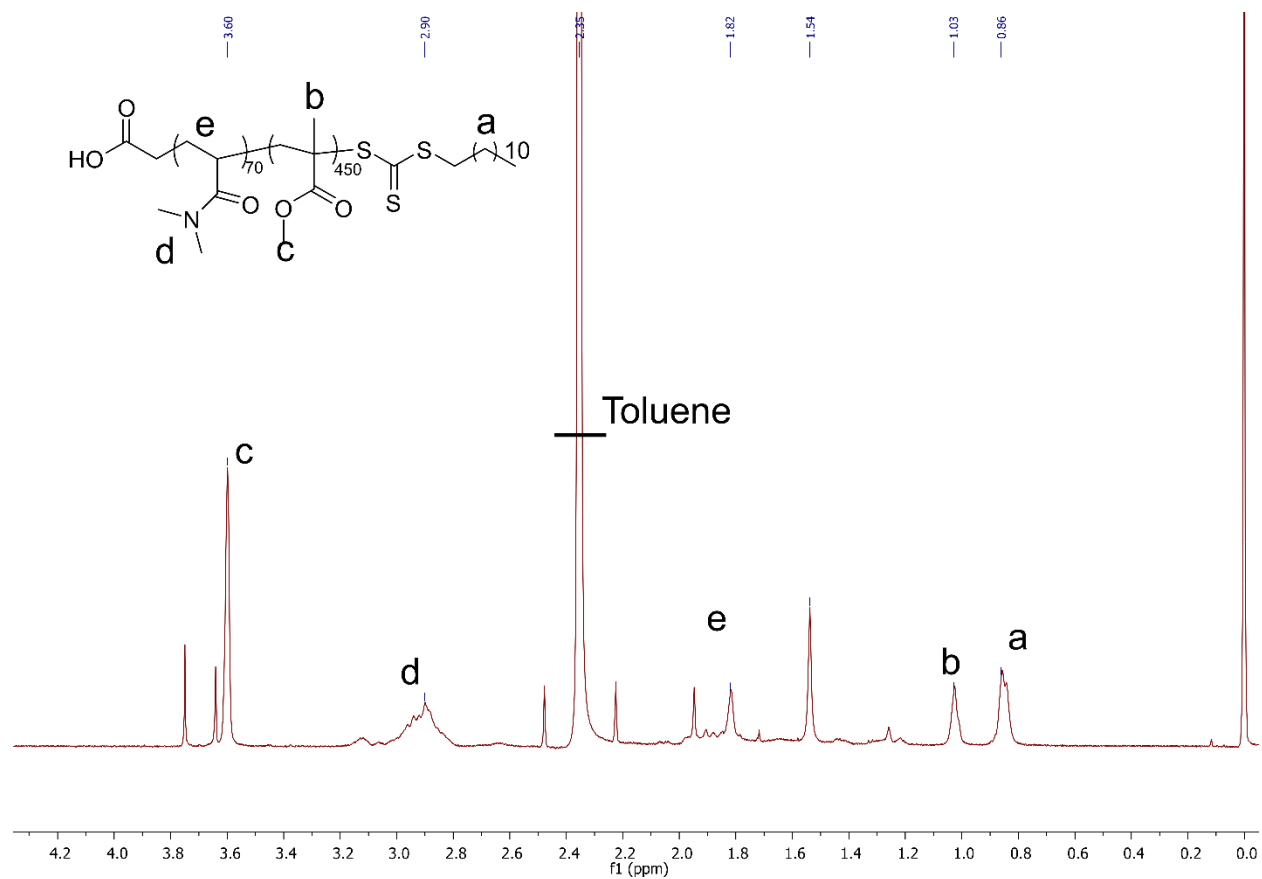


Figure S17: ^1H NMR spectrum of PDMA₇₀-b-PMMA₄₅₀ Solvent: CDCl₃ (TMS)

UV-Vis Data:

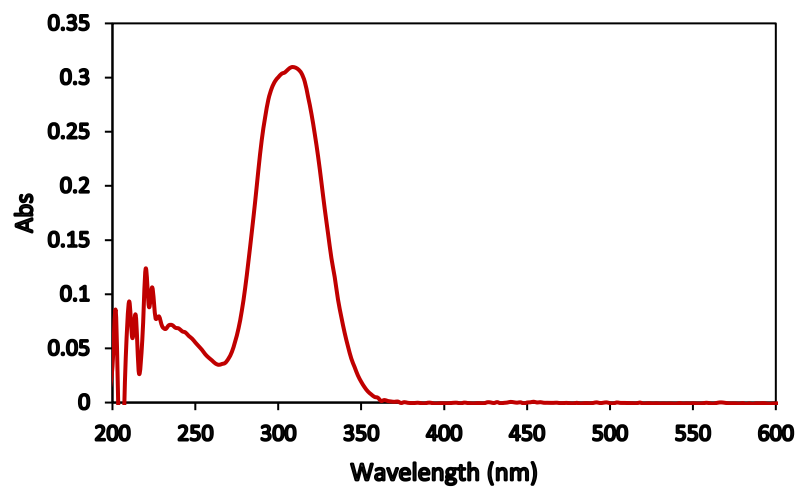


Figure S18: UV-Vis Absorbance Spectra of CTA1

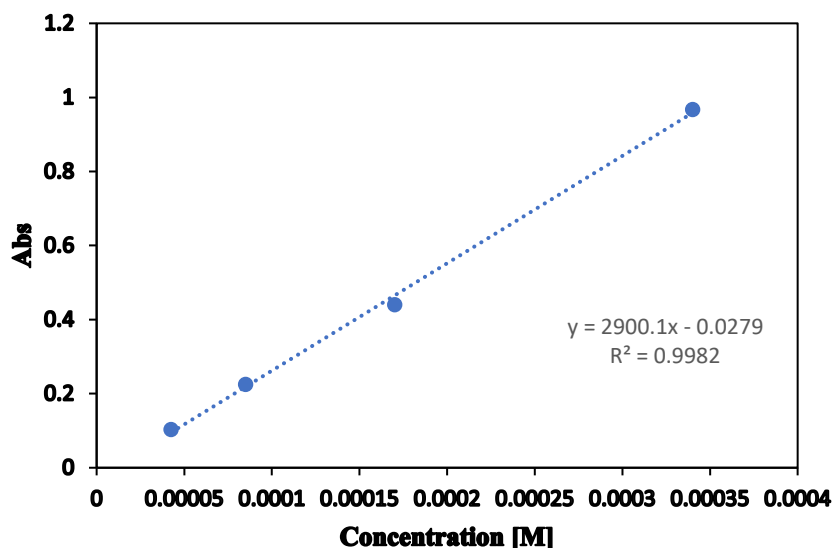


Figure S19: Calibration curve of CTA1 increasing concentration. ($\lambda_{\text{max}} = 309 \text{ nm}$)

References

- (1) Put, M. W. P. van de; Patterson, J. P.; Bomans, P. H. H.; Wilson, N. R.; Friedrich, H.; Benthem, R. A. T. M. van; With, G. de; O'Reilly, R. K.; Sommerdijk, N. A. J. M. Graphene Oxide Single Sheets as Substrates for High Resolution CryoTEM. *Soft Matter* **2015**, *11* (7), 1265–1270. <https://doi.org/10.1039/C4SM02587C>.
- (2) Patterson, J. P.; Robin, M. P.; Chassenieux, C.; Colombani, O.; O'Reilly, R. K. The Analysis of Solution Self-Assembled Polymeric Nanomaterials. *Chem. Soc. Rev.* **2014**, *43* (8), 2412–2425. <https://doi.org/10.1039/C3CS60454C>.
- (3) Oesterhelt, F.; Rief, M.; Gaub, H. E. Single Molecule Force Spectroscopy by AFM Indicates Helical Structure of Poly(Ethylene-Glycol) in Water. *New J. Phys.* **1999**, *1*, 6–6. <https://doi.org/10.1088/1367-2630/1/1/006>.
- (4) Ianaro, A.; Wu, H.; Rijt, M. M. J. van; Vena, M. P.; Keizer, A. D. A.; Esteves, A. C. C.; Tuinier, R.; Friedrich, H.; Sommerdijk, N. A. J. M.; Patterson, J. P. Liquid–Liquid Phase Separation during Amphiphilic Self-Assembly. *Nat. Chem.* **2019**, *11* (4), 320–328. <https://doi.org/10.1038/s41557-019-0210-4>.
- (5) Flory, F. 1953 *Principles Of Polymer Chemistry*.
- (6) Sato, T.; Takahashi, R. Competition between the Micellization and the Liquid–Liquid Phase Separation in Amphiphilic Block Copolymer Solutions. *Polym. J.* **2017**, *49* (2), 273–277. <https://doi.org/10.1038/pj.2016.110>.

