

Supplementary Information

for

**Solvent Quality Shape Control in Continuous Flow Block Copolymer Self-
Assembly**

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Table of Contents

1. Characterization methods	3
1.1 Size exclusion chromatography (SEC)	3
1.2 Nuclear magnetic resonance spectroscopy (NMR).....	3
1.3 Scanning Transmission Electron Microscopy (STEM)	3
1.4 Small Angle Neutron Scattering (SANS)	3
2. Polymer Synthesis.....	4
2.1 Poly(2-Hydroxy ethyl acrylate) homopolymer (PHEA).....	4
2.2 Poly(2-Hydroxy ethyl acrylate)- <i>b</i> -poly(styrene) (PHEA- <i>b</i> -PS) block copolymer	5
3. Small angle neutron scattering (SANS) data	6
3.1 Fitting parameters	6
3.2 Scattering length density (SLD) calculations	6
3.3 The equations for each model used to fit SANS data	7
3.3.1 Ellipsoid model.....	7
3.3.2 Cylinder model.....	8
3.4 Pair-distance distribution functions.....	8
4. Time stability of the SANS data	10
5. Scanning Transmission Electron Microscopy Images	10
6. References	11

1. Characterization methods

1.1 Size exclusion chromatography (SEC)

Analysis of the polymers was performed on a PSS SECcurity2GPC system operated by PSS WinGPC software, equipped with a SDV 5.0 μm guard column (50 x 8 mm), followed by three SDV analytical 5.0 μm columns with varying porosity (1000 \AA , 100000 \AA and 1000000 \AA) (50 x 8 mm) and a differential refractive index detector using tetrahydrofuran (THF, RCI Labscan, 99.9%) as the eluent at 40 $^{\circ}\text{C}$ with a flow rate of 1 $\text{mL}\cdot\text{min}^{-1}$. The SEC system was calibrated using linear narrow polystyrene standards ranging from 682 to $2.52 \times 10^6 \text{ g mol}^{-1}$ PS ($K = 14.1 \times 10^{-5} \text{ dL g}^{-1}$ and $\alpha = 0.70$).

1.2 Nuclear magnetic resonance spectroscopy (NMR)

Proton nuclear magnetic resonance ($^1\text{H-NMR}$) was undertaken using a Bruker Avance III 400 which operated at 400 MHz, using deuterated solvents in the form of chloroform (CDCl_3).

1.3 Scanning Transmission Electron Microscopy (STEM)

All Scanning Transmission Electron Microscopy (STEM) images were taken on the Magellan 400 FEGSEM. The Magellan 400 FEGSEM is an extreme high resolution (XHR) instrument equipped with a monochromator allowing improved resolution at low accelerating voltages. This system is fitted with a multi-sample holding station allowing for analysing multiple samples at once without compromising the vacuum. For STEM a special STEM detector is used with the system. The system consists of a FEG; 5-axis piezo stage; IR chamber camera; oil-free pumping system; retractable DBS annular BSE detector; retractable STEM3 detector; quick loader; beam deceleration; in-lens detectors; NavCam; plasma cleaner; cryo-trap; Bruker Quantax 400 X-ray analysis system and 30mm^2 SDD with super light element window (slew).

The STEM samples were prepared on lacey carbon film coated 300 mesh copper grids. The nanoaggregate solution was prepared by mixing polymer solution (concentration of polymer in THF was 5 mg/mL) and water in flow and 3 μL of the sample was drop casted on to the grid. After 1 minute, it was blotted with a filter paper without touching the grid to remove excess solvent. Then the staining of nanoparticles was done using a 0.75% w/w aqueous solution of uranyl acetate, where 3 μL of the staining agent was drop casted onto the sample loaded grid and after 15 seconds, the excess stain was carefully removed by blotting using a filter paper. It was dried in the fume hood overnight ensuring that all the solvent was removed from the particles, so that it would not dissolve the carbon layer on the grid.

1.4 Small Angle Neutron Scattering (SANS)

Small-Angle Neutron Scattering (SANS) measurements were undertaken on the Bilby instrument^{1,2} at the Australian Centre for Neutron Scattering, Australian Nuclear Science and Technology Organisation (ANSTO), Lucas Heights, NSW, Australia. Samples were prepared using D_2O and THF mixture as the aqueous phase and measured at a concentration of 1 mg/mL for 2 hours at room temperature in 1 mm thick quartz window cells with 0.95mm spacer inserted, reducing the path-length to 0.05mm.

Data was collected in monochromatic mode, using wavelength of 6 \AA with 10% nominal resolution. Distance to the rear detector was 18.5m, and to the left/right and top/bottom curtains 3m and 4m, respectively. The distance to the beam for the left, right, top and bottom curtains were 0.2m, 0.385m, 0.04m and 0.04m, respectively. q -range used for data analysis covers from 0.0026\AA^{-1} to 0.254\AA^{-1} .

Raw scattering data was reduced using standard instrument procedure. Scattering of the empty cell as well as aqueous background has been subtracted. SANS data were analysed using the SasView software (<http://www.sasview.org>) using the sphere, cylinder and ellipse models presented by Guinier and Fournet.³

2. Polymer Synthesis

2.1 Poly(2-Hydroxy ethyl acrylate) homopolymer (PHEA)

In a classical reaction, 10 g of HEA (20eq), 1.51 g of DOPAT (1eq) and 35mg of AIBN (0.05 eq) were dissolved in 18 mL of n-butanol in a round bottom flask. The solution was purged for 15 min with Argon gas and placed in a 100°C oil bath for 15 min. After the polymerization a small amount was taken for NMR analysis (Figure S1). The polymer was precipitated in cold diethyl ether and dried overnight.

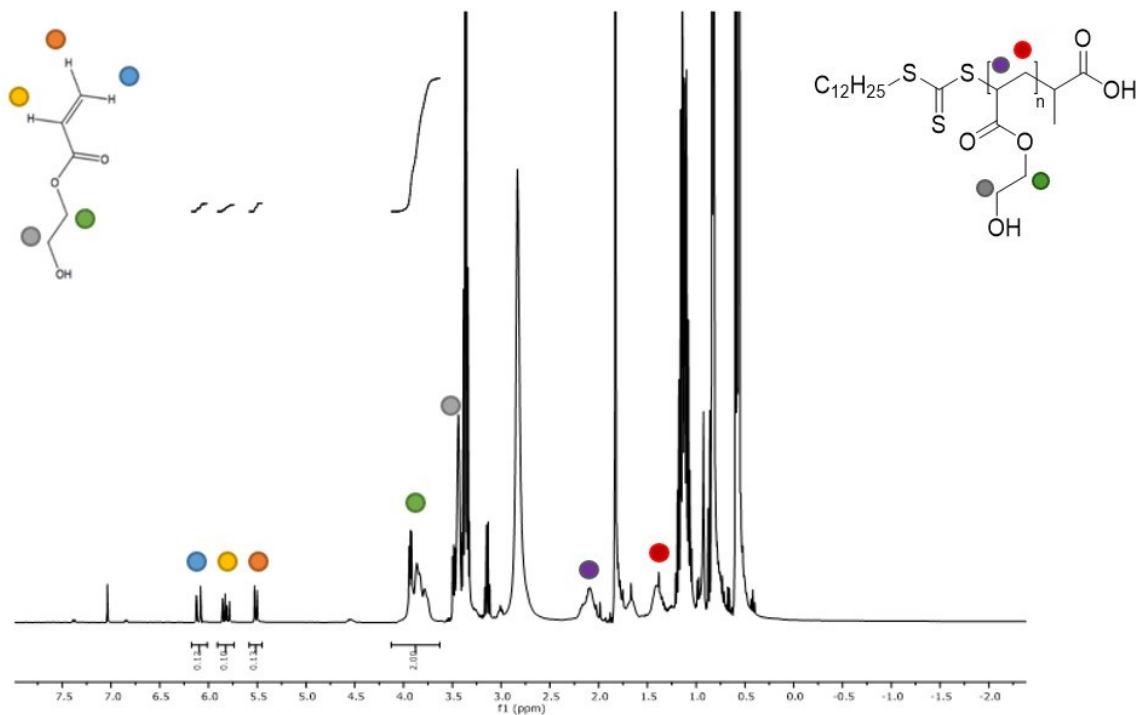


Figure S1: Conversion calculation via ^1H NMR for HEA homopolymer in CDCl_3

Average of peaks blue - yellow - green = 0.11

Conversion = $(1 - 0.11) \times 100 = 89\%$

2.2 Poly(2-Hydroxy ethyl acrylate)-b-poly(styrene) (PHEA-*b*-PS) block copolymer

In a classical reaction, 1.925g of HEA macro-RAFT (1eq), 15.4 g of Styrene (100eq) and 6.3 mg of AIBN (0.5eq) were dissolved in n-butanol in a round bottom flask, a small amount of DMF was added as internal standard for calculation of conversion via NMR. The solution was purged for 15 min with Argon gas and placed in an 80°C oil bath for 5 hours. The polymerization was stopped at around 50% conversion to avoid styrene autopolymerisation. A small amount was taken for NMR conversion (Figure S2) and the polymer was precipitated in cold methanol and dried overnight.

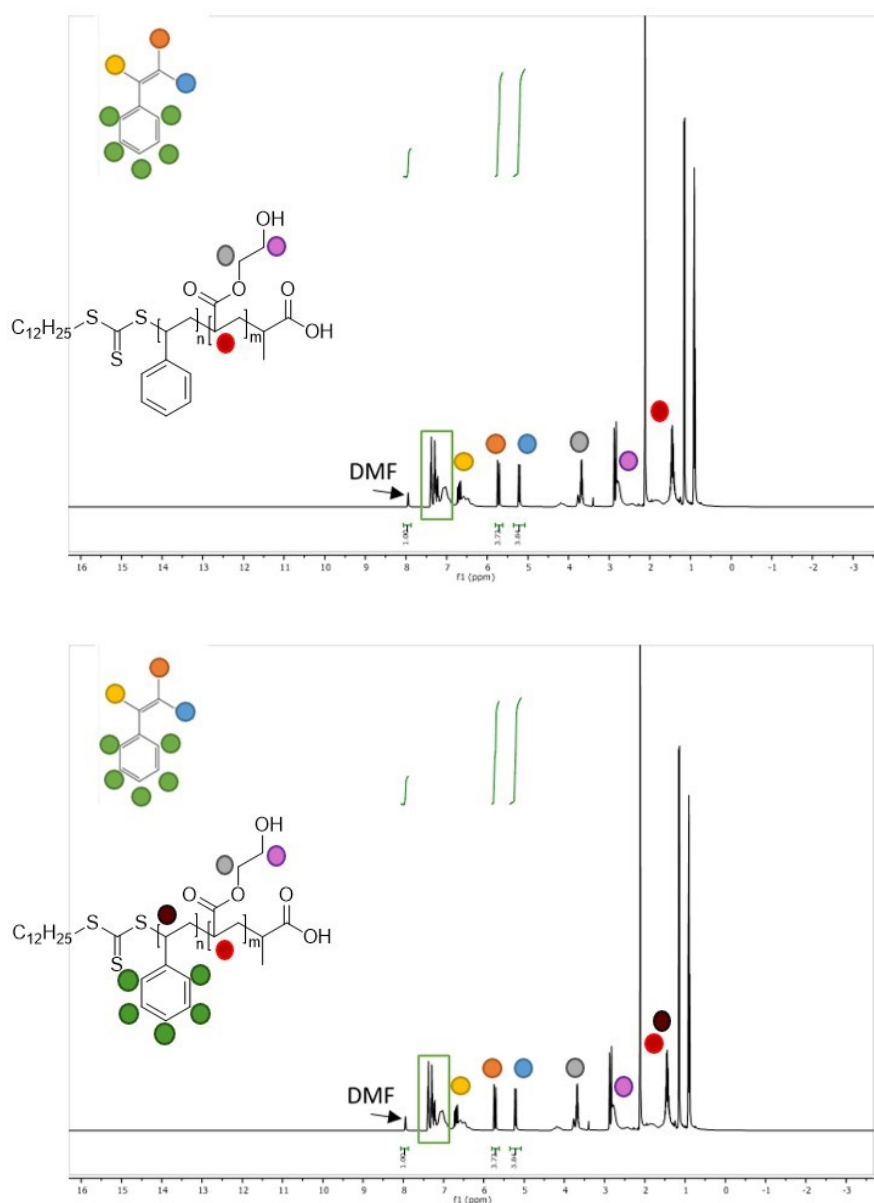


Figure S2: Conversion calculation via ¹H NMR for styrene in CDCl₃. The top spectrum shows the mixture before block extension, the bottom after.

Internal standard DMF = 1

Average of peaks (blue-orange) at T₀ = f6.56

Average of peaks (blue-orange) = f3.75

$$\text{Conversion} = \left(1 - \frac{3.75}{6.56}\right) \times 100 = 43\%$$

3. Small angle neutron scattering (SANS) data

3.1 Fitting parameters

Table S1 below shows all the fitting parameters used in SasView software in fitting the SANS data for all the experiments performed at different solvent ratios in the synthesis of nanoaggregates for the four different block copolymer. They mainly include scale, background, SLD and SLD for the solvent (background). Ellipsoid and cylinder models showed better fits with the data.

THF/D ₂ O ratio	Shape	Radius (polar) / nm	Radius (equatorial) / nm	scale	background	SLD, background	SLD, polymer (limits 1.1 and 1.2)
PHEA20-b-PS60 (A)							
3.2/0.8	Ellipsoid	31.7 ± 0.1	81.1 ± 0.5	0.0823	0.039	1.414	1.1
2.5/1.5	Ellipsoid	30.7 ± 0.1	67.5 ± 0.3	0.0045	0.189	2.49	1.1
2/2	Ellipsoid	27.6 ± 0.1	56.8 ± 0.2	0.0025	0.178	3.26	1.2
1.5/2.5	Ellipsoid	24.1 ± 0.1	57.8 ± 0.2	0.0012	0.377	4.03	1.1
THF/D ₂ O ratio	Shape	Radius / nm	Length / nm	scale	background	SLD, background	SLD, polymer (limits 1.1 and 1.2)
PHEA40-b-PS300 (B)							
3.2/0.8	Cylinder	22.3 ± 0.15	77.8 ± 2	0.028	0.09	1.414	1.1
2.5/1.5	Cylinder	22.3 ± 0.2	62.1 ± 1.5	0.0013	0.087	2.49	1.1
2/2	Cylinder	19.8 ± 0.15	56.1 ± 1.2	0.0006	0.061	3.26	1.1
1.5/2.5	Cylinder	18.2 ± 0.15	50.3 ± 1.1	0.00035	0.411	4.03	1.1
PHEA60-b-PS80 (C)							
3.2/0.8	Cylinder	13.5 ± 0.1	87.8 ± 3	0.0665	0.259	1.414	1.1
2.5/1.5	Cylinder	13.4 ± 0.1	43.3 ± 0.7	0.0036	0.08	2.49	1.1
2/2	Cylinder	13.1 ± 0.1	31.3 ± 0.6	0.0017	0.1177	3.26	1.2
1.5/2.5	Cylinder	15.2 ± 0.1	18.2 ± 0.3	0.0011	0.348	4.03	1.1
PHEA100-b-PS150 (D)							
3.2/0.8	Cylinder	18.5 ± 0.1	118 ± 6	0.082	0.308	1.414	1.1
2.5/1.5	Cylinder	18.7 ± 0.1	58 ± 0.8	0.002	0.382	2.49	1.1
2/2	Cylinder	18.5 ± 0.1	51 ± 0.6	0.001	0.451	3.26	1.1
1.5/2.5	Cylinder	22.5 ± 0.14	27.8 ± 0.3	0.0006	0.262	4.03	1.1

Table S1: The fitting parameters used for SANS data in the determination of particle size information

3.2 Scattering length density (SLD) calculations

SLD for the solvent (background) and the polymer were calculated as shown below. SLD Calculator in SasView software was mainly used for the calculation of SLDs.

For the SLD (background):

$$\text{THF SLD} = 1.83\text{e-}07 \text{ \AA}^{-2}$$

$$\text{D}_2\text{O SLD} = 6.39\text{e-}06 \text{ \AA}^{-2}$$

Solvent ratio	3.2/0.8	2.5/1.5	2/2	1.5/2.5
Ratio of THF	3.2	2.5	2	1.5
Ratio of D ₂ O	0.8	1.5	2	2.5
Fraction of THF	0.8	0.625	0.5	0.375
Fraction of D ₂ O	0.2	0.375	0.5	0.625

Mean SLD of solvent	(Fraction of THF*THF SLD) + (Fraction of D ₂ O*D ₂ O SLD)			
	1.42e-06 Å ⁻²	2.51e-06 Å ⁻²	3.29e-06 Å ⁻²	4.06e-06 Å ⁻²

Table S2: The calculation of SLD of the background at different solvent ratios

For the SLD (polymer):

Density of HEA = 1.011 g/mL

Density of styrene = 0.909 g/mL

Density of DoPAT RAFT agent = 1.093 g/mL

Polymer	PHEA20-b-PS60	PHEA40-b-PS300	PHEA60-b-PS80	PHEA100-b-PS150
Ratio of HEA	20	40	60	100
Ratio of Styrene	60	300	80	150
Ratio of DoPAT	1	1	1	1
Fraction of HEA	0.25	0.117	0.426	0.398
Fraction of Styrene	0.74	0.87	0.567	0.597
Fraction of DoPAT	0.01	0.0029	0.0071	0.004
Mean density	(Fraction of HEA*density of HEA) + (Fraction of styrene*density of styrene) + (Fraction of DoPAT*density of DoPAT)			
	0.936	0.922	0.954	0.95
Molecular formula	C596H670O62S3	C2616H2750O122S3	C956H1150O182S3	C1716H2030O302S3
SLD polymer (calculated)	1.15e-06 Å ⁻²	1.20e-06 Å ⁻²	1.14e-06 Å ⁻²	1.16e-06 Å ⁻²
SLD polymer (fitted)	1.1e-06 Å ⁻²	1.1e-06 Å ⁻²	1.1e-06 Å ⁻²	1.1e-06 Å ⁻²

Table S3: The calculation of SLD of different block copolymers used in the study

Based on calculated polymer SLDs, we decided to fit the data within a specific range, in this case 1.1-1.2e-6 Å⁻². We used a range rather than an exact value, since the forementioned amount of trapped solvent, as well as density of the polymers itself, are not easy to calculate precisely. The best fit values are given in the table. Yet, they cannot be taken as a real SLD value. The reason being that for the fitting, the value of the SLDs and that of the “scale” are strongly coupled, and without fixing one, the second cannot be determined unambiguously (and hence the exact volume fraction remains uncertain).

3.3 The equations for each model used to fit SANS data

3.3.1 Ellipsoid model

For Ellipsoid model, the equations for the output of 2D scattering intensity function is given by Feigin and Svergun (1987).^{4,5}

(<https://www.sasview.org/docs/user/models/ellipsoid.html>)

$$P(q,\alpha) = \frac{scale}{V} F^2(q,\alpha) + background \quad (1)$$

where,

$$F(q,\alpha) = \Delta\rho V \frac{3\sin(qr) - qr \cos(qr)}{(qr)^3} \quad (2)$$

for

$$r = [R_e^2 \sin^2 \alpha + R_p^2 \cos^2 \alpha]^{\frac{1}{2}} \quad (3)$$

A = angle between the axis of the ellipsoid and q

$$V = \text{volume of the ellipsoid} = \left(\frac{4}{3}\right)\pi R_p R_e^2 \quad (4)$$

R_p = polar radius along the rotational axis of the ellipsoid

R_e = equatorial radius perpendicular to the rotational axis of the ellipsoid

Δρ (contrast) = scattering length density difference between the scatterer and the solvent.

3.3.2 Cylinder model

For cylinder model, the equations for the output of 2D scattering intensity function is given by Guinier (1955).⁶⁻⁸

(<https://www.sasview.org/docs/user/models/cylinder.html>)

$$I(q) = \frac{\text{scale}}{V} \int_0^{\frac{\pi}{2}} F^2(q, \alpha) + \text{background} \quad (5)$$

Where,

$$F(q, \alpha) = 2(\Delta\rho)V \frac{\sin\left(\frac{1}{2}qL\cos\alpha\right) J_1(qR\sin\alpha)}{\frac{1}{2}qL\cos\alpha \quad qR\sin\alpha} \quad (6)$$

α = angle between the axis of the cylinder and q

V = πR²L = volume of the cylinder (7)

L = length of the cylinder

R = radius of the cylinder

Δρ (contrast) = scattering length density difference between the scatterer and the solvent

J₁ = first order Bessel function

3.4 Pair-distance distribution functions

A pair-distance distribution function ($p(r)$) analysis of the data offers useful insights into the average shape and size of these aggregates in real space.⁹

$p(r)$ is a Fourier-transform of the $I(q)$ and hence can be calculated without any assumptions on the shape of the particles in the dilute solutions:

$$p(r) = \frac{r^2}{2\pi^2} \int_0^{\infty} q^2 I(q) \frac{\sin qr}{qr} dq$$

$p(r)$ functions for all data sets for different block copolymers are shown below in Figure S3.

The trend observed in the changes of $p(r)$ patterns for each series corresponds well to that observed in the fitting parameters.

To note, for spherical or elongated particles with a ratio larger than ~ 1:3, it is straightforward to correlate maximum of the $p(r)$ and D_{max} (maximum dimension of a particle) with the radii and/or lengths of the particle in

solution. Though for particles with a ratio of diameters/cross-sections close to 1.5-2, the maximum of $\rho(r)$ and D_{max} are not easy to interpret. Therefore for the latter, the comparison of the observed trends in $\rho(r)$ shall be the only meaningful outcome, rather than estimation of the maximum cross-section or D_{max} .

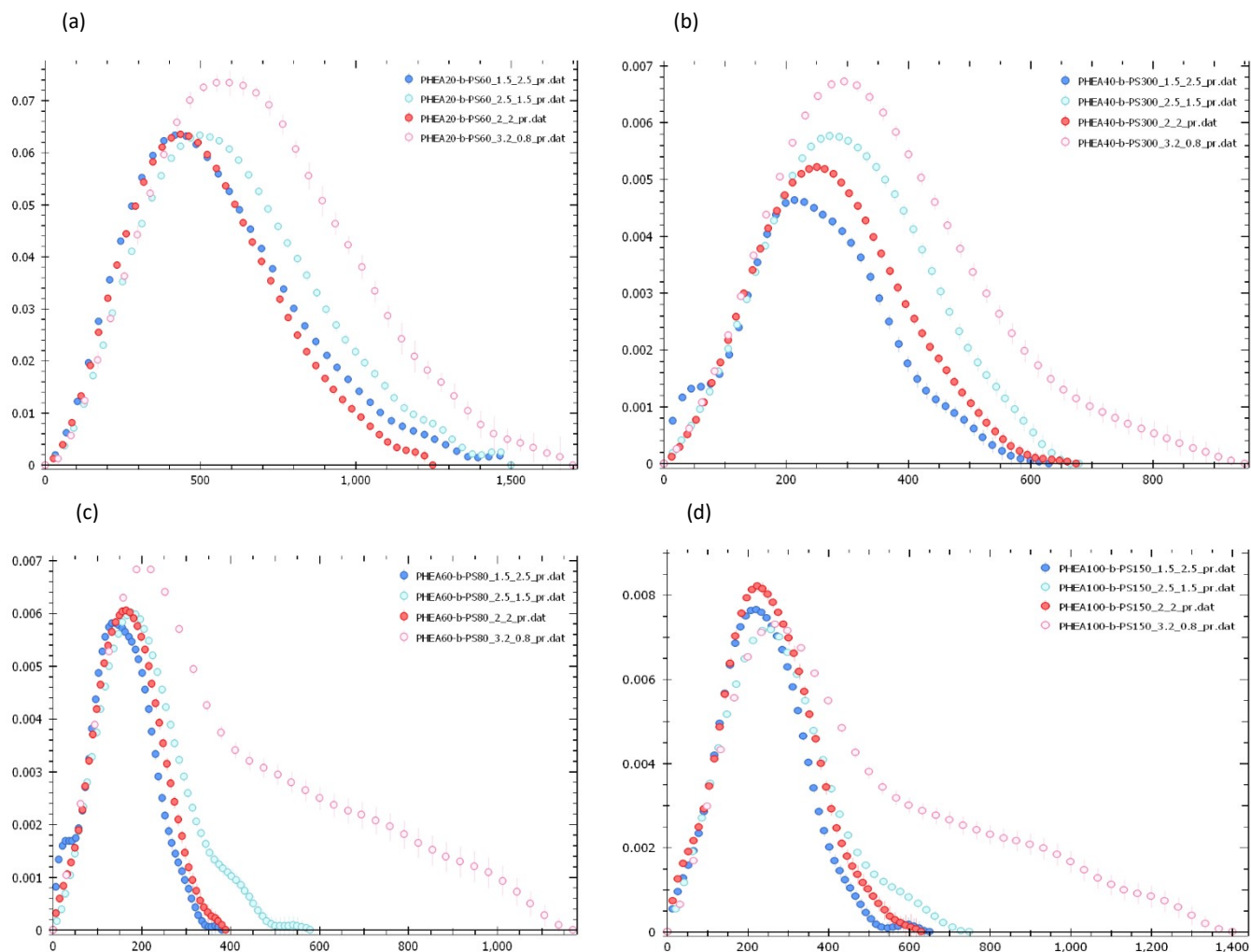


Figure S3: $p(r)$ function for nanoaggregates made with different block copolymers at different solvent ratios. (a) PHEA20-b-PS60 (b)PHEA40-b-PS300 (c) PHEA60-b-PS80 (d) PHEA100-b-PS150

4. Time stability of the SANS data

As indicated in the manuscript, SANS traces were obtained over 10 min periods. The Figure below shows an overlap of very first and last 100 sec slices of the data, demonstrating the time stability of the sample during data collection.

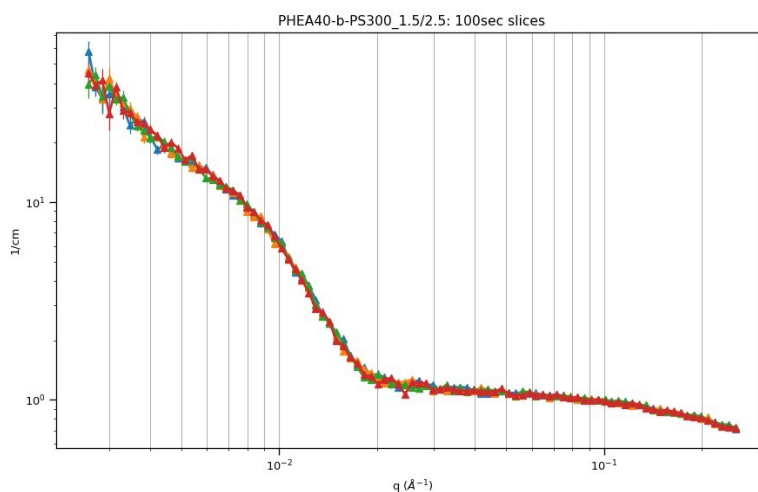


Figure S4: Overlap of 100 sec slices of SANS scattering data, demonstrating no change of the scattering during data collection time.

5. Scanning Transmission Electron Microscopy Images

STEM images taken for the sample PHEA40-b-PS300 are shown below. They were taken in bright field (BF) mode of detection at an accelerating voltage of 30.00 kV and are showing large sized particles (250 nm and 110 nm) as compared to much smaller particle sizes determined via SANS measurements.

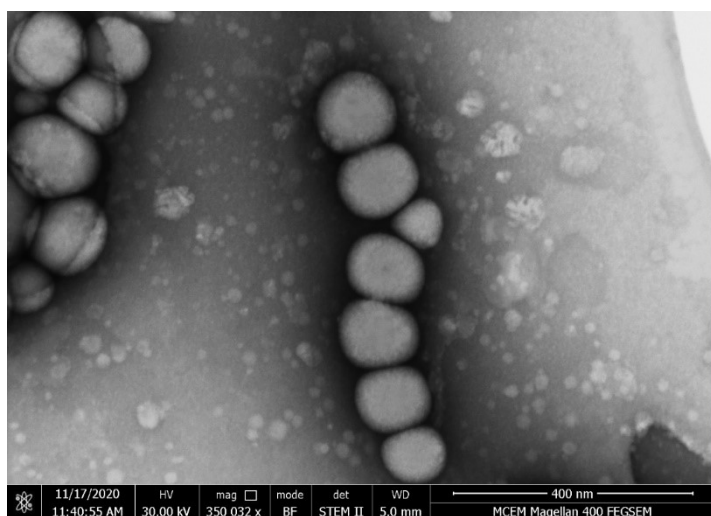
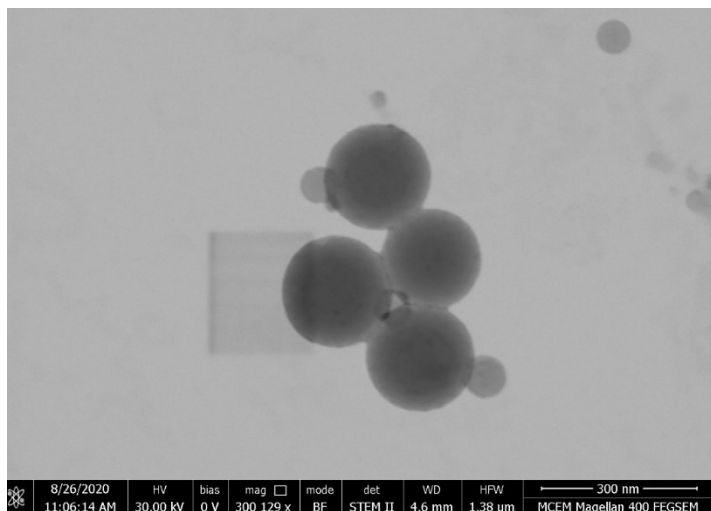


Figure S5: STEM images of the sample PHEA40-b-PS300; top image is at 2.5/1.5 THF to water ratio and bottom image is at 3.2/0.8 THF to water ratio.

6. References

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