Supplementary Material (ESI) for Chemical Science

Supporting information

for

Cylindrical Micelles from the Living Crystallization-Driven Self-Assembly of Poly(lactide)-Containing Block Copolymers

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Materials and Methods

Chemicals were used as received from Aldrich, Fluka and Acros. *L*-lactide, *D*-lactide and *DL*-lactide were donated by Purac and further purified/dried over 4Å molecular sieves in dichloromethane solution before being dried under vacuum and sublimed. Lactides were stored in a nitrogen-filled glove box. Tetrahydropyran acrylate (THPA) was prepared as described previously and stored below 4 °C.[1] AIBN (2,2'-azo-bis(isobutyronitrile)) was recrystallized twice from methanol and stored in the dark at 4 °C. Dry dichloromethane was obtained by passing over a column of activated alumina using an Innovative Technologies solvent purification system. (-)-Sparteine was dried over CaH_2 and distilled prior to use and 1-(3,5-bis(trifluoromethyl)phenyl)-3-cyclohexyl-thiourea was prepared and dried as previously reported.[2] ^1H NMR and ^{13}C NMR spectra were recorded on a

Bruker DPX-400 spectrometer in $CDCl_3$ unless otherwise stated. Chemical shifts are given in ppm downfield from TMS. Size exclusion chromatography/Gel Permeation chromatography (GPC) measurements in THF were conducted on a system comprised of a Varian 390-LC-Multi detector suite fitted with differential refractive index (DRI), light scattering (LS) and ultra-violet (UV) detectors equipped with a guard column (Varian Polymer Laboratories PLGel 5 μM, 50 × 7.5 mm) and two mixed D columns (Varian Polymer Laboratories PLGel 5 μM, 300 × 7.5 mm). The mobile phase was tetrahydrofuran with 5% triethylamine eluent at a flow rate of 1.0 mL min^{-1}, and samples were calibrated against Varian Polymer laboratories Easi-Vials linear poly(styrene) standards (162 - 2.4×10^5 $g.mol^{-1}$) using Cirrus v3.3. GPC measurements in DMF were conducted on a system comprised of a Varian 390-LC-Multi detector suite fitted with differential refractive index (DRI), visometer and ultra-violet (UV) detectors equipped with a guard column (Varian Polymer Laboratories PLGel 5 μM, 50 × 7.5 mm) and two mixed D columns (Varian Polymer Laboratories PLGel 5 μM, 300 × 7.5 mm). The mobile phase was dimethylformamide with 1 mg/mL LiBr eluting at a flow rate of 1.0 mL min^{-1}, and samples were calibrated against Varian Polymer laboratories Easi-Vials linear poly(methyl methacrylate) standards (162 - 2.4×10^5 $g.mol^{-1}$) using Cirrus v3.3. Infrared spectroscopy was recorded on a Perkin Elmer, Spectrum 100 FT-IR Spectrometer. High Resolution Mass Spectrometry (HR-MS) were conducted on a Bruker UHR-Q-TOF MaXis with electrospray ionisation. Hydrodynamic diameters ($D_{h,app}$) and size distributions of the nanostructures in aqueous solutions were determined by dynamic light scattering (DLS). The DLS instrumentation consisted of a Malvern Zetasizer NanoS instrument operating at 25 °C or 65 °C with a 4 mW He-Ne 633-nm laser module. Measurements were made at a detection angle of 173° (back scattering), and Malvern DTS 5.02 software was used to analyze the data. Multi angle DLS measurements were performed on a Malvern Instruments Autosizer 4800, equipped with an APD detector and a Malvern 7132 50 ns 16-bit digital auto-correlator, using a 50mW green laser beam at 532 nm. These measurements were carried out over an angle range of 30-130° in 10°

stepwise increments. Toluene was used as a calibration standard and the data was collected in triplicate.

Samples for TEM analysis were prepared by glow discharge of carbon coated grid followed by drop deposition of a solution of sample and air drying. All samples were then examined with a transmission electron microscope (JEOL TEM-1200), operating at 100 kV. Average sizes of the micelles were determined from counting the sizes of at least 100 particles for 3 different images. Cryo TEM were performed by Dr Steve Furzeland and Derek Atkins, Unilever. Sample preparation was carried out using a CryoPlunge 3 unit (Gatan Instruments) employing a double blot technique. 3μl of sample was pipetted onto a plasma etched (15secs) 400 mesh holey carbon grid (Agar Scientific) held in the plunge chamber at approx 90% humidity. The samples were blotted, from both sides for 0.5, 0.8 or 1.0 second dependant on sample viscosity. The samples were then plunged into liquid ethane at a temperature of -170 °C. The grids were blotted to remove excess ethane then transferred, under liquid nitrogen to the cryo TEM specimen holder (Gatan 626 cryo holder) at -170 °C. Samples were examined using a Jeol 2100 TEM operated at 200KV and imaged using a Gatan Ultrascan 4000 camera and images captured using DigitalMicrograph software (Gatan). X-ray diffraction scans WAXD) were performed on a Panalytical X'Pert Pro MPD equipped with a curved Ge Johansson monocromator on the incident beam optics giving pure focussed Cu $K_{\alpha 1}$ radiation. The solid-state PiXcel detector has an active length of ~3.2° 2θ enabling fast data collection with excellent signal to noise. The samples were mounted on a piece of offcut Si, and aligned to the direct beam to ensure that the effect of height error in the scans was minimised. Standard "powder" 2θ-θ diffraction scans were made.

SANS Data Collection and Discussion

SANS experiments were conducted by Dr Sarah Rogers at the ISIS neutron facility, Rutherford Appleton Laboratories, on the time-of-flight LOQ diffractometer. The incident wavelength range of

2.2 – 10 Å gave rise to a Q-range of 0.009 – 0.249 $Å^{-1}$.[3] Absolute intensities for I(Q) (cm^{-1}) were determined within 5% by measuring the scattering from a partially deuterated polymer standard. Standard procedures for data treatment were employed.[4] Measurements were made at 25 °C and the sample was made up in D_2O and run in a 2 mm quartz cell. The FISH analysis program was used for data modeling.[5] The model used for data fitting employed a core shell rigid rod form factor. There are two structural parameters in this model; the outer radius and the aggregate length. The radius was allowed to float freely during the fitting procedure but as it was known that the length of the aggregate was too large to be resolved using LOQ this parameter was only allowed to move between 100 and 300 nm (using a value found from DLS). It was noted that the fit improved up to a length of around 150 nm but after this the length continued to increase with no significant improvement to the fit. Scale factor (SF) checks were performed to ensure that the values obtained from fitting were physically realistic. For the rod form factor the SF is proportional to the different in scattering length densities $(\rho_{surf} - \rho_{solv})^2$ and the particle volume fraction. To obtain good agreement of the SFs it was found that the polymer aggregates were fully hydrated and a volume fraction of 0.0005 (so this is around 0.5 mg/mL) was required. Further detail on the model used can be found in the FISH manual which can be downloaded from http://www.small-angle.ac.uk/small-angle/Software.html.

Polymerization Procedures

In all RAFT polymerizations three freeze-pump-thaw cycles were performed on the reaction vessel for the removal of oxygen and were released and sealed in an ampoule under nitrogen before being immersed in an oil bath at the required temperature. ROP polymerizations were carried out in a glove box under an inert nitrogen atmosphere at ambient temperature. Molecular weights and polydispersity indices were measured by GPC analysis, with ^{1}H NMR spectroscopy used for the determination of end group functionality and molecular weight by careful integration of the polymer backbone to the end group signals.

Synthesis of dodecyl 4-(hydroxymethyl) benzyl carbonotrithioate CTA, 1

Dodecanethiol (1.53 mL, 6.4 mmol) was dissolved in acetone, potassium phosphate (1.5 g, 7.0 mmol) and carbon disulfide (1.15 mL, 20 mmol) were added and the solution mixture was stirred at room temprature for 2 hours. Subsequently, 4-(chloromethyl)benzyl alcohol (1 g, 6.4 mmol) was added to the yellow solution and the reaction was left for 72 hours. Afterwards the solvent was evaporated and the remaining solid was dissolved in dichloromethane. The organic layers were then washed with 0.1 M HCl (100 mL), 3 × DI water (100 mL) and then a saturated brine solution (100 mL). The organic phase was dried over magnesium sulfate, filtered and concentrated *in vacuo*. The crude product was purified by flash chromatography eluting with 3:2 hexane : ethyl acetate to yield the desired product (R_f = 0.55 in 3:2 hexane/ethyl acetate) as a yellow solid (2.1 g, 81%). ^{1}H NMR ($CDCl_3$, ppm, 400 MHz): δ = 7.30-7.20 (m, 4H, Ar*H*), 4.61 (s, 2H, SCH_2Ph), 4.51 (s, 2H, $PhCH_2OH$), 3.32-3.25 (t, 2H, $^3J_{H\text{-}H}$ = 7.2 Hz, CH_2SCS), 1.68-1.55 (m, 2H, CH_2CH_2SCS), 1.53-1.40 (s, 1H, -OH), 1.38-1.27 (m, 2H CH_3CH_2), 1.27-1.08 (s, 16H, $CH_3CH_2(CH_2)_8CH_2CH_2$), 0.85-0.75 (t, 3H, $^3J_{H\text{-}H}$ = 7.2 Hz, CH_3). ^{13}C NMR ($CDCl_3$, ppm, 400 MHz): δ = 129.5, 129.3, 65.0, 41.0, 37.1, 31.9, 29.6, 29.5, 29.1, 28.9, 28.0. ν_{max}/cm^{-1} 3492-3293 (O-H), 1583 (aromatic), 1469 (aromatic). HR-MS (MaXis) *found* 421.1669, *cald* 421.1664 (M+, 100 %).

Representative procedure for the synthesis of PLA

In a 20 mL scintillation vial equipped with a stir bar, a solution of the thiourea organocatalyst (14.3 mg, 34.5 μmol), (-)-sparteine (0.17 μmol, 0.039 mg) and **1** (10 mg, 0.15 μmol) in dry CH_2Cl_2 (0.2 mL) was added to a solution of L-lactide (140 mg, 0.97 mmol) in dry CH_2Cl_2 (0.8 mL). The reaction mixture was stirred for 3 h (for DP = 15-70) before the solution was reduced under vacuum to yield a light yellow solid. The conversion was determined by ^{1}H NMR spectroscopy. The polymer was precipitated from $CHCl_3$ into petroleum ether and dried to a constant weight to afford a triothiocarbonate end functionalized poly(lactide). The degree of polymerization and end group retention were determined by ^{1}H NMR spectroscopy and MALDI TOF MS while the molecular weight and PDI were determined by GPC. ^{1}H NMR ($CDCl_3$, ppm, 400 MHz): δ = 5.18-5.05 (q, 1H, backbone), 4.56-4.53 (t, 4H, end group), 4.51-4.48 (s, 2H, end group), 3.31-3.30 (s, 3H, end group), 3.32-3.26 (t, 2H, end group), 2.95-2.88 (m, 2H, end group), 1.53-1.49 (d, 3H, backbone).

Representative procedure for the synthesis of PTHPA-*b*-PLA

The THPA monomer (300 eq), the poly(lactide) macro CTA (1 eq) and AIBN (0.1 equiv) were disolved in 0.5 ml of $CHCl_3$ and transferred in an oven dried vial with a stirrer bar. The vial was degassed (three times) and sealed under nitrogen, and the polymerizations were heated at 65 °C for 7 h. The polymer was then dissolved in a minimum amount of tetrahydrofuran and purified by precipitation twice into ice cold hexane to afford a pure diblock copolymer PLA-*b*-PTHPA. The degree of polymerization and end group retention were determined by ^{1}H NMR spectroscopy while the molecular weight and polydispersity were determined by GPC. ^{1}H NMR ($CDCl_3$, ppm, 400 MHz): δ = 6.02-5.90 (br, 1H, PTHPA backbone) 5.18-5.05 (q, 1H, PLLA backbone), 2.10-1.48 (d, 8H, backbone of both blocks). ν_{max}/cm^{-1} 2954 (C-C stretch), 2920 (C-C stretch), 2850 (C-C stretch), 1758 (C=O stretch of PLA backbone), 1736 (C=O stretch of PTHPA)

Synthesis of PAA-*b*-P(*L*-LA) (2), PAA-*b*-P(*D*-LA) (3) and PAA-*b*-P(*DL*-LA) (4)

PLA-*b*-PTHPA (0.2 mg) was added to a round-bottom flask equipped with a stirrer bar and a needle on top, followed by tetrahydrofuran (3 mL). Deionized water was added (1 mL), followed by glacial acetic acid in a catalytic amount (20 μL). The mixture was allowed to stir at 60 °C for 15 h. The polymer was freeze dried for 2 days before being characterized by ^{1}H NMR and IR spectroscopy. ^{1}H NMR (DMSO, ppm, 400 MHz): δ = 12.50-11.98 (br, O-H), 5.22-5.04 (q, 1H, PLLA backbone), 1.53-1.49 (d, 3H, PLLA backbone), ν_{max}/cm^{-1}. 3630-2840 (O-H stretch + C-O strech), 2954 (C-C stretch), 2920 (C-C stretch), 2850 (C-C stretch), 1758 (C=O stretch of PLLA backbone), 1707 (C=O stretch of PAA acid groups).

Self assembly of 2 - 4 by direct dissolution to from 5 - 7

2 (2.5 mg) was dissolved in nanopure water (10 mL), that had been filtered through a 0.22 µm nylon filter, in a 20 mL scintillation vial equipped with a stirrer bar to prepare a 0.25 mg/mL solution of **2** in water. The vial was sealed with a screw cap and the solution was allowed to stir at 65 °C for the required time before cooling by emerging the vial in liquid nitrogen. The resultant particles were characterized by DLS and TEM analysis.

Solvent switch self assembly of 3

3 (0.01 g) was dissolved in 10 mL of acetone before being subsequently added dropwise to filtered nanopure water (30 mL). The solution was left overnight in the fume hood with an open top to remove acetone. The resultant particles were characterized by DLS and TEM analysis.

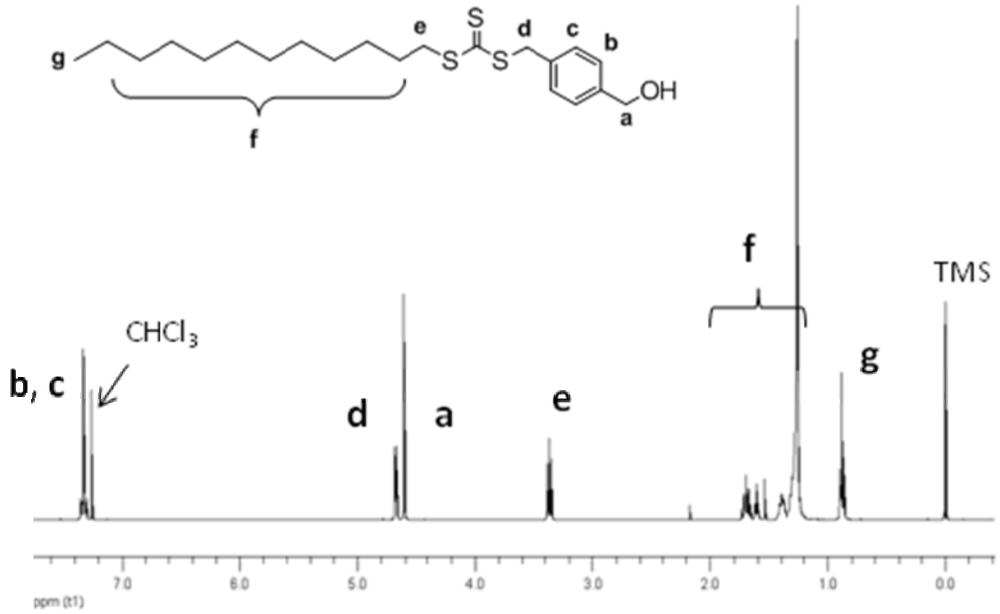


Figure S1 – ^{1}H NMR spectrum of CTA **1** in CDCl$_3$ (400 MHz, 293 K).

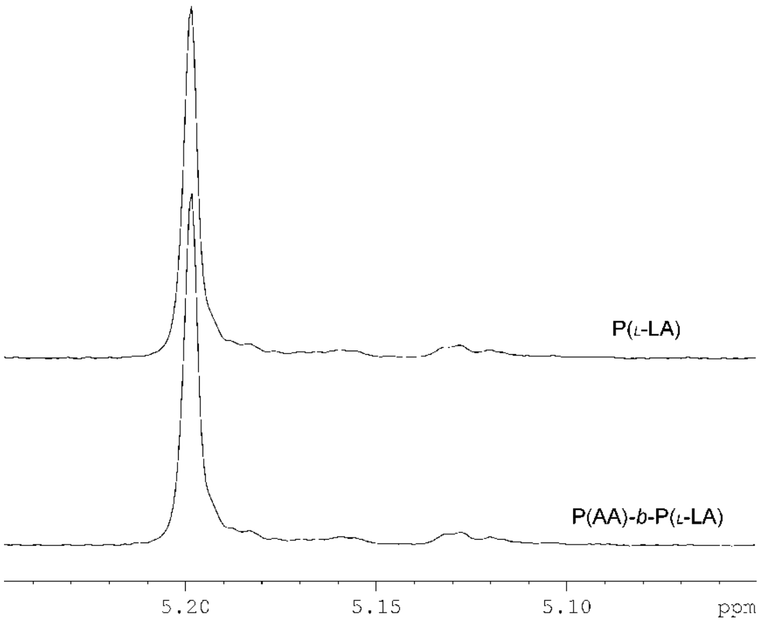


Figure S2 – Homonuclear decoupled ^{1}H NMR spectrum of (a) P(***L***-LA) and (b) P(***L***-LA)-*b*-PAA in *d*$_6$-DMSO (500 MHz, 293 K).

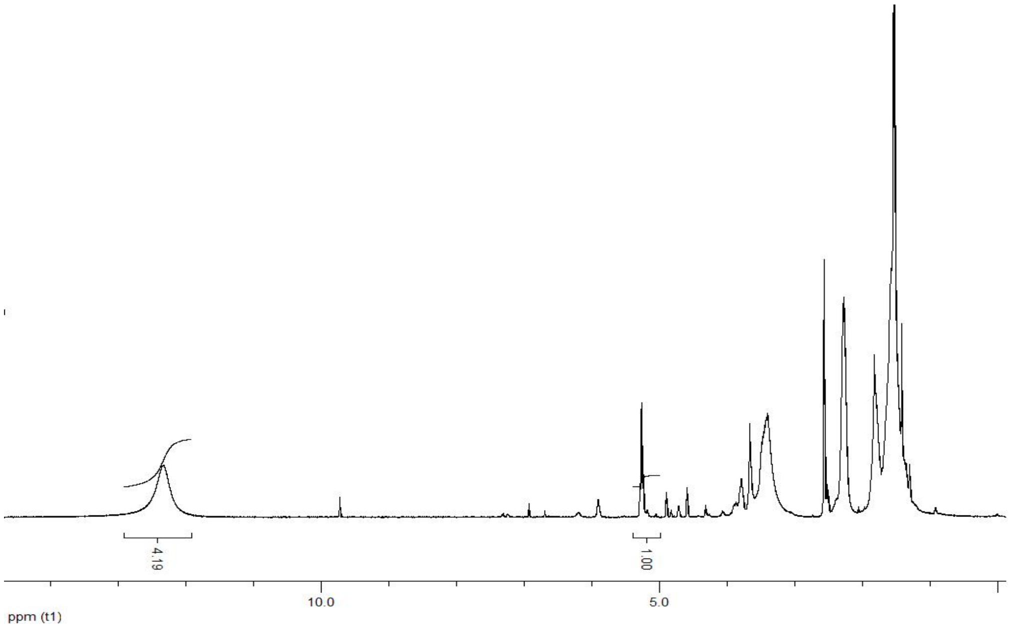


Figure S3 – ^{1}H NMR spectrum for PAA-*b*- P(***L***-LA), **2**, in $CDCl_3$ (400 MHz, 293 K).

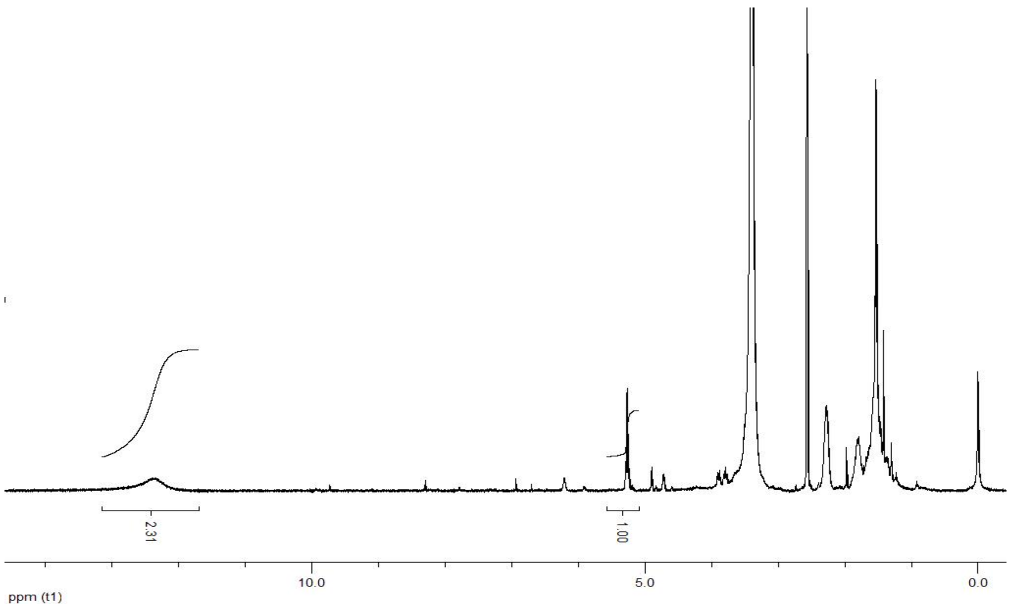


Figure S4 – ^{1}H NMR spectrum for PAA-*b*-P(***D***-LA), **3**, in $CDCl_3$ (400 MHz, 293 K).

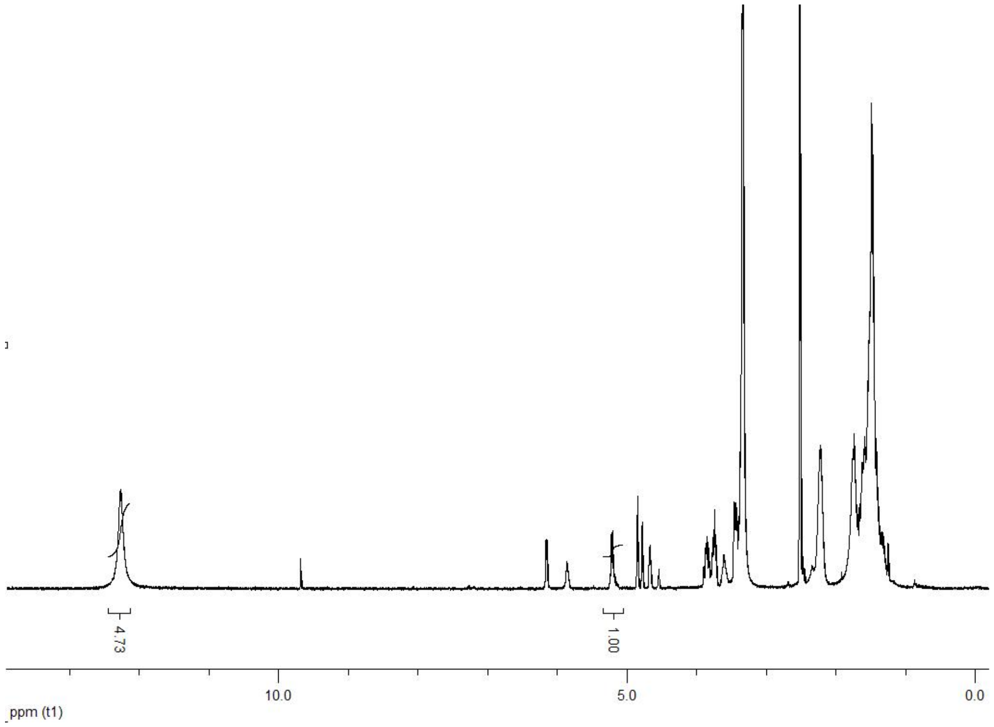
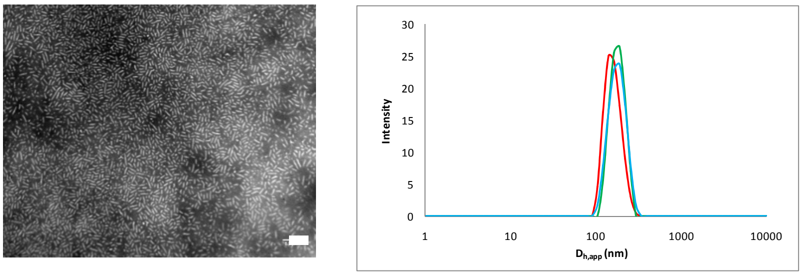


Figure S5 – ^{1}H NMR spectrum for PAA-*b*-P(***DL*** -LA), **4**, in $CDCl_3$ (400 MHz, 293 K).

Micelle

5



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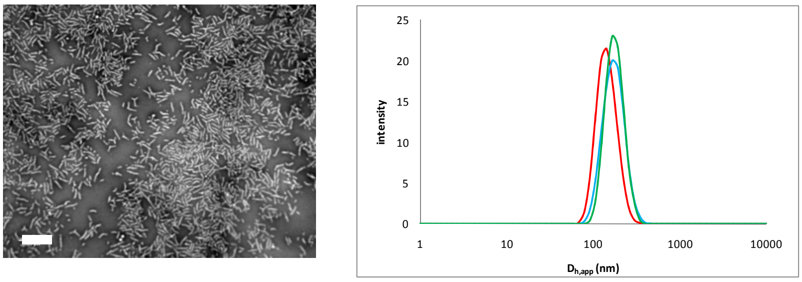

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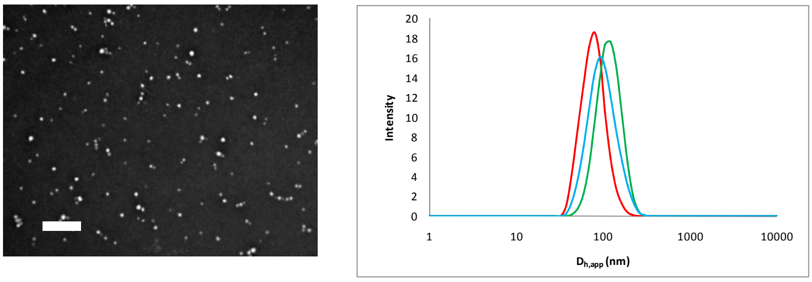

Figure S6 – TEM data and DLS data (at 173^{o}) for micelles **5-7** upon preparation at 65 °C for 1 hour (red, number average; green, intensity average; blue, volume average), scale bar = 500 nm.

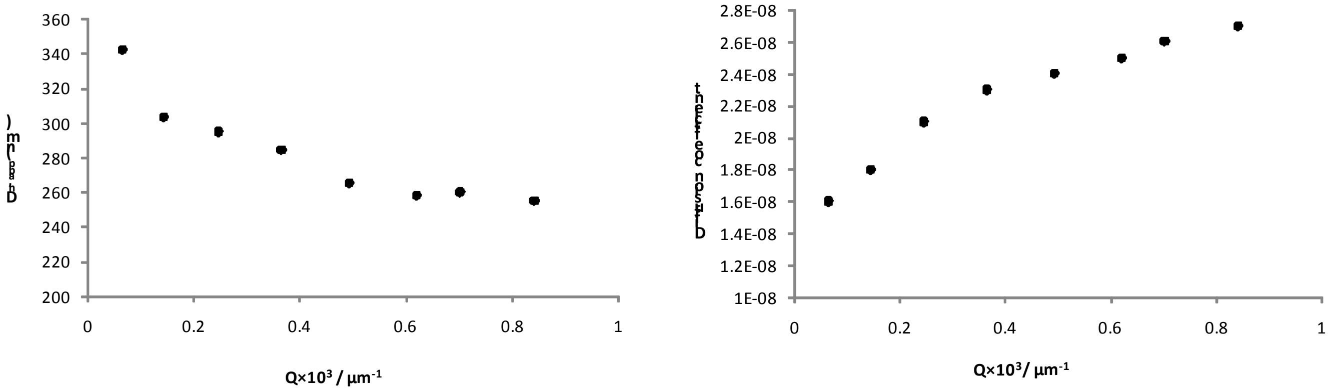

Figure S7 – Angular dependence of the particle radius (LHS) and self diffusion coefficient (RHS) for micelle **5.**

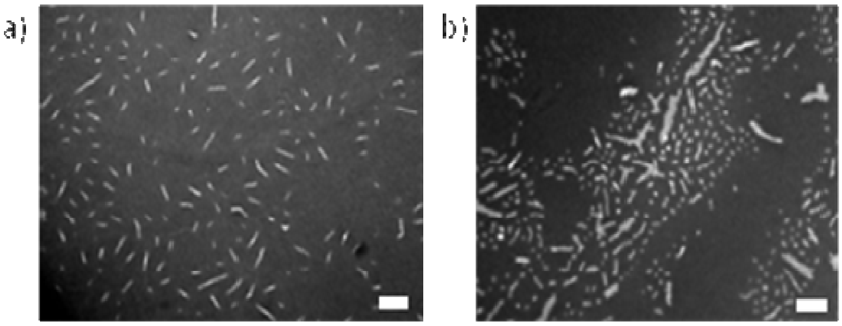

Figure S8 – TEM data for the self-assembly of polymer **3** for a) 20 mins and b) 1 hour, scale bar = 300 nm.

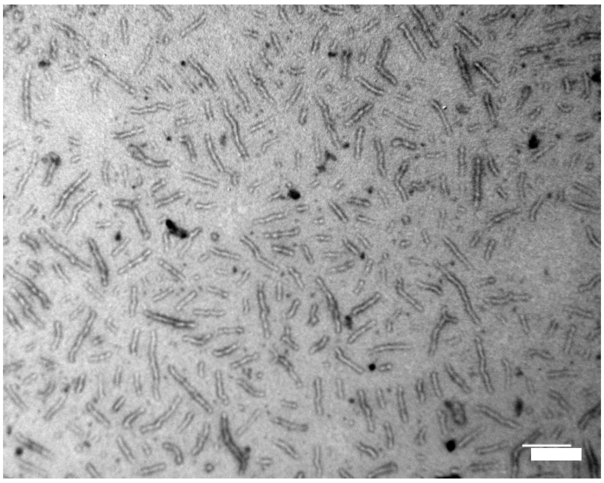

Figure S9 – Positively stained TEM image for micelle **6,** scale bar = 200 nm.

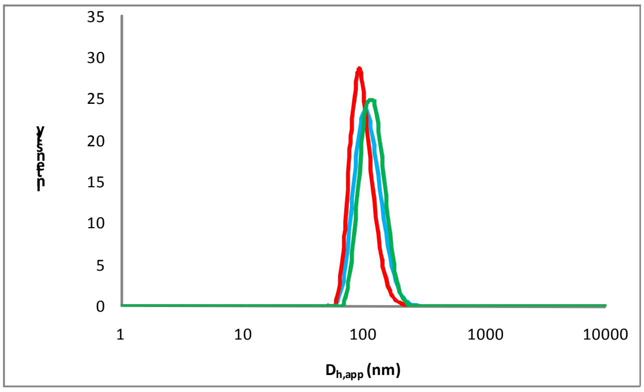

Figure S10 – DLS data (at 173^{o}) for polymer **3** assembled using solvent switch method (red, number average; green, intensity average; blue, volume average) .

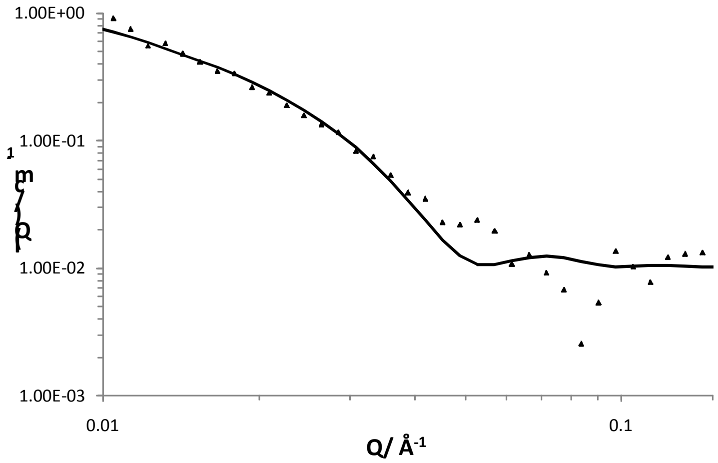

Figure S11 – SANS data for micelles **6**. The fit at high Q is poor due to the resolution limitations of the instrument on the length scale of the cylinders. It should be noted that the fit using a spherical model was very poor which further confirmed the non-spherical shape of the nanostructures.

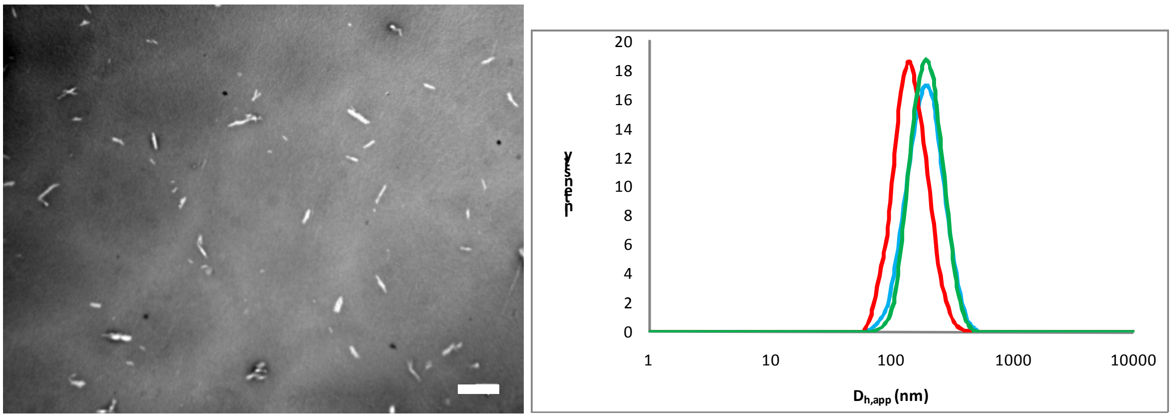

Figure S12 – TEM and DLS for micelle **9** prepared from polymer **8** (red, number average; green, intensity average; blue, volume average), scale bar = 300 nm.

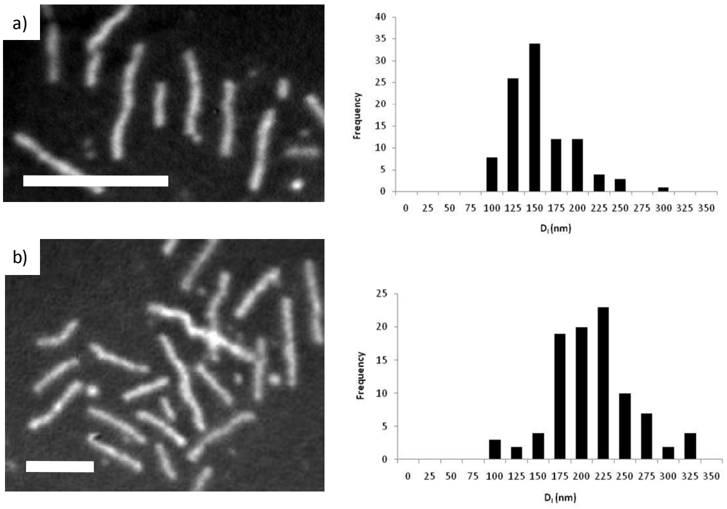

Figure S13 – TEM images and histograms for cylindrical micelles prepared from self assembly of PAA_{300}-*b*-P(*L*-LA)$_{32}$, **2a**, at 65 °C in H_2O, 0.25 mg/mL, a) 10 min, b) sample a) heated for a further 50 min. Scale bar = 200 nm.

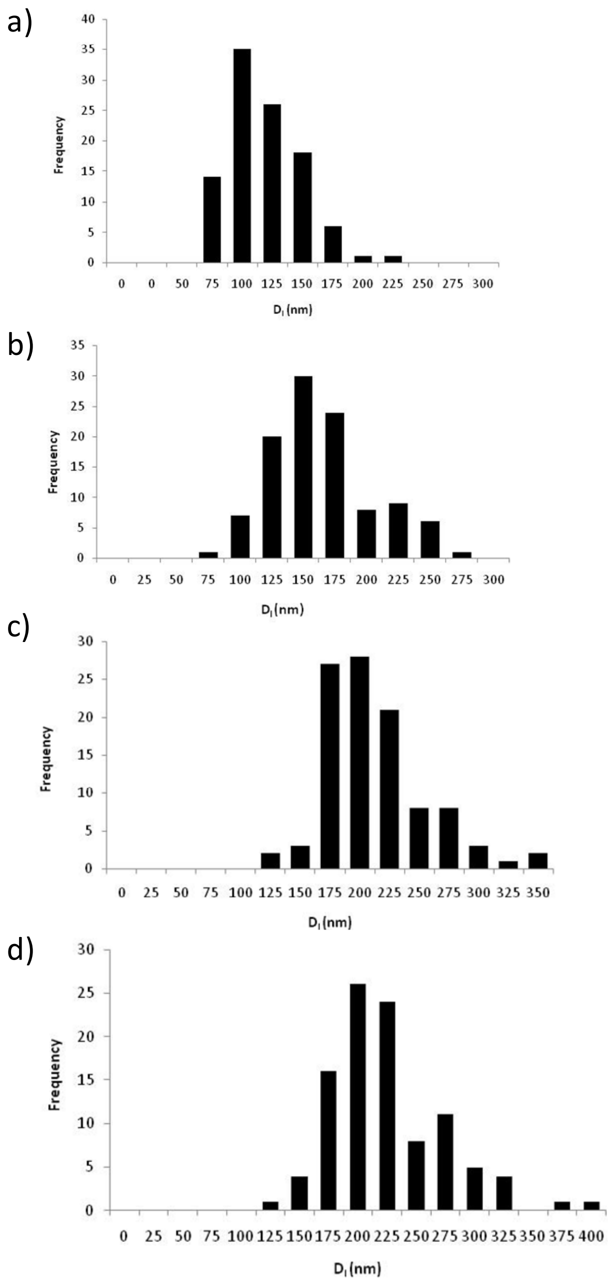

Figure S14 – Histograms for cylindrical micelles prepared from self assembly of PAA$_{300}$-*b*-P(*L*-LA)$_{32}$, **2a**, at 65 °C in H_2O, 0.25 mg/mL, a) 10 min, b) 30 min c) 60 min, d) 90 min.

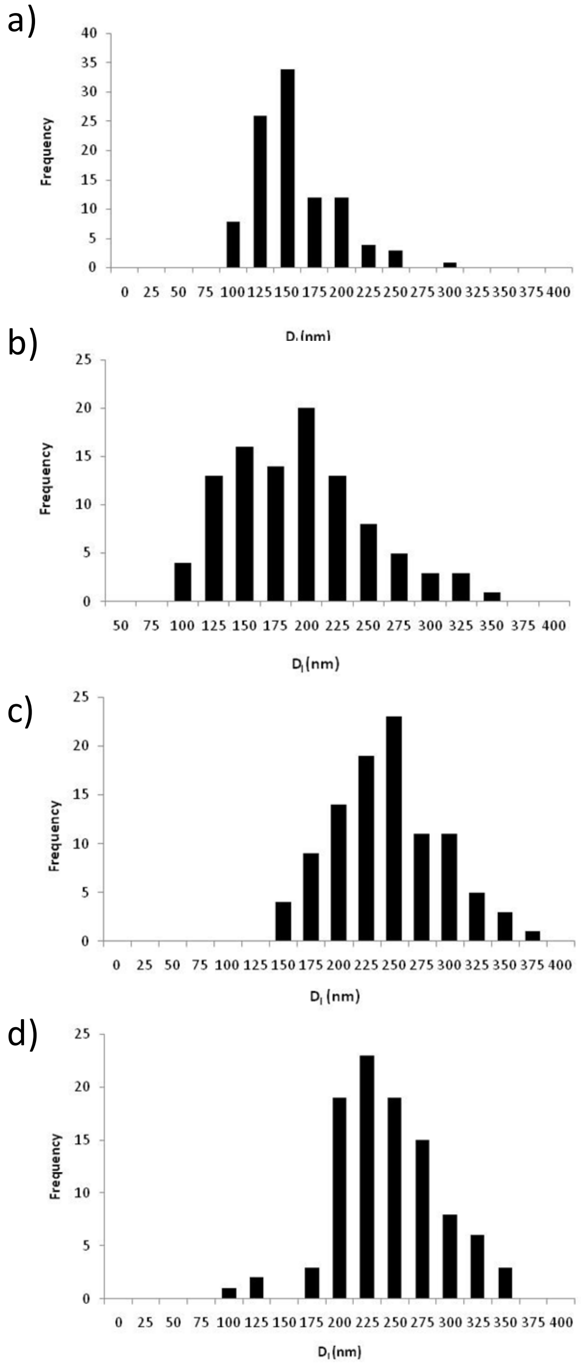

Figure S15 – Histograms for cylindrical micelles prepared from self assembly of PAA$_{300}$-*b*-P(*L*-LA)$_{32}$, **2a**, at 65 °C in H_2O, 0.5 mg/mL, a) 10 min, b) 30 min c) 60 min, d) 90 min.

References

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