Polymeric nanocylinders by combining block copolymer self-assembly and nanoskiving

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MATERIALS AND METHODS

Materials

All of the reagents were from Sigma-Aldrich and were used as received. N,N-dimethylacrylamide (DMA) and styrene were passed through an activated alumina column to remove the inhibitors and stored at –20 °C for future use.

^{1}H NMR Spectroscopy

All NMR spectra were recorded from a Varian INOVA 300 MHz or a Brüker Avance III 500 MHz Spectrometer with $CDCl_3$ as solvent at room temperature. Chemical shifts are relative to the TMS peak at 0.00 ppm.

Size-exclusion chromatography (SEC)

SEC was performed in THF using a Waters Styragel guard column and 3 Waters Styragel columns (HR6, HR4, and HR1) in series with an available of 100–10,000,000 g.mol^{-1}. The columns are contained in an Agilent 1260 Infinity liquid chromatograph equipped with a Wyatt Dawn Heleos II multiangle light scattering detector and a Wyatt Optilab T-rEX refractive index detector.

Transmission Electron Microscopy (TEM)

To investigate the microstructure of the shear-oriented specimens: ultrathin sections (ca. 70–100 nm) of the polymeric monoliths were cut using a Leica EM UC6 Ultramicrotome at –120 °C. Although both materials are already glassy at room temperature, cryomicrotomy helped to achieve the thickness desired for TEM imaging. The cut sections were placed on 400 mesh copper grids and subsequently stained with RuO_4 vapor for ~5 min by exposure to a 0.5% aqueous solution. For the nanocylinders after dissolution, 2-3 drops of the solution were placed

on TEM grids with a Formvar® supporting film (~3 nm) and after complete evaporation of the solvent, or quick absorption of the solvent with a piece of cleaning paper under the grid, stained with RuO_4 vapor for ~5 min by exposure to a 0.5% aqueous solution. All of the TEM images were obtained with a FEI Tecnai G2 Spirit BioTWIN transmission electron microscope, operated at 120 kV.

Small-angle X-ray scattering (SAXS)

All experiments were performed at the Sector 5-ID-D beamline of the Advanced Photon Source (APS) at Argonne National Laboratories, maintained by the Dow-Northwestern-Dupont Collaborative Access Team (DNDCAT). The source produces X-rays with 0.70 Å wavelengths. The sample to detector distance was fixed to 7.491 m. Scattering intensity was monitored using a Mar 165 mm diameter CCD detector operating with a resolution of 2048 by 2048. The two dimensional scattering patterns were azimuthally integrated to afford one-dimensional profiles presented as spatial frequency (q) versus scattered intensity.

Dynamic Light Scattering (DLS)

Size distributions of the nanocylinders were investigated by DLS, in cyclohexane for PS-PLA and water for PDMA-PS samples. The solutions were passed through 0.45 μm filters into glass tubes. Light scattering was carried out in a Brookhaven BI-200SM DLS system equipped with a Mini L-30 HeNe laser operating at 637 nm, and a BI-NDO detector. The sample tube was immersed in decalin. Experiments were performed at room temperature. Intensity correlation functions were recorded at scattering angle 90°, with an aperture size of 400 nm, and converted to size distributions using the CONTIN analysis program provided by the Brookhaven software.

Self-assembly, channel die alignment and thermal annealing

Polymer films were prepared by casting from chloroform solutions. The polymer films were collected and dried in a vacuum oven at 80 °C for the PS-PLA sample, and 130 °C for PDMA-PS samples, for 18 h. ~0.5 g of the polymer films were cut into small pieces (~0.5 cm^2) and placed in the center of a home-built channel die 2 mm wide and 5 cm long. The channel die was placed in a laboratory press at 130 ºC for the PS-PLA sample, and 160 ºC for the PDMA-PS samples, and the compression was started after 15 min. The sample was pressed for 1–2 mm every 10 min manually. Compression stopped when the melted polymers reached the ends of the channel die. The channel die was left in the laboratory press for 15 min without further pressing. The aligned specimens were left in the channel die and annealed under vacuum at 130 °C for the PS-PLA sample, and 160 ºC for the PDMA-PS samples, for 60 h. The aligned material was removed from the die with sample thickness of ~2 mm. In summary, the time required was 18 hours for solvent casting and drying the films, followed by 63 hours for channel die alignment and annealing, for theoretically about 6×10^{15} particles.

Nanoparticle preparation by nanoskiving

A Leica EM UC6 Ultramicrotome equipped with a diamond knife was used for sectioning the polymer specimens. The aligned specimen, with dimensions of $\sim 2 \times 2 \times 10$ mm^3, was mounted on the microtome arm. After the surface of the sample was leveled using a razor blade and smoothed with a glass knife, sections with the desired thicknesses were collected into a water-boat on the diamond knife. The microtome was set on the automatic setting with a 4 mm/s cutting speed. During the sectioning, some polymer sections were picked-up using an eyelash stick to prevent exceeding accumulation at the surface of the water and transferred into a plastic centrifuge tube. Sectioning was stopped after 1000 sections. The sections were then recovered by

dumping the boat into the same plastic tube. The samples were then lyophilized to remove the water. Finally, solutions for DLS analysis were prepared by dissolving dry sections in 1 ml of the proper solvent, cyclohexane for PS-PLA and water for PDMA-PS samples. Further dilutions were used for TEM characterization. At a rate of 25 sections/min, the nanoparticle fabrication rate is estimated to 6×10^{10} particles/min for PS-PLA and 9×10^{10} particles/min for PDMA-PS. Freeze-drying (overnight) and dissolution (30 min) steps are independent from the number of sections and are not included in the rate calculations.

POLYMER SYNTHESIS

PS-PLA: Following a previously reported protocol,[1] a hydroxyl-terminated polystyrene (PS-OH) was synthesized by anionic polymerization, and subsequently used as macroinitiator to polymerize D,L-lactide by ring opening transesterification polymerization. Addition of PLA to the PS block was performed in a glove-box by mixing 3.27 g of PS-OH, 1.921 g of D,L-lactide, 20.0 μL of 1,8-diazabicycloundec-7-ene (DBU) with 35 mL of dichloromethane (anhydrous) at room temperature. The reaction was terminated with a spatula tip of benzoic acid (stirring 1-2 min) after 65 minutes. The polymer was precipitated into ~ 400ml of cold methanol (-20 to -30 °C). The D,L lactide conversion was determined to be 82.3%. M_{nNMR}= 60 kg/mol, f_{PLA}= 0.26 (calculated using ρ_{PLA}=1.25 g/cm^3,[2] ρ_{PS}=1.04g/cm^3 [3]). M_{nSEC}= 57 kg/mol Đ =1.01. ^{1}HNMR (CDCl$_3$, 300 MHz): δ = 1.25−1.55 (br, CH$_2$-PS), 1.55−1.7 (m, CH$_3$-PLA), 1.7-2 (br, CH-PS), 5.1-5.3 (m, CH-PLA), 6.4−6.7 (br m, ArH-PS), 6.85-7.25 (br m, ArH-PS) ppm.

PDMA-PS: To a 200 mL round-bottom flask equipped with a Teflon stirring bar was added DIBTTC (346 mg, 0.95 mmol), AIBN (16 mg, 0.098 mmol), and 30 mL of DMF. Next, DMA (30 mL, 291 mmol) was added and the flask was sealed and the mixture was degassed by

bubbling nitrogen at room temperature for 2.5 hours. Subsequently, the reaction vessel was submerged into a thermostated oil bath at 70 °C for one hour. The polymerization was quenched by immediately placing the flask into liquid nitrogen and opening it to air. The obtained viscous bright yellow reaction mixture was diluted by adding 50 mL of methylene chloride, and subsequently the polymer was precipitated in four liters of ice-cold hexane/diethyl ether 50:50 (v/v). The yellow solid was isolated via filtration and dissolved in 200 ml of CH_2Cl_2 and precipitated in four liters of ice-cold cyclohexane. After reprecipitation in another four liters of ice-cold cyclohexane followed by filtration, the resulting PDMA-CTA powder was dried in a vacuum oven at 40 °C for one week (~16 g, 67% yield). M_n= 25 kg/mol (80% conversion), Đ =1.10. ^{1}HNMR (CDCl$_3$, 500 MHz): δ = 1.1−1.9 (br m, CH_2), 2.4−2.9 (br m, CH), 2.9−3.4 (br m, CH_3) ppm. The product was stored under vacuum, in a desiccator, at room temperature until further use.

To synthesize the final block copolymer, AIBN (3.6 mg, 0.022 mmol), the PDMA-CTA (5.99 g, 0.24 mmol), styrene (20 mL, 174 mmol) and 28 mL of DMF were mixed in a 200 ml round-bottom flask equipped with a Teflon stirring bar. The flask was sealed and the mixture was degassed under inert nitrogen at room temperature for one hour. Subsequently, the reaction vessel was submerged into a preheated, stirring oil bath maintained at 70 °C. After 31 hours, the reaction was quenched by immediately placing the flask into liquid nitrogen and opening it to air. 50 mL of CH_2Cl_2 was added to the mixture, and subsequently the polymer was precipitated in four liters of ice-cold hexane/diethyl ether 75:25 (v/v). After filtration, the polymer was redissolved in 150 mL of CH_2Cl_2 followed by precipitation in four liters of ice-cold hexane. After another precipitation in ice-cold hexane, the product was dried in a vacuum oven at 40 °C for one week (~6.5 g, 73% yield). M_n(PS)= 12 kg/mol (15% conversion), f_{PS}= 0.36 (calculated

using ρ_{PDMA}=1.21 g/cm^3 [4], ρ_{PS}=1.04g/cm^3 [3]), Đ =1.12. ^{1}HNMR (CDCl$_3$, 500 MHz): δ = 1.1−2.75 (br m, CH$_2$-PDMA and PS, CH- PDMA and PS), 2.75−3.85 (br m, CH$_3$-PDMA), 6.3−6.9 (br m, ArH-PS), 6.9-7.25 (br m, ArH-PS) ppm. The final block copolymer was stored under vacuum, in a desiccator, at room temperature.
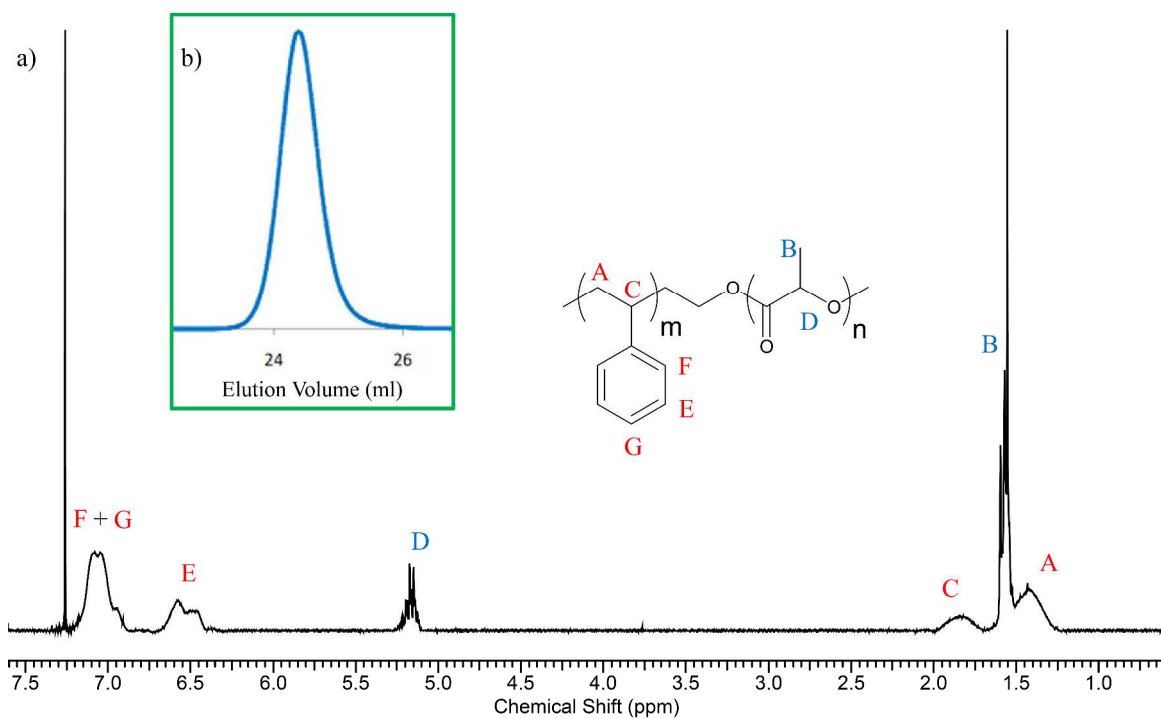
Figure S1. a) ^{1}H-NMR spectrum of the PS-PLA block copolymer (300 MHz, CDCl$_3$). b) SEC trace for the PS-PLA block copolymer (eluent: chloroform, at room temperature).
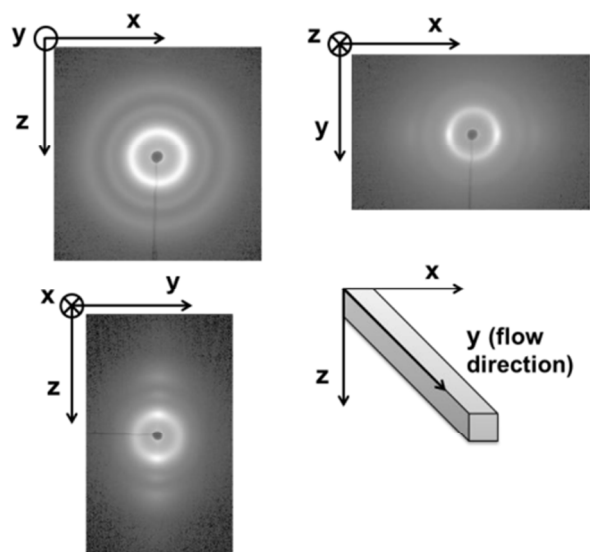

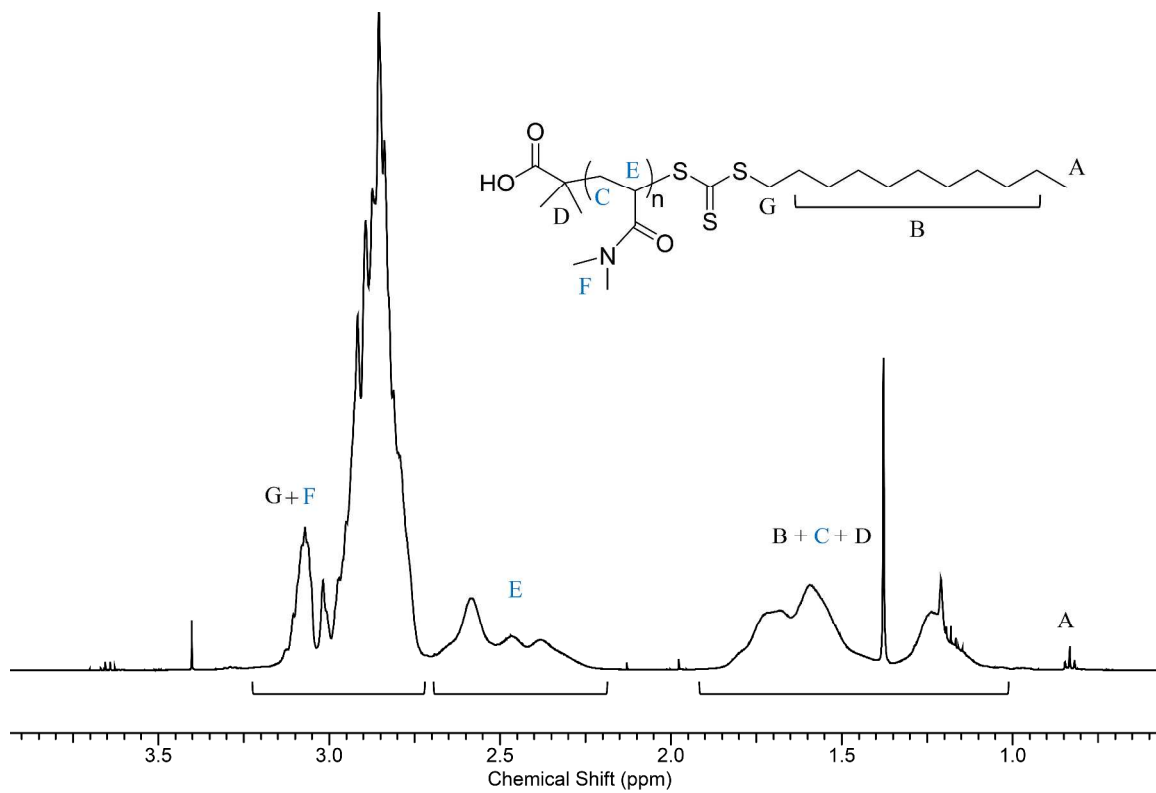**Figure S2.** 2D Synchrotron SAXS patterns of shear-oriented PS-PLA block copolymer.

Figure S3. ^{1}H-NMR spectrum of the PDMA Macro-CTA (500 MHz, CDCl$_3$). Non-assigned peaks at 1.4, 1.9, 3.4, and 3.65 ppm correspond to residual solvents (cyclohexane, diethyl ether, dimethylformamide and diethyl ether, respectively)
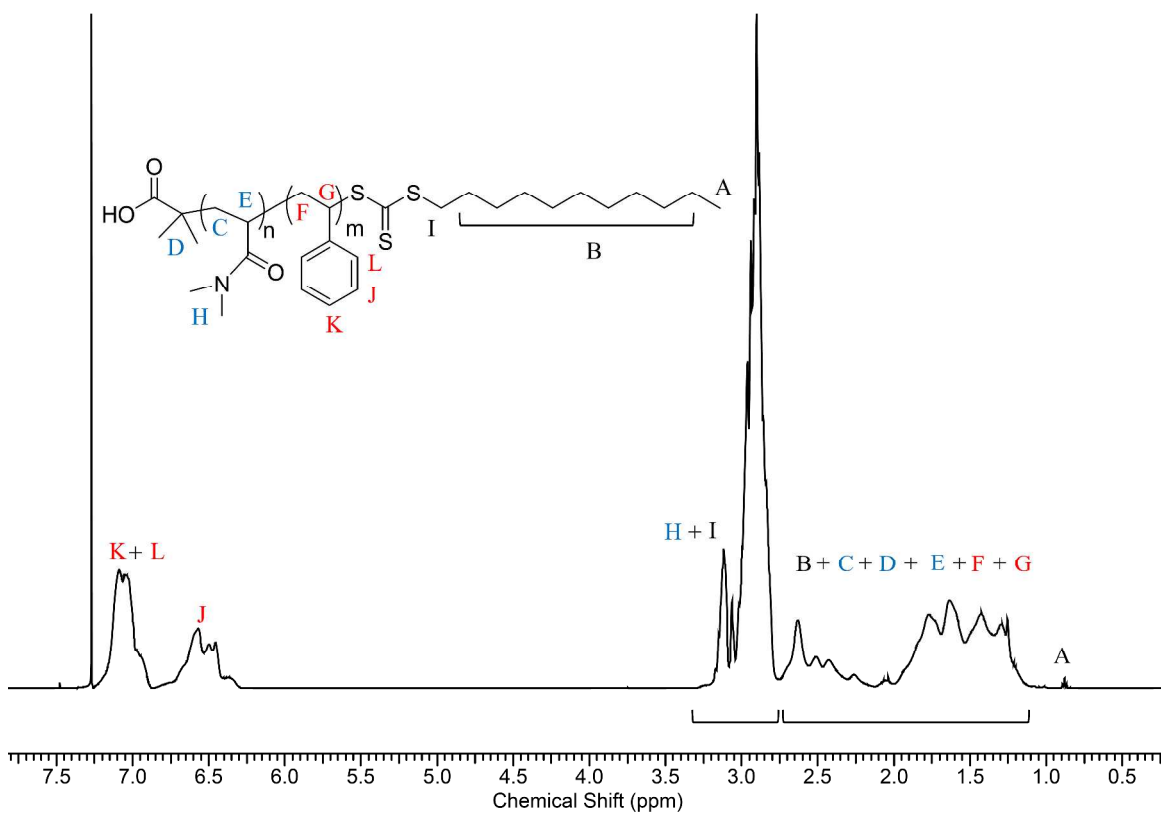
Figure S4 . ^{1}H-NMR spectrum of the PDMA-PS block copolymer (500 MHz, CDCl$_3$)
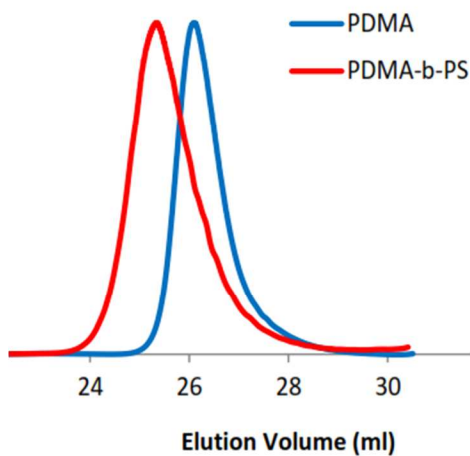

Figure S5. SEC traces for the PDMA macro-CTA and the resulting PDMA- PS copolymer (eluent: THF, at room temperature).
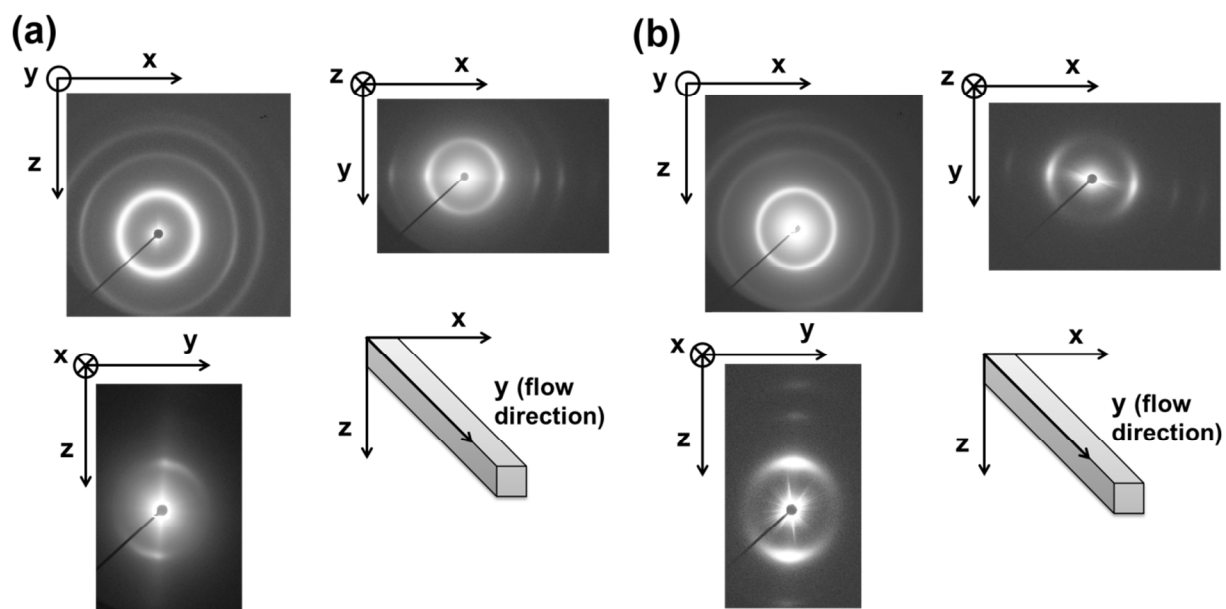
Figure S6. 2D Synchrotron SAXS patterns of shear-oriented PDMA-PS block copolymer (a) Sample A and (b) Sample B.
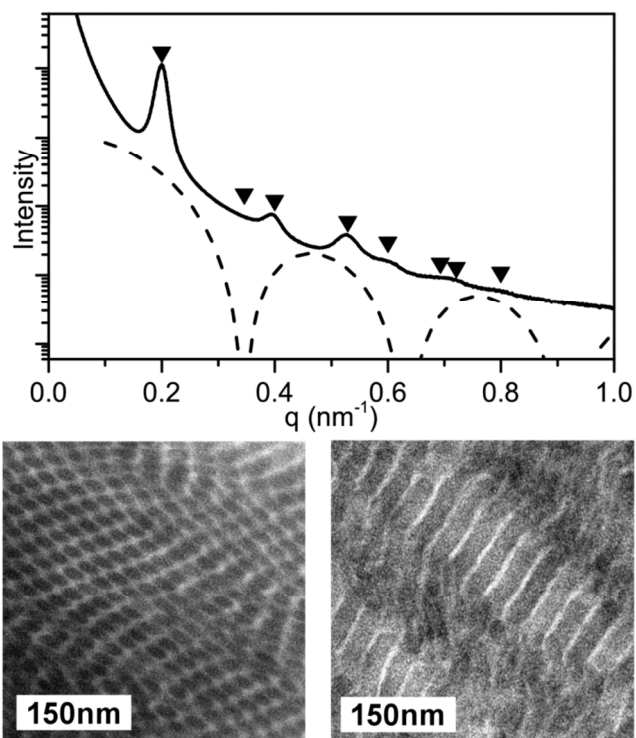
Figure S7. (Solid line) Experimental 1D synchrotron SAXS profile of shear-oriented PDMA-PS sample B at 25°C and (dashed line) simulated form factor scattering curve generated for cylinders with a 10.8 nm radius (value extracted from the experimental SAXS profile). The triangle symbols indicate the expected reflections for a cylindrical morphology ($\sqrt{1}$; $\sqrt{3}$; $\sqrt{4}$; $\sqrt{7}$; $\sqrt{9}$; $\sqrt{12}$; $\sqrt{13}$; $\sqrt{16}$). Corresponding TEM images of the material, obtained (left) perpendicularly and (right) parallel to the shear direction (PS domains were stained by RuO_4 vapors).
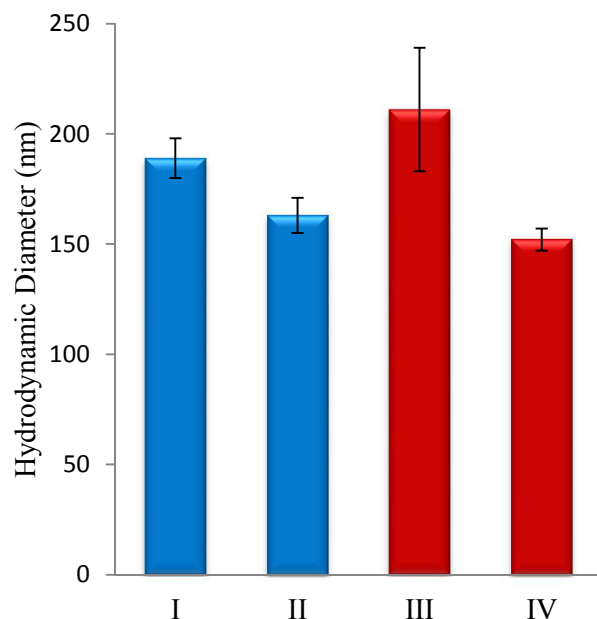
Figure S8. Apparent size distribution of the PS nanocylinders dispersion in water by DLS, the error bars represent standard deviations for ten measurements. Scattering detection angle is 90° and λ= 637 nm. For the concentrations, see Table1. The theoretical thickness of the sections was set at 200 nm. I) Sample A, II) Sample A after sonication, III) Sample B, and IV) Sample B after sonication.
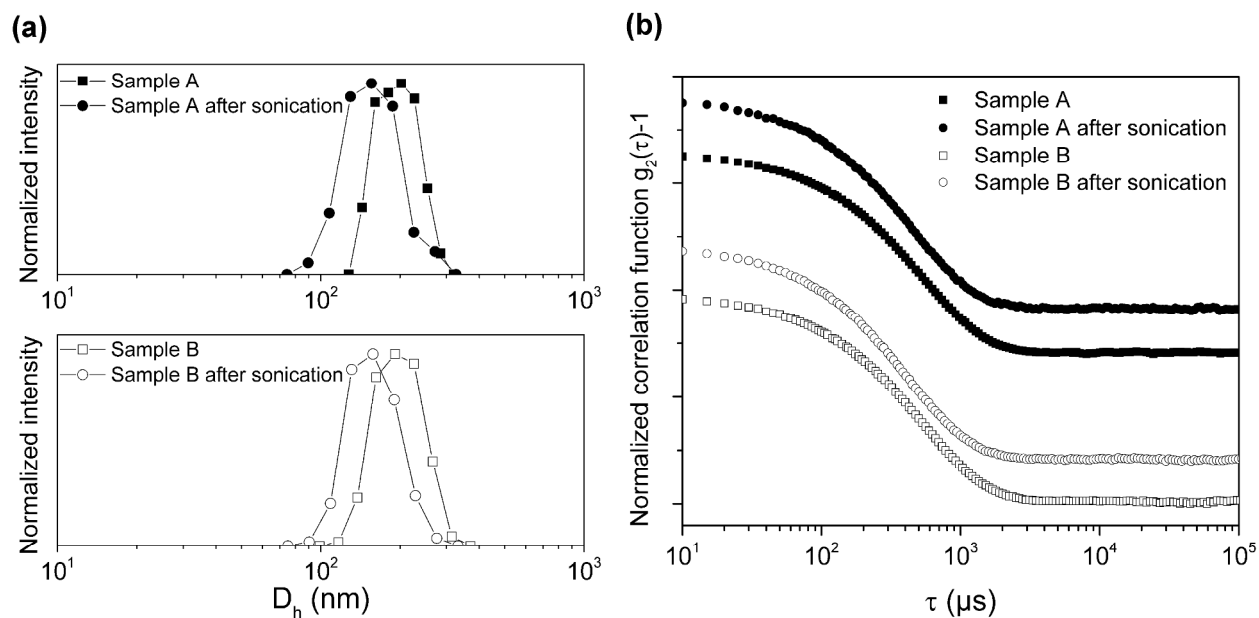

Figure S9. (a) DLS size distribution profiles of the PS nanocylinders dispersions in water for samples A and B, before and after sonication. (b) Associated correlation functions. Scattering detection angle is 90° and λ= 637 nm. For the concentrations, see Table1. The theoretical thickness of the sections was set at 200 nm.

Table S1. DLS characterization summary of the PS nanocylinders with PDMA coronae in water, for ten measurements for each sample. The laser beam wavelength was 637 nm and the scattered light was detected at 90°. I) Sample A, II) Sample A after sonication, III) Sample B, and IV) Sample B after sonication.

Sample	$D_h{}^a$ (nm)	Peak Position (nm)[b]	Rel. Var. [c]	Conc. Wt.%[d]
I	189 ± 9	201.4 ± 14.6	0.074 ± 0.043	0.09
II	163 ± 8	166.7 ± 12.8	0.119 ± 0.050	0.03
III	211 ± 28	204.5 ± 30.3	0.073 ± 0.066	0.09
IV	152 ± 5	154.2 ± 11.0	0.064 ± 0.041	0.03

[a] Average hydrodynamic diameters determined by the CONTIN analysis. [b] Position at which the highest scattering intensity occurs. [c] Particle dispersity (average value of the relative variances for 10 measurements). [d] Approximate weight percent of the polymer in solution.
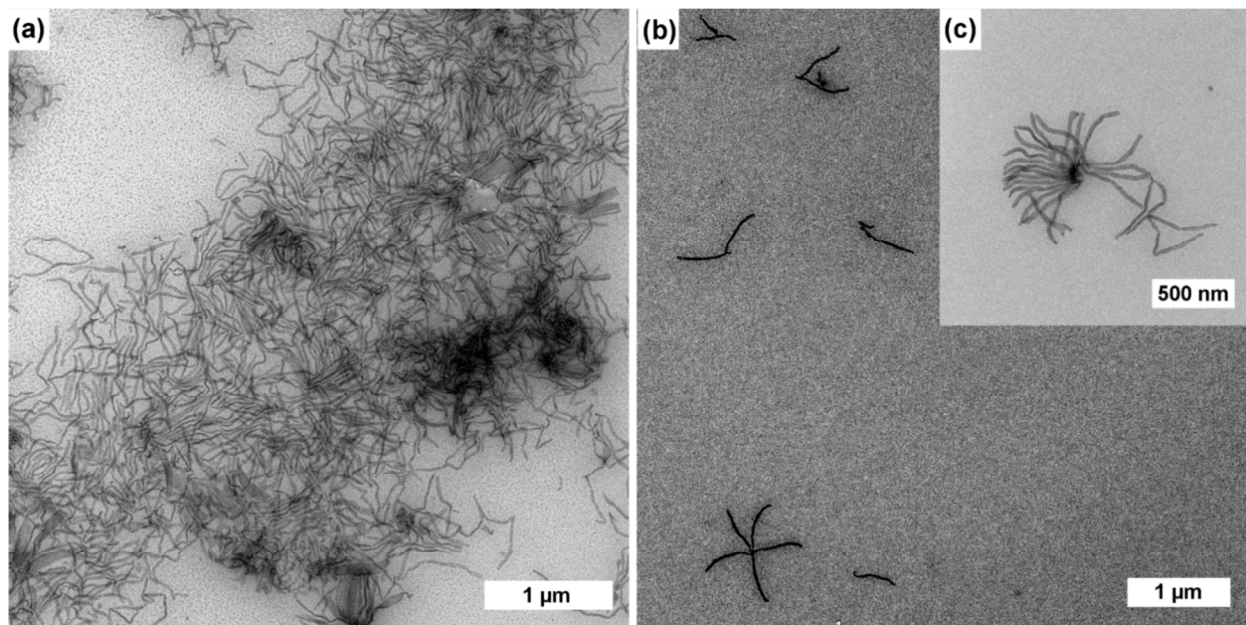

Figure S10. TEM image of PDMA-PS nanoparticles (sample B) casted from an aqueous solution (0.2 g.L^{-1}). The nanoparticles were stained with RuO$_4$. (a) Sample prepared through complete evaporation of the water in a droplet placed on the grid. (b) Sample prepared by absorbing the excess of solution with a piece of paper placed under the grid. (c) Close-up view of end-aggregated cylinders, same sample preparation than b).
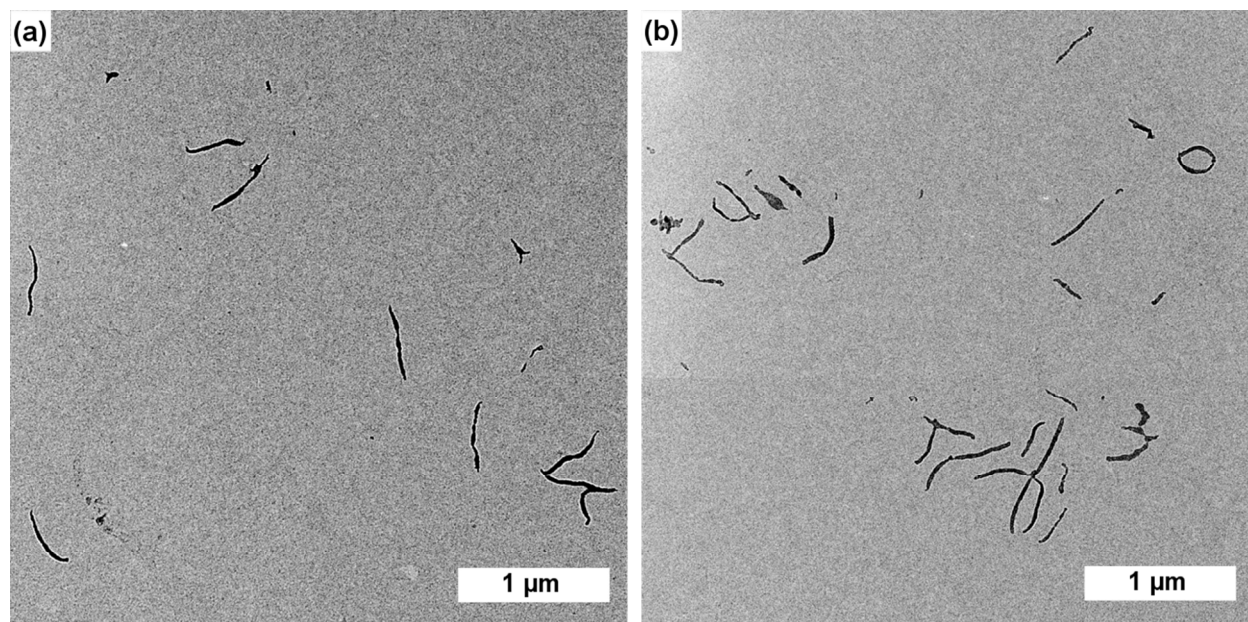
Figure S11. (a) and (b) TEM images of PS-PDMA nanoparticles (sample B) casted from an aqueous solution (0.2 g.L^{-1}) after 5min of sonication. The carbon/Formvar-coated TEM grids were treated with air plasma to improve their hydrophilicity prior to use. The nanoparticles were stained with RuO$_4$. The sample was prepared by absorbing the excess of solution with a piece of paper placed under the grid.
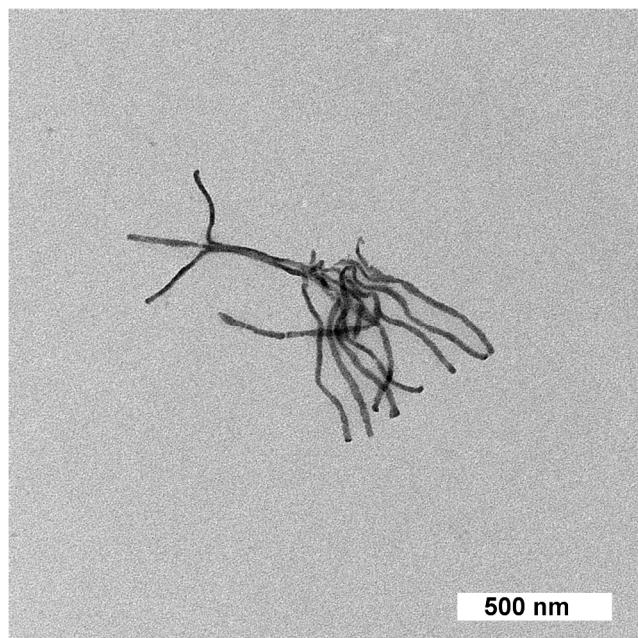
Figure S12. TEM image of PDMA-PS nanoparticles (sample B) casted from an aqueous solution (0.2 g.L^{-1}). The carbon/Formvar-coated TEM grids were treated with air plasma to improve their hydrophilicity prior to use. The theoretical thickness of the sections was set on 300 nm and the nanoparticles were stained with RuO$_4$. Sample prepared by absorbing the excess of solution with a piece of paper placed under the grid.

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