Supporting Information

Uniform, Length-Tunable Antibacterial 1D Diblock Copolymer Nanofibers

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1. Supplementary Materials and Methods

General considerations

PEG_{530} homopolymer was purchased from Polymer Source and used without purification, after drying via vacuum desiccation over phosphorous pentoxide. The synthesis and characterization of $PFTMC_{16}$-*b*-$PDMAEMA_{131}$ has been previously reported by Street et al.,[S1] and the synthesis and characterization of $PFTMC_{18}$-*b*-PEG_{530} has been reported by Garcia-Hernandez et al.[S2,S3] PFTMC homopolymer degrees of polymerization (DP_n) were determined by matrix-assisted laser desorption/ionization (MALDI) mass spectrometry and block copolymer DP_n were determined by integrations of coronal block peaks in ^{1}H NMR relative to peaks of the PFTMC block. RAFT-CTA was dried via vacuum desiccation over phosphorus pentoxide prior to use. All other reagents and solvents were purchased from Sigma-Aldrich (Canada), Combi-Blocks (USA), VWR (Canada), or Fisher Scientific (Canada) and used without further purification. Solvents for self-assembly were HPLC grade and were filtered through polytetrafluorethylene (PTFE) or nylon filters with a pore size of 200 nm before use.

Instrumentation

Gel permeation chromatography (GPC)

Gel permeation chromatograms were acquired on a Malvern OMNISEC triple-detector (refractive index, UV-Vis photodiode detector, light scattering detector and viscometer) chromatograph. Prepared samples were of 1 mg/mL concentration in HPLC grade THF, and were filtered through a PTFE filter with a 200 nm pore size prior to measurement. The eluent used was Triethylamine/THF (1 % *v/v*) at a flow rate of 1 mL/min. Two columns were employed, the first of grade T3000 and the second of grade T5000, operated at 35 °C. Universal calibration was constructed using a polystyrene standard.

Ultrasonication

Micelle sonication was carried out using either a Fisherbrand 112xx series advanced ultrasonic cleaner (FB-11203), operated in sweep mode at 80 % power and 37 MHz at 15 °C, or using a Hielschur UP100H sonication probe (100 W total output power) at 80% power.

Transmission electron microscopy (TEM)

TEM images were obtained on a JEOL 1011 microscope equipped with an 11 Megapixel CCD camera, operated at 80 kV. Nanoparticle solutions (3.5 µL, 1 – 4 mg/mL) were drop-casted onto a carbon-coated copper grid. Uranyl acetate solution (8 µL) in EtOH (3 wt %) was subsequently drop-casted on top, and the grids were left to dry overnight. Copper grids (400 mesh) were purchased from Ted Pella. Carbon films (ca. 6 nm) were prepared by carbon sputtering mica sheets with a Leica ACE 600 carbon coater. The carbon films were deposited onto copper grids via floatation on water and the grids were allowed air dry.

For micelle length analysis, a minimum of 200 nanofibers were traced manually using the FIJI software package. The number average micelle length (L_n) or width (W_n) and weight average micelle length (L_w) were calculated using eq. S1-2 from the individual contour lengths (L_i) of the micelles. Here, N_i is the number of micelles of length L_i, and n is the number of micelles examined in each sample. The distribution of micelle lengths is characterized by $Đ = L_w/L_n$.

$$L_n = \frac{\sum_{i=1}^{n} N_i L_i}{\sum_{i=1}^{n} Ni} \qquad L_w = \frac{\sum_{i=1}^{n} N_i L_i^2}{\sum_{i=1}^{n} NiLi} \qquad \text{(eq. S1-2)}$$

Dynamic Light Scattering (DLS) and ζ-Potential Measurements

Dynamic light scattering (DLS) and *ζ*-potential experiments were carried out using a Malvern Zetasizer Pro. Aqueous sample of nanofibers (100 ug/mL, 700 uL, 25 °C) were prepared in 5 mM NaCl and added into a folded capillary cell (DTS1070 type). For DLS, the correlation function was acquired in real time and analysed by Cumulant analysis. This process allowed the diffusion coefficients for the nanoparticles to be determined, and these were expressed as the effective hydrodynamic radius (R_H) using the Stokes-Einstein relationship for coated spheres in water (Refractive Index = 1.33, Dispersant Viscosity = 0.887, Dispersant Dielectric Constant = 78.5) with core properties of polystyrene latex (Refractive Index = 1.590, Absorption = 0.010). For *ζ*-potential measurements, the Smoluchowski approximation was used. A minimum of five measurements per sample were taken, consisting of between 10 and 100 cycles per run. The average *ζ*-potential was calculated from the individual measurements taken, with error represented as σ.

2. Self-Assembly Procedures

The composition of all solvent mixtures is given as *v:v*. Low dispersity nanofibers and nanospheres of PFTMC$_{16}$-*b*-PDMAEMA$_{131}$ were prepared by the procedures outlined below, which are based on those developed by Street et. al.,[S1] while low dispersity nanofibers of PFTMC$_{18}$-*b*-PEG$_{530}$ were prepared according to the procedure by Garcia-Hernandez et. al.[S2,S3] All nanofibers were ultimately transferred into distilled water *via* dialysis or *via* syringe-pump infusion and evaporation as reported by Garcia-Hernandez et. al.[S2]

General self-nucleation procedure. A solution of diBCP dissolved in THF (unimer) (20 mg/mL – 200 mg/mL) was diluted with an appropriate amount of THF. Subsequently, unimer solution was slowly added via a micropipette into a selective solvent (MeOH) to yield solutions which had final diBCP concentrations between 1 mg/mL – 10 mg/mL with 10:90 THF:MeOH solvent ratios. Each solution was manually shaken for ~10 s, agitated using a vortex mixer for ~10 s, and left to age at 22 °C for 24 h for PFTMC-*b*-PDMAEMA$_{131}$ or heated to 70 °C for 3 h for PFTMC$_{18}$-*b*-PEG$_{530}$. The resulting length-disperse nanofibers were analyzed via TEM.

General preparation of seed nanofibers. Length disperse nanofibers (1 mg/mL – 10 mg/mL, 10:90 THF:MeOH) were sonicated for at least 3 h using a Hielschur UP100H sonication probe at a temperature between 0 °C and 22 °C or using a Fisherbrand 112xx series advanced

ultrasonic cleaner (FB-11203) for 3 h at 10 °C in sweep mode at 37 mHz and 80 % power. The resulting seed nanofibers were analyzed by TEM.

General procedure for the preparation of low dispersity, controlled length nanofibers via seeded growth (living CDSA). For seeded growth assemblies with $m_{unimer}/m_{seed} \leq 10$: aliquots of unimer (20 mg/mL in THF) were added to diluted identical composition seed nanofiber solutions (0.1 mg/mL – 1 mg/mL) in MeOH. The self-assembly solutions (THF content: 10 – 20% in MeOH) were manually shaken for ~15 s and aged for 24 h at 22 °C.

For seeded growth assemblies with $m_{unimer}/m_{seed} > 10$: aliquots of unimer (20 mg/mL) were added in intervals of 10 m_{unimer}/m_{seed} every 24 h. The self-assembly solutions were manually shaken for ~15 s and aged for 24 h at 22 °C.

General procedure for the preparation of nanospheres

Nanospheres of PFTMC$_{16}$-*b*-PDMAEMA$_{131}$ were prepared via dialysis of a unimer solution of BCP in THF into deionized water over 24 h. Dialysis membranes were purchased from Sigma Aldrich with a molecular weight cutoff of 12,000 – 14,000 Da. Dialysis clips were purchased from Spectrum Chemical. The dialysate was exchanged a minimum of three times, ensuring that all of the organic solvent had been removed. The resulting nanospheres were left overnight and analyzed via TEM.

3. Antibacterial assays

All bacteria work was performed in a biological safety cabinet (BSC) using sterile technique. All materials were autoclaved and disinfected with 70 % ethanol prior to being placed in the BSC. Clear, sterile, 96-well plates were purchased from Corning. Lysogeny broth (LB) was used for streak plates and liquid culturing. *Escherichia coli* (*E. coli*) W3110 was used for all experiments. The buffer was prepared by weighing out (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (HEPES) into a glass bottle, filling with distilled water, autoclaving, and balancing to pH 7.4. Kinetic growth curves were fitted using GraphPad Prism and the logistic growth model. Minimum inhibitory concentration curves were fitted using GraphPad Prism and the Lambert and Pearson method.[S4] All bacterial experiments were repeated in triplicate on a minimum of three separate occasions, and the results were combined for analysis.

Preparation of bacteria for assays. From a stock solution of *E. coli* W3110 that was kept at -80 °C, a single loop was used to make a streak plate (LB agar). This was repeated. These plates were placed in an incubator at 37 °C for 24 h. From these two initial streak plates, a second propagation was performed. A disposable loop was used to select one colony from each, and streaked onto another plate, respectively. The new plates were incubated at 37 °C for 24 h. Subsequently, they were moved into a 4 °C fridge. To grow a liquid culture, a single colony was selected from a plate using a disposable loop. This was placed in 5 mL of LB broth contained in a 15 mL Falcon tube. The loop was swirled in the broth until the colony was visibly deposited in the broth. The falcon tube was placed in an incubator at 37 °C with orbital shaking at 200 rpm. The culture was allowed to grow for 24 h. From the initial culture, 25 µL of solution was taken and added to a second 15 mL falcon tube containing 5 mL of LB broth. This new culture was placed in an incubator at 37 °C with orbital shaking at 200 rpm for 24 h.

Plating of bacteria and nanofibers for kinetic growth assays. Liquid bacterial cultures were diluted in LB to a maximum volume of 3 mL and to an absorbance of 0.600 using an OD meter and a polystyrene cuvette. Once the appropriate dilutions were determined, they were performed on a larger scale to give at least 5 mL of *E. coli* and LB in a sterile reservoir. 10 mL of 40 mM HEPES buffer was added to a separate sterile reservoir. Using a multichannel pipette, 50 µL of buffer was added to each well, excluding the third column. The first row (A1-12) and the last row (H1-12) were topped up to 100 µL of buffer to be used as blanks and to prevent evaporation. The first column was also used as a buffer blank. To three wells in the third column (B3, C3, D3) 20 µL of 1 mg/mL material to be tested was added, and 20 µL of 1 mg/mL of a second material to be tested was added to the next three (E3, F3, G3). Each of these wells was then diluted with 80 µL of buffer solution. From the third column, 50 µL was taken from each well using a multichannel pipette and placed into the next column in order to dilute the material in half. This was repeated until the full plate was diluted. Each time, the material was pipetted up and down three times to ensure proper mixing. Subsequently, 50 µL of HEPES buffer was added to the second column (B-G2). Diluted *E. coli* in LB (50 µL) was added into each well, including the second column as a control (HEPES, broth, and bacteria). Therefore, the final volume in each well was 100 µL.

Cytation 5 plate reading for kinetic assays. Absorbance measurements (100 µL of sample) were obtained with the Biotek Cytation 5 multimode plate reader and were conducted at 37 °C

in a Corning 96-well plate (clear plates, with the lid). The samples were excited at 600 nm and the absorbance values were recorded. Two readings were taken, 10 minutes apart. The reading at 10 minutes was used over the reading at 0 minutes as the blanks were consistent at this time point. After the 10-minute reading, the 96-well plate was placed back in an incubator at 37 °C with orbital shaking at 200 rpm.

4. Supplementary Tables

Table S1. Summary of molar mass data for PDMAEMA$_{91}$-CTA, PFTMC$_{16}$-*b*-PDMAEMA$_{131}$, and PFTMC$_{18}$-*b*-PEG$_{530}$ polymers.[S1,S2] Data for PFTMC$_{18}$-*b*-PEG$_{530}$ is reproduced with permission from the American Chemical Society and data for PFTMC$_{16}$-*b*-PDMAEMA$_{131}$ is reproduced with permission from the Royal Society of Chemistry.[S1, S2]

Polymer	M_n (g/mol) GPC	M_w (g/mol) GPC	$Đ_M$ GPC	DP_n NMR	M_n (g/mol) NMR	DP_n MALDI-TOF
PDMAEMA$_o$	49,500	59,400	1.20	o = 91	14,710	-
PFTMC$_m$-*b*-PDMAEMA$_q$	9,700	15,000	1.55	m = 20 q = 131	24,881	m = 16
PFTMC$_n$-*b*-PEG$_s$	26,600	30,300	1.14	n = 18 s = 530	27,921	n = 18

Table S2. Hydrodynamic radius (R_H) and ζ-potential of PFTMC$_{16}$-*b*-PDMAEMA$_{131}$ nanofibers of different lengths, and nanospheres, recorded in 5 mM NaCl.

Morphology	Length in THF/MeOH (nm) via TEM	Length in water (nm) via TEM	Diameter in water (nm) via TEM	$Đ$ in water via TEM	R_H in 5 mM NaCl (nm) via DLS	ζ-potential in 5 mM NaCl (mv)
Nanofiber	112 ± 35	107 ± 34	-	1.10	39 ± 0.6	+13.4 ± 0.7
Nanofiber	351 ± 69	377 ± 78	-	1.04	59.5 ± 0.8	+15.7 ± 0.4
Nanofiber	701 ± 153	593 ± 222	-	1.14	77.5 ± 1.5	+16.1 ± 0.2
Nanosphere	-	-	15 ± 3	1.05	65 ± 0.85	+7.4 ± 0.2

5. Supplementary Figures

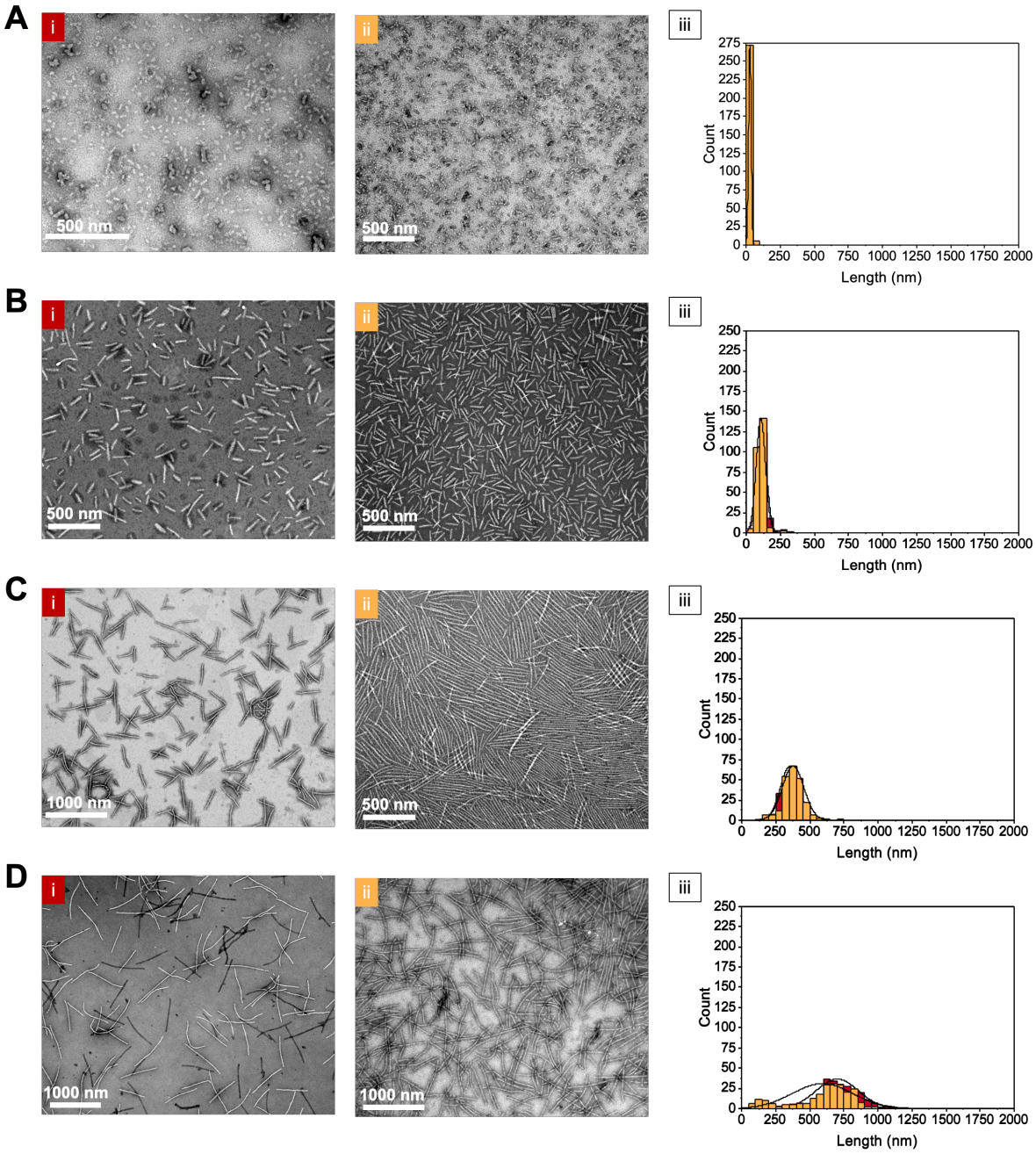

Figure S1. TEM micrographs of PFTMC$_{16}$-*b*-PDMAEMA$_{131}$ nanofibers. All samples were stained using a 3 wt% uranyl acetate solution in EtOH. Figures are colour coded where red represents the sample in organic solvents and yellow represents the sample after transfer into water for both the TEM micrographs and the corresponding histograms. (A) i) Seed nanoparticles after sonication using a Hielschur UP100H sonication probe (100W total output power) at 80% power (L_n = 28 nm, Đ = 1.20) in THF:MeOH (9:1) and ii) after dialysis into

water (L_n = 27 nm, $Đ$ = 1.12). iii) Representative histogram of seed dispersity as measured in organic solvents (red) and water (yellow). (B) i) Length controlled PFTMC$_{16}$-*b*-PDMAEMA$_{131}$ nanofibers (L_n = 112 nm, $Đ$ = 1.09) in THF:MeOH and ii) after dialysis into water (L_n = 107 nm, $Đ$ = 1.10) iii) Representative histogram of the nanofiber sample dispersity. (C) i) PFTMC$_{16}$-*b*-PDMAEMA$_{131}$ nanofibers (L_n = 351 nm, $Đ$ = 1.04) in THF:MeOH and ii) after transfer into water (L_n = 377 nm, $Đ$ = 1.04) iii) Representative histogram of the sample dispersity. (D) i) PFTMC$_{16}$-*b*-PDMAEMA$_{131}$ nanofibers (L_n = 701 nm, $Đ$ = 1.05) in THF:MeOH and ii) after transfer into water (L_n = 593 nm, $Đ$ = 1.14). iii) Representative histogram of sample dispersity before and after dialysis. A small population of nanofibers centred around 200 nm was observed after dialysis due to small amounts of fragmentation occurring.

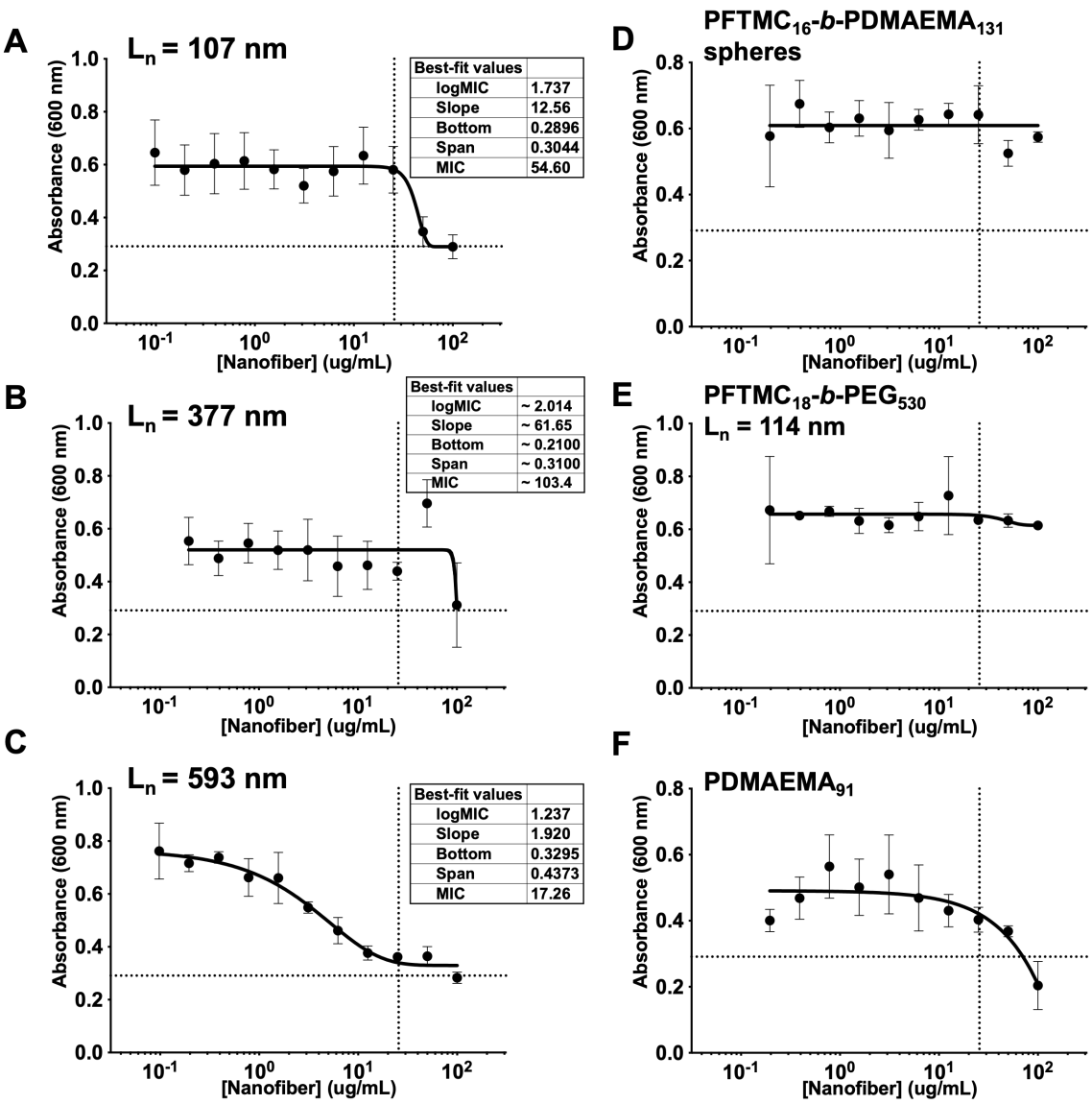

Figure S2. MIC curves as determined by the Lambert Pearson method using GraphPad Prism of nanofibers and materials tested against *E. coli* W3110. (A) Varying concentrations of $PFTMC_{16}$-*b*-$PDMAEMA_{131}$ nanofibers of L_n = 107 nm fibers ($Đ$ = 1.10) (B) L_n = 377 nm fibers ($Đ$ = 1.04) (C) L_n = 593 nm fibers ($Đ$ = 1.14) (D) $PFTMC_{16}$-*b*-$PDMAEMA_{131}$ nanospheres of D_H = 130 nm ($Đ$ = 1.05) (E) $PFTMC_{18}$-*b*-PEG_{530} nanofibers of L_n = 114 nm ($Đ$ = 1.06) (F) $PDMAEMA_{91}$ homopolymer.

6. References

S1. S. T. G. Street, Y. He, R. L. Harniman, J. D. Garcia-Hernandez, I. Manners, *Polym. Chem.,* 2022, DOI: 10.1039/D2PY00152G.

S2. J. D. Garcia-Hernandez, S. T. Street, Y. Kang, Y. Zhang and I. Manners, *Macromolecules*, 2021, **54**, 5784–5796.

S3. J. D. Garcia-Hernandez, H. C. Parkin, Ren, Y., Zhang, Y., I. Manners, *Polym. Chem.,* 2022, in revision.

S4. R. J. W. Lambert and J. Pearson, *J. Appl. Microbiol.*, 2000, **88**, 784–790.