Supporting Information

Hydrophobic Cargo Loading at the Core-Corona Interface of Uniform, Length-Tunable Aqueous Diblock Copolymer Nanofibers with a Crystalline Polycarbonate Core

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Supplementary Materials and Methods

General considerations

Methoxy PEGs were purchased from Polymer Source (Montreal, Canada). FTMC was synthesized according to the procedure reported by Finnegan et al.[1] 2-cyano-5-hydroxypentan-2-yl ethyl carbonotrithioate was prepared according to the procedure reported by Arno et al.[2] The PEGs and RAFT-CTAs were 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 as received unless otherwise noted. Solvents for

self-assembly were of HPLC grade and were filtered through polytetrafluoroethylene (PTFE), nylon, or cellulose membranes with a pore size of 200 nm before use. All reactions were carried out in an MBraun 200B glove box under a nitrogen atmosphere or using standard Schlenk line techniques. RAFT polymerizations were performed in custom-made Schlenk-vials to fit dry heating blocks. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) was dried over CaH_2, and purified by distillation under reduced pressure. Anhydrous solvents were dried and purified using an MBraun Grubbs/Dow solvent purification system.[3]

Instrumentation

Ultrasonication

Micelle sonication was carried out using a Fisherbrand 112xx series advanced ultrasonic cleaner (FB-11203). The instrument was operated in sweep mode at 80 % power and 37 MHz at 10 °C.

NMR Spectroscopy

^{1}H NMR spectra were obtained using a Bruker 500 MHz spectrometer with CD_2Cl_2 (^{1}H NMR: δ = 5.32 ppm), or DMSO-d_6 (^{1}H NMR: δ = 2.50 ppm) as the solvents. Chemical shifts are quoted in parts per million, with spectra referenced to the residual solvent peak. Multiplicities are abbreviated as brs (broad singlet), s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), m (multiplet) and *app.* (apparent) or combinations thereof.

Gel permeation chromatography (GPC)

GPC was conducted on a Malvern OMNISEC chromatograph equipped with a refractive index (RI), UV/Vis photodiode detector array, light scattering detector and viscometer. Triethylamine/THF (1% *v/v*) was used as the eluent, with the flow rate set at 1 mL/min. The columns used were grade T3000, followed by T5000 (Viscotek) at a constant temperature of 35 °C. The calibration (universal) of the RI detector was carried out using polystyrene standards (Viscotek). Samples were prepared at 1 mg/mL in eluent and filtered through a PTFE membrane filter, pore size = 200 nm.

Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS)

MALDI-TOF MS measurements performed using a Bruker Ultraflextreme running in linear mode. Samples were prepared using a *trans*-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene]malononitrile matrix (20 mg/mL in THF) and the polymer sample (2 mg/mL in THF), mixed in a 10:1 (*v*/*v*) ratio. Approximately 3 μL of the mixed solution was deposited onto a stainless-steel sample plate and allowed to air dry.

Transmission electron microscopy (TEM)

TEM images were obtained on a JEOL 1011 microscope with an 11 Megapixel CCD camera, operated at 80 kV. Samples were prepared by drop-casting 7.5 μL of the micelle solution onto a carbon-coated copper grid, followed by drop-casting 10 μL of uranyl acetate in EtOH (3 wt %). Sample concentrations for TEM analysis were either 0.5 or 0.1 mg/mL based on nanofiber concentration. Copper grids (400 mesh) were purchased from Ted Pella, and carbon films (ca. 6 nm) were prepared on mica sheets by carbon sputtering with a Leica ACE 600 carbon coater. The carbon films were deposited onto the copper grids by floatation on water and the carbon-coated grids were allowed to dry in air.

For micelle contour length analysis, a minimum of 150 nanofibers in several images were traced manually using the ImageJ software package developed at the US National Institute of Health. 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 measurements of the contour lengths (L_i) of individual micelles, where 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 $Đ = L_w/L_n$.

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

Spectroscopic Experiments

UV-Vis spectra were recorded at 25°C by using a Biotek Cytation 5 multimode plate reader or a PTI QM40 fluorometer.

Biotek Cytation 5 multimode plate reader. Fluorescence measurements (0.1 mL of sample) were obtained with the Biotek Cytation 5 multimode plate reader and were conducted at 25°C in a Costar 96-well plate (clear bottom, black sides, and with the lid). The samples were excited at 540 nm and the emission was recorded from 570 – 700 nm. The bottom optics was used with a gain of 100 (a.u.). To determine the excitation wavelength to use, excitation scans were recorded with the emission detected at 665 nm, with excitation wavelengths scanned from 400 - 650 nm. The emission of NR from the loaded nanofibers was studied in H_2O and in MeOH. Analysis of the NR content in the nanofibers was determined by measuring the fluorescence of NR in MeOH or H_2O after the PNSL process, and compared against a calibration curve (Figure S17A-B and S20 B-C). The loaded nanofibers, or non-encapsulated NR in water, were freeze dried and resuspended in MeOH (0.9 mL) for spectroscopic analysis (Figures S15, 16, 19).

PTI QM40 fluorometer. Fluorescence measurements (1 mL of sample) were obtained by using a PTI QM40 fluorometer at 25°C in a quartz glass cuvette (PCS8501 type, 10.0 mm light path). The slits were set to 2 nm bandpass. All measurements were corrected for the fluctuations of the lamp intensity and transmission of the optics. The samples were excited at 540 nm and the emission was recorded from 570 - 700 nm. To determine the excitation wavelength to use, excitation scans were recorded with the emission detected at 665 nm, with excitation wavelengths scanned from 400 - 650 nm. The emission of NR from the loaded nanofibers was studied in H_2O and in MeOH. Analysis of the NR content in the nanofibers (from loading and release studies) was determined by measuring the fluorescence of NR in MeOH or H_2O after the PNSL process, and compared against a calibration curve (Figure S20B-C). The loaded nanofibers or non-encapsulated NR in water, were freeze dried and resuspended in MeOH (1.5 mL) for spectroscopic analysis (Figure S20A).

Fluorescence lifetime measurements on the NR-loaded nanofibers in H_2O were obtained using a Hamamatsu Compact fluorescence lifetime spectrometer C11367 with a Xenon flash lamp at 25°C

in a quartz glass cuvette. The samples were excited at 590 nm, and the fluorescence lifetimes were recorded in the range of 610 – 640 nm via the single wavelength measurement mode (in line with the corresponding emission peak). The pre-set fluorescent lifetime measurement range is below 10 ns. The fluorescence lifetime time constants were obtained by single or multiexponential fitting of the fluorescence decay curves by Quantaurus-Tau software via deconvolution processing. When a multi-exponential decay is present in a sample the contribution (or fraction) of the decay component is proportional to the $A_x\tau_x$ product of the respective component.[4]

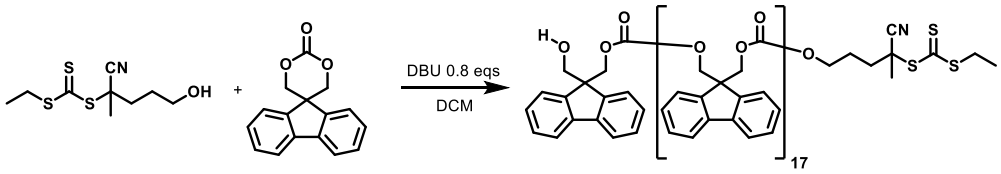
Dynamic Light Scattering (DLS)

Dynamic light scattering (DLS) experiments were carried out using a Zetasizer Pro. Samples of different polymer concentrations were prepared in filtered solvents by passing through a 0.45 μm membrane filter into an optical quartz glass cuvette (PCS8501 type, 10.0 mm light path). The correlation function was acquired in real time and analyzed with a function capable of modelling multiple exponentials (Cumulant analysis). This process enabled the diffusion coefficients for the component particles to be extracted, and these were subsequently expressed as the intensity weighted mean hydrodynamic size ($R_{h,z}$) by using the Stokes-Einstein relationship for coated spheres in THF (Refractive Index = 1.41, Dispersant Viscosity = 0.455, Dispersant Dielectric Constant = 7.5), MeOH (Refractive Index = 1.33, Dispersant Viscosity = 0.548, Dispersant Dielectric Constant = 33), or H_2O (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).

Synthetic Procedures

Synthesis of PFTMC$_{18}$-CTA

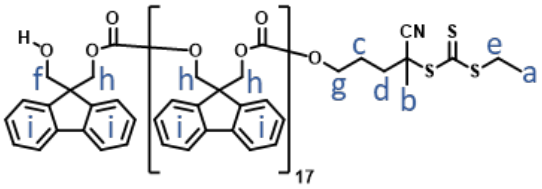
Scheme S1. Synthesis of PFTMC$_{18}$-CTA by ring-opening polymerization of FTMC



To a solution of 2-cyano-5-hydroxypentan-2-yl ethyl carbonotrithioate[2] in anhydrous CH_2Cl_2 (500 µL, 100 mg/mL, 0.2 mmol, 1.0 eq), DBU (24 µL, 24.4 mg, 0.16 mmol, 0.8 eq) was added in an oven-dried round bottom flask equipped with a magnetic stirring bar. To the stirring solution, FTMC (1.01 g, 4.0 mmol, 20 eq) in anhydrous CH_2Cl_2 (6 mL) was added, and the reaction mixture was stirred at room temperature for 1 h, before the reaction mixture was quenched by the addition of benzoic acid (100 mg). The crude product was purified by precipitation into ice-cold diethyl ether three times, followed by precipitation into ice-cold MeOH three times, and drying *in vacuo* to yield PFTMC$_{18}$-CTA as a yellow solid (1.0 g, 95 %).

MALDI-TOF MS $[M]^+$ found: 4729.3, DP_n: 18.

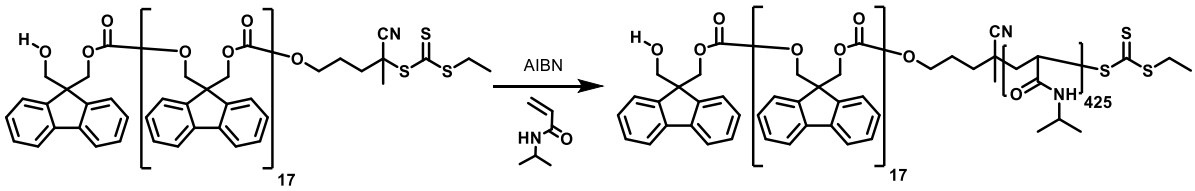
GPC: M_n = 4,800, $Đ_M$ = 1.17.



^{1}H NMR (500 MHz, CD_2Cl_2) δ 7.78 – 7.69 (36H, m, Hi), 7.61 – 7.47 (36H, m, Hi), 7.44 – 7.34 (36H, m, Hi), 7.32 – 7.19 (36H, m, Hi), 4.50 – 4.27 (70H, m, Hh), 4.07 (2H, t, J = 5.2 Hz, Hg), 3.70 (2H, d, J = 6.4 Hz, Hf), 3.31 (2H, dd, J = 7.4 Hz, He), 2.22 – 2.13 (1H, m, Hd), 2.06 – 1.98 (1H, m, Hd), 1.87 (2H, h, Hc), 1.80 (3H, s, Hb), 1.31 (3H, t, J = 7.4 Hz, Ha). The ^{1}H ratio of g/f indicates the CTA capping efficiency. ^{1}H integration is based on 'f' (HOCH_2).

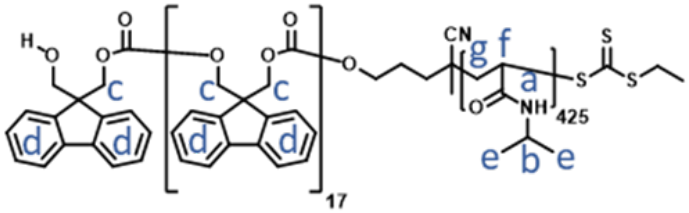
Synthesis of PFTMC$_{18}$-*b*-PNIPAM$_{425}$

Scheme S2. Synthesis of PFTMC$_{18}$-*b*-PNIPAM$_{425}$ by RAFT polymerization.



PFTMC$_{18}$-CTA (50 mg, 0.004 mmol, 1 eq), *N*-isopropylacrylamide (192 mg, 1.7 mmol, 400 eq), and AIBN (0.2 mg, 0.001 mmol, 0.3 eq) were dissolved in dioxane (4 mL) in a custom-made schlenk-vial followed by four freeze-pump-thaw cycles. The vial with the reaction mixture was placed in a preheated dry heating block and heated to 70 °C for 18 h. The reaction was quenched by submersion in liquid nitrogen. The product was precipitated three times in ice-cold diethyl ether. The product was dried *in vacuo* to yield PFTMC-*b*-PNIPAM as a white-yellow solid. GPC analysis indicated the presence of a small amount of water-initiated PFTMC homopolymer (as evidenced by a lower M_w shoulder with increased absorbance at 268 nm via UV/Vis). Water-initiated PFTMC homopolymer cannot subsequently polymerize other monomers via RAFT polymerization. The PFTMC homopolymer was removed via flash chromatography (hexane/ethyl acetate, 40:60 to ethyl acetate, then CH_2Cl_2:MeOH:H_2O, 65:35:2 to elute the diblock copolymer). The residual silica-gel was removed via dissolution in THF (50 mL), followed by centrifugation (4500 rpm, 10 mins) and decantation five times. The resulting polymer was dried and precipitated in ice-cold diethyl ether to yield PFTMC$_{18}$-*b*-PNIPAM$_{425}$ as a white-yellow solid (175 mg, 91%).

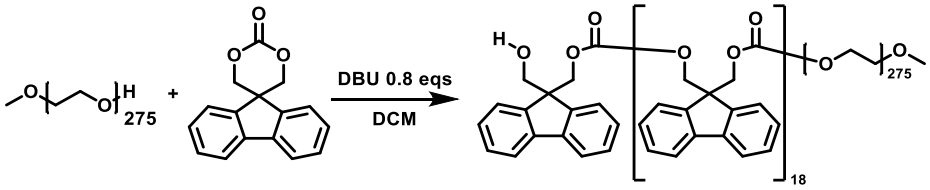
GPC: M_n = 31,800, $Đ_M$ = 1.76. This value is likely overestimated due to GPC column absorption effects (see main text).



^{1}H NMR (500 MHz, DMSO-d_6) δ 7.99 – 6.72 (556H, m, Ha + Hd), 4.44 – 4.17 (70H, m, Hc), 4.06 – 3.58 (425H, m, Hb), 2.43 – 0.67 (m, 3735H, He + Hf + Hg). ^{1}H integration is based on 'c' (OCH_2C) from the PFTMC, matching the value on the homopolymer.

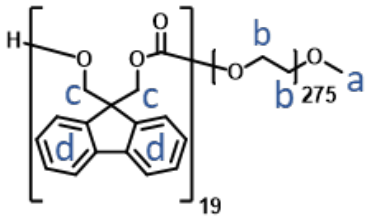
Synthesis of PFTMC$_{19}$-*b*-PEG$_{275}$

Scheme S3. Synthesis of PFTMC$_{19}$-*b*-PEG$_{275}$ by organocatalytic ring-opening polymerization.



PEG$_{275}$ (500 mg, 0.04 mmol, 1 eq) and DBU (5.2 µL, 5.3 mg, 0.03 mmol, 0.8 eq) were dissolved in the minimum volume of anhydrous CH_2Cl_2 in a round bottom flask equipped with a magnetic stirring bar. To the stirring solution, FTMC (219 mg, 0.87 mmol, 20 eq) in anhydrous CH_2Cl_2 (1.5 mL) was added, and the reaction mixture was stirred at room temperature for 3 h, before the reaction mixture was quenched by the addition of benzoic acid (20 mg). The crude product was purified by precipitation into ice-cold diethyl ether three times. The resulting solid was dried *in vacuo* to yield PFTMC$_{19}$-PEG$_{275}$ as a white solid (689 mg, 96%).

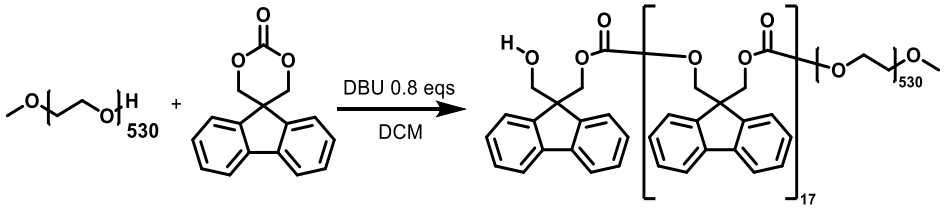
GPC: M_n = 15,200, $Đ_M$ = 1.07.



^{1}H NMR (500 MHz, CD_2Cl_2) δ 7.84 – 7.68 (38H, m, Hd), 7.63 – 7.47 (38H, m, Hd), 7.46 – 7.32 (38H, m, Hd), 7.32 – 7.20 (38H, m, Hd), 4.47 – 4.17 (76H, m, Hc), 3.77 – 3.42 (1102H, m, Hb), 3.34 (3H, s, Ha). ^{1}H integration is based on 'a' (CH_2OCH_3) from the terminal methyl group of the PEG.

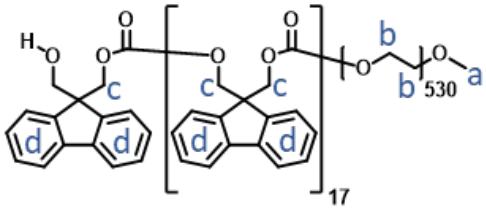
Synthesis of PFTMC$_{18}$-*b*-PEG$_{530}$

Scheme S4. Synthesis of PFTMC$_{18}$-*b*-PEG$_{530}$ by organocatalytic ring-opening polymerization.



PEG$_{530}$ (500 mg, 0.02 mmol, 1 eq) and DBU (2.9 µL, 2.9 mg, 0.02 mmol, 0.8 eq) were dissolved in the minimum volume of anhydrous CH_2Cl_2 in a round bottom flask equipped with a magnetic stirring bar. To the stirring solution, FTMC (122 mg, 0.49 mmol, 20 eq) in anhydrous CH_2Cl_2 (1 mL) was added, and the reaction mixture was stirred at room temperature for 3 h, before the reaction mixture was quenched by the addition of benzoic acid (10 mg). The crude product was purified by precipitation into ice-cold diethyl ether three times and dried *in vacuo* (515 mg, 83 %). 300 mg of the collected white solid was then dissolved in 20 mL of THF in a 50 mL centrifuge with a stir bar. 10 mL of hexane was added to the stirring solution and centrifuged. To the supernatant an extra 6 mL of hexanes was added, stirred, and centrifuged. The resulting solid was dried *in vacuo* to yield PFTMC$_{18}$-PEG$_{530}$ as a white solid (210 mg, 70%).

GPC: M_n = 26,600, $Đ_M$ = 1.14.



^{1}H NMR (500 MHz, CD_2Cl_2) δ 7.84 – 7.68 (37H, m, Hd), 7.63 – 7.47 (37H, m, Hd), 7.46 – 7.32 (38H, m, Hd), 7.32 – 7.20 (37H, m, Hd), 4.47 – 4.17 (73H, m, Hc), 3.77 – 3.42 (2120H, m, Hb), 3.34 (3H, s, Ha). ^{1}H integration is based on 'a' (CH_2OCH_3) from the terminal methyl group of the PEG.

Self-Assembly Procedures

Composition of all solvents given in *v:v*

Self-nucleation of PFTMC$_{18}$-*b*-PNIPAM$_{425}$. PFTMC$_{18}$-*b*-PNIPAM$_{425}$ (8 mg) were placed in a vial, followed by addition of a mixture of 15:85 THF:MeOH (4 mL). The resulting solution (2 mg/mL in 15:85 THF:MeOH) was manually shaken for ~15 s and heated for 3 h at 75 °C. The solution was cooled to 20 °C over 3 h, and aged for 48 h. The resulting solution contained morphologically pure nanofibers with disperse lengths, as analyzed via TEM.

Preparation of PFTMC$_{18}$-*b*-PNIPAM$_{425}$ seed nanofibers. PFTMC$_{18}$-*b*-PNIPAM$_{425}$ disperse nanofibers (4 mL, 2 mg/mL, in 15:85 THF:MeOH) were fragmented by ultrasonication for 3 h at 0 °C. The resulting seed nanofibers were analyzed by TEM (L_n = 46 nm, $Đ$ = 1.17, σ = 19 nm).

Preparation PFTMC$_{18}$-*b*-PNIPAM$_{425}$ nanofibers of controlled length and low dispersity by seeded growth (living CDSA). For seeded growth assemblies with $m_{unimer}/m_{seed} \leq 10$: aliquots of PFTMC$_{18}$-*b*-PNIPAM$_{425}$ unimer (10 mg/mL in THF), equivalent to corresponding m_{unimer}/m_{seed}, were added to diluted seed nanofiber solutions in MeOH (200 µL). The self-assembly solutions in MeOH (0.1 mg/mL, THF content: 10 – 20% in MeOH) were manually shaken for ~15 s and aged for 48 h at 20 ºC.

For seeded growth assemblies with m_{unimer}/m_{seed} 20, 30, and 40: aliquots of PFTMC$_{18}$-*b*-PNIPAM$_{425}$ unimer (10 mg/mL in THF), equivalent to corresponding m_{unimer}/m_{seed}, were added to diluted seed nanofiber solution in MeOH (100 µL). The unimer was added in intervals of 10 m_{unimer}/m_{seed} every 24 h. The self-assembly solutions (0.1 mg/mL, THF/MeOH 10:90) were manually shaken for ~15 s and aged 48 h at 20 ºC.

Preparation of 125 nm PFTMC$_{18}$-*b*-PNIPAM$_{425}$ nanofibers of controlled length and low dispersity by seeded growth (living CDSA). To a diluted seed solution (L_n = 46 nm, $Đ$ = 1.17, σ = 19 nm, 2 mL, 0.615 mg/mL, in MeOH), PFTMC$_{18}$-*b*-PNIPAM$_{425}$ unimer (10 mg/mL in THF, m_{unimer}/m_{seed} equivalent to 1.7) was added in two portions (2 × 104.5 µL), 24 h apart. The self-assembly solution (~2.2 mL, 1.5 mg/mL, THF:MeOH 12:88) was manually shaken for ~15 s after

each addition, and then aged for 48 h at 20 ºC. The resulting nanofibers were analyzed by TEM (L_n = 127 nm, $Đ$ = 1.03, σ = 22 nm).

Preparation of 125 nm PFTMC$_{19}$-*b*-PEG$_{275}$ nanofibers of controlled length and low dispersity by seeded growth (living CDSA). To a diluted seed solution (L_n = 30 nm, $Đ$ = 1.22, σ = 14 nm, 2.7 mL, 0.414 mg/mL, in MeOH), PFTMC$_{19}$-*b*-PEG$_{275}$ unimer (10 mg/mL in THF, m_{unimer}/m_{seed} equivalent to 3.1) was added in two portions (2 × 173.3 µL), 24 h apart. The self-assembly solution (~3.05 mL, 1.5 mg/mL, THF:MeOH 15:85) was manually shaken for ~15 s after each addition, and then aged for 48 h at 20 ºC. The resulting nanofibers were analyzed by TEM (L_n = 127 nm, $Đ$ = 1.06, σ = 32 nm).

Preparation of 125 nm PFTMC$_{18}$-*b*-PEG$_{530}$ nanofibers of controlled length and low dispersity by seeded growth (living CDSA). To a diluted seed solution (L_n = 32 nm, $Đ$ = 1.25, σ = 16 nm, 2.7 mL, 0.435 mg/mL, in MeOH), PFTMC$_{18}$-*b*-PEG$_{530}$ unimer (10 mg/mL in THF, m_{unimer}/m_{seed} equivalent to 2.9) was added in two portions (2 × 189 µL), 24 h apart. The self-assembly solution (~3.05 mL, 1.5 mg/mL, THF:MeOH 15:85) was manually shaken for ~15 s after each addition, and then aged for 48 h at 20 ºC. The resulting nanofibers were analyzed by TEM (L_n = 114 nm, $Đ$ = 1.06, σ = 28 nm).

Nile Red (NR) loading of diBCP nanofibers.

Preparation of low dispersity nanofibers loaded with NR. Vials containing either of the three diBCP nanofiber solutions of a given length (i.e. 125 or 114 nm nanofibers) were prepared by adding THF, MeOH and NR to obtain final solutions containing nanofibers (0.1 mg/mL) and NR (10, 1, 0.1, or 0.01 *w*/*w*) in MeOH:THF (15:85, 0.9 – 2 mL). The resulting solutions were placed in an orbital shaker (300 rpm), and H_2O (same volume as prepared nanofiber solution e.g. 0.9 mL) was infused into the vials via syringe pump at a rate of 100 µL/min. The vials were left uncapped but protected from light in the orbital shaker for 12 h, before any residual organic solvent was evaporated by gently blowing air for 30 min. Finally, the solutions were passed through a Nylon syringe-filter (0.45 µm pore size) and made up to a final volume in H_2O equal to the originally prepared nanofiber solution (e.g. 0.9 mL, as measured by weight, using the density of water). For quantification of the loaded NR, analysis of aliquots of the loaded nanofibers in H_2O (0.2 or 1 mL)

were freeze dried and resuspended in MeOH to an equal volume of the aliquot taken in MeOH (e.g. 0.45 or 1 mL).

Control experiments with NR. Control experiments to investigate NR removal via filtration were carried out with by transferring the NR (equivalent to 1 wt %) to H_2O via the PNSL method, followed by a gentle flow of air (30 min). Samples with and without filtration through a Nylon syringe-filter (0.45 μm pore size) were made up to a final volume in H_2O equal to the originally prepared solution (as measured by weight, using the density of water). For spectroscopic analysis, 1 mL of the resulting solutions were freeze-dried and resuspended in MeOH (1 mL).

Supplementary Tables

Table S1. Summary of molecular weight data for PFTMC$_{18}$-CTA, PFTMC$_{18}$-*b*-PNIPAM$_{425}$, PFTMC$_{19}$-*b*-PEG$_{275}$ and PFTMC$_{18}$-*b*-PEG$_{530}$ polymers.

Polymer	M_n (g/mol) GPC	M_w (g/mol) GPC	$Đ_M$ GPC	DP_n NMR	DP_n MALDI-TOF
PFTMC$_m$	4,800	5,600	1.17	m =18	18
PFTMC$_{18}$-*b*-PNIPAM$_n$	31,800	55,900	1.76	n = 425	-
PFTMC$_o$-*b*-PEG$_p$	15,200	16,200	1.07	o = 19 p = 275	-
PFTMC$_q$-*b*-PEG$_r$	26,600	30,300	1.14	q = 18 r = 530	-

Table S2. Statistical analysis of contour length measurements for PFTMC$_{18}$-*b*-PNIPAM$_{425}$ diBCP nanofibers prepared via seeded growth at 20 °C, in DMSO:MeOH solvent mixtures (DMSO = 10 – 20 %), measured via TEM. The number of nanofibers measured is represented by n, and σ represents the standard deviation of the measured length, and L_n/eq corresponds to the L_n of the nanofibers per mass equivalent of triBCP in the sample.

Length	m_{unimer}/m_{seed}								
	Seeds	2.5	2.5[a]	5	10	20	30	40	40[a]
n	172	156	153	158	193	150	151	150	155
L_n (nm)	46	163	165	278	455	787	1222	1729	1798
L_w (nm)	54	171	174	296	482	837	1270	1814	1837
Đ	1.17	1.04	1.05	1.06	1.06	1.06	1.04	1.04	1.02
L_n/eq (nm)	46	47	47	46	42	38	40	42	44
σ (nm)	19	36	36	71	106	198	243	385	267
σ / L_n	0.41	0.22	0.22	0.26	0.23	0.25	0.20	0.22	0.16

[a] Measured 12 months after the sample was originally prepared.

Supplementary Figures

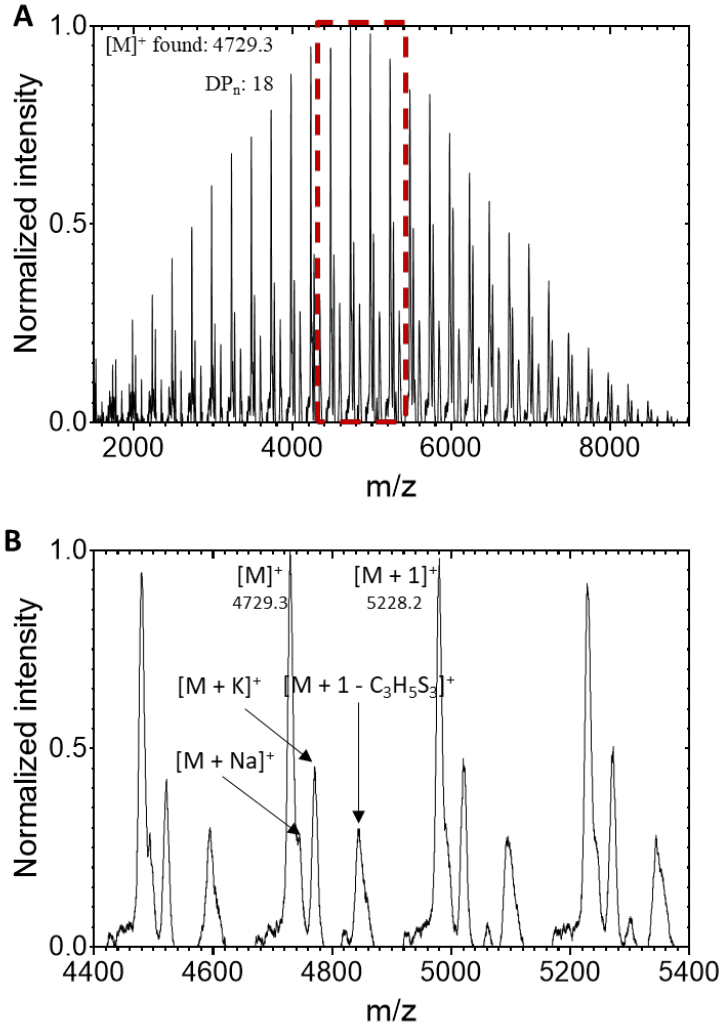


Figure S1. MALDI-TOF MS spectra of $PFTMC_{18}$. The red square represents the area expanded in B. Reproduced from ref [5].

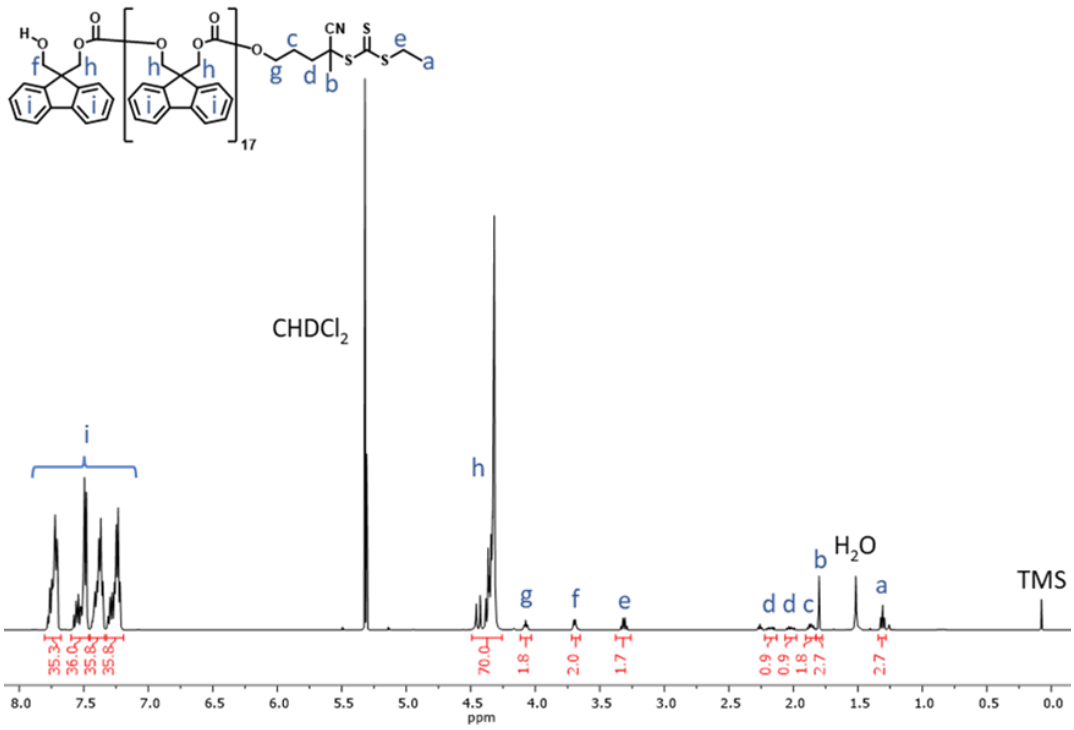


Figure S2. ^{1}H NMR spectra (in CD_2Cl_2) of $PFTMC_{18}$-CTA. Reproduced from ref [5].

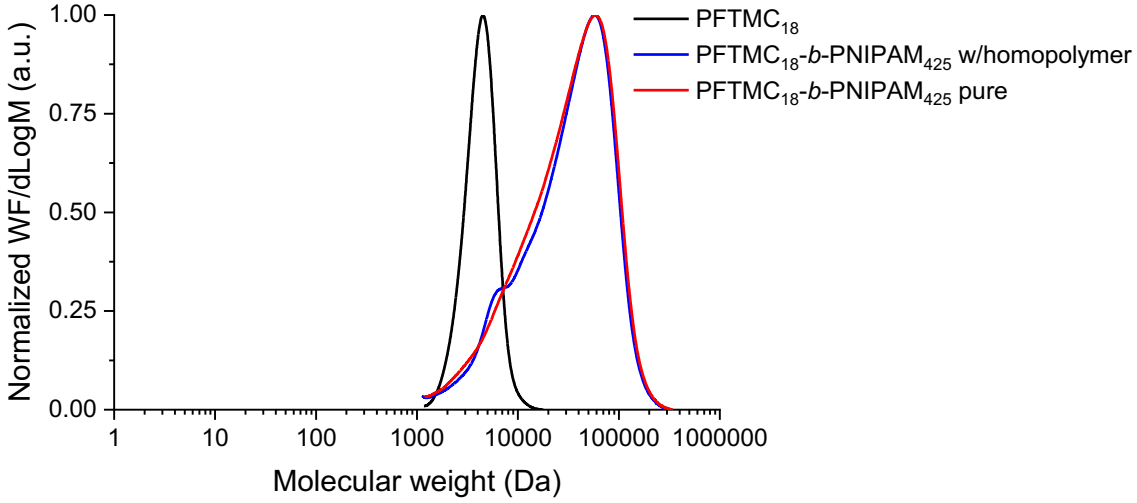


Figure S3. GPC (refractive index) traces of in triethylamine/THF (1% *v/v*) 1 mL min^{-1}, at 35 °C of $PFTMC_{18}$ homopolymer capped with the CTA (black trace, 1 mg/mL), $PFTMC_{18}$-*b*-$PNIPAM_{425}$ diBCP before (blue trace, 1 mg/mL), and after flash column chromatography (red trace, 1 mg/mL). The y-axis reflects the distribution of weight fractions by molecular weight. The GPC trace of the diBCP was unchanged when a lower concentration was used (0.25 mg/mL).

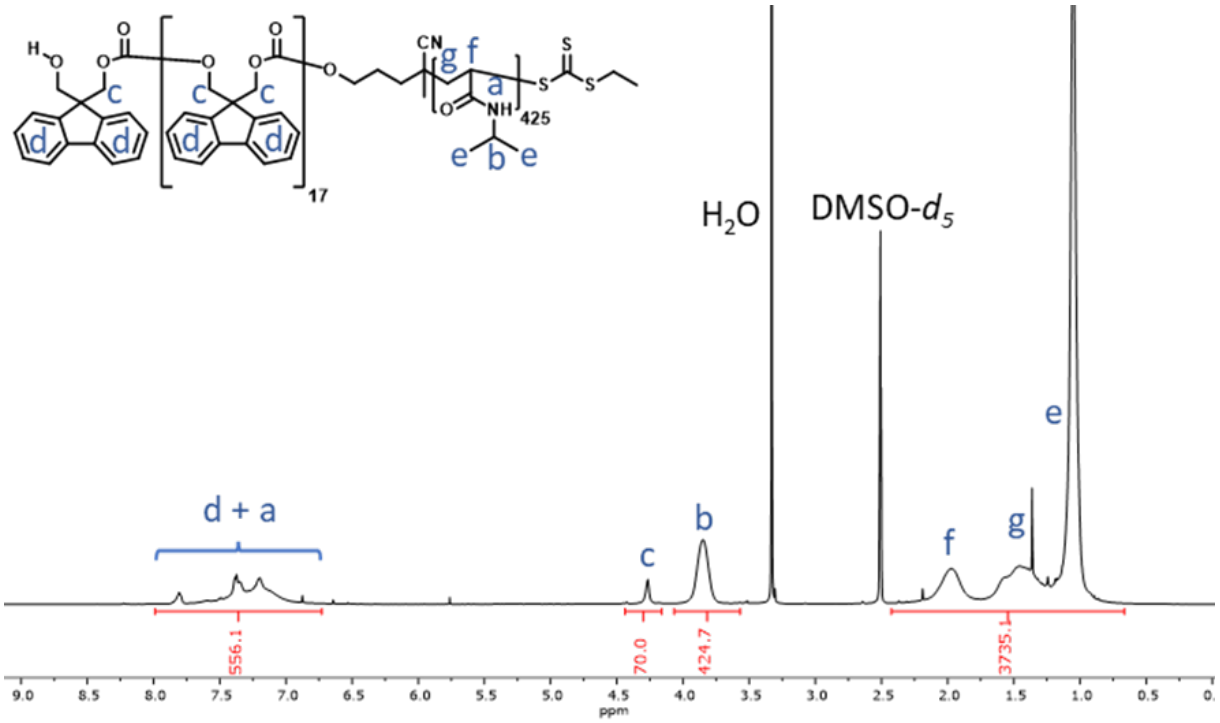


Figure S4. ^{1}H NMR spectra (in DMSO-d_6) of purified PFTMC$_{18}$-*b*-PNIPAM$_{425}$. Reproduced from ref [5].

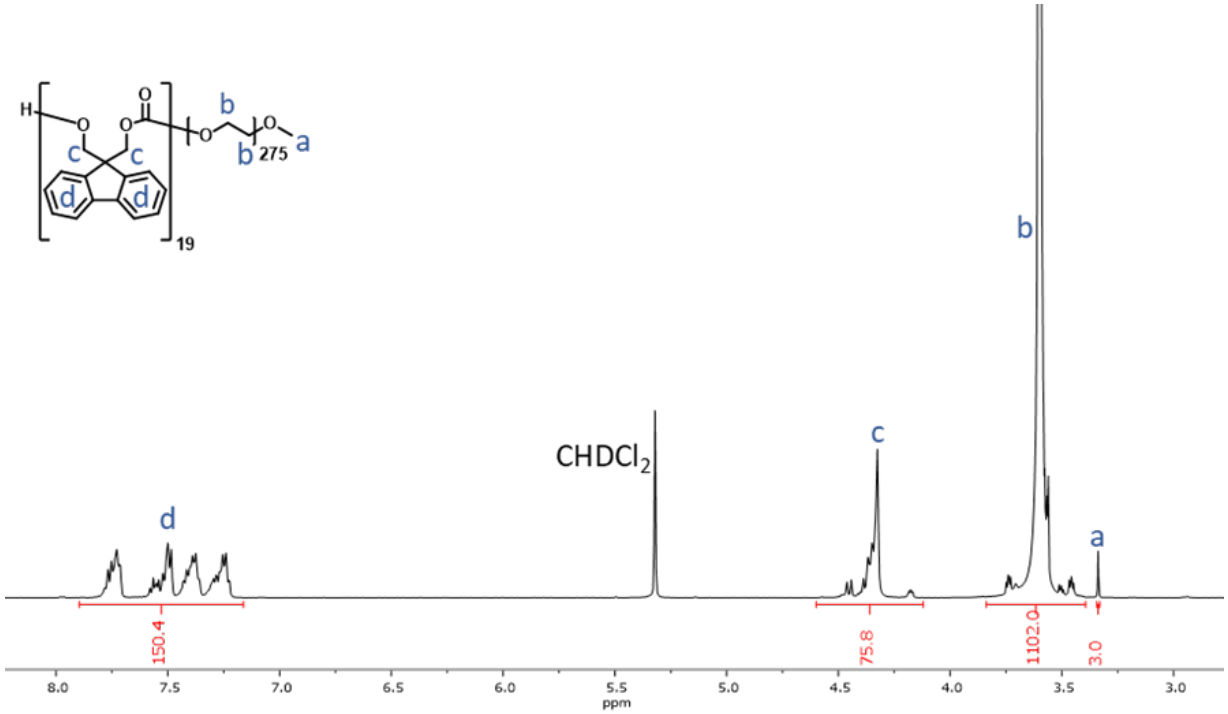


Figure S5. ^{1}H NMR spectra (in CD_2Cl_2) of PFTMC$_{19}$-*b*-PEG$_{275}$.

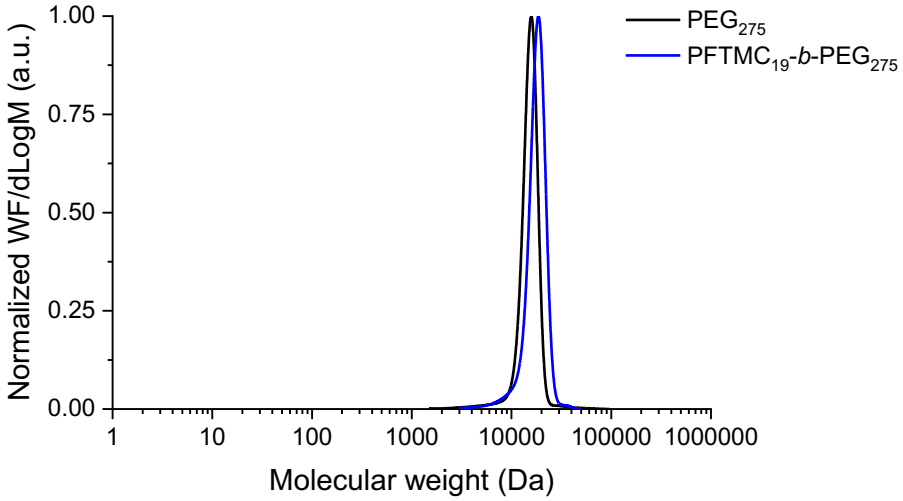


Figure S6. GPC (refractive index) traces eluted in triethylamine/THF (1% *v/v*), 1 mL/min, at 35 °C of PEG_{275} homopolymer (black trace, 1 mg/mL), $PFTMC_{19}$-*b*-PEG_{275} diBCP (blue trace, 1 mg/mL). The y-axis reflects the distribution of weight fractions by molecular weight.

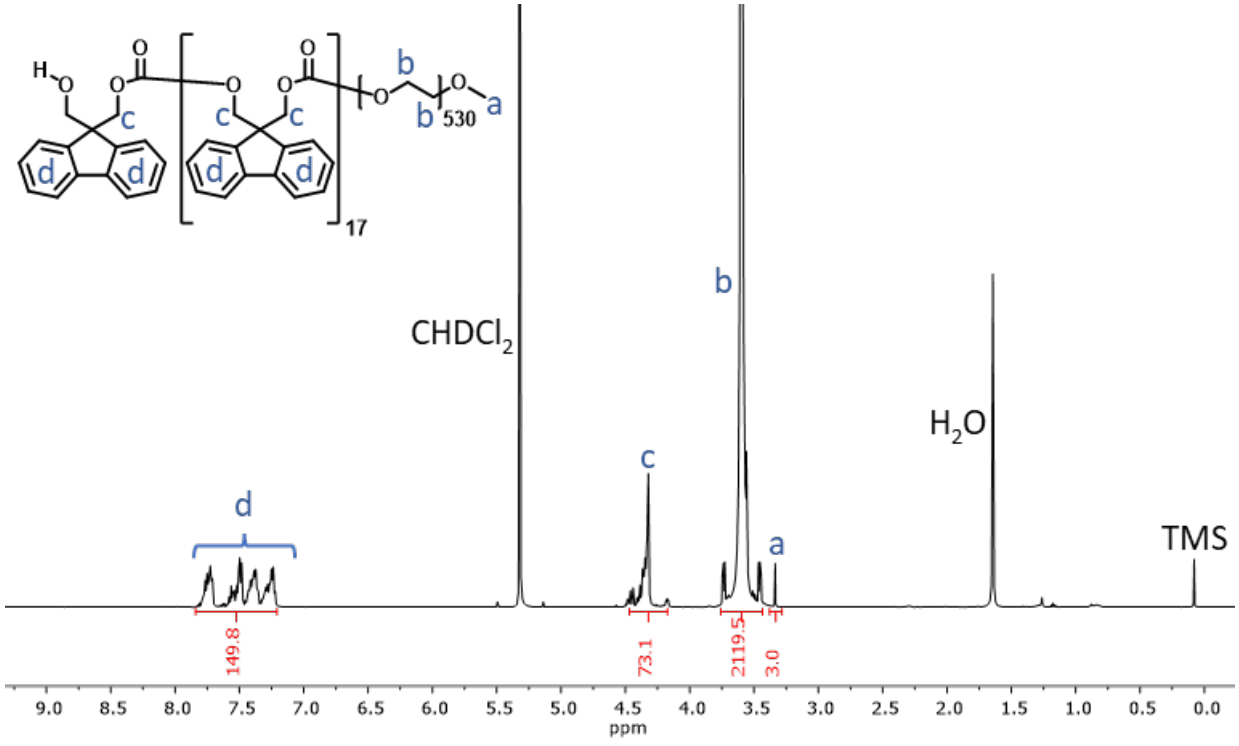


Figure S7. ^{1}H NMR spectra (in CD_2Cl_2) of $PFTMC_{18}$-*b*-PEG_{530}. Reproduced from ref [5].

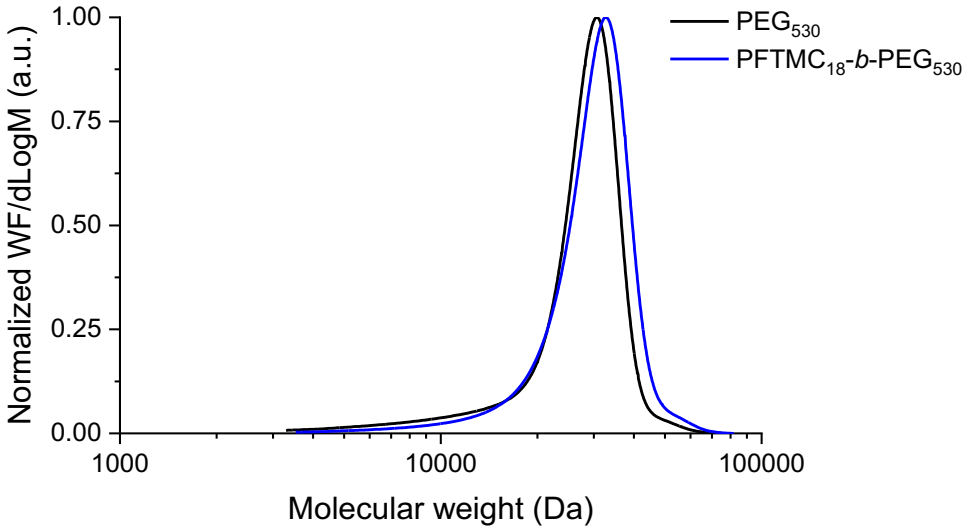


Figure S8. GPC (refractive index) traces eluted in triethylamine/THF (1% *v/v*), 1 mL/min, at 35 °C of PEG_{530} homopolymer (black trace, 1 mg/mL), $PFTMC_{18}$-*b*-PEG_{530} diBCP (blue trace, 1 mg/mL). The y-axis reflects the distribution of weight fractions by molecular weight.

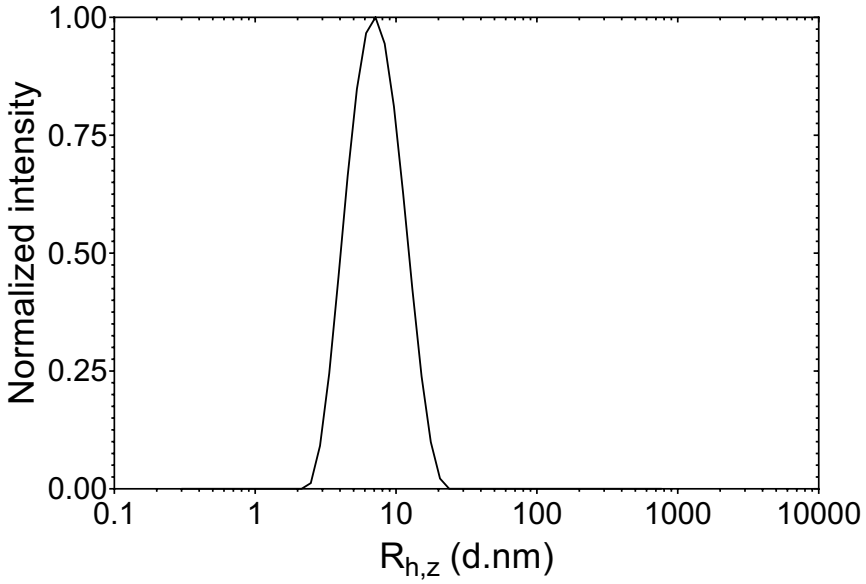


Figure S9. DLS of the $PFTMC_{18}$-*b*-$PNIPAM_{425}$ diBCP in tetrahydrofuran (THF); $R_{h,z}$ = 9.3 nm, σ = 2.22 nm.

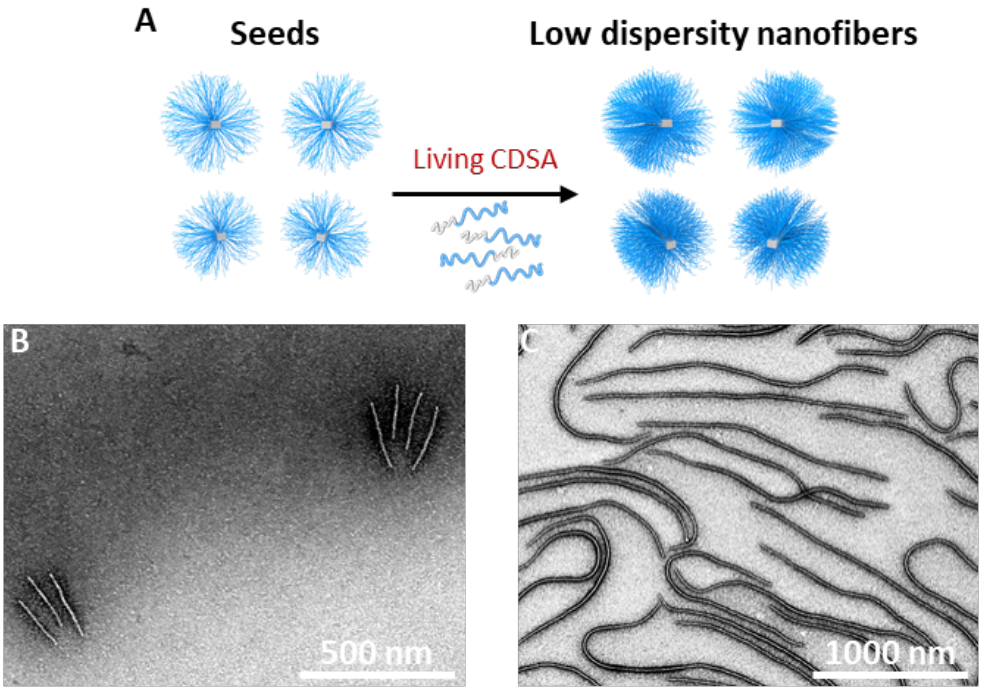


Figure S10. A) Schematic representation of the generation of low-dispersity nanofibers made from PFTMC$_{18}$-*b*-PNIPAM$_{425}$. B-C) TEM micrographs of nanofibers prepared via Living CDSA at various m_{unimer}/m_{seed} ratios added to solutions of seed nanofibers. m_{unimer}/m_{seed} = B) 2.5:1 C) 40:1.

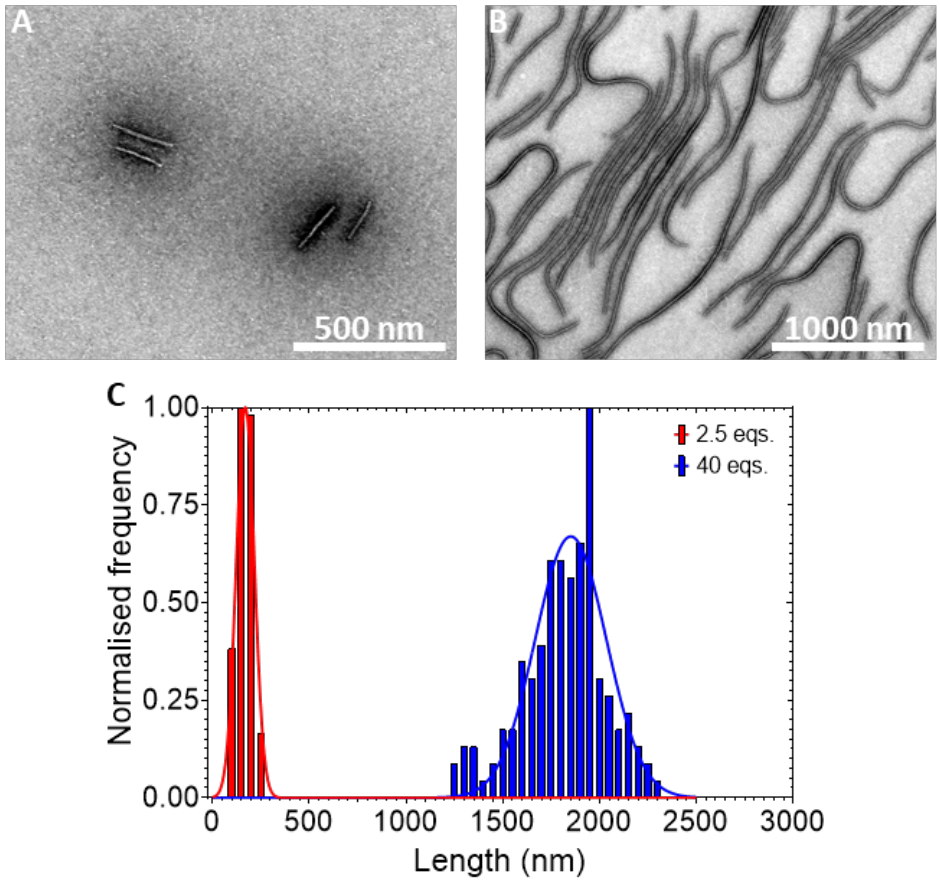


Figure S11. TEM micrographs of nanofibers prepared through seeded-growth after 12 months of the addition of unimer in THF to nanofiber seed solutions (L_n = 46 nm, $Đ$ = 1.17, σ = 19 nm, in THF:MeOH 15:85 *v/v*) at: A) 2.5:1 m_{unimer}/m_{seed} ratio (L_n = 167 nm, $Đ$ = 1.05, σ = 37 nm), and B) 40:1 m_{unimer}/m_{seed} ratio (L_n = 1798 nm $Đ$ = 1.02, σ = 267 nm). C) Contour length histograms of the nanofibers in A (red) and B (blue).

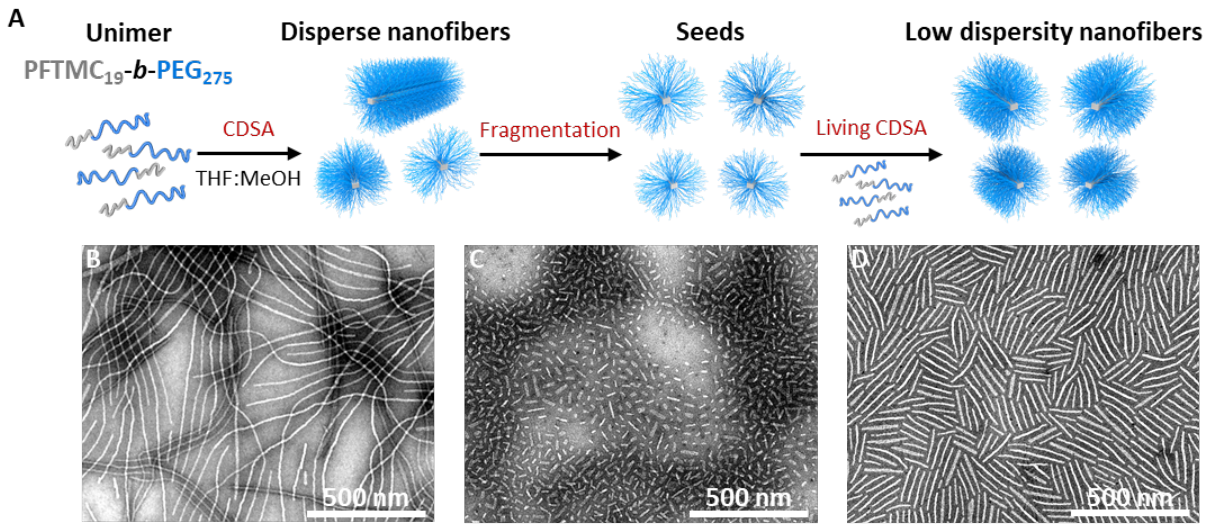


Figure S12. A) Schematic representation of the generation of low dispersity PFTMC$_{19}$-*b*-PEG$_{275}$ nanofibers through the living CDSA method. B) TEM of disperse PFTMC$_{19}$-*b*-PEG$_{275}$ nanofibers (1.5 mg/mL) prepared in THF:MeOH (15:85 *v/v*), after heating at 70 °C for 3 h, and aged for 48 h. C) TEM of seed nanofibers (L_n = 30 nm, $Đ$ = 1.22, σ = 14 nm) prepared through sonication of the disperse nanofibers (from B, in THF:MeOH) for 3h at 0 °C. D) TEM micrograph of low dispersity nanofibers (L_n = 127 nm, $Đ$ = 1.06, σ = 32 nm) prepared through seeded-growth by addition of unimer in THF to the nanofiber seed solution at: 3.1:1 m_{unimer}/m_{seed} ratio.

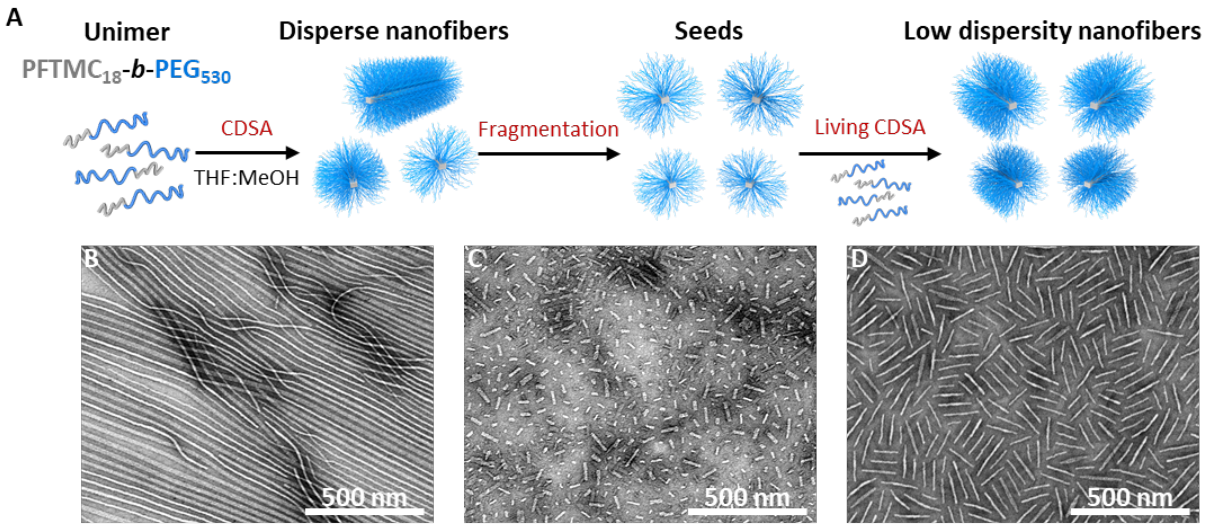


Figure S13. A) Schematic representation of the generation of low dispersity PFTMC$_{18}$-*b*-PEG$_{530}$ nanofibers through the living CDSA method. B) TEM of disperse PFTMC$_{18}$-*b*-PEG$_{530}$ nanofibers (1.5 mg/mL) prepared in THF:MeOH (15:85 *v/v*), after heating at 70 °C for 3 h, and aged for 48 h. C) TEM of seed nanofibers (L_n = 32 nm, $Đ$ = 1.25, σ = 16 nm) prepared through sonication of the disperse nanofibers (from B, in THF:MeOH) for 3 h at 0 °C. D) TEM micrograph of low dispersity nanofibers (L_n = 114 nm, $Đ$ = 1.06, σ = 28 nm) prepared through seeded-growth by addition of unimer in THF to the nanofiber seed solution at: 3.1:1 m_{unimer}/m_{seed} ratio.

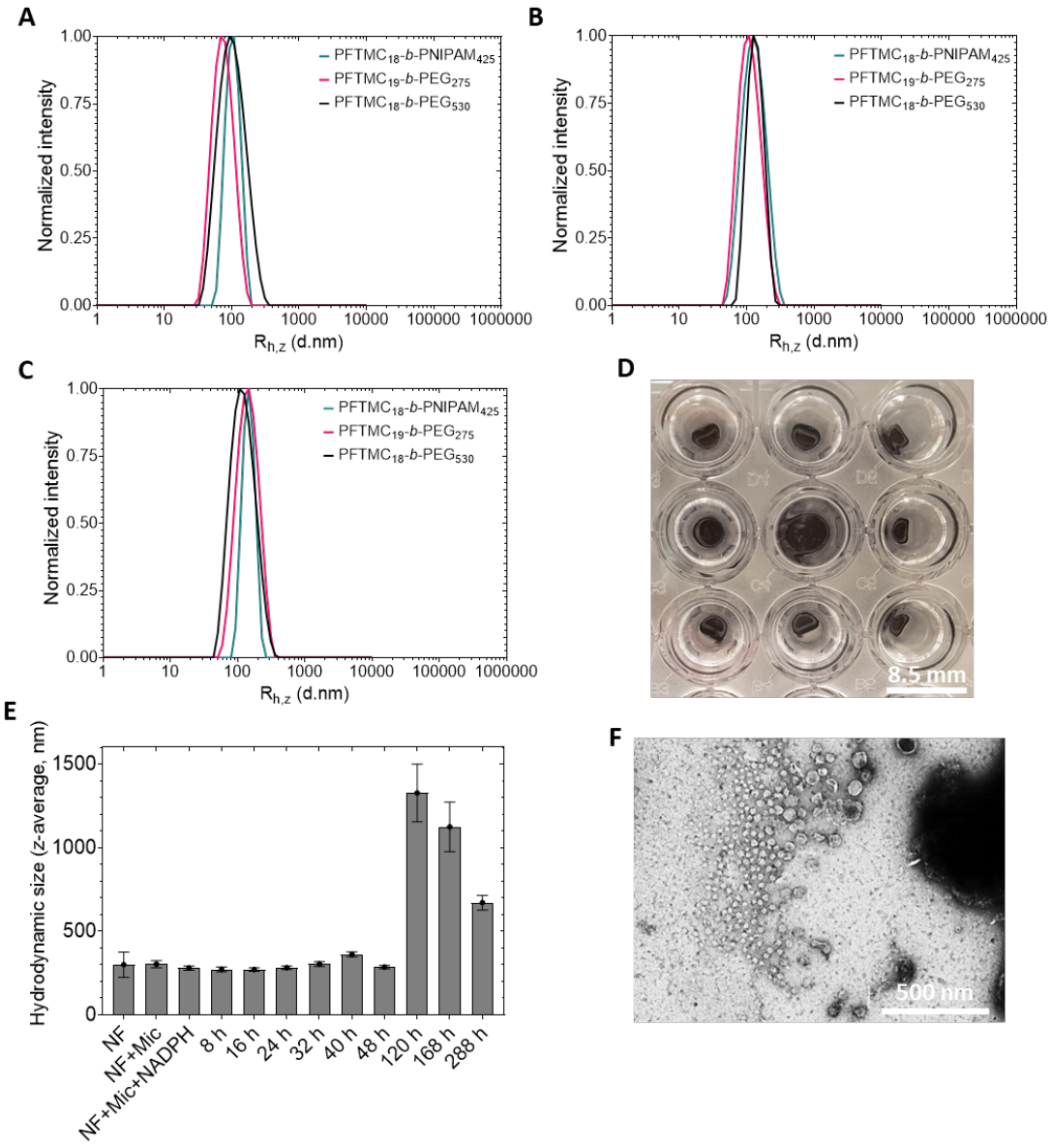


Figure S14. A) DLS analysis in MeOH 15:85 v/v; PFTMC$_{18}$-*b*-PNIPAM$_{425}$ ($R_{h,z}$ = 101 nm, $Đ$ = 1.05, green trace), PFTMC$_{19}$-*b*-PEG$_{275}$ ($R_{h,z}$ = 73 nm, $Đ$ = 1.19, pink trace), and PFTMC$_{18}$-*b*-PEG$_{530}$ ($R_{h,z}$ = 91 nm, $Đ$ = 1.17, dark trace). B) DLS analysis immediately after loading and filtration of the nanofibers loaded at 1 wt % of NR in H_2O; PFTMC$_{18}$-*b*-PNIPAM$_{425}$ ($R_{h,z}$ = 110 nm, $Đ$ = 1.18), PFTMC$_{19}$-*b*-PEG$_{275}$ ($R_{h,z}$ = 103 nm, $Đ$ = 1.14), and PFTMC$_{18}$-*b*-PEG$_{530}$ ($R_{h,z}$ = 127 nm, $Đ$ = 1.06, dark trace). C) DLS analysis after 6 months of the nanofibers loaded at 1 wt % of NR in H_2O; PFTMC$_{18}$-*b*-PNIPAM$_{425}$ ($R_{h,z}$ = 127 nm, $Đ$ = 1.19, green trace. $L_{n(\text{loaded})}$ = 116 nm by TEM), PFTMC$_{19}$-*b*-PEG$_{275}$ ($R_{h,z}$ = 136 nm, $Đ$ = 1.21, pink trace. $L_{n(\text{loaded})}$ = 133 nm by TEM), and

PFTMC$_{18}$-*b*-PEG$_{530}$ ($R_{h,z}$ = 108 nm, $Đ$ = 1.29, dark trace. $L_{n(\text{loaded})}$ = 125 nm by TEM). D) Digital images of the vials containing loaded (via PNSL, NR 1 wt %) and filtered nanofibers in water after 6 months. The image shows clear aqueous solutions with no signs of precipitation by the nanofibers at the bottom of the vials, the dark objects in each vial result from the lids at the top of the vials. The vials were placed in a well-plate and the images were recorded from the bottom; PFTMC$_{18}$-*b*-PNIPAM$_{425}$ (1st column), PFTMC$_{19}$-*b*-PEG$_{275}$ (2nd column), and PFTMC$_{18}$-*b*-PEG$_{530}$ (3rd column). E) Time-dependant microsome degradation assay of PFTMC$_{19}$-*b*-PEG$_{275}$ nanofibers (1μM, L_n = 127 nm by TEM Figure 3E) measured by DLS at 37 °C in phosphate buffer (1 mL) supplemented with microsomes (from liver, pooled, rat (Sprague-Dawley), 0.5 mg/mL) and Coenzyme II reduced tetrasodium salt (NADPH, 1 mM). The PFTMC$_{19}$-*b*-PEG$_{275}$ nanofibers remained stable for up to 48 h. The hydrodynamic radius of the nanofibers was ca. two times larger in PBS ($R_{h,z}$ = 250 nm) than in H_2O ($R_{h,z}$ = 136 nm, Figure S14B), this may arise from a difference in the ionic strength of the medium or the viscosity of the media.[6] The distinct $R_{h,z}$ value of microsomes was removed from the chart. NF: PFTMC$_{19}$-*b*-PEG$_{275}$ nanofibers; Mic: microsomes; NADPH: Coenzyme II reduced tetrasodium salt. F) TEM micrograph of the solution containing the nanofibers under enzymatic conditions after 120 h (5 days). The sphere-like aggregates may correspond to PFTMC fractions that aggregate in aqueous media. The dark ill-defined aggregates interact well with the staining solution (in EtOH) suggesting the presence of PEG homopolymer or PEG-microsome aggregates.

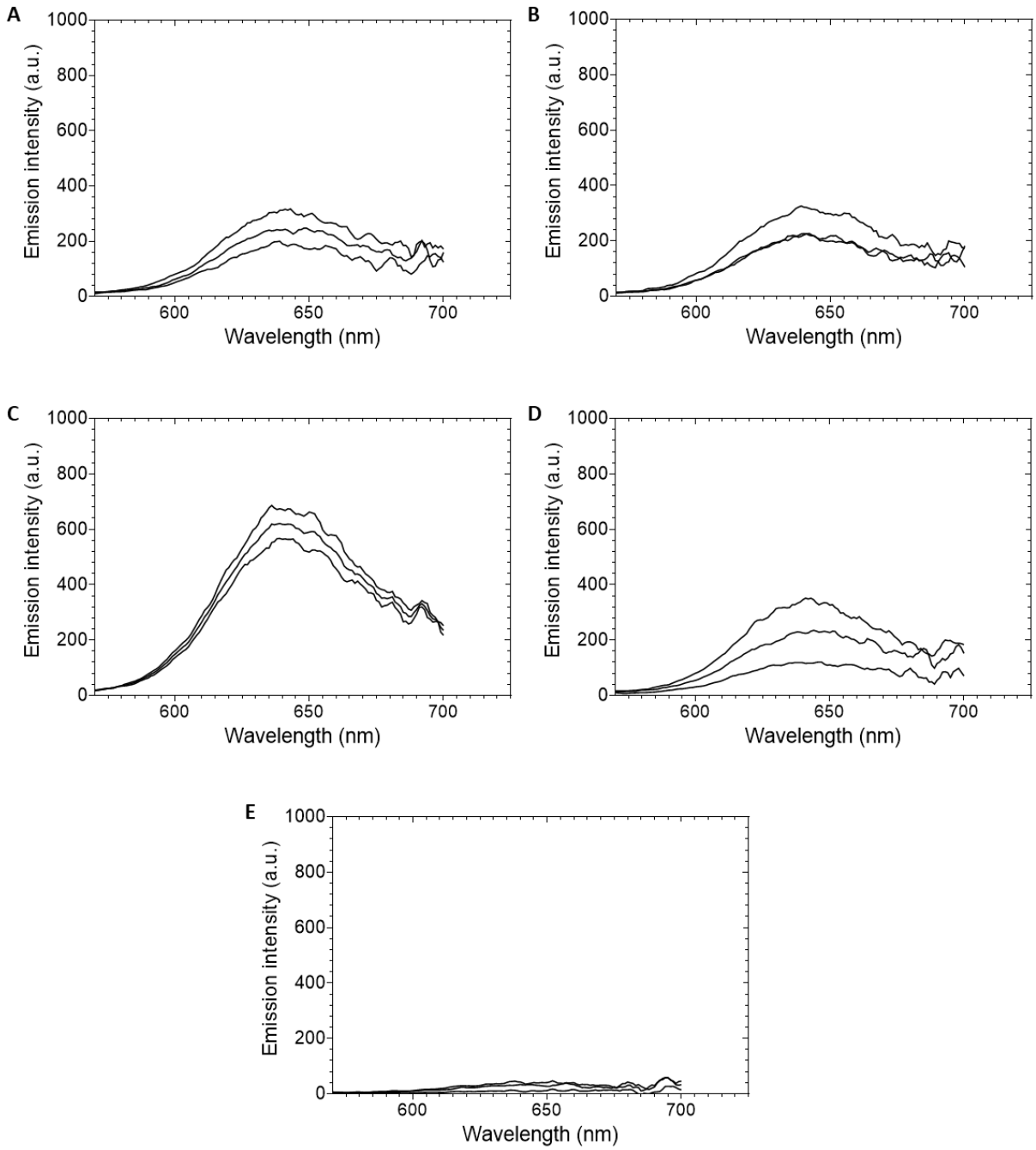


Figure S15. Emission spectra (in MeOH) of the PFTMC$_{18}$-*b*-PNIPAM$_{425}$ (L_n = 127 nm, $Đ$ = 1.03, σ = 22 nm) preformed nanofibers loaded with NR via PNSL at different concentrations, followed by filtration: A) 10 wt %, B) 5 wt %, C) 1 wt %, D) 0.5 wt %, and E) 0.1 wt %. Each experiment was conducted in triplicate. λ_{ex} = 540 nm.

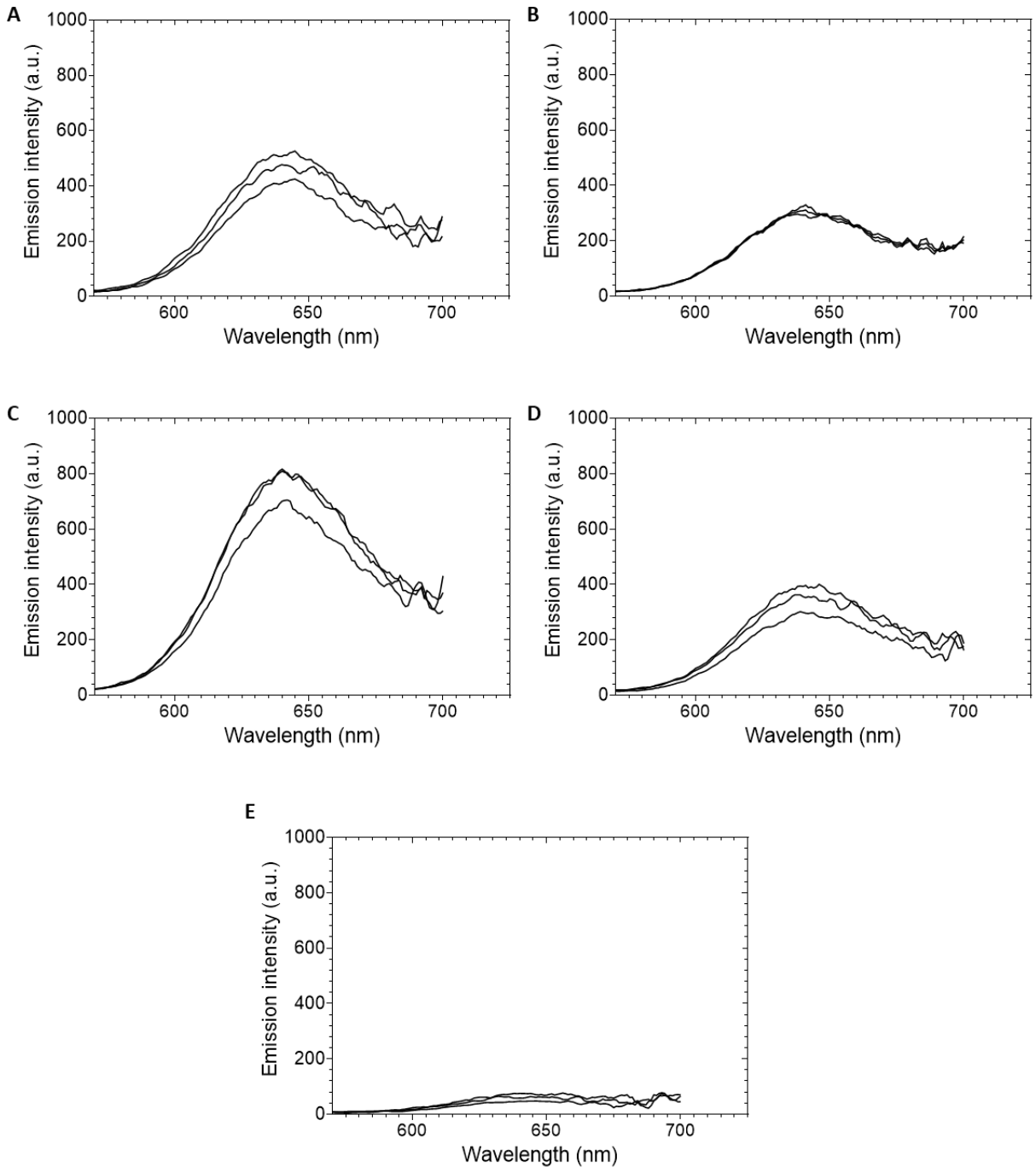


Figure S16. Emission spectra (in MeOH) of the $PFTMC_{19}$-*b*-PEG_{275} (L_n = 127 nm, $Đ$ = 1.06, σ = 32 nm) preformed nanofibers loaded with NR via PNSL at different concentrations, followed by filtration: A) 10 wt %, B) 5 wt %, C) 1 wt %, D) 0.5 wt %, and E) 0.1 wt %. Each experiment was conducted in triplicate. λ_{ex} = 540 nm.

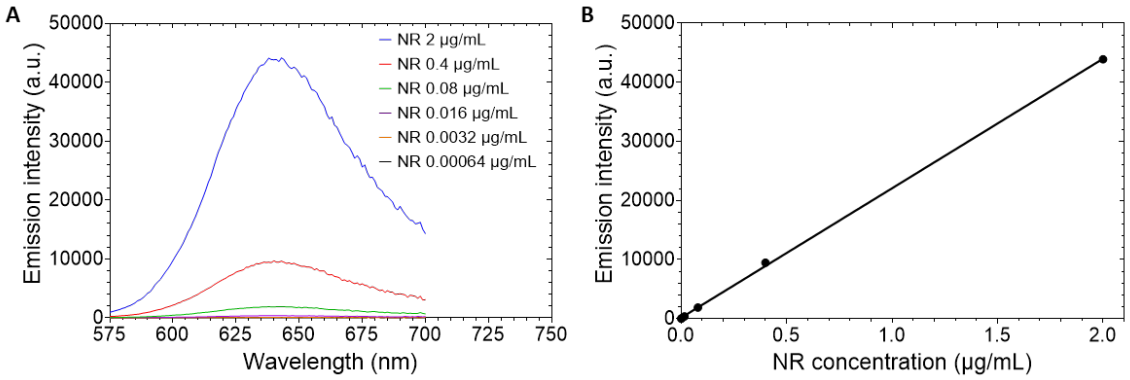


Figure S17. A) Emission data for NR at different concentrations (in MeOH). This was used to prepare the calibration curve in B. The emission intensity for the calibration curves was recorded with λ_{ex} = 540 nm. B) Calibration curve of A. The equation of the line and the coefficient of determination for the calibration curve at $\lambda_{ex\text{-}max}$ = 640 nm is as follows: $y = 146.1 + 2.19\times10^4\,x$; $r^2 = 0.9997$. The equation was used to quantify the loading of NR in the nanofibers from the Figures S15 and S16.

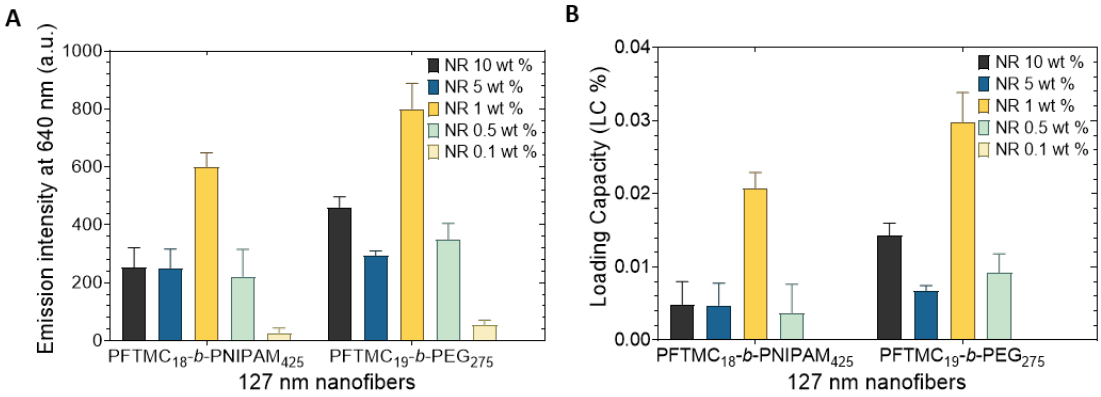


Figure S18. Loading properties of L_n = 127 nm nanofibers made from PFTMC$_{18}$-*b*-PNIPAM$_{425}$ ($Đ$ = 1.03, σ = 22 nm, Figure 3B), and PFTMC$_{19}$-*b*-PEG$_{275}$ ($Đ$ = 1.06, σ = 32 nm, Figure 3E) with different initial quantities of NR (1 – 10 wt %) used in the loading process; A) Nile Red loaded in ng per mg of diBCP. A) Emission intensity, and B) Loading Capacity (LC %) of diBCP nanofibers. The EE % (in Figures 3I, 4D) and LC % were calculated as follows: $EE\,\% = \frac{Mass\ of\ Drug\ Added - Mass\ of\ Non\text{-}Encapsulated\ Drug}{Mass\ of\ Drug\ Added} \times 100$, e.g. 127 nm PFTMC$_{19}$-*b*-PEG$_{275}$ nanofibers loaded at 1 wt %; $EE\,\% = \frac{900\ ng - 873\ ng}{900\ ng} \times 100 = 3.0\,\%$, and $LC\,\% = \frac{Mass\ of\ Encapsulated\ Drug}{Mass\ of\ Nanofibers} \times 100$, e.g. 127 nm PFTMC$_{19}$-*b*-PEG$_{275}$ nanofibers loaded at 1 wt %; $LC\,\% = \frac{27\ ng}{90{,}000\ ng} \times 100 = 0.03\%$.

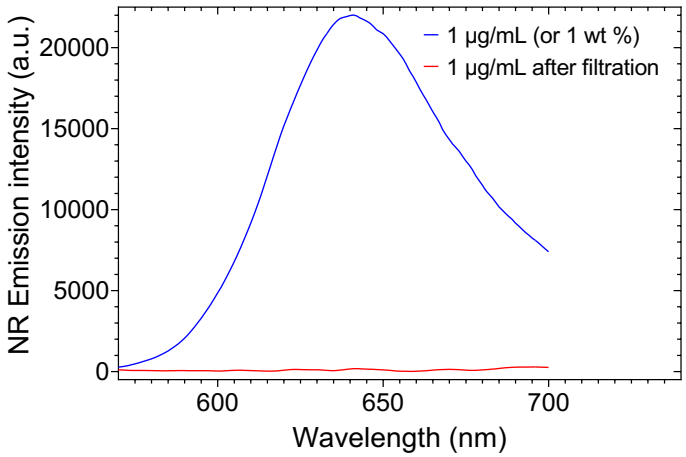


Figure S19. Validating syringe-filtration as a purification process to remove nonbounded NR. Emission of NR at 1 µg/mL after transfer to water via the PNSL method, freeze-drying, and resuspension in MeOH for spectroscopic analysis (blue line), and emission of NR at 1 µg/mL after transfer to water via the PNSL method *with filtration*, freeze-drying, and resuspension in MeOH for spectroscopic analysis (red line).

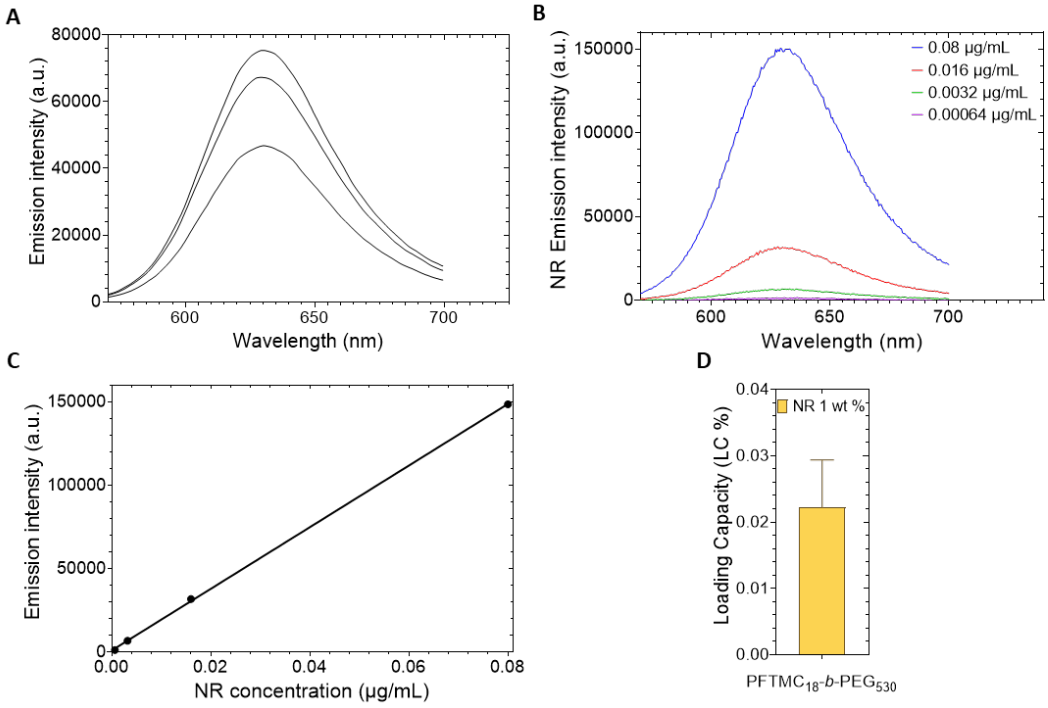


Figure S20. A) Emission spectra (in MeOH) of the PFTMC$_{18}$-*b*-PEG$_{530}$ (L_n = 114 nm, $Đ$ = 1.06, σ = 28 nm) preformed nanofibers loaded with NR via PNSL at 1 wt % NR, followed by filtration. B) Emission of NR at different concentrations (in MeOH) to prepare the calibration curve in C. The emission intensity for the calibration curves was recorded at λ_{ex} = 540 nm. C) Calibration curve of B. The equation of the line and the coefficient of determination for the calibration curve at $\lambda_{ex\text{-}max}$ = 640 The equation of the line and the coefficient of determination for the calibration curve at $\lambda_{ex\text{-}max}$ = 640 nm is as follows: B) $y = 855.1 + 1.85\times10^6$ x; $r^2 = 0.9998$. The equation was used to quantify the loading of NR in the nanofibers from A. D) Loading capacity of PFTMC$_{18}$-*b*-PEG$_{530}$ nanofibers loaded at 1 wt % of NR.

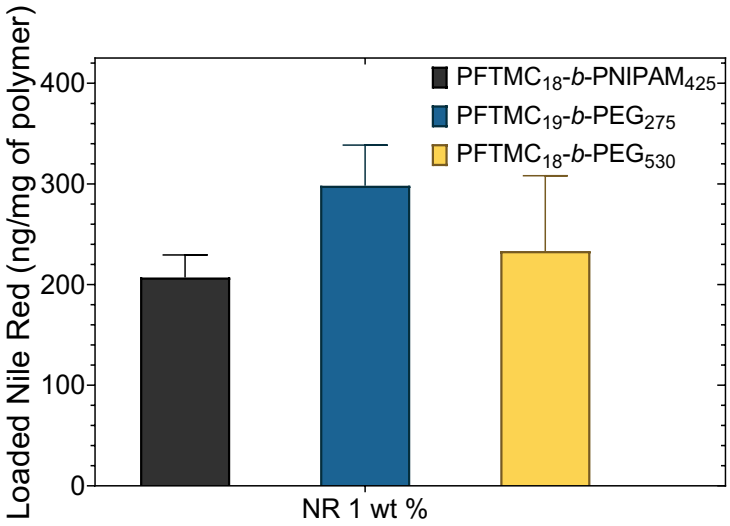


Figure S21. Loaded NR (in ng/mg of polymer) determined by fluorometry of the three diBCP nanofiber systems utilized in this work (Figure 3H, 4D); $PFTMC_{18}$-*b*-$PNIPAM_{425}$, $PFTMC_{19}$-*b*-PEG_{275}, and $PFTMC_{18}$-*b*-PEG_{530} diBCP. A Welch's analysis of variance (ANOVA, GraphPad Prism 9, V 9.2.0) test of the data of this figure indicates that there are no significant differences among the means ($p < 0.05$).

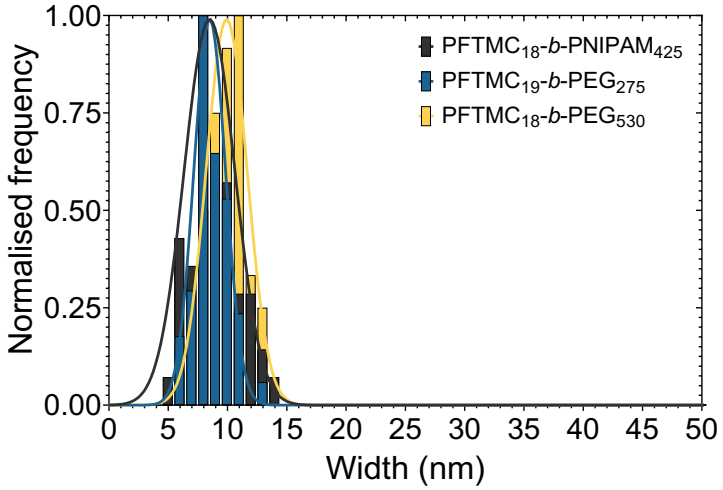


Figure S22. Contour width histograms of nanofibers from TEM micrographs of $PFTMC_{18}$-*b*-$PNIPAM_{425}$ W_n = 8.9 nm ($Đ$ = 1.06, σ = 2.1 nm, dark, from Figure 3B), $PFTMC_{19}$-*b*-PEG_{275} W_n = 8.6 nm ($Đ$ = 1.03, σ = 1.5 nm, blue, from Figure 3E), and $PFTMC_{19}$-*b*-PEG_{530} W_n = 9.8 nm ($Đ$ = 1.03, σ = 1.6 nm, yellow, from Figure 4A). The nanofibers used for width measurements were cast from 15:85 THF:MeOH. Number of counted nanofibers, $n \geq 50$.

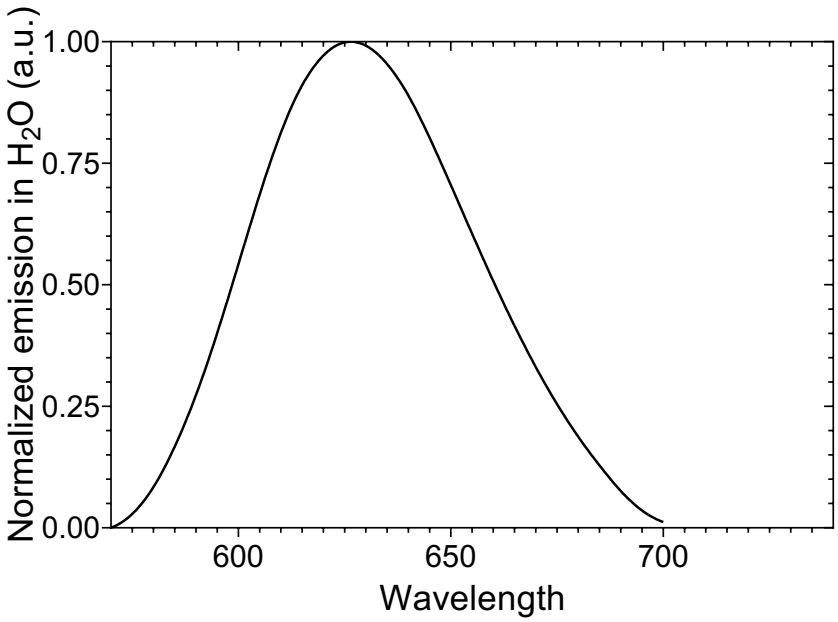
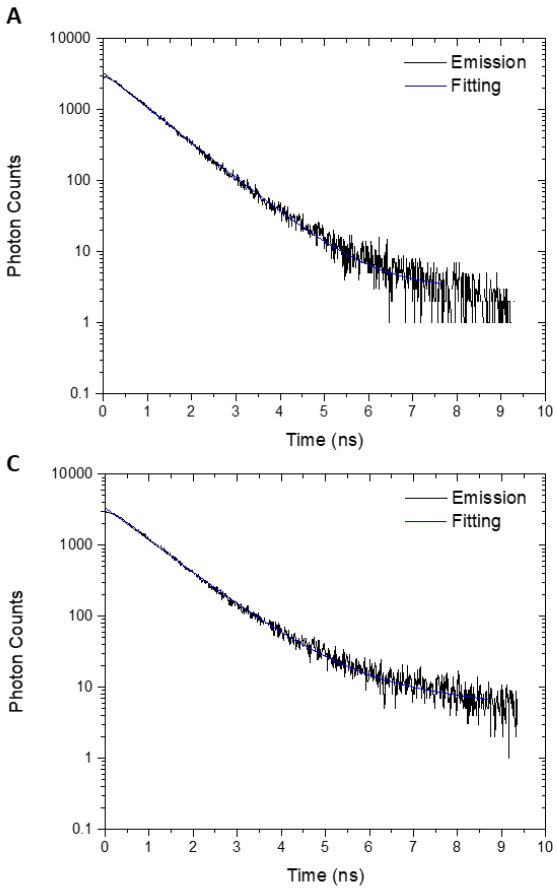


Figure S23. Emission spectra of NR loaded (at initial 1 wt %) by $PFTMC_{18}$-*b*-PEG_{530} diBCP nanofibers (0.1 mg/mL) in water.



D

Nanofiber System	PFTMC$_{18}$-*b*-PNIPAM$_{425}$	PFTMC$_{19}$-*b*-PEG$_{275}$	PFTMC$_{18}$-*b*-PEG$_{530}$
τ_1 (ns)	0.88	1.01	0.94
A_1	3343.59	2596.39	3404.26
τ_2 (ns)	-	3.16	8.80
A_2	-	585.56	20.02

Figure S24. Fluorescence lifetime measurements in H_2O of nanofibers loaded with NR at 1 wt % via the PSNL method: A) PFTMC$_{18}$-*b*-PNIPAM$_{425}$ (L_n = 127 nm, $Đ$ = 1.03, σ = 22 nm), B) PFTMC$_{19}$-*b*-PEG$_{275}$ (L_n = 127 nm, $Đ$ = 1.06, σ = 32 nm), and C) PFTMC$_{18}$-*b*-PEG$_{530}$ (L_n = 114 nm, $Đ$ = 1.06, σ = 28 nm). D) Summary of fluorescence lifetime measurements recorded in the range of 610 – 640 nm with λ_{ex} = 590 nm.

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