Supplementary Information

One polymer composition, various morphologies: the decisive influence of conditions on the polymerization-induced self-assembly (PISA) of *N*-acryloyl thiomorpholine

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Table of Contents

Experimental Procedures

Materials and Methods

All chemicals and solvents were purchased from Sigma-Aldrich, Merck, and Acros Organics and if not mentioned, were used without further purification. NAT and 2-(Butylthiocarbonothioylthio)propanoic acid (PABTC) were synthesized *via* previously reported procedures.[1, 2] 1,4-dioxane and NAM were treated 24 h with inhibitor remover resin prior to use. ^{1}H-NMR was performed at room temperature on a Bruker AC 300 MHz spectrometer. Size-exclusion chromatography (SEC) of the polymers was performed on a Shimadzu system equipped with an SCL-10A system controller, an LC-10AD pump, a RID-10A refractive index detector and a PSS SDV column with *N*,*N*-dimethylacetamide (DMAc) + 0.21% LiCl as eluent. The column oven was set to 50 °C. Differential scanning calorimetry (DSC) experiments were performed on a Netzsch DSC 204 F1 Phoenix under a nitrogen atmosphere with a heating rate of 20 K min^{-1} from -20 to 300 °C, if not indicated differently. Three cycles were recorded for each sample. The glass transition temperature (T_g) values are reported for the second heating run. DLS was performed on a ZetaSizer Nano ZS (Malvern, Herrenberg, Germany) equipped with a He–Ne laser operating at a wavelength of λ = 633 nm. Counts were detected at an angle of 173°. The particle size was approximated as the effective diameter (Z-average) obtained by the cumulants method assuming a spherical shape. All measurements were conducted at 25 °C in semi-micro cuvettes after equilibration times of 30 s in triplicate. Every measurement included 10 runs, in which every run took 30 seconds. Apparent hydrodynamic radii were calculated using the Stokes-Einstein Equation (1):

$$R\mathrm{h} = \frac{kT}{6\pi\eta D} \qquad (1)$$

R_h = hydrodynamic radius, k = Boltzmann constant, T = absolute temperature, η = viscosity of the sample and D = apparent translational diffusion coefficient.

General procedure for the homopolymerization of NAM

A microwave vial (20 mL) was charged with a magnetic stirrer and PABTC (1.13 mL of a 0.5 M solution in 1,4-dioxane, 0.567 mmol), NAM (2 g, 14.2 mmol), VA-044 solution (91.6 µL of a 20 mg mL^{-1} in Milli-Q water, 5.67 µmol), 1,3,5-trioxane (5 mg) as an internal standard was added. The mixture was dissolved in deionized H_2O (2.09 mL), the vial was sealed with a rubber septum and deoxygenated by a stream of bubbled nitrogen for 15 min. The vial was then suspended in a preheated oil bath at 70 °C and allowed to stir for 6 h until no monomer could be detected by ^{1}H-NMR (D_2O). Upon completion, the solution was cooled to room temperature and opened to air. The concentration of the solution was determined gravimetrically to be $6.50 \cdot 10^{-2}$ mmol g^{-1}. ^{1}H-NMR was performed after lyophilization in $CDCl_3$.

^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 3.90-3.05 (m, morpholine), 2.93-2.18 (m, backbone), 2.05-1.02 (m, backbone), 0.92 (t, ^3J 7.3, CH_3).

SEC (eluent: DMAc + 0.21% LiCl, PS-standard): M_n: 3,800 g mol^{-1}, M_w: 4,200 g mol^{-1}, Đ = 1.10.

General procedure for the chain extension with NAT

A microwave vial (5 mL) was charged with a magnetic stirrer and mCTA PNAM (256 mg of a $7.95 \cdot 10^{-2}$ mmol g^{-1} solution in H_2O, 20.4 µmol). NAT (80 mg, 509 µmol), VA-044 solution (33.9 µL of a 20 mg mL^{-1} in Milli-Q water, 2.04 µmol), 1,3,5-trioxane (5 mg) as an internal standard was added. The mixture was dissolved in deionized H_2O (2.51 mL), the vial was sealed with a rubber septum and deoxygenated by a stream of bubbled nitrogen for 20 min. The vial was then suspended in a preheated oil bath at 70 °C and allowed to stir for 1 h until no monomer could be detected by ^{1}H-NMR (D_2O). Upon completion, the solution was cooled to room temperature, opened to air and analyzed by SEC. Subsequently, the micelle dispersion was purified by dialysis (MWCO: 3.5 – 5 kDa) against deionized water for three days including four water exchanges. The concentration of the dispersion was determined gravimetrically (n = 3) after lyophilization. ^{1}H-NMR was performed after lyophilization in $CDCl_3$.

^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 4.25-3.14 (m, morpholine), 2.98-2.19 (m, backbone), 2.02-1.01 (m, backbone), 0.95 (t, ^3J 9.3, CH_3).

SEC (eluent: DMAc + 0.21% LiCl, PS-standard): M_n: 7,700 g mol^{-1}, M_w: 8,600 g mol^{-1}, Đ = 1.12.

Degradation kinetics

Polymer stock solutions of samples **P7**, **9** and **11** were diluted with PBS (10 mM, pH: 7.4) to a volume of 500 µL and a concentration of 2 mg mL^{-1}. After filtration through a 0.2 µm PA syringe filter, 500 µL of a 0.2 M H_2O_2 solution was added and the degradation process monitored by time-resolved DLS count rate measurements. Simultaneously, the hydrodynamic diameter was determined for every time point by the cumulants method. The kinetics were proceeded at 37 °C and a measurement point was recorded every 5 min. The relative count rate was calculated as the mean count rate divided by the maximum value.

(Cryo)-TEM investigations

Due to the high T_g of PNAT, cryo-TEM measurements could be circumvented for the majority of samples. Cryo-TEM was used for a number of polymers as a control. The measurements were performed on an FEI Tecnai G² 20 platform with a LaB_6 filament at 200 kV acceleration voltage. Samples were prepared on carbon coated TEM grids (Plano) or Quantifoil grids (R2/2) which were both treated with Ar plasma prior to use for hydrophilization and cleaning. 15 µL of the solutions were blotted onto the carbon coated films for TEM investigation, while for cryo-TEM measurements 8.5 µL of the solution were vitrified on Quantifoil grids using a Vitrobot Mark IV system. Liquid ethane was used as a cryogen. Samples were transferred to a Gatan 626 cryo holder and were maintained at a temperature < -175 °C during the entire process. All images were acquired with a Mega View (OSIS, Olympus Soft Imaging Systems) or an Eagle 4k CCD camera, respectively.

Supplementary Characterization Data

Table S1. Overview of the prepared samples including polymerization conditions and characterization data.

Exp.	Polymer	T [°C]	c [M]	[M]/[CTA]	[CTA]/$[I]_0$	Time [min]	M_n[a] [kg mol^{-1}]	M_n[b] [kg mol^{-1}]	Đ[b]	D_H[c] [nm]	PDI[c]	Morph.[d]
P1	$PNAM_{25}$	70	6.5	25	100	420	3.8	4.1	1.10	-	-	-
P2	$PNAM_{25}$-*b*-$PNAT_5$		0.2	5	10	60	4.6	4.3	1.10	-	-	-
P3	$PNAM_{25}$-*b*-$PNAT_{10}$			10			5.7	5.1	1.10	-	-	-
P4	$PNAM_{25}$-*b*-$PNAT_{25}$	50	0.2	25	10	240	7.7	6.6	1.25	21	0.08	S
P5			0.6					6.6	1.26	31	0.12	S
P6			1					6.6	1.28	42	0.14	S / sW
P7		70	0.2			60		7.5	1.12	31	0.09	S
P8			0.6					7.5	1.13	46	0.15	S / sW
P9			1					7.3	1.12	93	0.18	W
P10		90	0.2			10		7.5	1.13	44	0.06	S
P11			0.6					7.4	1.13	109	0.16	V
P12			1					7.6	1.12	569	0.67	L

[a] Calculated based on $[M]_0/[CTA]_0$ × monomer conversion. [b] Determined by SEC (Eluent: DMAc + 0.21 wt% LiCl, PS-calibration) [c] Determined by DLS measurements of the purified self-assembled structures (three technical replicates, c: 1 mg mL^{-1}). [d] Morphology judgment based on (cryo)-TEM and DLS investigations. The morphologies were categorized as follows: spheres (**S**), short worms (**sW**), worms (**W**), vesicles (**V**) and lamellae (**L**). Exp.: experiment, T: reaction temperature, c: monomer concentration, Morph.: morphology.

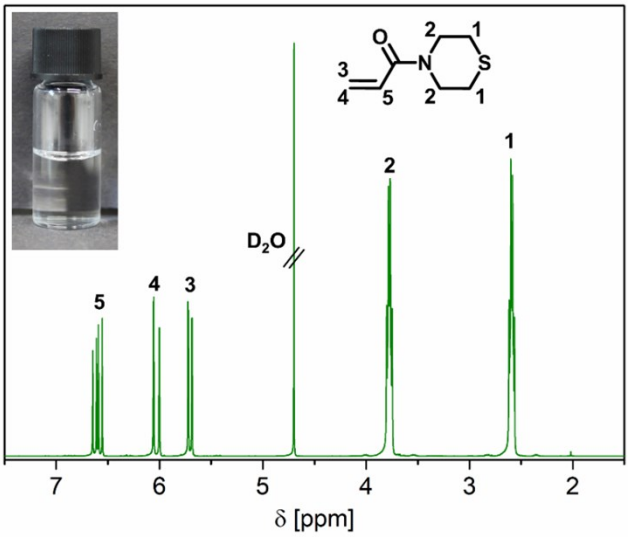


Figure S1. ^{1}H-NMR and photograph of NAT in D_2O at 100 mg mL^{-1}.

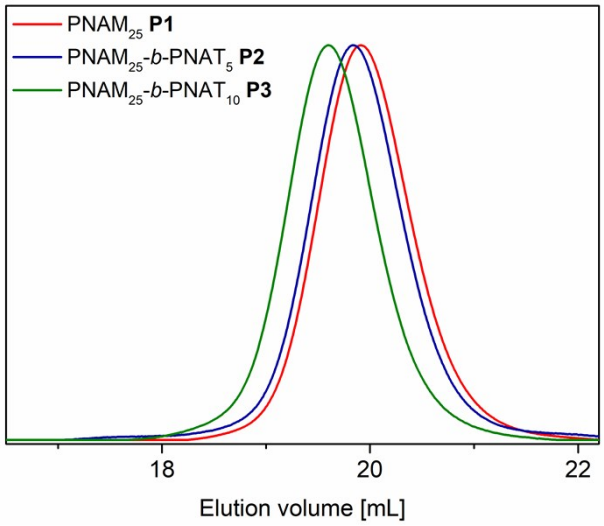


Figure S2. Overview of the SEC curves of the synthesized polymers. (Eluent: DMAc + 0.21% LiCl, PS-calibration).

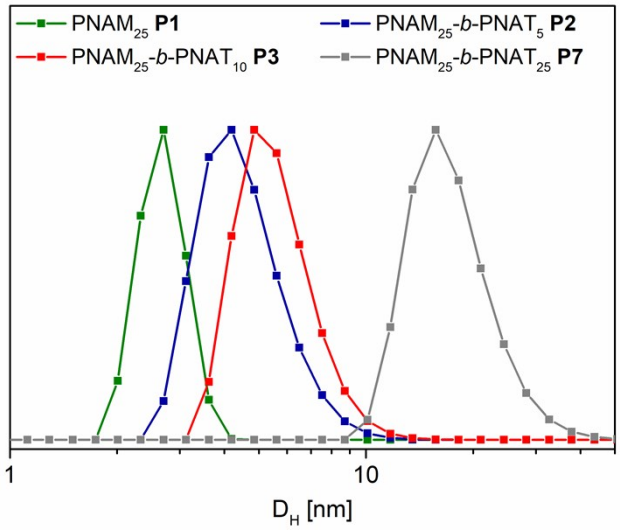


Figure S3. Number weight size distribution of PNAM$_{25}$-*b*-PNAT$_n$ based samples of increasing DP of PNAT (0.2 M, 70 °C); DLS: three technical replicates, c: 1 mg mL^{-1}.

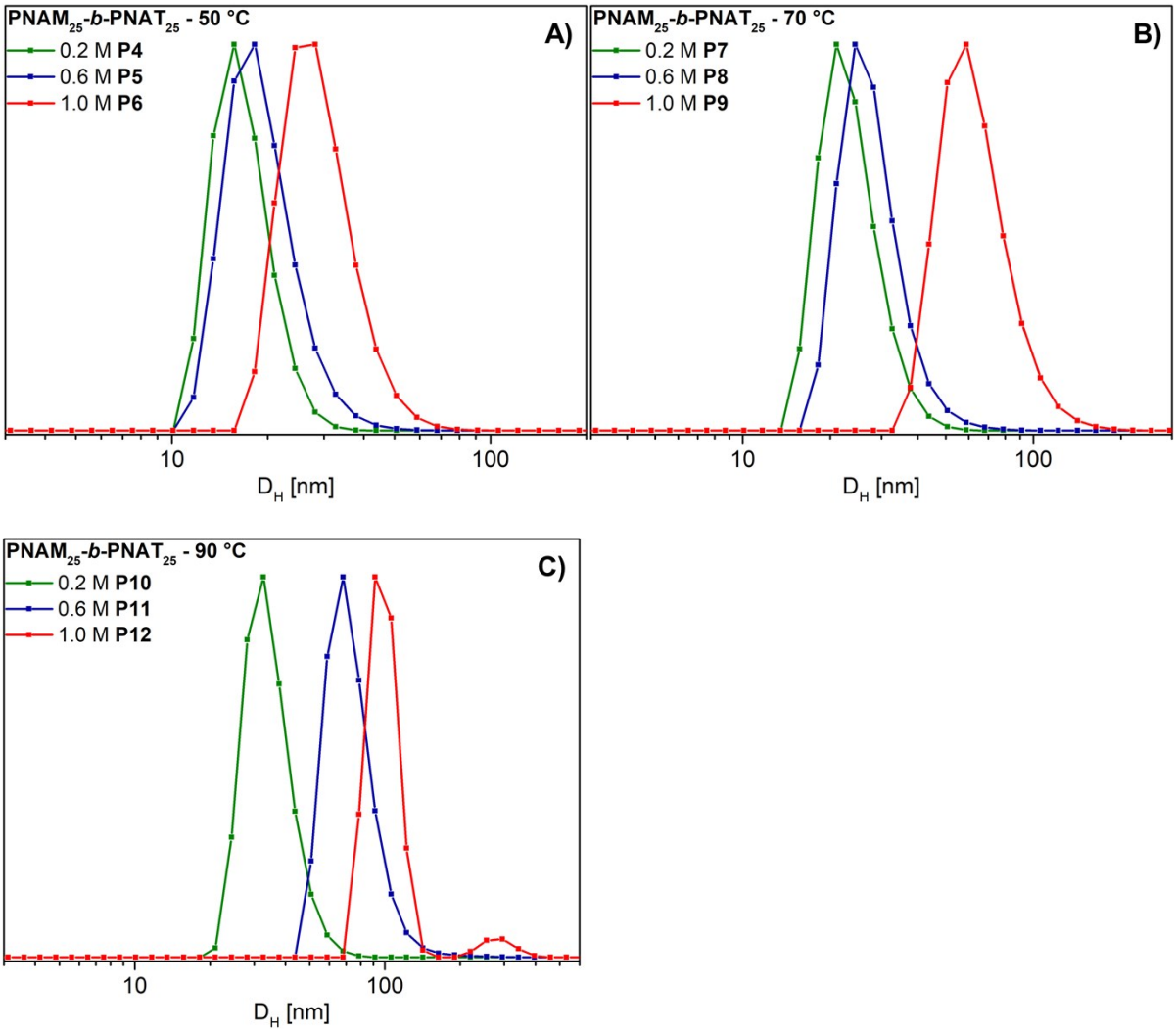


Figure S4. Overview of number weight size distributions of the prepared micelles determined by DLS (c: 1 mg mL^{-1}).

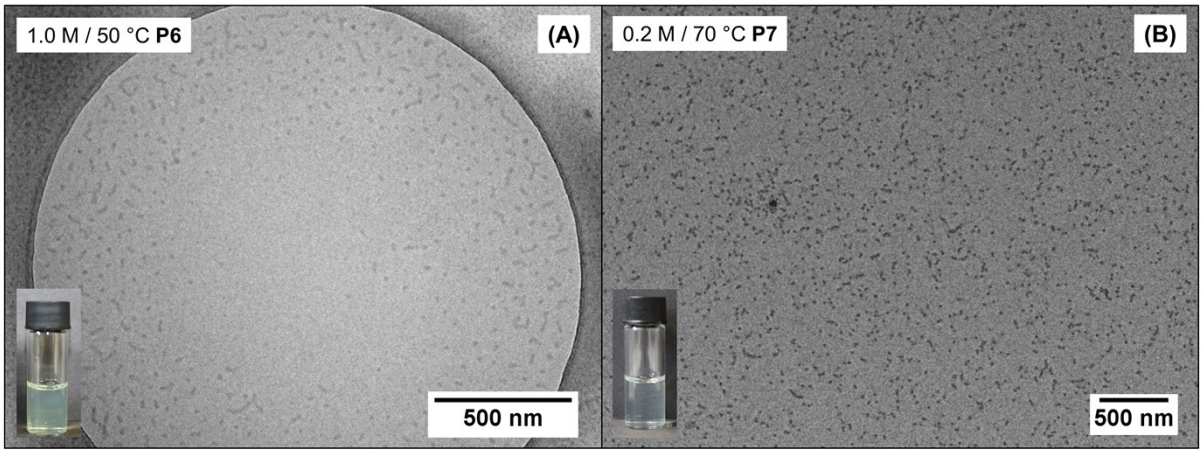


Figure S5. TEM images acquired after dialysis. A). **P6** prepared at 1 M and 50 °C. B) **P7** prepared at 0.2 M and 70 °C.

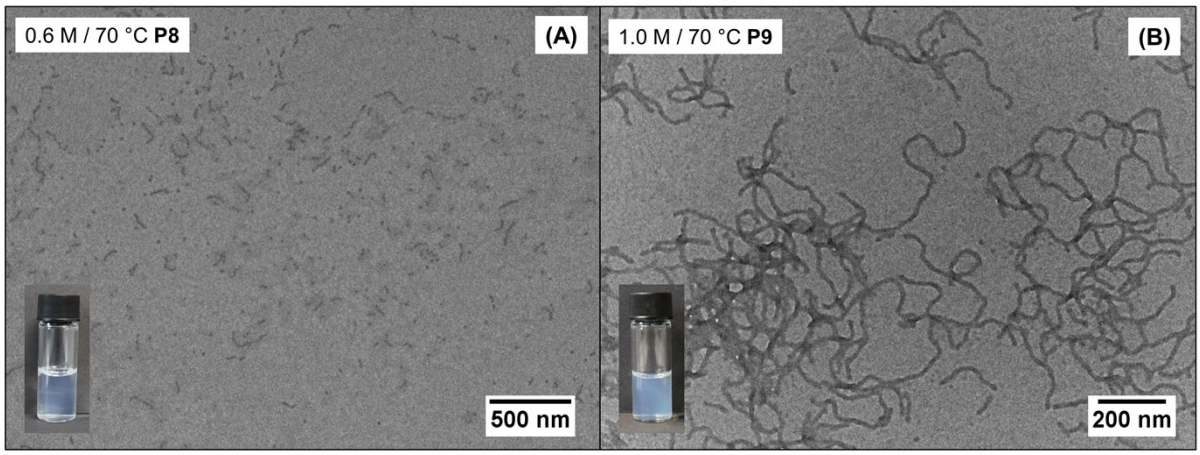


Figure S6. TEM images acquired after dialysis. A). **P8** prepared at 0.6 M and 70 °C. B) **P9** prepared at 1 M and 70 °C.

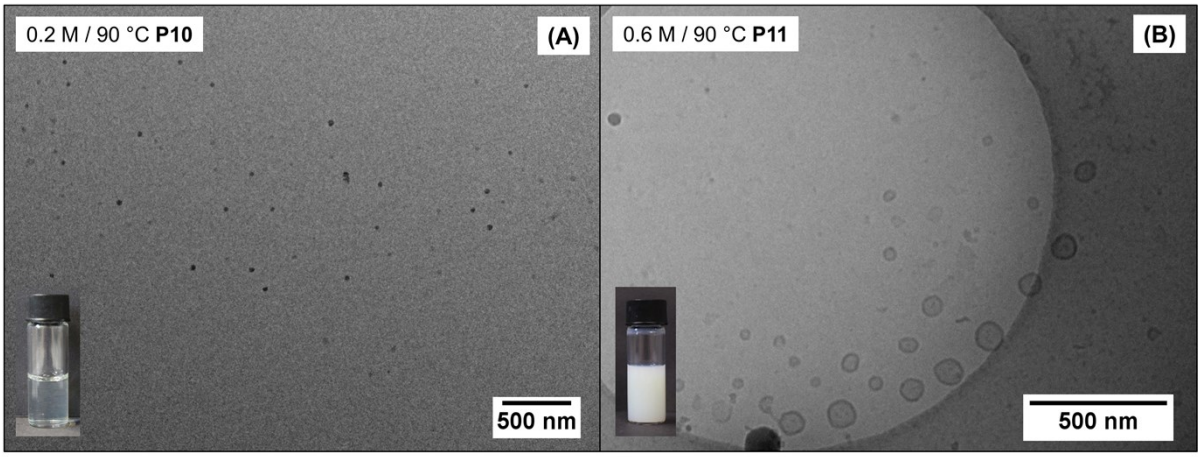


Figure S7. TEM images acquired after dialysis. A). **P10** prepared at 0.2 M and 90 °C. B) **P11** prepared at 0.6 M and 90 °C.

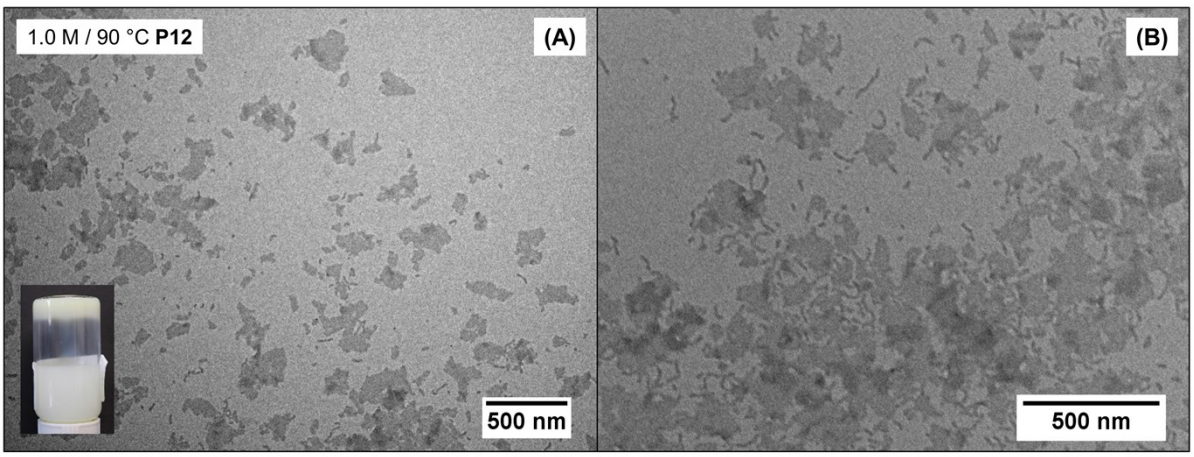


Figure S8. TEM images of **P12** prepared at 1 M and 90 °C acquired after dialysis. A). 500 nm magnification. B) 500 nm magnification.

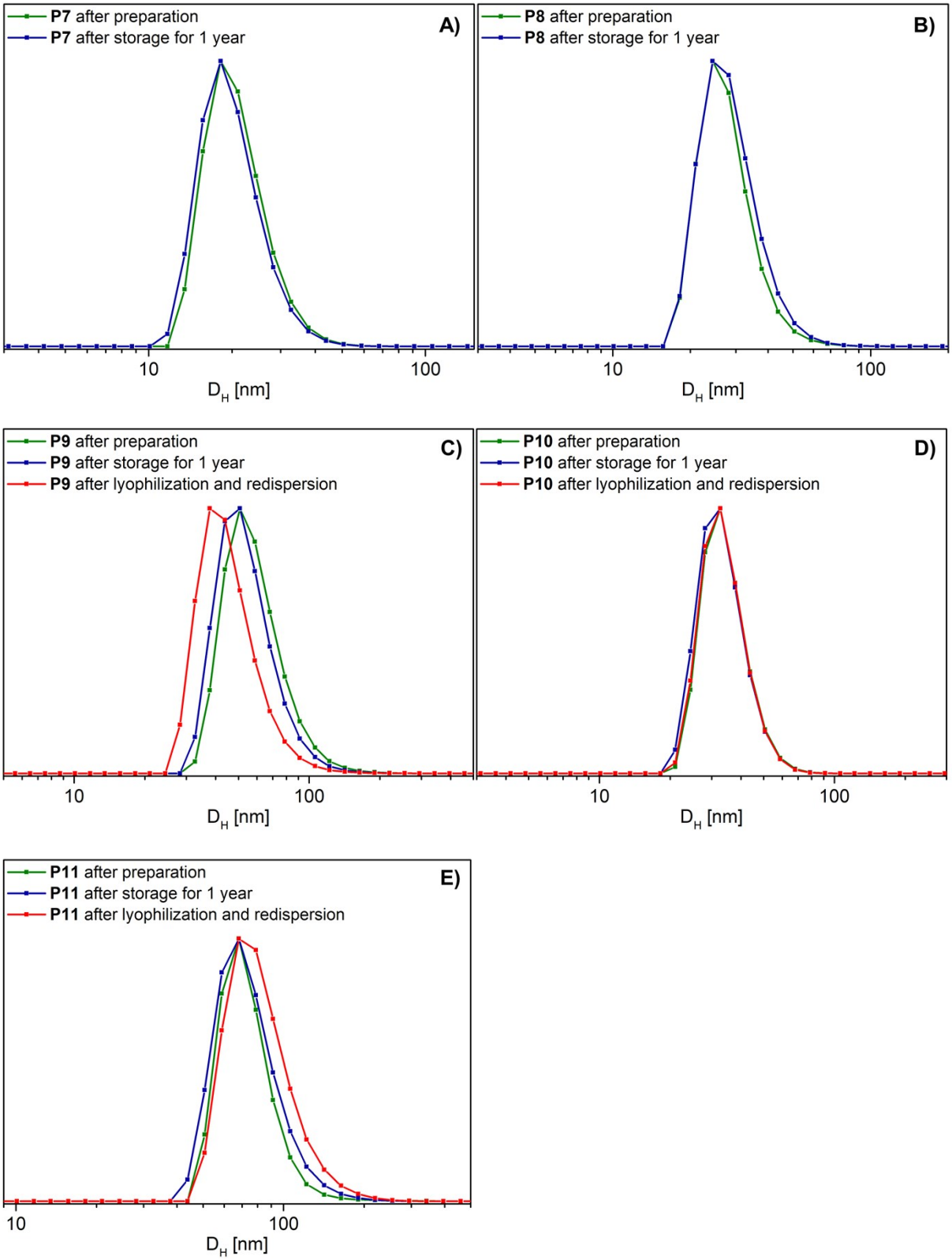


Figure S9. Stability test of aqueous block copolymer dispersions by DLS (number weight size distributions, c: 1 mg mL^{-1}).

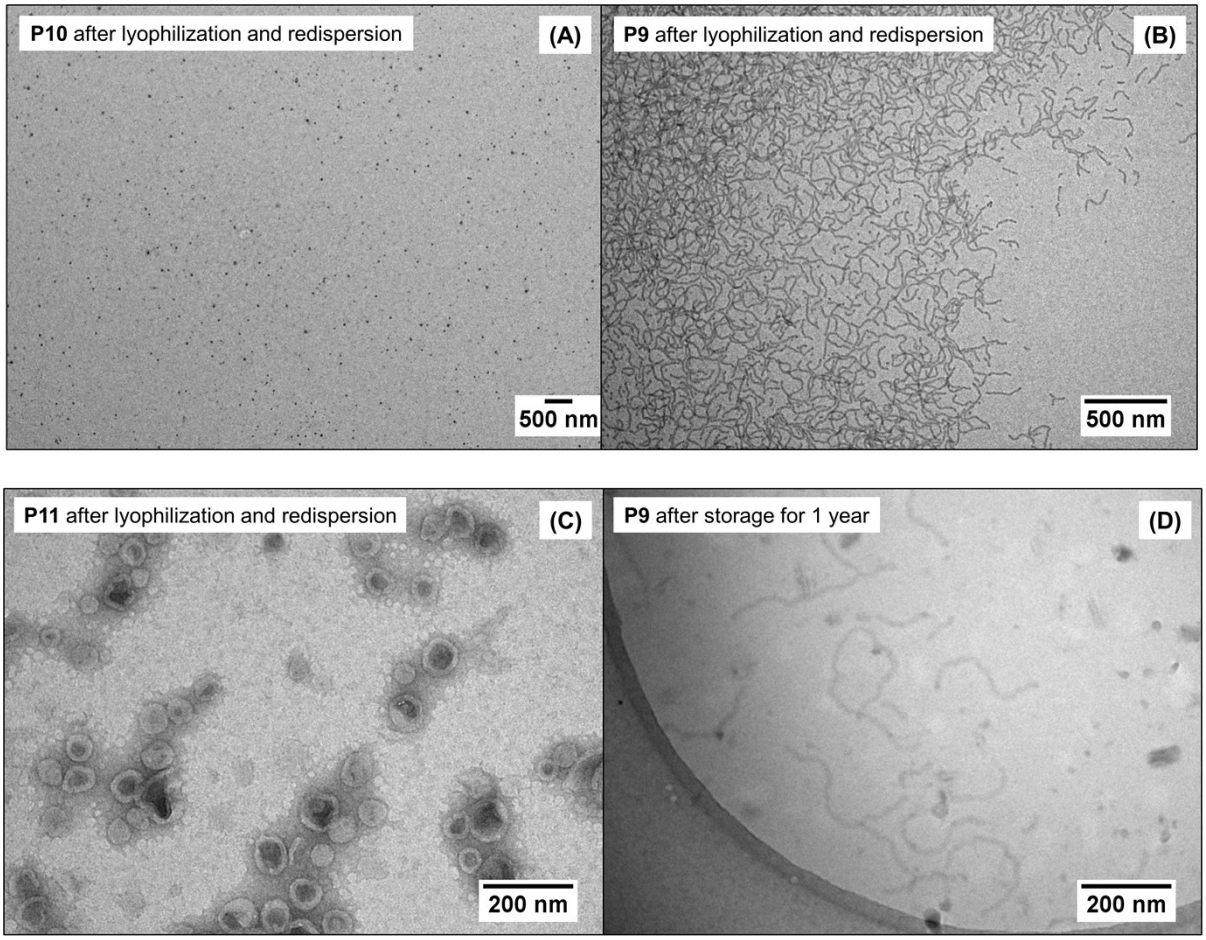
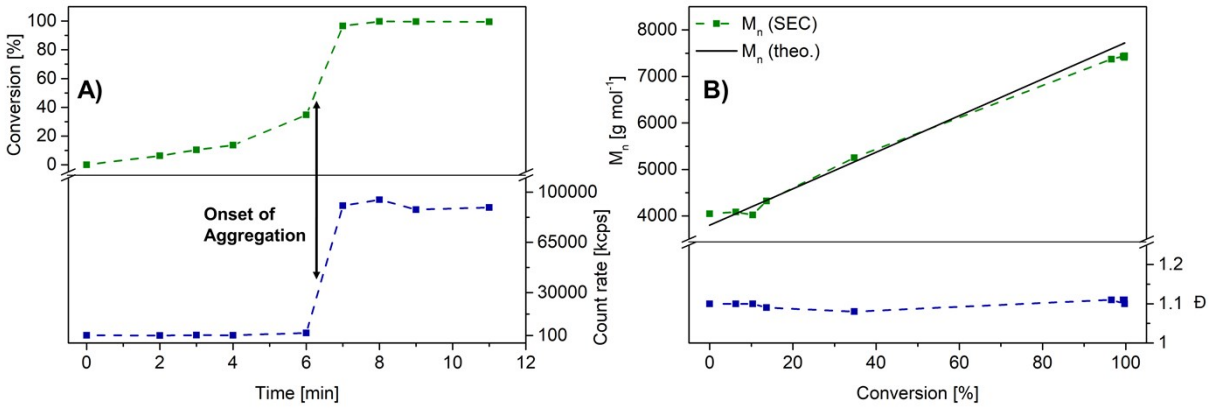


Figure S10. Stability test of samples **P9**-**10**. A) TEM analysis of sample **P10** after lyophilization and redispersion. B) TEM analysis of sample **P9** after lyophilization and redispersion. C) TEM analysis of sample **P11** after lyophilization and redispersion. D) cryo-TEM analysis of sample **P9** after storage for 1 year.



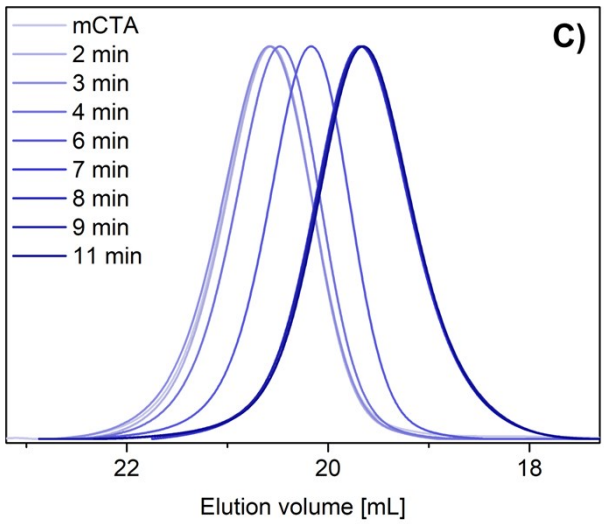


Figure S11. Kinetic studies of the PISA of **P9** at 70 °C and 0.2 M. A) Dependence of monomer conversion and DLS count rate on reaction time. B) Evolution of theoretical M_n, M_n and Đ determined by SEC vs. conversion. C) Evolution of the size distribution analyzed by SEC over reaction time. (Eluent: DMAc + 0.21% LiCl, PS-calibration)

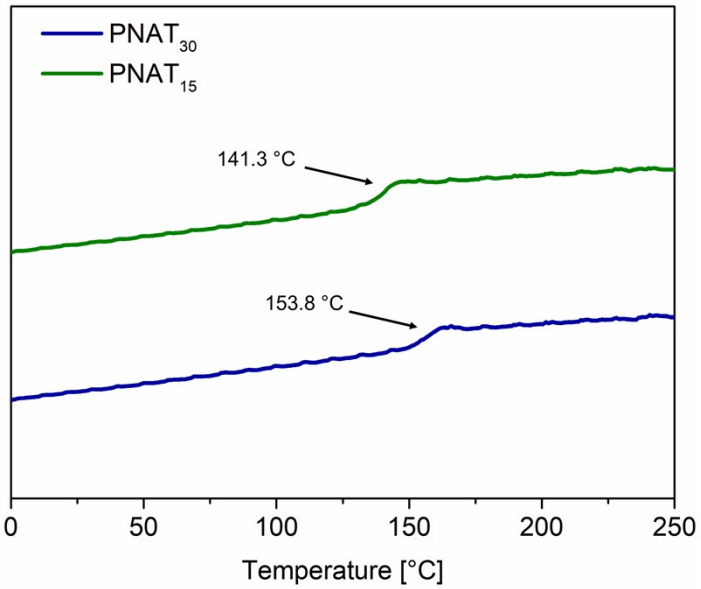


Figure S12. DSC thermograms of $PNAT_{15}$ and $PNAT_{30}$ homopolymers (2nd heating run, 20 K min^{-1}).

References

[1] J. Yi, S. H. Goh and A. T. S. Wee, *Macromolecules*, **2001**, *34*, 4662-4665.

[2] S. C. Larnaudie, J. C. Brendel, K. A. Jolliffe and S. Perrier, *J. Polym. Sci. A: Polym. Chem.*, **2016**, *54*, 1003-1011.