Electronic Supplementary Material (ESI) for Polymer Chemistry.

SUPPORTING INFORMATION

Responsive tertiary amine methacrylate block copolymers: Uncovering temperature-induced shape-shifting behaviour

Zahn Stanvliet,[a] Yiyi Deng,[b] Dietmar Appelhans,[b] Silvia Moreno,[b] Susanne Boye,[b] Jens Gaitzsch [*b] and Albena Lederer [*a,b]

[a]*Stellenbosch University, Department of Chemistry and Polymer Science, Private Bag X1, Matieland, 7602, South Africa.*
[b]*Leibniz-Institut für Polymerforschung Dresden e.V., Hohe Str. 6, 01069 Dresden, Germany.*

Content

SI1. Block copolymers and self-assembly of polymersomes

Synthesis

mPEG-Br macroinitiator and cross-linker dimethylmaleic imidobutyl methacrylate (DMI) and the general polymerisation process of block copolymers PEG-PDEA, PEG-PDEA-PDMI and PEG-PDEA-PDMA-PDMI was adapted from earlier works.[1] Briefly, mPEG-Br macroinitiator, 2,2'-bipyridine, monomers and 2-butanone were charged into a Schlenk tube. The compounds were completely dissolved into 2-butanone and then frozen in the liquid nitrogen. Subsequently, CuBr was added and the mixture was degassed using three freeze-pump-thaw-cycles, backfilled with argon and heated up to 50°C. After polymerising overnight, the reaction mixture was diluted with THF and filtrated over a neutral aluminium oxide column to remove Cu. The collected solution was then dialysed against acetone for four times (regenerated cellulose, MWCO=2 kDa). Afterwards, the solvent was concentrated under reduced pressure and the final product was dried in vacuo to give aimed block copolymer. The amount of each substance used in each polymerisation was listed below.

For PEG_{45}-*b*-$PDEA_{115}$ (always eq./mmol/mg): 1/0.05/108 (PEG-Br), 2/0.1/15.6 (2,2'-bipyridine), 1/0.05/7.2 (CuBr), 100/5/785.6 (DEA), 1.5 mL 2-butanone. Yield: 571 mg (61 %)

For $PDEA_{45}$-*b*-($PDEA_{83}$-*s*-$PDMI_{18}$) (always eq./mmol/mg): 1/0.025/54 (PEG-Br), 2/0.1/7.8 (2,2'-bipyridine), 1/0.025/3.6 (CuBr), 70/1.75/275 (DEA), 15/0.375/99.5 (DMI), 0.75 mL 2-Butanone. Yield: 167 mg (39 %)

For PEG_{45}-*b*-($PDEA_{48}$-*s*-$PDMA_{31}$-*s*-$PDMI_{27}$) (always eq./mmol/mg): 1/0.05/108 (PEG-Br), 2/0.1/15.6 (2,2'-bipyridine), 1/0.05/7.2 (CuBr), 27/1.35/210 (DMA, 49/2.43/449.5 (DEA), 24/1.2/315 (DMI), 1.5 mL 2-Butanone. Yield: 725 mg (67 %)

Final compositions as by 1H NMR:

PEG-PDEA: 45-115. Molar Mass: 20200 g/mol

PEG-PDEA-PDMI: 45-83-18. Molar mass: 20000 g/mol

PEG-PDEA-PDMA-PDMI: 45-48-31-27. Molar Mass: 23000 g/mol

Characterization

^{1}H NMR of the polymers, depending on their composition (500 MHz, δ in ppm, all multiplets):

3.63, 3.37, 1.13 (all from PEG-Br, all polymers)

4.04, 2.55, 2.27, 1.80-1.96, 1.90, 1.04, 0.86 (all from PDMAMA, only PEG-PDEA-PDMA-PDMI)

4.13, 2.71, 2.59, 1.05, 0.89 (all from PDEAMA, for all polymers)

3.91, 3.51, 1.80-1.96, 1.61, 1.04, 0.86 (all from PDMIBM, only PEG-PDEA-PDMI and PEG-PDEA—PDMA-PDMI)

SEC results:

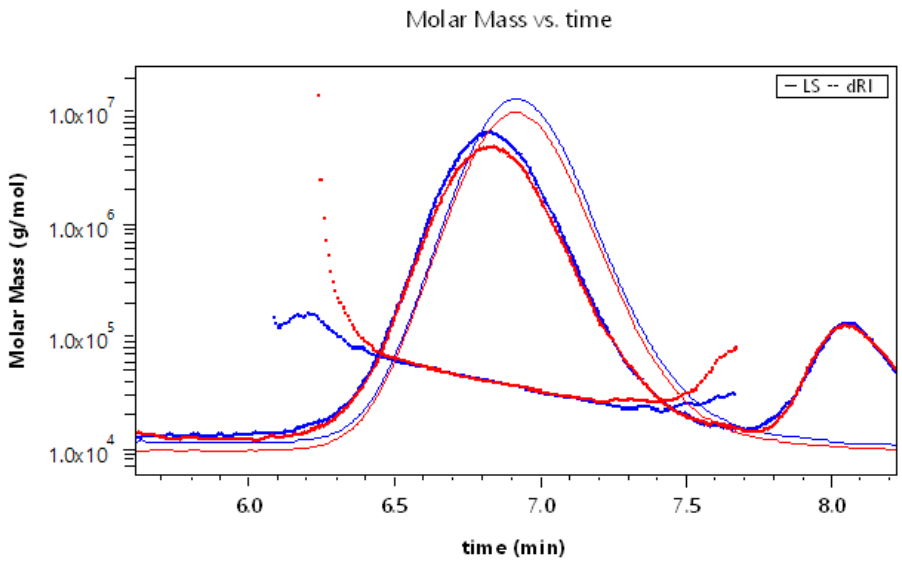

Figure SI1: SEC chromatogram of PEG-PDEA-PDMA-PDMI. Solvent: DMAc + 3 g/L LiCl, c_{BCP} = 4 mg/mL.

Table SI1: SEC results of two injections of the same polymer (PEG-PDEA-PDMA-PDMI).

PEG-PDEA-PDMA-PDMI	M_n [g/mol]	M_w [kg/mol]	Đ (M_w/M_n)
Sample 1 (Blue)	34 200	37 700	1.10
Sample 2 (Red)	35 700	38 900	1.09

Self-assembly

The pH-switch approach was based on a previously published protocol.[1] Polymersome formation was achieved by changing the solution pH from acidic to basic with the slow addition of NaOH. The BCPs were dissolved in 0.01 M HCl (aq), at room temperature, to make up solutions with a BCP concentration of 1 mg/mL. The solutions were stirred for 2 h, before being filtered over a 0.2 µm nylon filter to remove residual particles. To initiate self-assembly, 1 M NaOH solution was added dropwise to increase the pH value to pH 5. After that, 0.1 M NaOH solution was added dropwise to further raise the pH level to pH 9. The solution was then stirred for 3 days at room temperature, in the dark, and covered with aluminium foil. A 0.8 µm nylon filter was used to filter the final suspension. Filtered samples were cross-linked from 40 s to 80 s in aliquots of 2 mL (in 20 mL vials) by UV radiation. The optimum time for each sample was determined by DLS measurements at pH 9 and pH 4.

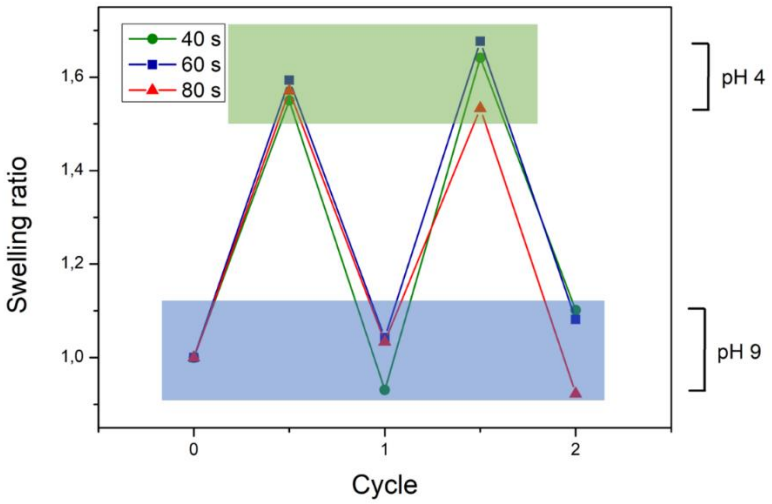

Figure SI2: Representation of swelling and shrinking behaviour of PEG-PDEA-PDMA-PDMI polymersomes in water for different cross-linking times. The change in size induced by switching the pH between 9 and 4. Diameters have been determined by DLS. Conditions: c_{BCP} = 1.0 mg/mL, in 10 mM NaCl.

Inducing morphology transition

To induce the morphological transition, the polymersome solution was placed in the fridge at 3 °C for 48 hours. Thereafter the samples were immediately cross-linked in aliquots of 2 mL (in 20 mL vials) by UV radiation.

Size and Swelling behaviour

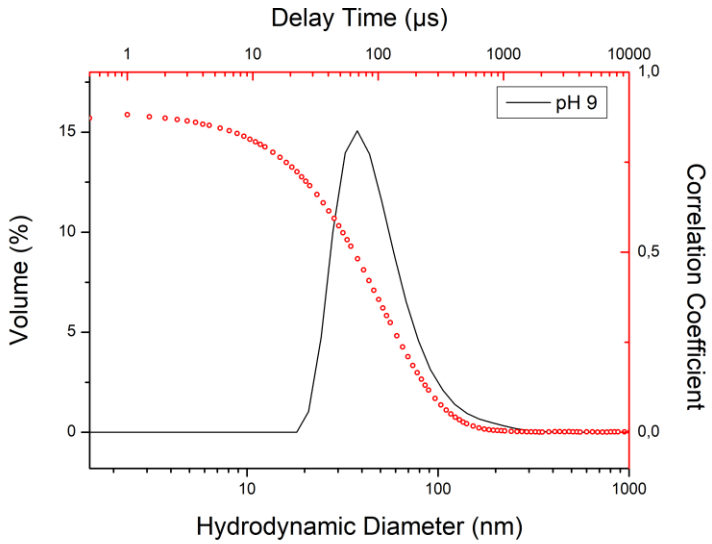

Figure SI3: Volume size distribution and correlation function of PEG-PDEA-PDMA-PDMI nanoparticles at pH 9. Obtained by DLS. Conditions: c_{BCP} = 1.0 mg/mL, in 10 mM NaCl.

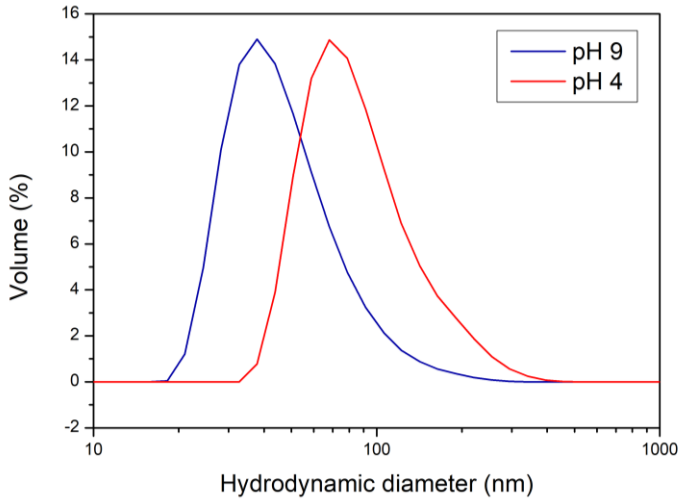

Figure SI4: Volume size distribution of PEG-PDEA-PDMA-PDMI nanoparticles at pH 9 and pH 4 in water. Obtained by DLS. Conditions: c_{BCP} = 1.0 mg/mL, in 10 mM NaCl.

Table SI2: Diameter (obtained from volume distribution) and swelling ratio (SR) of nanoparticles at pH 9 and 4 in water.

BCP	Diameter [nm]		SR*
	pH 9	pH 4	
PEG-PDEA-PDMA-PDMI	34.2	66.5	1.57

*Swelling ratio

The swelling ratio (SR) is used to compare nanoparticles based on different BCPs. It is calculated as follows:

$$SR_i = \frac{d(t_i)}{d(t_0,pH9)} \quad \text{(SI1)}$$

where $d(t_0, pH\ 9)$ is the initial measured diameter at pH 9 and $d(t_i)$ the measured diameter at any given time.

SI2. pH and temperature studies

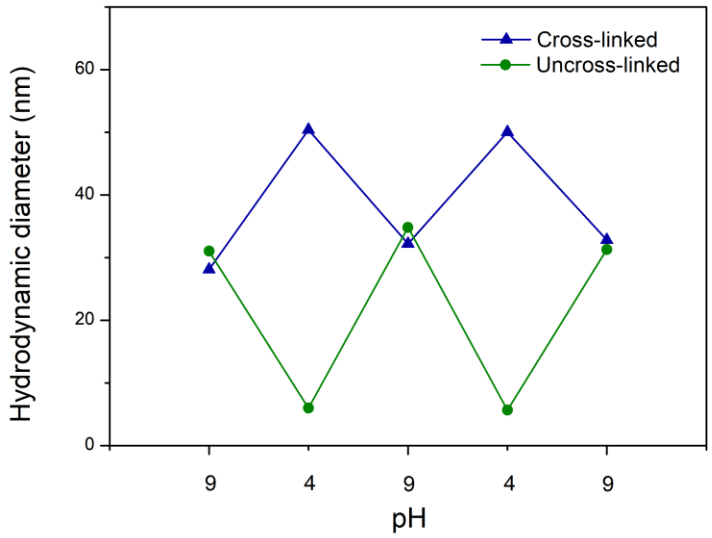

Figure SI5: Representation of the pH-responsive behaviour of PEG-PDEA-PDMA-PDMI polymersomes in water. The change in size is induced by switching the pH between 9 and 4. Diameters have been determined by DLS. Measurements were done in triplicate, taking the average of the peak maximum of the volume distribution. Conditions: c_{BCP} = 1.0 mg/mL, in 10 mM NaCl.

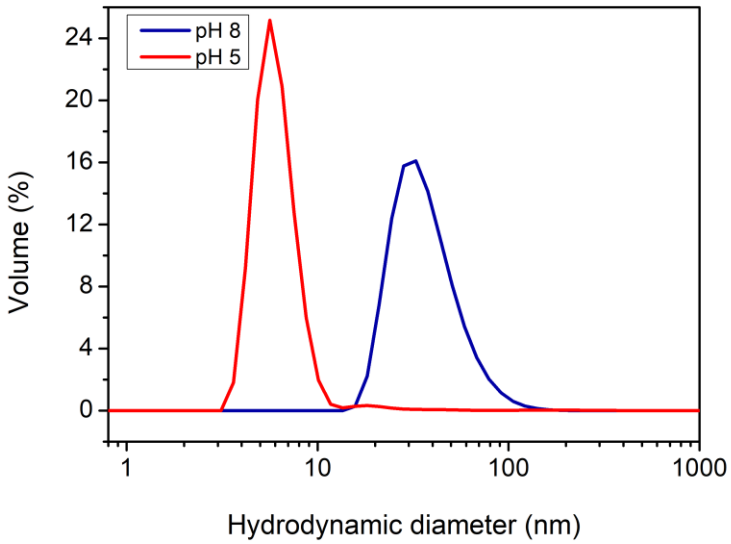

Figure SI6: Volume distribution of insufficient cross-linked PEG-PDEA-PDMA-PDMI nanoparticles at pH 8 and pH 4 in water.

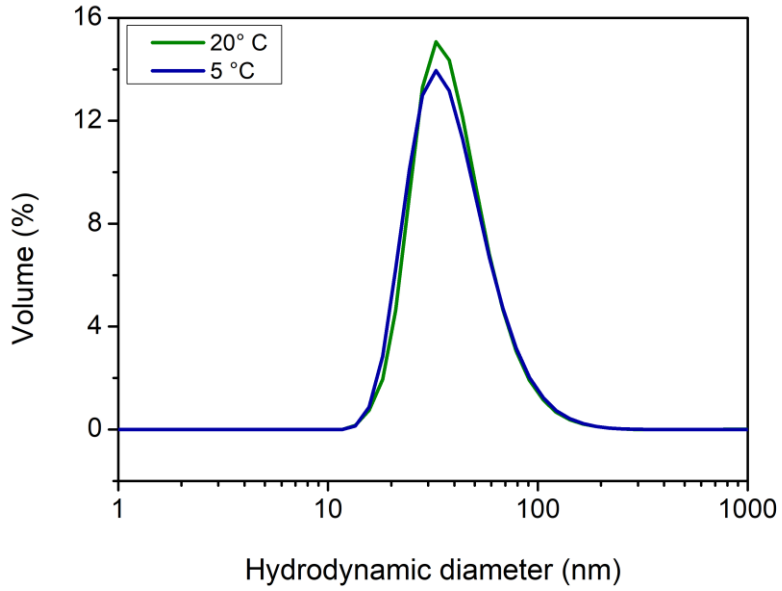

Figure SI7: Volume distribution of cross-linked (60 s) polymersomes of PEG-PDEA-PDMA-PDMI. Measurements were recorded at 3 °C. Conditions: c_{BCP} = 1.0 mg/mL, pH 9.

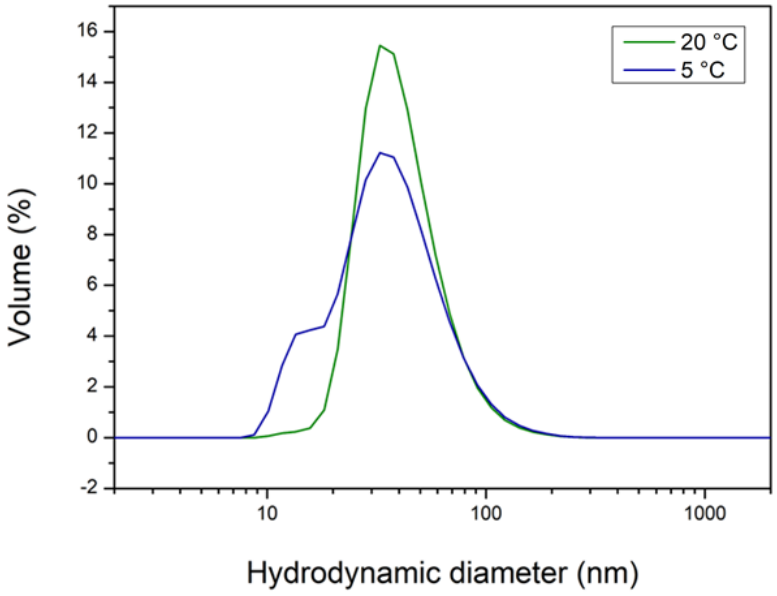

Figure SI8: Volume size distribution of uncross-linked PEG-PDEA-PDMA-PDMI polymersomes. Measurements were recorded at 5 °C and 20 °C. Conditions: c_{BCP} = 1.0 mg/mL, pH 9.

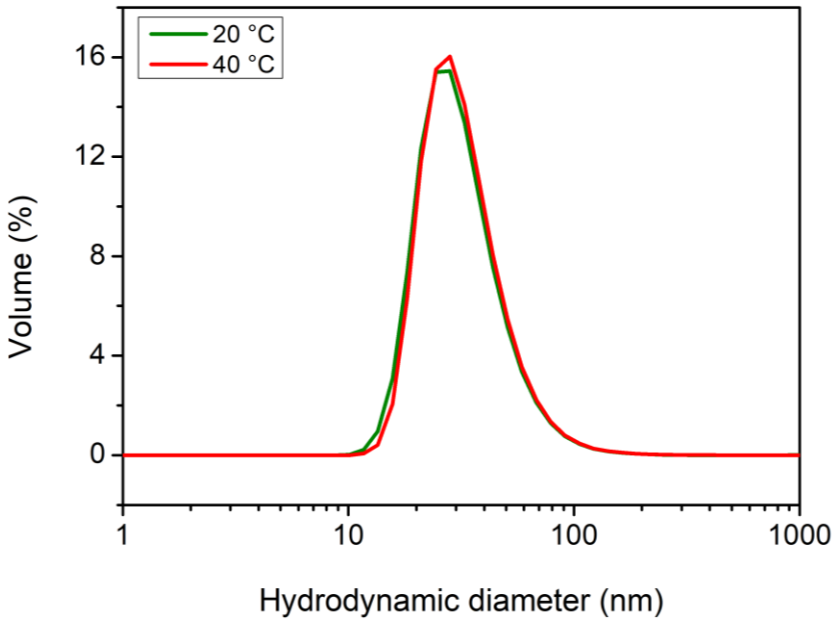

Figure SI9: Volume size distribution of uncross-linked polymersomes of BCP PEG-PDEA-PDMI. Measurements were recorded at 20 °C and 40 °C. Conditions: c_{BCP} = 1.0 mg/mL, pH 9.

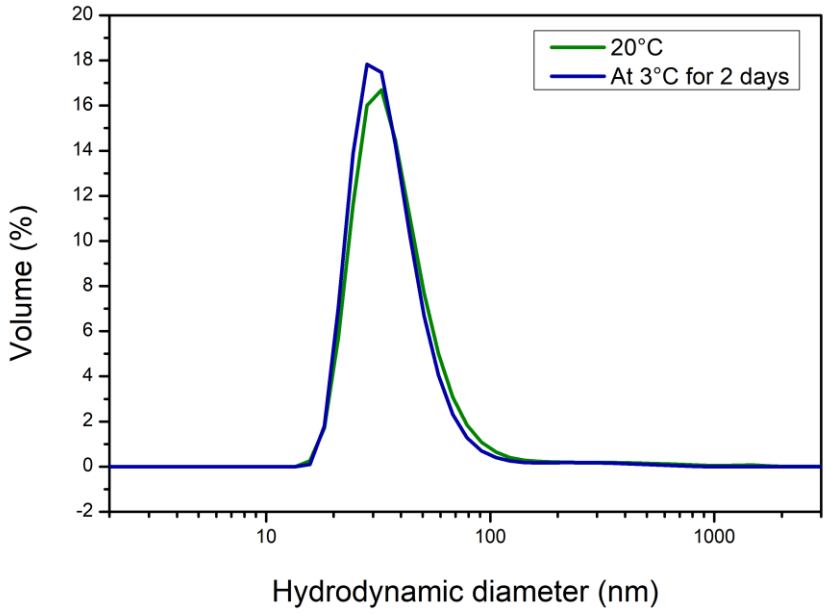

Figure SI10: Volume size distribution of uncross-linked polymersomes of BCP PEG-PDEA-PDMI. Measurements were recorded at 20 °C and 3 °C. Conditions: c_{BCP} = 1.0 mg/mL, pH 9.

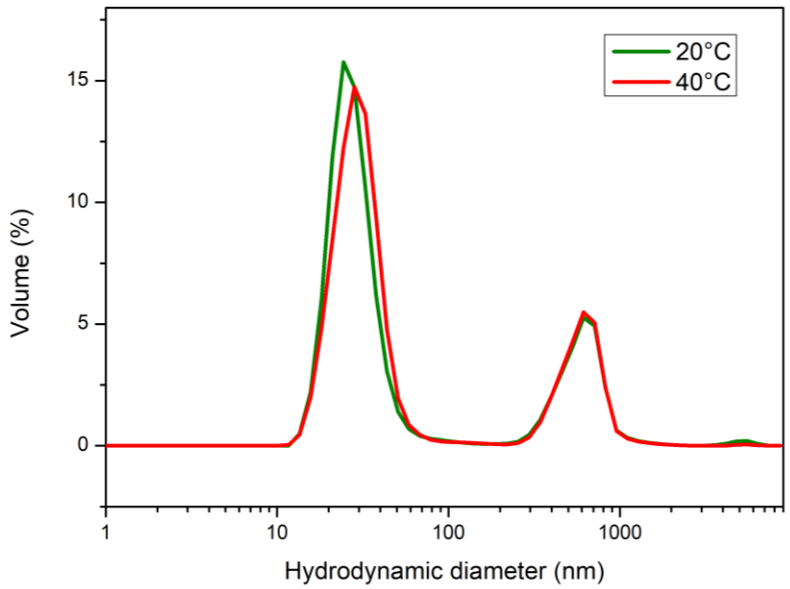

Figure SI11: Volume size distribution of the BCP PEG-PDEA which did not yield polymersomes but rather aggregates. Temperature was increased from 20 °C to 40 °C. Only displaying measurements recorded at 20 °C and 40 °C. Conditions: c_{BCP} = 1.0 mg/mL, pH 8.

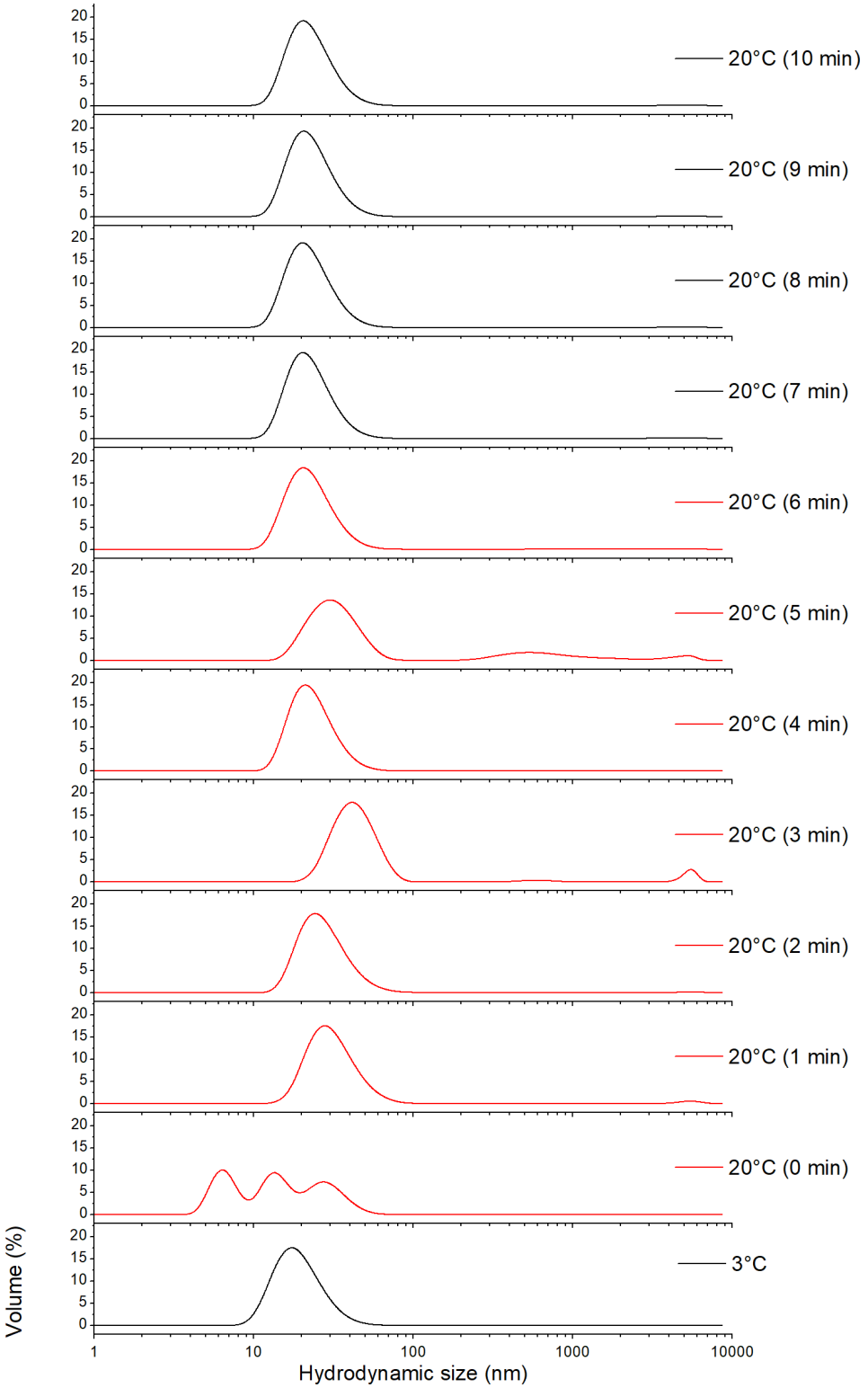

Figure SI12 Resulting size distributions of temperature study of unknown structure of PEG-PDEA-PDMA-PDMI cross-linked for 60 s. Sizes were monitored every 60 s for 10 min at 20 °C. Size distributions in red indicate phases in which the sample has not yet reached equilibrium at the specified temperature. Conditions: c_{BCP} = 1.0 mg/mL, pH 9.

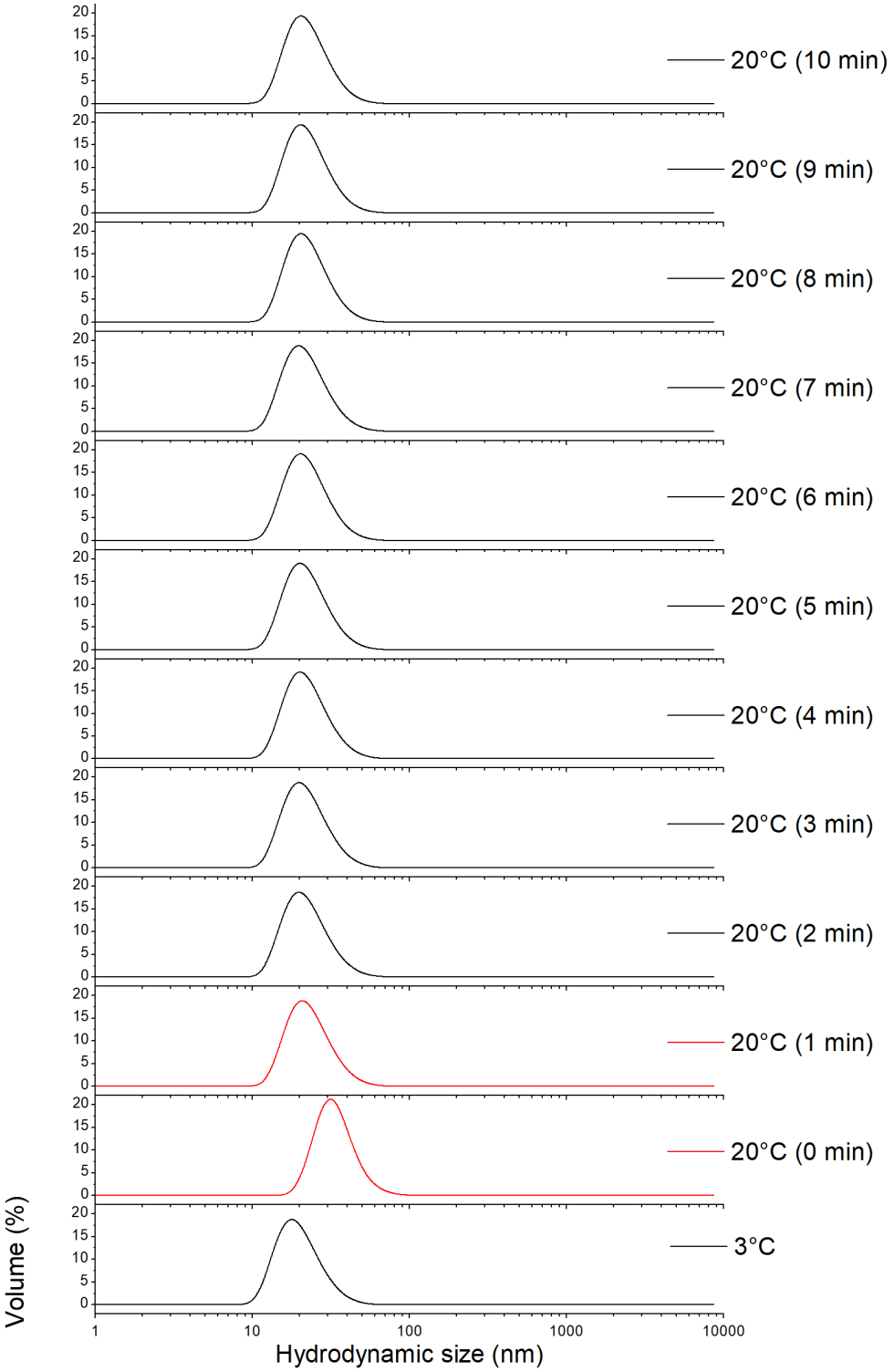

Figure SI13: Resulting size distributions of temperature study of unknown structure of PEG-PDEA-PDMA-PDMI cross-linked for 240 s. Sizes were monitored every 60 s for 10 min at 20 °C. Size distributions in red indicate phases in which the sample has not yet reached equilibrium at the specified temperature. Conditions: c_{BCP} = 1.0 mg/mL, pH 9.

SI3. AF4 analysis

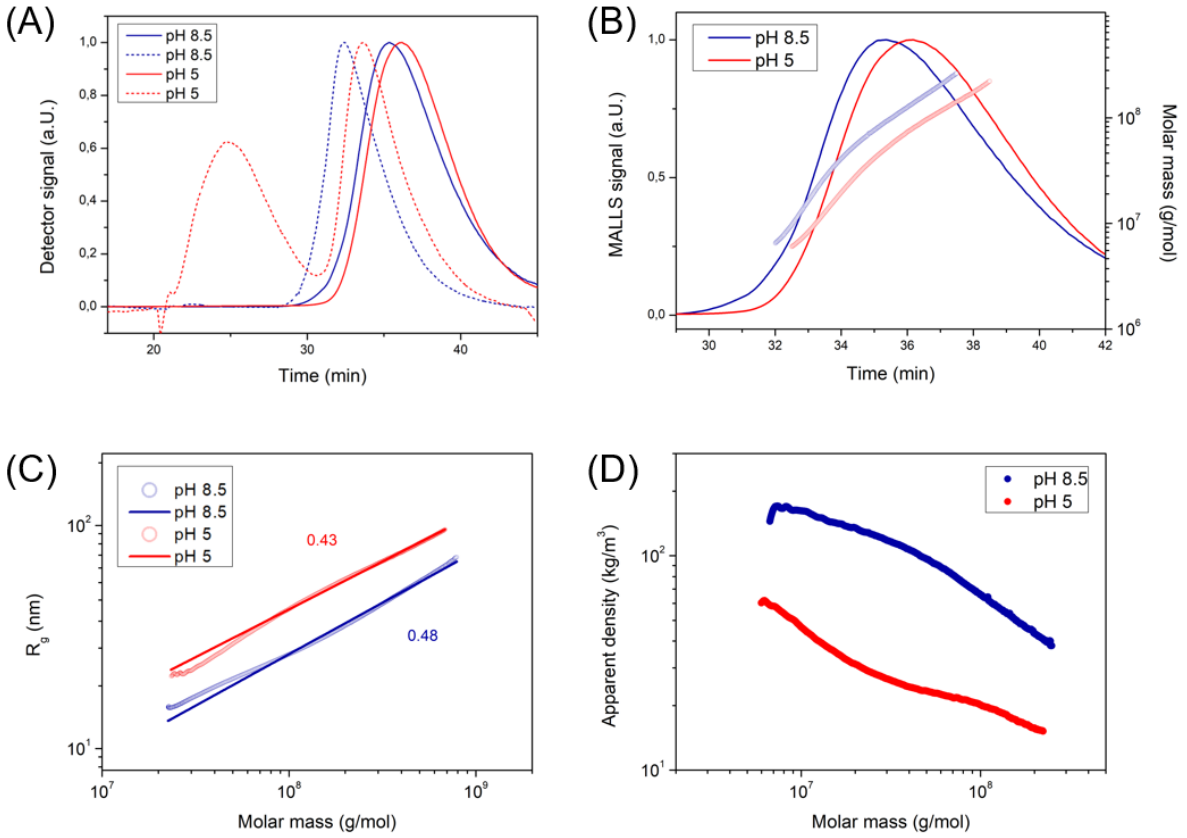

Figure SI14: pH-dependent structural parameters of cross-linked polymersomes at basic (pH 8.5, blue) and acidic (pH 5, red) conditions determined by AF4 LS. (A) AF4 fractograms of polymersomes. (B) Static light scattering signal and molar mass (M_w) distribution versus elution time. (C) Conformation analysis, indicating M_w vs radius (R_g). (D) Apparent density calculated according to M_w and R_g with the assumption of spherical shape. Conditions: c_{BCP} = 1.0 mg/mL, 10 mM NaCl.

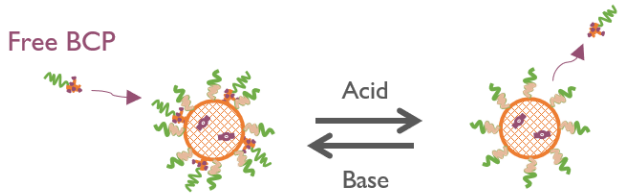

Figure SI15 Simplified schematic of BCP attachment and detachment to particle surface. Hypothesized to be the reason behind the hydrodynamic size decreased upon acidification and large scaling parameter.

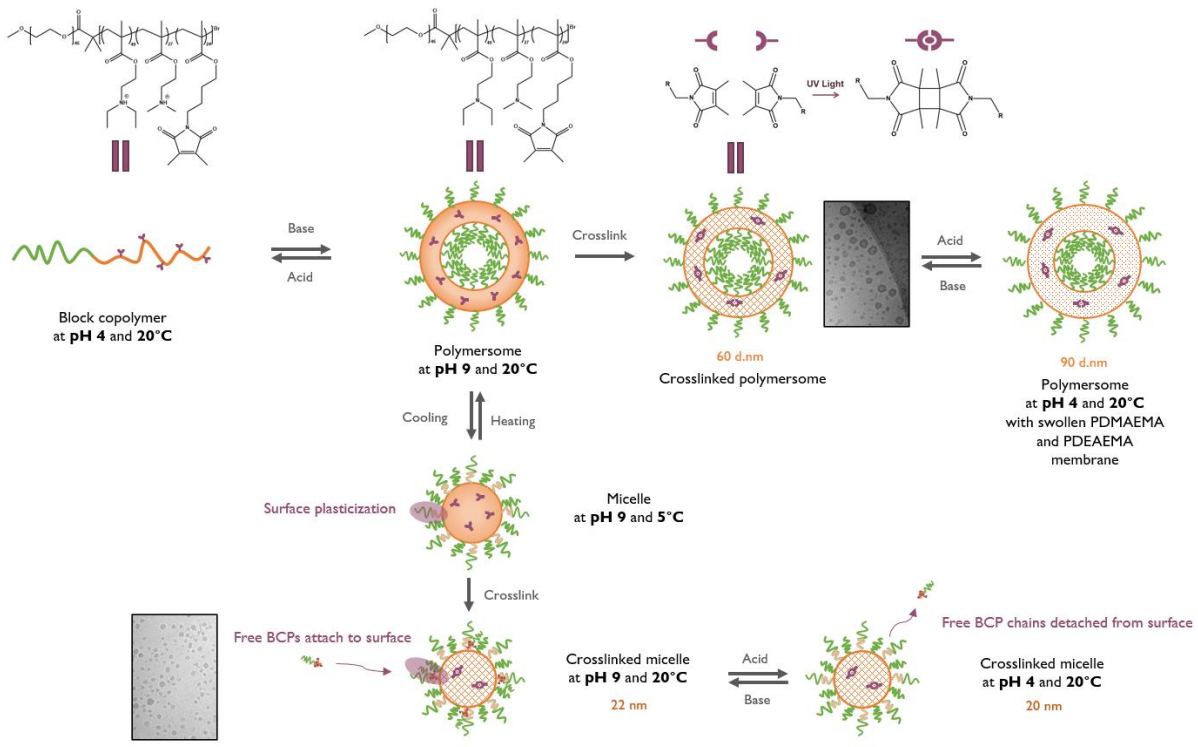

Figure SI16: Overview schematic of the temperature and pH responsivity of PEG-PDEA-PDMA-PDMI polymersomes.

Table SI3: AF4 results for *polymersomes* based on PEG-PDEA-PDMA-PDMI. Conditions: c_{BCP} = 1.0 mg/mL, eluent: 10 mM PBS.

BCP	Sample	M_n [kg/mol]	M_w [kg/mol]	Đ (M_w/M_n)	R_g [nm]	R_h [nm]	R_g/R_h*	SR
PEG-PDEA-PDMA-PDMI	pH 8.5	12 200	40 000	3.27	64.4	37.8	1.02	1.26
	pH 5.0	16 200	45 000	2.78	85.1	47.5	1.17	

* RI peak maximum

Table SI4: AF4 results for *micelle* structure of PEG-PDEA-PDMA-PDMI. Conditions: c_{BCP} = 1.0 mg/mL, eluent: 10 mM PBS.

BCP	Sample	M_n [kg/mol]	M_w [kg/mol]	Đ (M_w/M_n)	R_g [nm]	R_h [nm]	R_g/R_h*
PEG-PDEA-PDMA-PDMI	pH 8.5	7 500	10 000	1.33	24.6	21.5	1.01
	pH 5.0	3 800	4 000	1.07	18.8	19.7	0.92

* RI peak maximum

SI4. Scaling properties calculation via AF4

The scaling law is as follows:[2]

$$R_g = K \cdot (M_w)^{\nu} \quad \text{(SI2)}$$

where R_g is the radius of gyration and M_w the molecular weight. By plotting R_g against M_w, known as the scaling plot, the scaling exponent can be obtained from the slope. It enables the interpretation of shape and conformation of the polymersome. For an ideal hard sphere in a good solvent $\nu = 0.33$. For a linear random coil, $\nu = 0.50$ in theta conditions and $\nu = 0.60$ in a good solvent.[3] Any values of ν higher than 0.6 refer to other anisotropic structures, e.g. rigid rods ($\nu = 1.00$) or elongated conformations.[4,5] Though, this value is additionally strongly dependent on the nature of the particle's surface. Values lower than 0.3 would correspond to spherical particles with a smooth, well-defined surface.

The apparent density ρ_{app}, is determined as follows:[6]

$$\rho_{app} = \frac{M_w}{V(R_g)} q \quad \text{(SI3)}$$

where $V(R_g)$ is a sphere's volume with radius R_g. and q is defined by:[7]

$$q = \frac{V_{sphere}(R_g)}{V_{sphere}(r)} = \left(\frac{3}{5}\right)^{1.5} \quad \text{(SI4)}$$

SI5. Experimental details

SEC

Analysis was performed using a PolarGel-M column (300 x 7.5 mm), differential refractive index (dRI) detector (K-2301, Knauer, DE) and a MiniDAWN TREOS II detector (Wyatt Technology, U.S.A.). Measurements were monitored by an Agilent HPLC-Pump 1200 series (Agilent Technologies). The column oven temperature was kept at 25 °C and 25 µL of 4.0 mg/mL sample in *N,N*-dimethylacetamide (DMAc) with 3 g/L of lithium chloride (LiCl) was injected into the column. A flow rate of 1 mL/min was used.

Dynamic light scattering

Offline DLS measurements were made with a Zetasizer Nano ZS device (Malvern Instruments, Worcestershire, U.K). A BCP concentration of 1 mg/mL was used for all measurements. Each set consisted of five measurements with the number of acquisitions and acquisition time set to automatic depending on the sample quality. Measurement sets were averaged to give the intensity-average particle size distributions. If not stated otherwise, samples were analyzed at 20 °C employing a 632.8 nm helium-neon laser and the non-invasive back-scatter technique (NIBS) with a scattering angle of 173°. Disposable micro cuvettes were used for all measurements.

UV lamp for cross-linking

For the purpose of cross-linking polymersomes, an OmniCure S2000 UV curing light system (Lumen Dynamics Group Inc., Canada) with a high-pressure mercury lamp (0.35 W/cm2, UV light between 320-500 nm) was employed.

Asymmetric flow field-flow fractionation

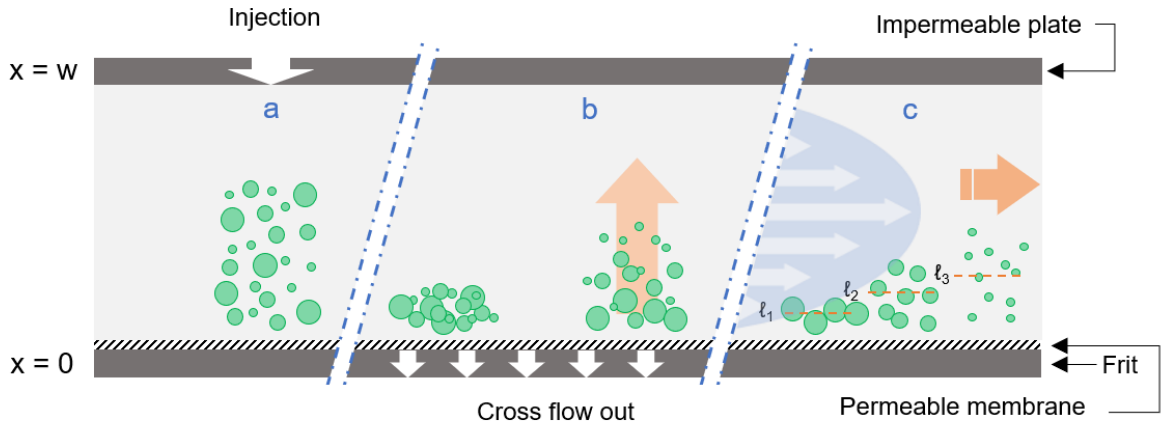

Figure SI17: Simplified scheme of an AF4 channel operating in the normal mode. It depicts a) sample injection, b) sample focusing, and c) separation of the particle "clouds" with mean layer thickness l_n.

On an Eclipse DUALTEC system (Wyatt Technology Europe, Germany), AF4 measurements were performed with a mobile phase of PBS (10 mM) and NaN_3 (0.02% (w/v)) to inhibit the growing of bacteria and algae. The solvent pH used for the measurements were adjusted to 6.5, 7.4, and 8.5. The poly(tetrafluoroethylene) channel spacer had a thickness of 490 µm, and the channel's length and

breadth were 26.5 cm and 0.6 - 2.1 cm, respectively. As an accumulation wall (Superon GmbH, Germany), regenerated cellulose membrane (10 kDa molecular cut-off) was used. To regulate flows, an Agilent Technologies 1260 series isocratic pump with a vacuum degasser was utilized. The detection system included a MALLS detector (DAWN HELEOS II, Wyatt Technology, Germany) conducting measurements at a wavelength of 660 nm with an online DLS detector (QELS module, Wyatt Technology, USA) that is an add-on device attached to the MALS's 99° angle, a diode array detector (SPD-M20, Shimadzu), and an absolute refractive index (RI) detector (Optilab T-rEXm Wyatt Technology, US) conducting measurements at a wavelength of 660 nm. Every injection was carried out using an autosampler (1260 series, Agilent Technologies, Deutschland GmbH). All operations were conducted at ambient temperature. The injected load was 100 µL and 300 µL, and the sample concentration was approximately 1 mg/mL. Data was collected and processed using Astra 7.3 software (Wyatt Technologies, USA).

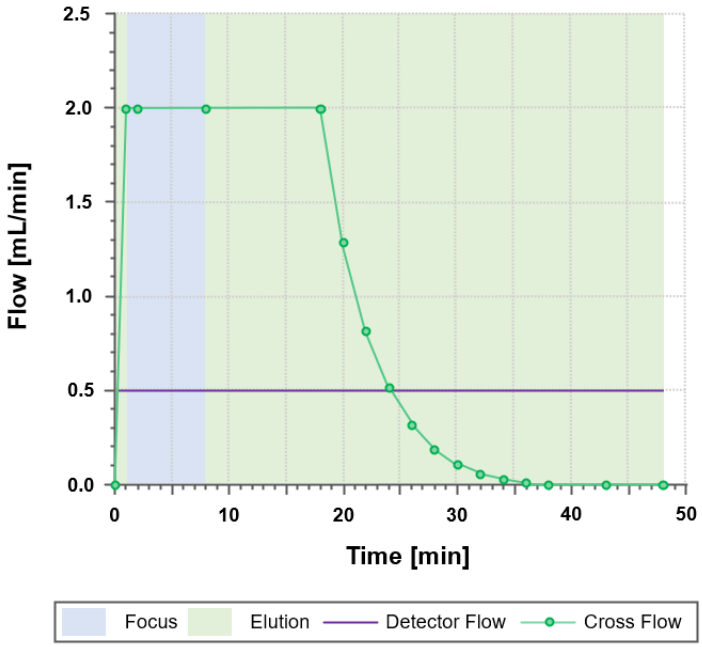

Figure SI18: Flow profile for separation method.

The crossflow rate (F_x) profile was optimized. For all AF4 procedures, the detector flow rate (F_c) was set at 0.5 mL/min. For separation, an exponential F_x gradient from 2.0 to 0 mL/min within 20 minutes was utilized after an isocratic step with a Fx of 2.0 mL/min for 10 min (Figure 3.1). The final step continued for 10 minutes without F_x (0 mL/min). Three injections with an injection volume of 100 µL were performed followed by fourth injection with an injection volume of 300 µL. M_w and R_g of polymersomes were calculated from the MALLS signal using detectors 7 – 16 applying a Zimm fit.

Cryo-TEM

Cryo-TEM pictures were obtained using a Libra 120 microscope (Carl Zeiss Microscopy GmbH, Oberkochen, Germany) and an acceleration voltage of 120 kV. A small volume (4 µL) of the polymersome solution was dropped onto copper grids covered in holey carbon foil (so-called Qunatifoil type R3.5/1) to create the samples. Using a Leica GP (Grid Plunging) device, the grids were instantly frozen in liquid ethane at -178 °C after being wiped with filter paper. Every image was captured in a bright field at -172 °C.

SI6. References

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