Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information

Vesicles of double hydrophilic pullulan and poly(acrylamide) block copolymers: A combination of synthetic- and bio-derived blocks

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Synthesis of alkyne terminated pullulan

Depolymerization of pullulan: Pullulan was depolymerized according to a procedure reported in literature.[1] In a dry, argon purged 100 mL round bottom Schlenk flask commercially available pullulan (4.0 g) was dissolved in aqueous hydrochloric acid solution (80 mL, 0.025 mol·L^{-1}). The flask was sealed and immersed in a pre-heated oil bath at 85 °C. The solution was stirred for 2.5 h and the depolymerization reaction was stopped by putting the flask into an ice bath. The

cooled reaction mixture was placed in a 10.000 MWCO dialysis tube and extensively dialyzed against deionized water for three days. The solution was lyophilized to afford depolymerized pullulan as colorless solid (3.0 g, 75% recovery, $M_{n,app,SEC}$ = 14000 g mol^{-1}, pullulan standard in acetate with 20% MeOH, $Đ$ = 1.8)

Alkyne functionalization of pullulan: Alkyne terminated pullulan was synthesized according to a derived procedure reported by Schatz et. al.[2] In a dry, argon purged 100 mL round bottom Schlenk flask pullulan (1.5 g, 0.144 mmol, 1.0 eq.) was dissolved in acetate buffer solution (50 mL, 50 mM). Propargyl amine (0.92 mL, 14.4 mmol, 100.0 eq.) was added and the flask was immersed into a pre-heated oil bath at 50 °C. Sodium cyanoborohydride (0.226 g, 3.6 mmol, 25.0 eq.) was added and the mixture was stirred for 96 hours with a repeated daily addition of sodium cyanoborohydride of 0.226 g (3.6 mmol, 25.0 eq.). The reaction mixture was intensively dialyzed against deionized water (SpectraPor 3.5 kD MWCO tube) for three days and lyophilized to afford alkyne terminated pullulan (1.25 g, 83% recovery, $M_{n,app,SEC}$ = 16000 g mol^{-1}, pullulan standard in acetate with 20% MeOH, $Đ$ = 1.8). The full conversion was confirmed by the disappearance of the anomeric proton signals of the reducing group in the ^{1}H-NMR spectrum (400 MHz, DMSO-d_6, 6.67 ppm and 6.32 ppm).

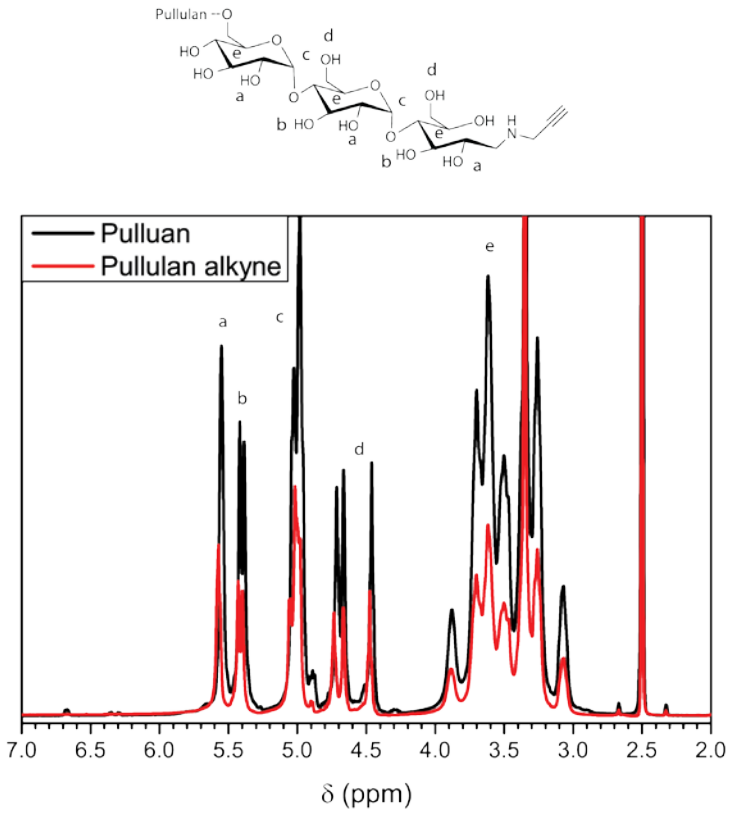

Figure S1. ^{1}H-NMR of pullulan before and after alkyne functionalization recorded at 400 MHz in DMSO-d_6.

Synthesis of azide functionalized RAFT chain transfer agent 2 dodecylthiocarbonylthio-2-methylpropanoic acid 3'-azido propyl ester

3-Azido-1-propanol:[3] Sodium azide (2.3 g, 35.4 mmol 1.5 eq.) was placed in a 100 mL round bottom flask and dissolved in a mixture of acetone (36 mL) and deionized water (6.0 mL). 3-Bromo-1-propanol (1.96 mL, 22.4 mmol, 1.0 eq.) was added. The mixture was refluxed for 18 h. Acetone was removed under reduced pressure. Deionized water (30 mL) was added. The aqueous phase was extracted with diethyl ether (3 x 30 mL). The combined organic phases were dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure to

give 3-azido-1-propanol as yellow oil (2.08 g, 20.6 mmol, 92% yield). ^{1}H NMR (400 MHz, $CDCl_3$ δ:) 3.75 (t, ^{3}J = 6.0 Hz, 2H,CH_2OH), 3.44 (t, ^{3}J = 6.6 Hz, 2H, N_3CH_2), 1.82 (p, ^{3}J = 6.3 Hz, 2H CH_2), FT-IR $\tilde{\nu}$ cm^{-1}: 2950, 2875 (CH_2), 2088 (N_3), 1455 (CH_2), 1292, 1259, 1045 (C-OH), 952, 900 (C-H).

Dodecylthiocarbonylthio-2-methylpropanoic acid:[3] In a 100 mL round bottom flask, dodecanethiol (5.0 mL, 21 mmol, 1.4 eq.) was dissolved in a suspension of K_3PO_4 (3.5 g, 16.5 mmol, 1.1 eq.) in acetone (60 mL) at ambient temperature. After stirring for 20 minutes at ambient temperature carbon disulfide (2.72 mL, 45 mmol, 3.0 eq.) was added. The reaction mixture was stirred additional 20 minutes at ambient temperature. 2-Bromo-2-methylpropionic acid (2.505 g, 15 mmol, 1.0 eq.) was added and the reaction was stirred overnight at ambient temperature. 1M HCl (200 mL) was added and the mixture was extracted with DCM (2 x 150 mL). The combined organic phases were washed with deionized water (75 mL), saturated aqueous brine solution (75 mL) and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure. The crude product was purified by recrystallization from hexane to afford dodecylthiocarbonylthio-2-methylpropanoic acid (5.2 g, 14.3 mmol, 95% recovery) as slightly yellow crystals. ^{1}H NMR (400 MHz, $CDCl_3$, δ:) 3.28 (t, ^{3}J = 7.6 Hz, 2H,), 1.72 (s, 6H, CH_3), 1.67 (t, ^{3}J = 7.1 Hz, 2H,); 1.38 (m, 2H,), 1.25 (s, 16H,), 0.88 (t, ^{3}J = 6.7 Hz, 3H, CH_3).

Dodecylthiocarbonylthio-2-methylpropanoic acid 3'-azidopropylester:[3] In a dry, argon purged 100 mL Schlenk flask dodecylthiocarbonylthio-2-methylpropanoic acid (2.5 g, 6.86 mmol, 1.0

eq.) was dissolved in dry DCM (40 mL). 3-Azidopropanol (1.73 g, 17.14 mmol, 2.5 eq.) and DMAP (0.34 g, 2.74 mmol, 0.4 eq.) was added and the stirred reaction mixture was cooled to 0 °C. A solution of DCC (2.82 g, 13.71 mmol, 2.0 eq.) in dry DCM (18 mL) was added drop wise to the cooled solution and stirred for an additional hour at 0 °C. The reaction mixture was allowed to warm to ambient temperature and stirred overnight. A precipitate was filtered off and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc/ Hexane = 1:20, R_f = 0.58) to afford dodecylthiocarbonylthio-2-methylpropanoic acid 3'-azido propyl ester (3.19 g, 70% yield, 7.13 mmol) as yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$, δ:) 4.17 (t, ^3J = 6.0 Hz, 2H, OCH_2), 3.35 (t, ^3J = 6.8 Hz, 2H,), 3.27 (t, ^{3}J = 7.5 Hz, 2H,), 1.89 (p, ^3J = 6.2 Hz, 2H,), 1.69 (s, 6H, CH_3), 1.65 (p, ^3J = 7.4 Hz, 2H,), 1.37 (m, 2H,), 1.25 (m, 16H,), 0.87 (t, ^3J = 7.0 Hz, 3H, CH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz, 300 K, δ:) 226 (13-C), 172.9 (17-C), 62.8 (18-C), 55.9 (14-C), 48.2 (20-C), 37.0 (12-C), 31.9 (3-C), 29.6 (5-C, 6-C, 7-C, 8-C), 29.5 (19-C), 29.4 (4-C), 29.1 (9-C), 28.9 (10-C), 28.0 (11-C), 25.4 (C-15, C-16), 22.7 (2-C), 14.1 (1-C). FT-IR ($\tilde{\nu}$ cm^{-1}): 2920 (CH_2), 2850 (S-CH_2), 2095 (N_3), 1733 (C=O), 1454 (C-H), 1253 (S-CH_2), 1153 (C-C), 1125, 1063 (trithiocarbonate), 814 (C-C).

Polymerization procedures

All polymerizations were performed in dry argon purged Schlenk tubes immersed in an oil bath. The polymerization mixture was degassed with at least three freeze-pump-thaw cycles, followed by purging with argon. A typical polymerization was conducted as follows.

Synthesis of PDMA.[4] In a dry argon purged 25 mL Schlenk tube, DMA (2.25 g, 22.7 mmol, 227.0 eq.) was dissolved in DMF (5.6 mL). Dodecylthiocarbonylthio-2-methylpropanoic acid 3'-azidopropylester (40.8 mg, 0.1 mmol, 1.0 eq.) and AIBN (3.0 mg, 0.018 mmol, 0.2 eq.) were added and the tube was sealed. The flask was degassed by four freeze-pump-thaw cycles and introduced into an oil bath at 60 °C. The reaction mixture was stirred for six hours. The polymerization was terminated by freezing the flask in liquid nitrogen and exposing the reaction mixture to air. PDMA (2.10 g, 0.125 mmol) was afforded as yellow solid after dialysis (MWCO 1000) against deionized water for 3 days and freeze drying. $M_{n,app,SEC}$ = 16800 g mol^{-1} (PS equivalents in NMP), $Đ$ = 1.26.

Synthesis of PEA.[4] In a dry argon purged 25 mL Schlenk tube, EA (1.0 g, 10.09 mmol, 174.0 eq.) was dissolved in DMF (3.2 mL). Dodecylthiocarbonylthio-2-methylpropanoic acid 3'-azidopropylester (23.6 mg, 0.058 mmol, 1.0 eq.) and AIBN (1.7 mg, 0.01 mmol, 0.17 eq.) were added and the tube was sealed. The flask was degassed by four freeze-pump-thaw cycles and immersed into an oil bath at 60 °C. The reaction mixture was stirred for six hours. The polymerization was terminated by freezing the flask in liquid nitrogen and exposing the reaction mixture to air. PEA (0.75 g 0.054 mmol) was afforded as yellow solid after dialysis (MWCO 1000) against deionized water for 3 days and freeze drying. $M_{n,app,SEC}$ = 13900 g mol^{-1} (PS equivalents in NMP), $Đ$ = 1.37.

Removal of PDMA-N_3 RAFT functionality[5]

AIBN (0.197 g, 1.167 mmol, 38.9 eq.) was dissolved in destabilized THF (60 mL) under vigorous stirring and heated to 60 °C. The mixture was stirred for 30 minutes at 60 °C and THF

(3x10 mL) was subsequently added to compensate solvent evaporation. PDMA-N_3 (0.5 g, 0.03 mmol, 1.0 eq.) was added to the solution and the reaction mixture was stirred for 90 minutes. Ascorbic acid (0.051 g, 0.291 mmol, 9.7 eq.) was added and the mixture was stirred additional 30 minutes. The reaction mixture was cooled to room temperature and THF was allowed to evaporate overnight. The colorless residue was dissolved in deionized water and dialyzed against water for three days. Remaining AIBN was removed by filtration and the solution was lyophilized to afford hydroxyl terminated PDMA-N_3 (0.350 g, 0.021 mmol, 70% recovery) as white powder.

Synthesis of Rhodamine B labelled PDMA-N_3

In a dry, argon purged 25 mL Schlenk flask PDMA-N_3 (0.1 mg, 0.006 mmol, 1 eq.) was dissolved in dry DMSO (7 mL). Hexylamine (0.002 mL, 0.015 mmol, 2.5 eq.) was added and the reaction mixture was stirred overnight at 50 °C. The mixture was cooled to room temperature and Rhodamine B ITC (0.013 g, 0.024 mmol, 4 eq.) was added. The reaction mixture was stirred overnight at 50 °C, cooled to room temperature, diluted with deionized water and dialyzed against deionized water for four days. The solution was lyophilized to afford Rhodamine B labelled PDMA-N_3 (63.0 mg, 63% recovery) as purple powder.

Synthesis of azidomethyl polystyrene resin

In a dry, argon purged 100 mL round bottom Schlenk tube chloromethyl polystyrene resin (10.0 g, 24 mmol, 1.0 eq.) was dissolved in dry DMSO (50 mL). Sodium iodide (10.8 g, 72

mmol, 3.0 eq.) and sodium azide (15.6 g, 240 mmol, 10.0 eq.) were added and the reaction mixture was moderately stirred for 48 hours at 80 °C. The afforded resin was filtered over a glass frit (pore size 3) and alternately washed with DCM (6 x 30 mL) and MeOH (6 x 30 mL). The purified resin was finally washed with diethyl ether (30 mL) and dried under vacuum to afford azidomethyl polystyrene resin (9.25 g, 22.2 mmol, 93% recovery) as a white solid. FT-IR ($\tilde{\nu}$ cm^{-1}): 3120, 2855, 2089 (N_3), 1509, 1450, 750, 697.

SLS measurements of block copolymers

In order to determine the radius of gyration (R_g) of Pull-*b*-PDMA and Pull-*b*-PEA, static light scattering experiments were conducted with concentration rows of 10 mg mL^{-1}, 5 mg mL^{-1}, 2.5 mg mL^{-1}, 1.25 mg mL^{-1} and 0.63 mg mL^{-1}. The data obtained from the light scattering experiments at scattering angles between 30° and 150° were plotted via a Guinier plot. The calculated values are shown in Table S3 for Pull-*b*-PDMA and Table S4 for Pull-*b*-PEA, respectively.

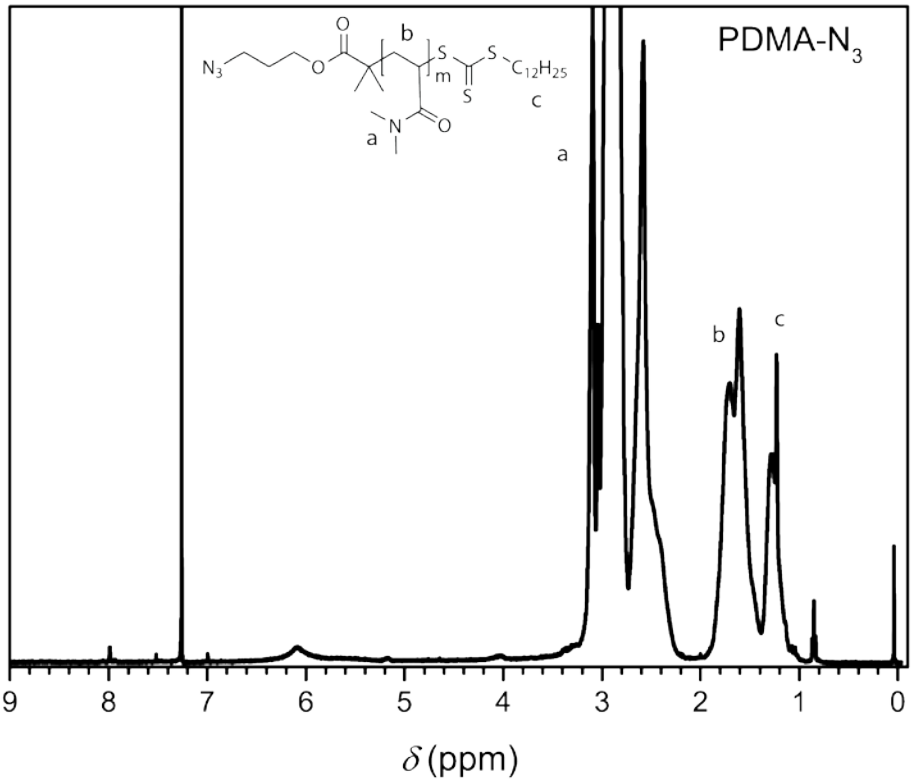

Figure S2. ^{1}H NMR of PDMA-N_3 recorded at 400 MHz in $CDCl_3$.

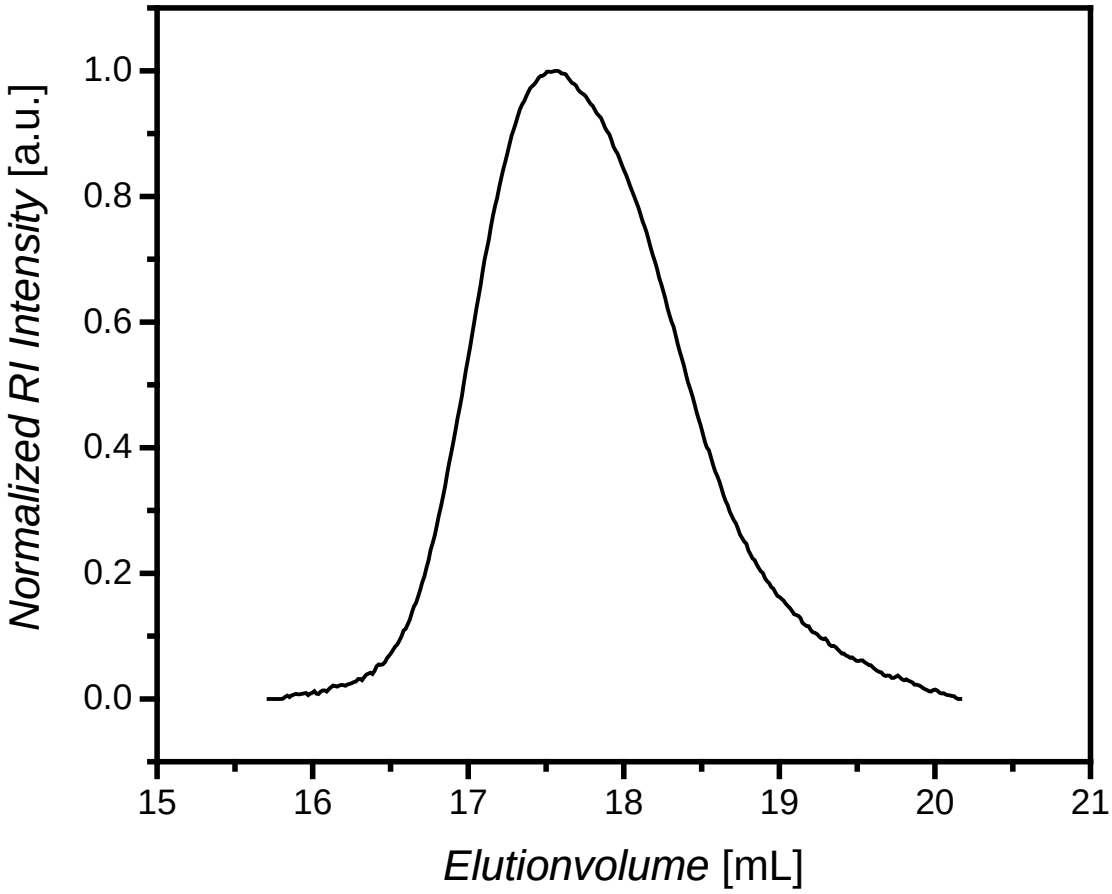

Figure S3. SEC trace of PDMA-N_3 measured in NMP at 70 °C against PS calibration.

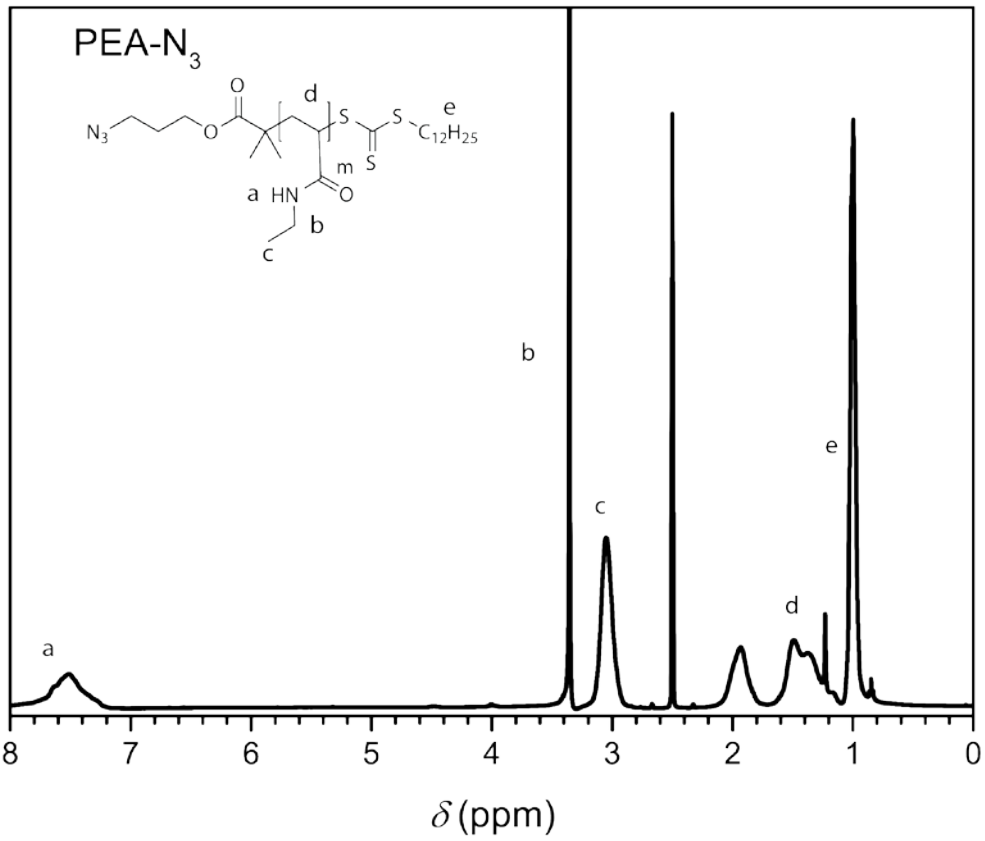

Figure S4. ^{1}H NMR of PEA-N_3 recorded at 400 MHz in DMSO-d_6.

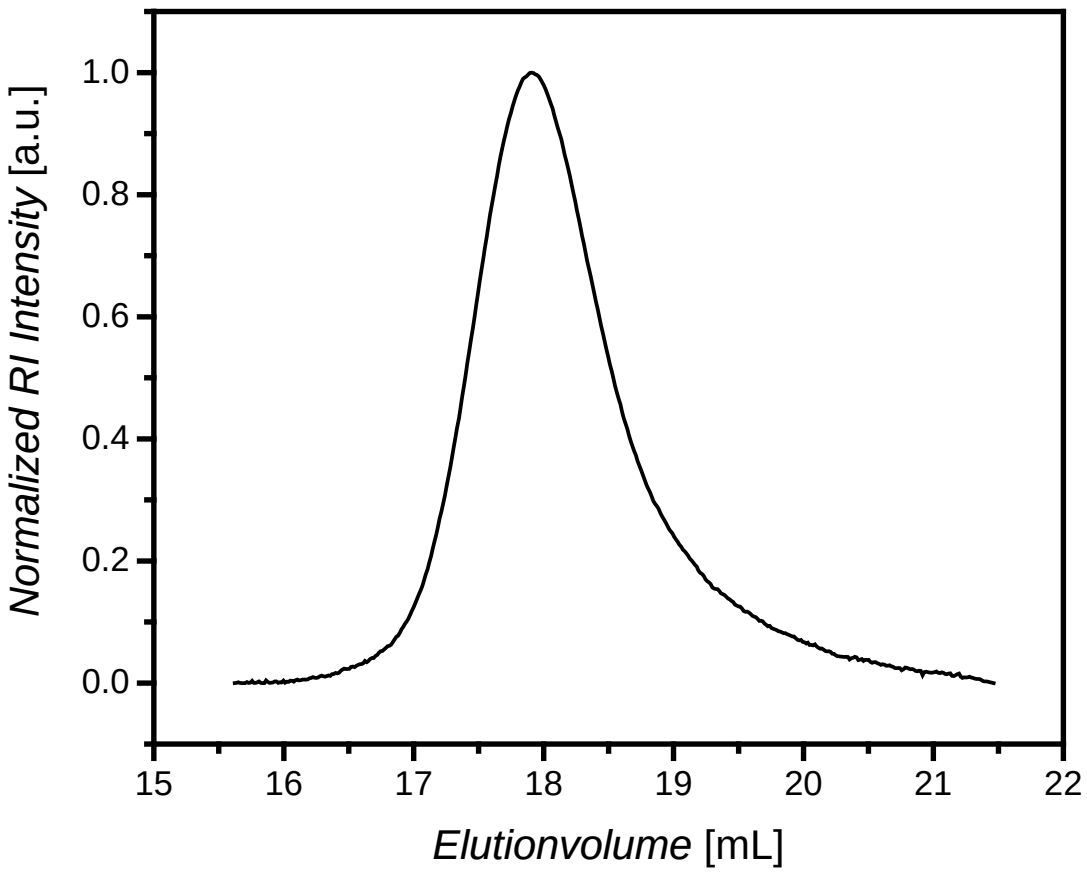

Figure S5. SEC trace of PEA-N_3 measured in NMP at 70 °C against PS calibration.

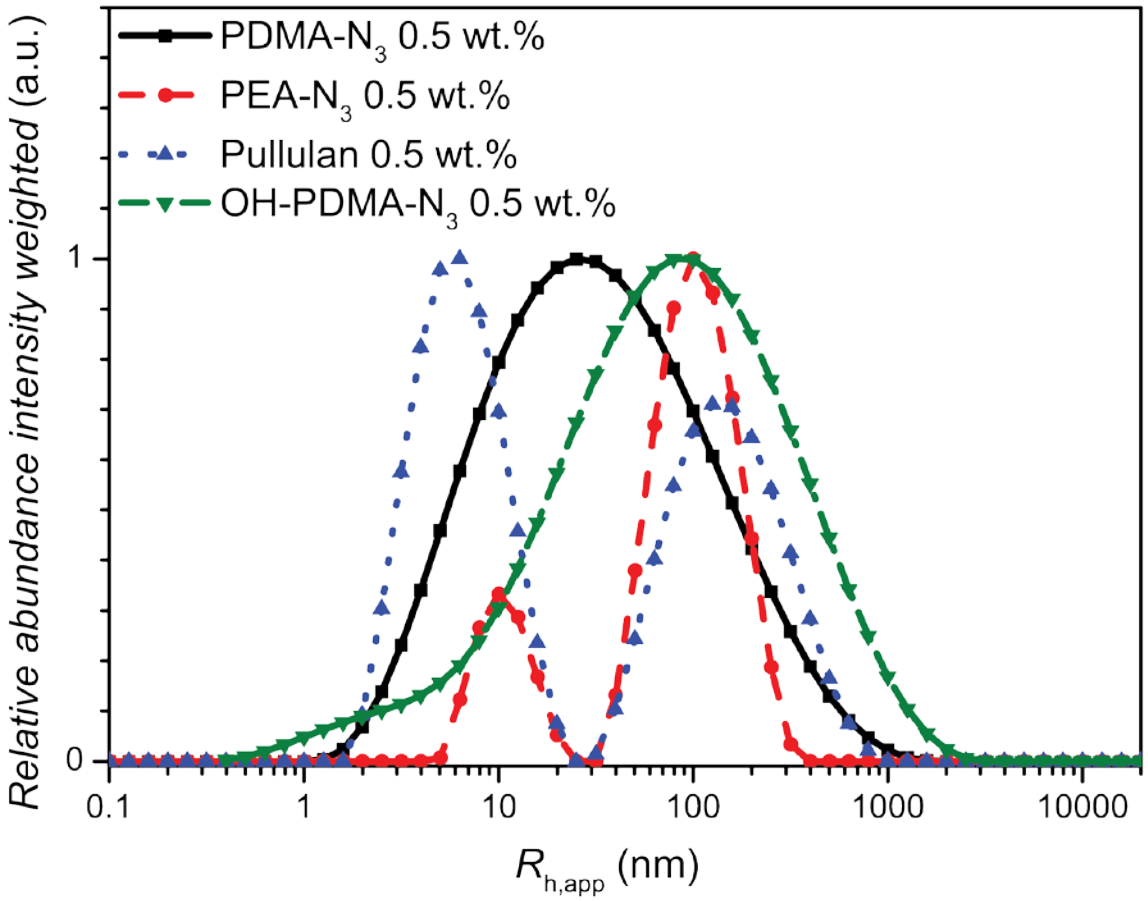

Figure S6. Intensity weighted size distributions of pullulan, PDMA-N_3 and PEA-N_3 in water measured via DLS at 25 °C.

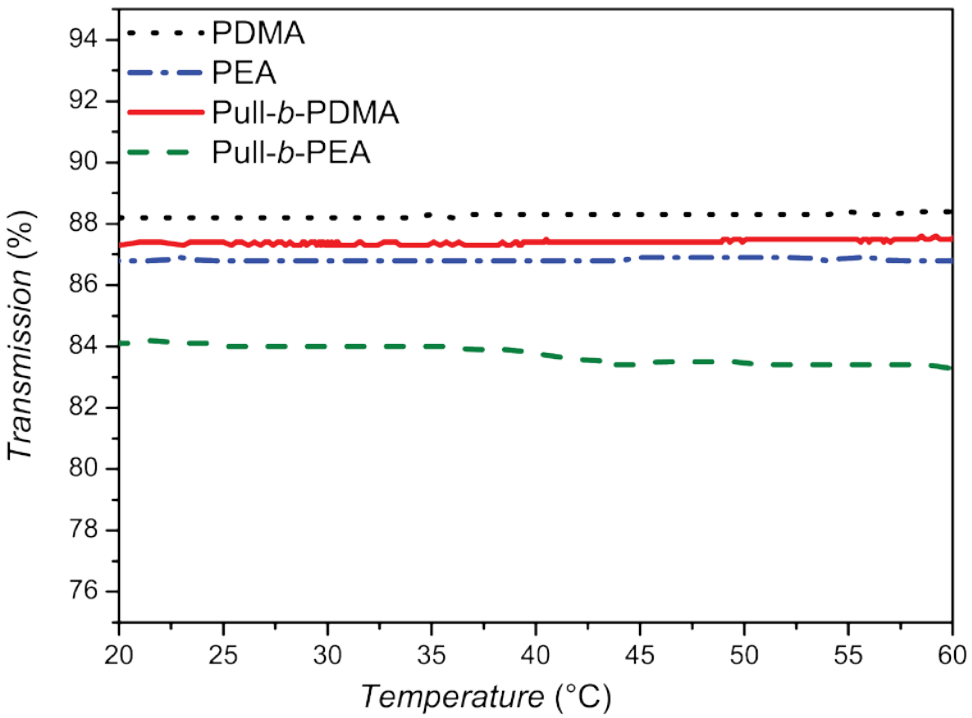

Figure S7. Turbidimetry measurements of PDMA and PEA homopolymers and the block copolymers Pull-*b*-PDMA and Pull-*b*-PEA.

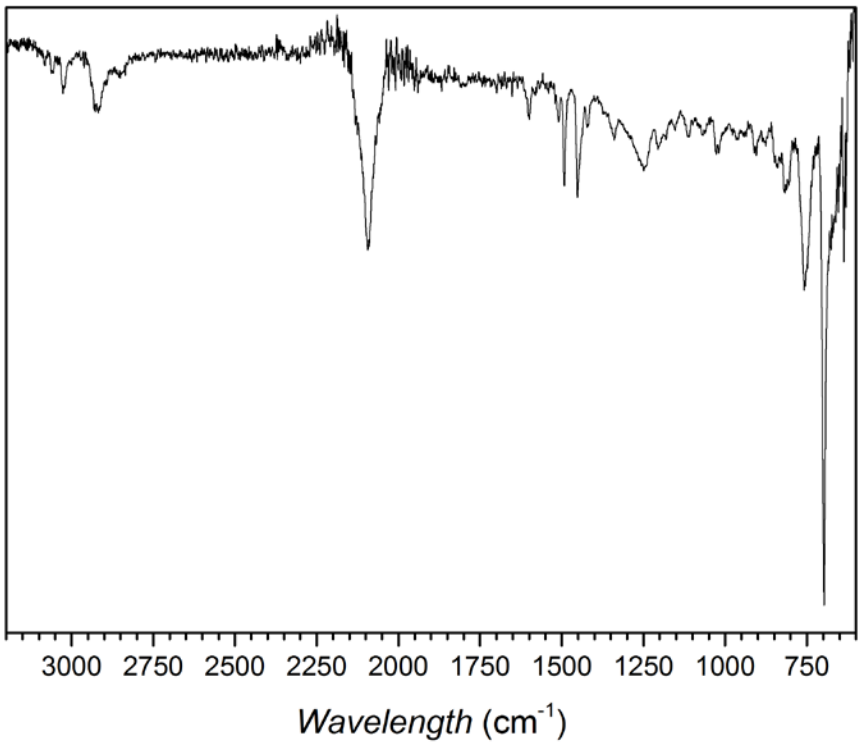

Figure S8. IR spectrum of azidomethyl polystyrene resin recorded at 25 °C.

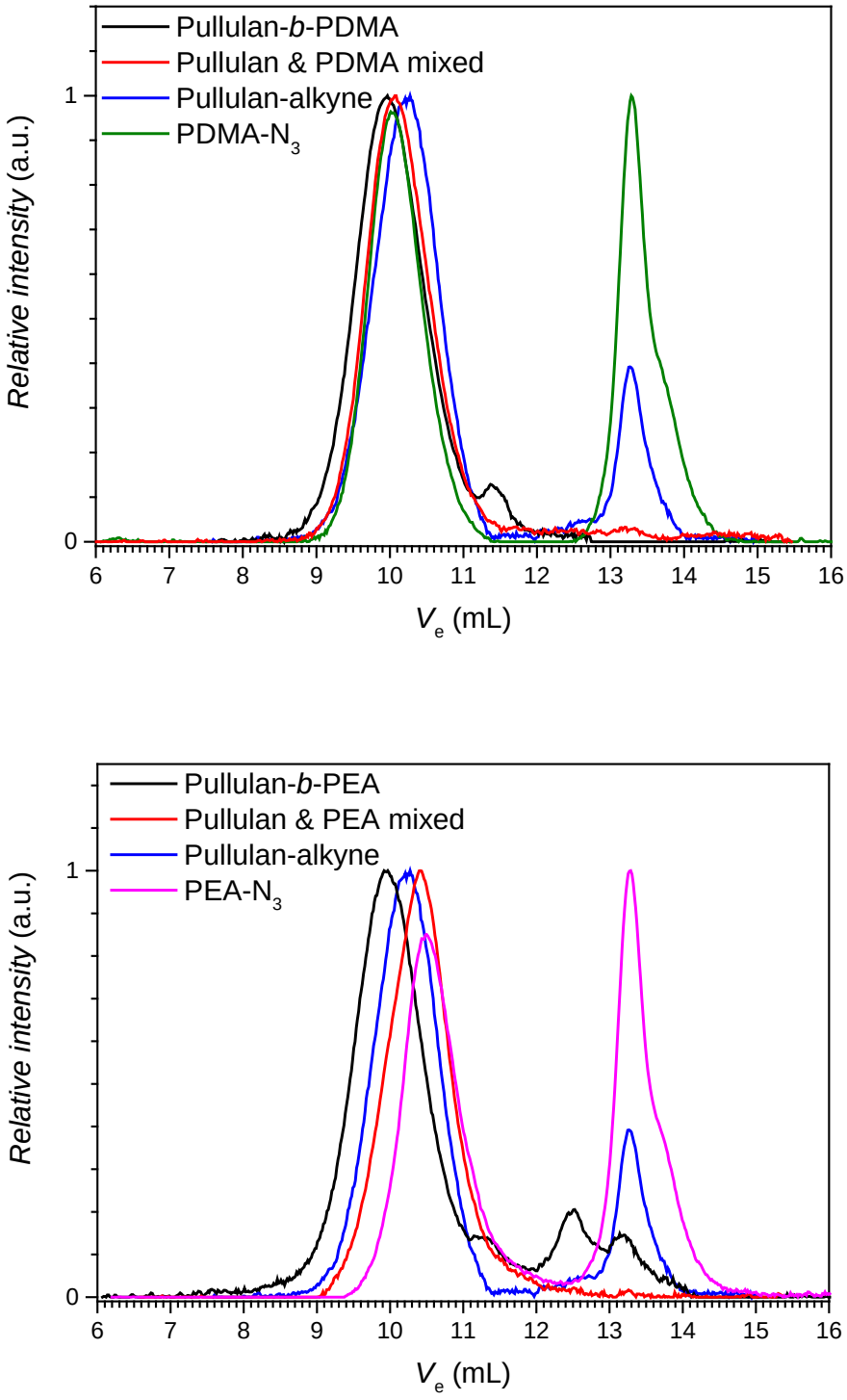

Figure S9. SEC traces of block copolymers measured in acetate buffer against pullulan calibration.

Table S1. Summary of SEC results.

Polymer	*SEC System*	$M_{n,app,SEC}$	*Đ*
Pullulan depolymerized	Water, pullulan calib.	14000	1.8
Pullulan alkyne	Water, pullulan calib.	16000	1.8
PDMA-N_3	NMP PS calib.	16800	1.26
PEA-N_3	NMP PS calib.	13700	1.37
Pull-*b*-PDMA	Water, pullulan calib.	21500	1.9
Pull-*b*-PEA	Water, pullulan calib.	26500	1.6
Pull-*b*-PDMA-RhB	Water, pullulan calib.	22600	2.7

Table S2. DLS summary of synthesized block copolymers.

Entry	**Polymer**	**Concentration (wt.%)**	**1st Peak (nm)**	**2nd Peak (nm)**
1	Pull-*b*-PDMA	0.1	4.0	79.4
		0.5	5.0	79.4
		1.0	5.0	100.0
2	Pull-*b*-PEA	0.1	5.0	125.9
		0.5	5.0	125.9
		1.0	4.0	158.5
3	Pull-*b*-PDMA-RhB	0.1	-	100.0

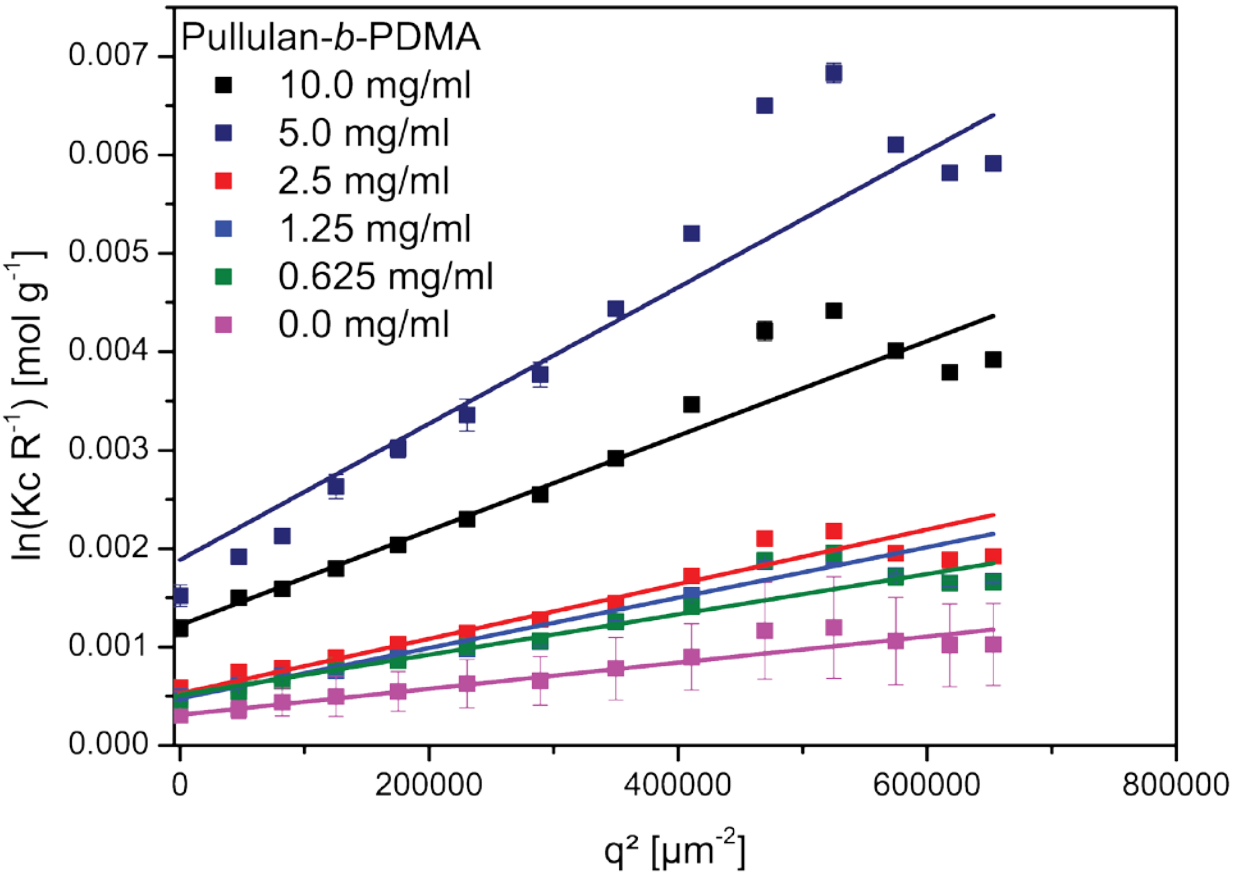

Figure S10. SLS Guinier plot of Pull-*b*-PDMA with extrapolation of c→0.

Table S3. Calculated values of the Quantities of Pull-*b*-PDMA determined via the Guinier plot.

Entry	Quantity	Value [unit]	Error [%]
1	M_w (c)	3.239e+06 [g/mol]	± 27.9
2	M_w (q^2)	3.239e+06 [g/mol]	± 8.35
3	A_2	6.684e-08 [mol dm³/g²]	± 42.2
4	R_g	1.081e+02 [nm]	± 7.1

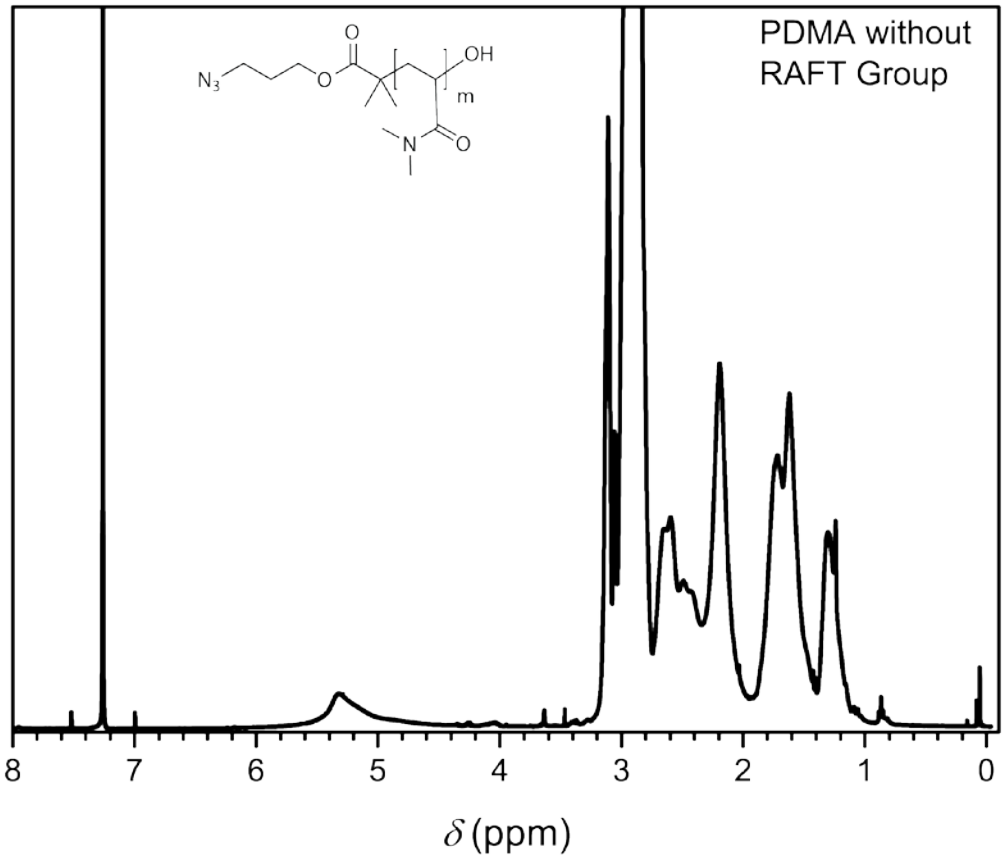

Figure S11. ^{1}H NMR of PDMA-N_3 without RAFT group recorded at 400 MHz in $CDCl_3$.

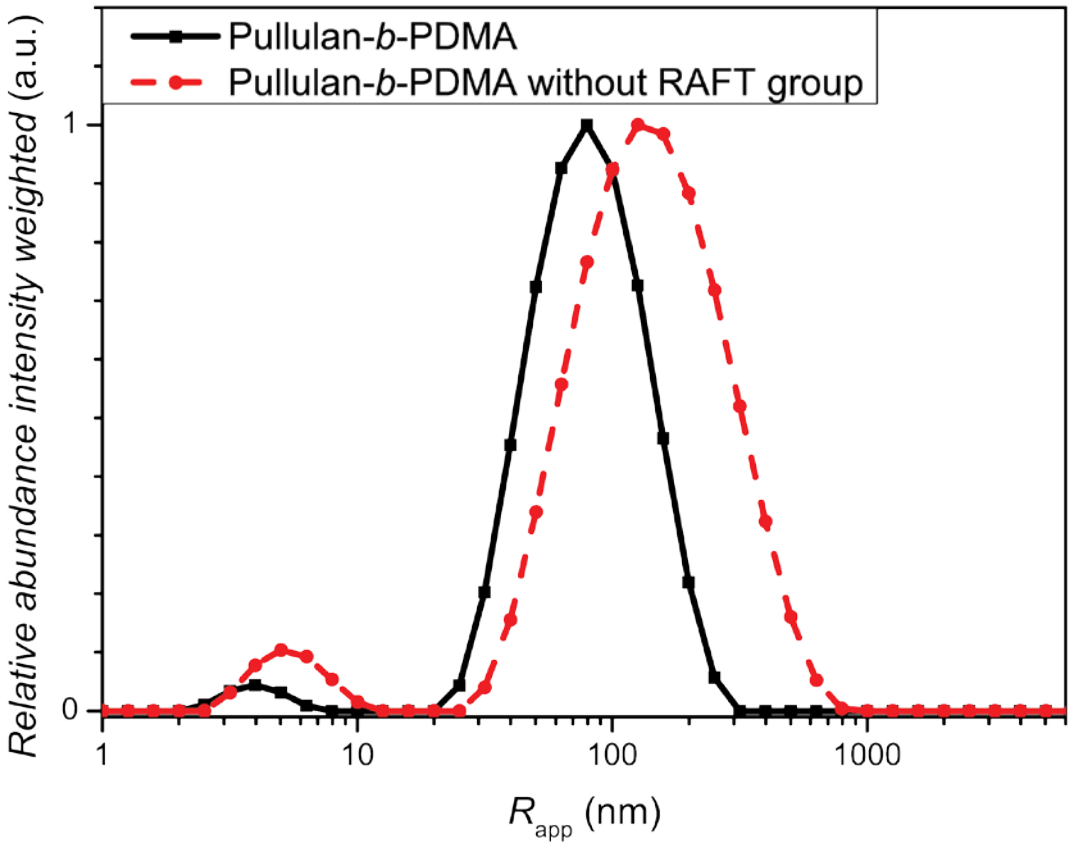

Figure S12. Intensity weighted particle size distributions of Pull-*b*-PDMA with and without RAFT group in water measured via DLS at 25 °C.

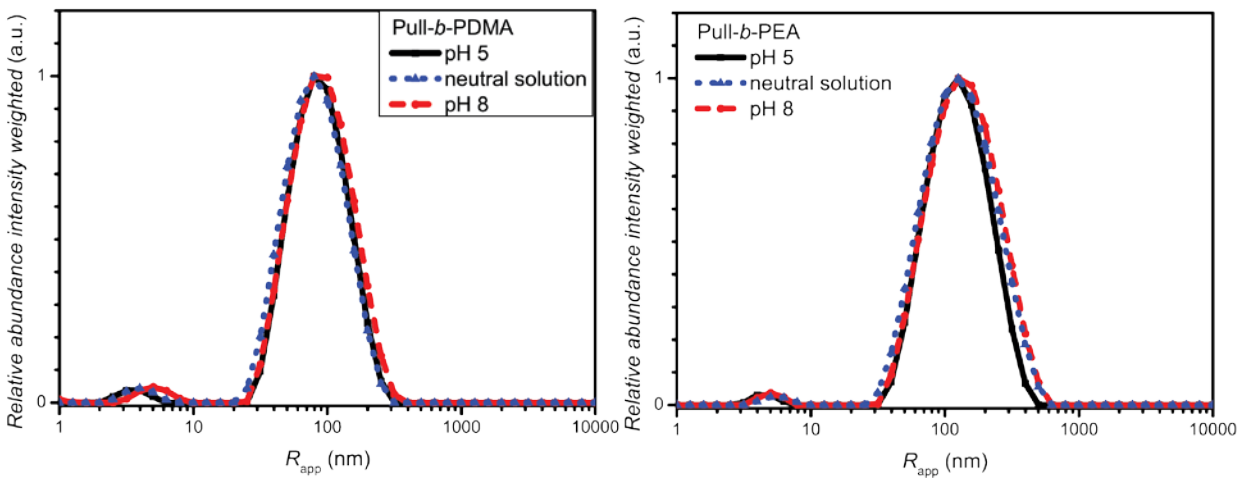

Figure S13. Intensity weighted particle size distributions of Pull-*b*-PDMA and Pull-*b*-PEA at different pH values, e.g. pH 5, pH 8 and neutral solution measured via DLS at 25 °C.

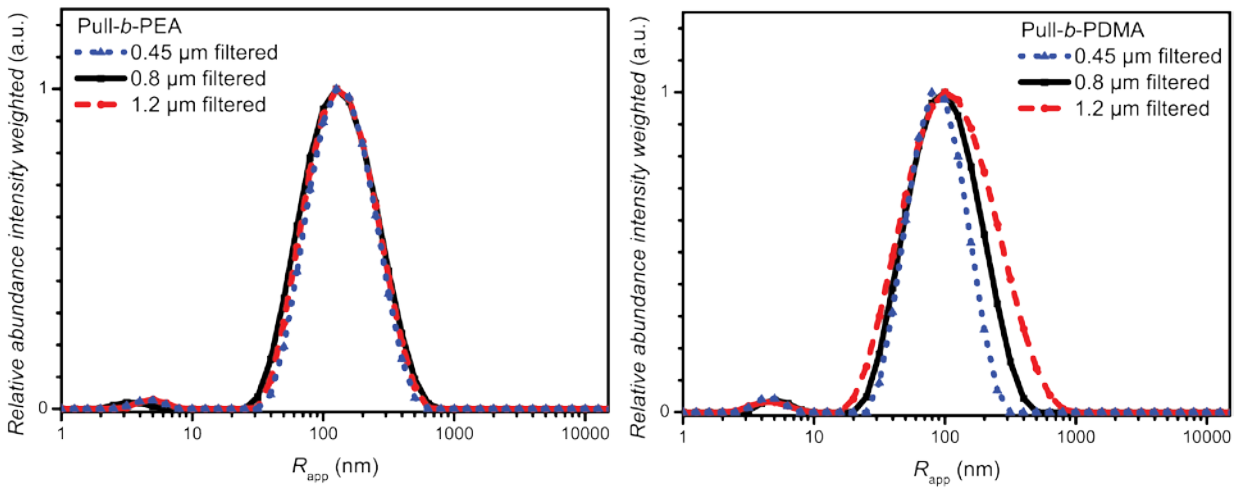

Figure S14. Intensity weighted particle size distributions of Pull-*b*-PDMA and Pull-*b*-PEA filtered with different CA filters, e.g. 0.45 µm, 0.8 µm and 1.2 µm measured via DLS at 25 °C, showing that no aggregates were removed by using 0.45 µm CA filters.

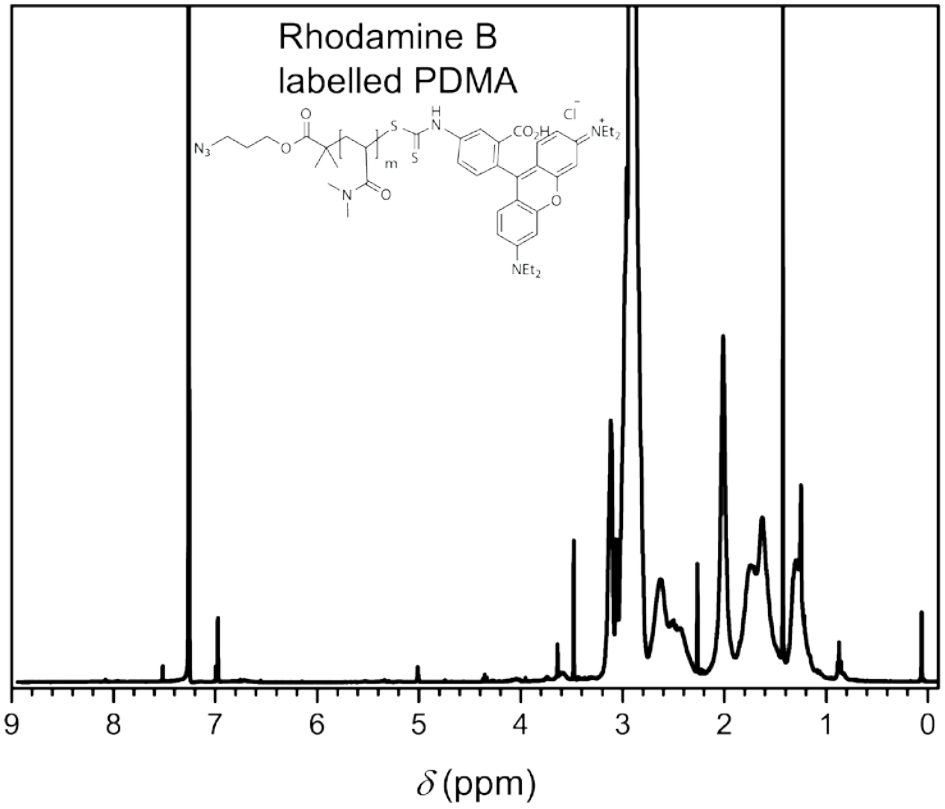

Figure S15. ^{1}H NMR of Rhodamine B labelled PDMA-N_3 recorded at 400 MHz in $CDCl_3$.

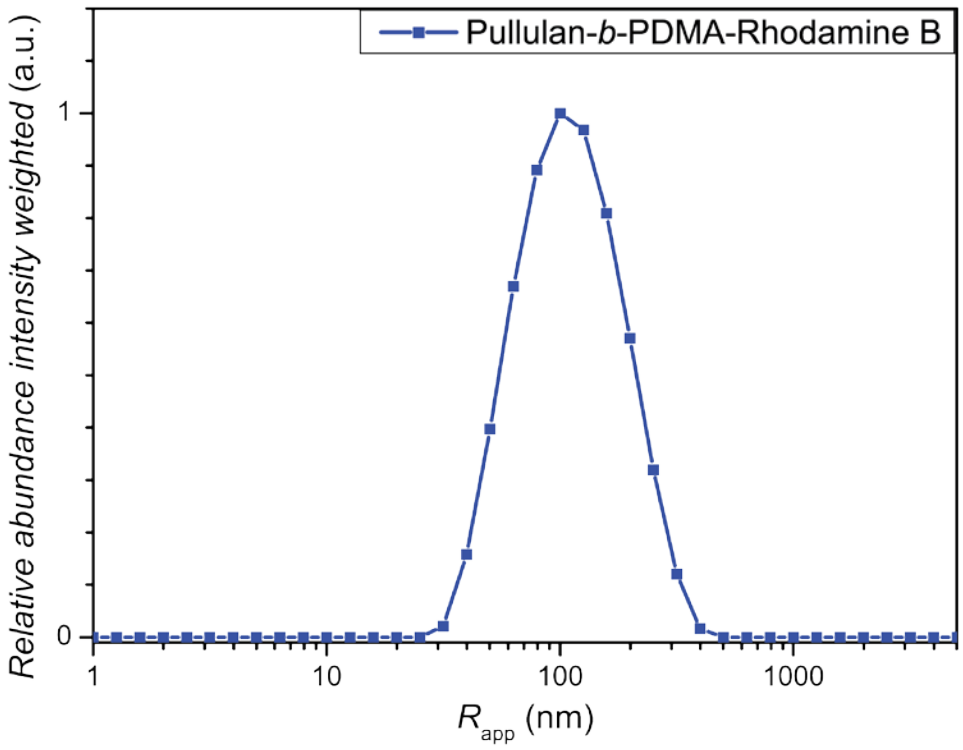

Figure S16. Intensity weighted particle size distributions of Pull-*b*-PDMA-RhB in water measured via DLS at 25 °C.

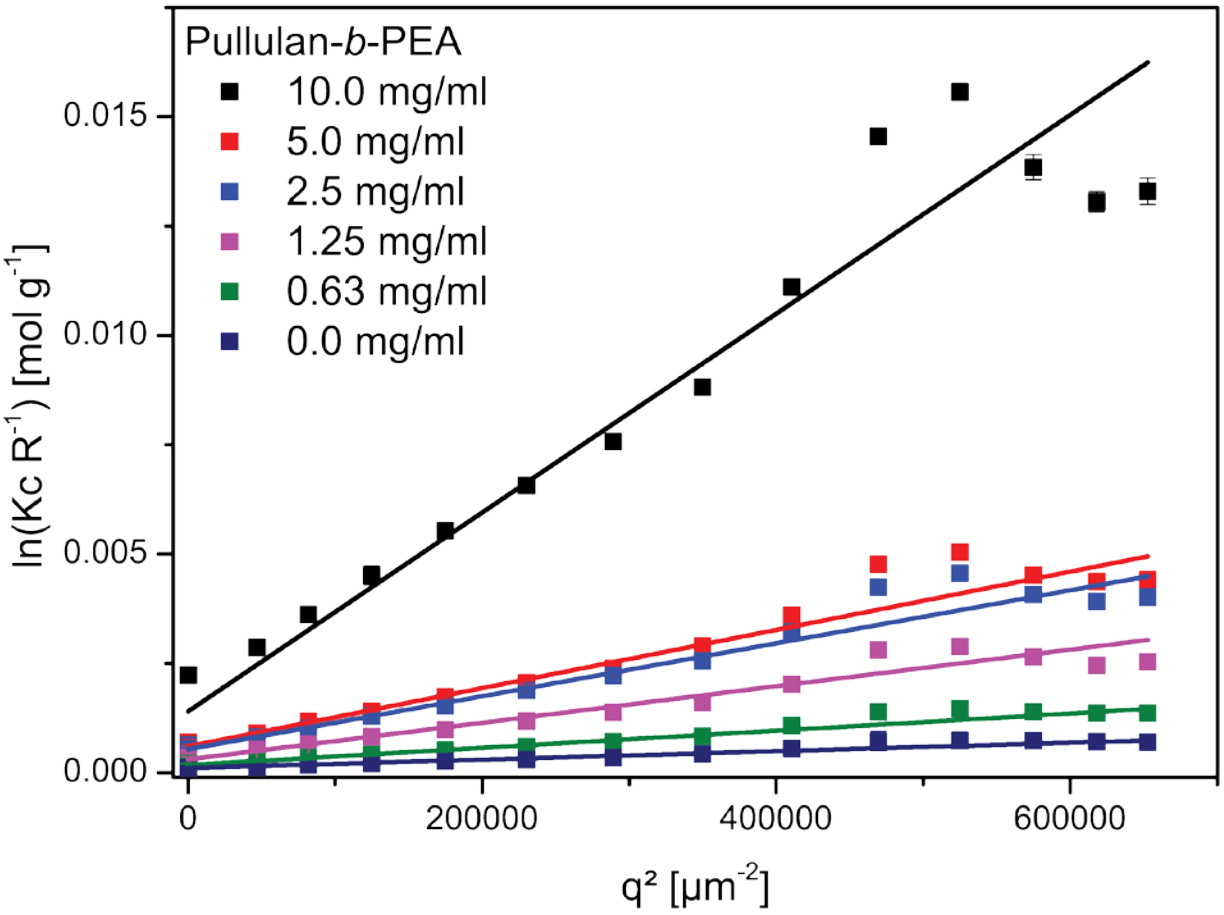

Figure S17. SLS Guinier plot of Pull-*b*-PEA with extrapolation of c→0.

Table S4. Calculated values of the Quantities of Pull-*b*-PEA determined via the Guinier plot.

Entry	Quantity	Value [unit]	Error [%]
1	M_w (c)	9.117e+06 [g/mol]	± 23.2
2	M_w (q^2)	9.117e+06 [g/mol]	± 5e-12
3	A_2	7.029e-08 [mol dm^3/g^2]	± 29.6
4	R_g	1.293e+02 [nm]	± 3e-12

1. L. Ilic, K. Jeremic and S. Jovanovic, *Eur. Polym. J.*, 1991, **27**, 1227-1229.
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4. B. V. K. J. Schmidt, M. Hetzer, H. Ritter and C. Barner-Kowollik, *Polym. Chem.*, 2012, **3**, 3064-3067.
5. B. V. K. J. Schmidt and C. Barner-Kowollik, *Polym. Chem.*, 2014, **5**, 2461-2472.