Electronic Supplementary Material (ESI) for Polymer Chemistry.

SUPPORTING INFORMATION

Synthesis of nucleobase functionalised block copolymers towards precision self-assembly

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1. Materials

Adenine (A, Sigma Aldrich ,>99%), Thymine (T, Sigma Aldrich ,>98%), Cytosine (C, Sigma Aldrich, >98%), Uracil (U, Sigma Aldrich, 99%), 2,6-Di-tert-butyl-4-methylphenol (BHT, Alfa Aesar, 99%), Potassium carbonate (K_2CO_3, Acros Organics, 99+%), Triethylamine (TEA, Acros Organics, 99%), Potassium tert-butoxide (KtBuO, Acros Organics, 98+ %), 1,4-butanediol diacrylate (BDDA, Sigma Aldrich, 90%), Formic acid (HCOOH, Thermo fisher, 97%), 1-dodecanethiol (Acros Organics, 98%) and Carbon disulphide (CS2 , Acros organics, 99.9%)were used as received. 2,2'-azobis(2-methylpropionitrile) (AIBN, Sigma-Aldrich, 98%) was recrystallized twice from methanol prior to use. poly(ethylene glycol) methyl ether acrylate (PEGMEA, Sigma-Aldrich) were deinhibited over a column of activated basic alumina prior to use.The RAFT agent 2-(dodecylthiocarbonothioylthio)propionic acid (DoPAT) was synthesised according to literature procedures.[1] All other solvents and chemicals used are obtained from commercial sources (Acros, Sigma-Aldrich and Thermo Fisher) and used as received.

2. Characterization

Nuclear Magnetic Resonance (NMR): Proton (^{1}H) NMR spectra were recorded in DMSO-d6 on a Bruker Avance III nanobay NMR spectrometer (9.4 Tesla magnet) with a 5mm broadband autotunable probe with Z-gradients and BACS 60 tube autosampler operating at 400.20 MHz. The system has variable temperature capabilities. NMR spectra are collected and analysed in MestReNova software. Monomer degradation analysis via 1H NMR spectra were recorded periodically at a range of temperatures on a Bruker Avance III nanobay NMR spectrometer equipped with a 9.4 T magnet and 5 mm BBFO probe, operating at 400.20 MHz (1H). Spectra of samples in DMSO-d6 were acquire using the standard 1H acquisition parameters: pulse program zg30 and 16 transients. ^{1}H DOSY NMR spectra were recorded at 298 K with an air flow of 400 L h^{-1} on a Bruker Avance III nanobay NMR spectrometer equipped with a 9.4 T magnet, GAB/2 gradient amplifier and 5 mm BBFO probe with z-gradient coil with maximum gradient strength of 50 G cm^{-1}, operating at 400.20 MHz (^{1}H)

Gel Permeation Chromatography (GPC):

Gel permeation chromatography (GPC) was performed on a system comprising a Shimadzu LC-20AT pump, a Shimadzu RID-20A refractive index detector, and an SPD-20A UV–visible detector. The GPC is equipped with a guard column (WAT054415) and 3× Waters GPC columns

(WAT044238, WAT044226, WAT044235, 300 mm × 7.8 mm). The eluent is DMF with 10 mM LiBr and eluted at 1 mL min^{-1} for 45 min in total. The samples were dissolved in DMF with 10 mM LiBr and filtered through 0.20 µm syringe filters. A calibration curve was obtained from poly(methylmethacrylate) (PMMA) standards (Agilent) ranging from 960 to 1 568 000 g mol^{-1}.

Dynamic light Scatering (DLS)

Particle size and size distributions of self-assembled micelles are measured using DLS. These are performed with Litesizer 500 together with Kalliope software (Anton Paar, USA) which uses a 40 mW 658 nm Laser and includes three measurement angles for particle size determination.

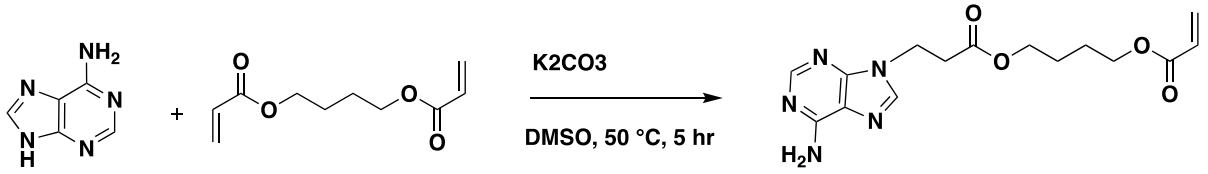
Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM) was conducted at 200 kV using a Tecnai T20 FEGTEM. TEM samples were prepared on 300 mesh continuous film copper grids at 0.1% w/w and stained with a 2% w/w uranyl acetate solution.

3. Synthetic Procedures

3.1. Synthesis of adenine acrylate monomer

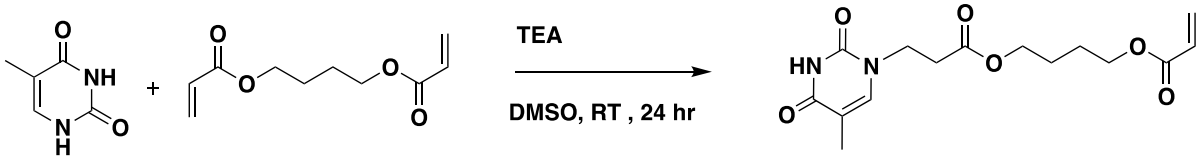
Adenine acrylate monomer (AAM) was synthesized according to literature.[2] Adenine (10.00 g, 1 eq.), K_2CO_3 (0.46 g, 0.04 eq.) and BHT (0.69 g, 0.04 eq.) were suspended in 200 mL DMSO. The mixture was heated to 50 °C and stirred for 1 h. BDDA (28.00 mL, 2 eq.) was added to the reaction mixture. After 5 h, the mixture was diluted with water (1500 mL) and washed with hexane (350 mL) to remove excess of BDDA followed by extraction with DCM (3 x 200 mL). The organic phase was dried with MgSO4, filtered and concentrated under reduced pressure. The residual mixture was purified by column chromatography using a $CHCl_3$/MeOH mixture (90/10 vol%). After removing the solvent and drying under vacuum 14.80 g of the pure product was obtained as a white solid (60%yield). 1H-NMR (600 MHz, DMSO-d6, ppm) δ 8.20 (s, 1H), 8.16 (s, 1H), 7.25(broad singlet, 2H), 6.36 (dd, J = 17.3, 1.7 Hz, 1H), 6.21 (dd, J = 17.3, 10.3 Hz,531H), 5.99 (dd, J = 10.3, 1.6 Hz, 1H), 4.44 (t, J = 6.7 Hz, 2H), 4.13–4.07 (m, 4H),3.02 (t, J = 6.8 Hz, 2H), 1.62 (m, 4H).



Scheme S1: *Reaction scheme for the synthesis of AAM*

3.2. Synthesis of thymine acrylate monomer

Thymine acrylate monomer was synthesized according to literature[2].Thymine (1.00 g, 1 eq.), TEA (0.22 mL, 0.20 eq.) and BHT (0.06 g, 0.04 eq.) were suspended in 20 mL DMSO. The mixture was stirred for 1 h at room temperature. BDDA (3.00 mL, 2 eq.) was added and the reaction mixture was stirred for 24 h. Then, the mixture was diluted with water (150 mL) and washed with hexane (35mL) to remove excess of BDDA followed by extraction with DCM (3 x 20 mL). The organic phase was dried with $MgSO_4$, filtered and concentrated under reduced pressure. The residual mixture was purified by column chromatography using a $CHCl_3$/MeOH mixture (95/5 vol%). After removing of the solvent and drying under vacuum 1.75 g of the pure product was obtained as a white solid (68% yield). 1H-NMR (600 MHz, DMSO-d6, ppm) δ 11.25 (broad singlet, 1H), 7.49 (d, J = 1.2 Hz,1H), 6.30 (dd, J = 17.3, 1.6 Hz, 1H), 6.18 (dd, J = 17.3, 10.3 Hz, 1H), 5.94 (dd,J = 10.3, 1.6 Hz, 1H), 4.11–4.05 (m, 4H), 3.84 (t, J = 6.8 Hz, 2H), 2.69 (t, J =6.8 Hz, 2H), 1.73 (d, J = 1.1 Hz, 3H), 1.62 (m, 4H).

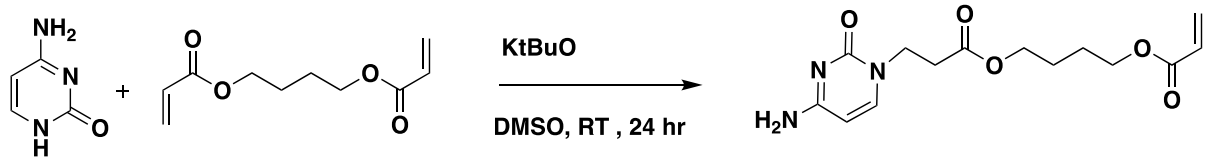


Scheme S2: *Reaction scheme for the synthesis of TAM*

3.3. Synthesis of cytosine acrylate monomer

Cytosine acrylate monomer was synthesized according to an adapted literature procedure. [2] Cytosine (4.00 g, 1 eq.), KtBuO (0.16 g, 0.04 eq.) and BHT (0.32 g,0.04 eq.) were suspended in 80 mL DMSO. The mixture was stirred for 1h at room temperature. BDDA (14.00 mL, 2 eq.) was added, and the reaction mixture was stirred for 24 h. Then, the mixture was diluted with water (600 mL) and washed with hexane (100 mL) to remove excess of BDDA followed by extraction with DCM (3 x 80 mL). The organic phase was dried with $MgSO_4$, filtered and concentrated under reduced pressure. The residual mixture was purified by column chromatography using a $CHCl_3$/MeOH mixture (93/7 vol%). After removing of the solvent and drying under vacuum 4.63 g of the pure product was obtained as a white solid (41% yield). 1H-NMR (600 MHz, DMSO-d6, ppm) δ 7.54 (d, J = 7.2 Hz,1H), 6.95 (broad doublet, J = 33.4 Hz,

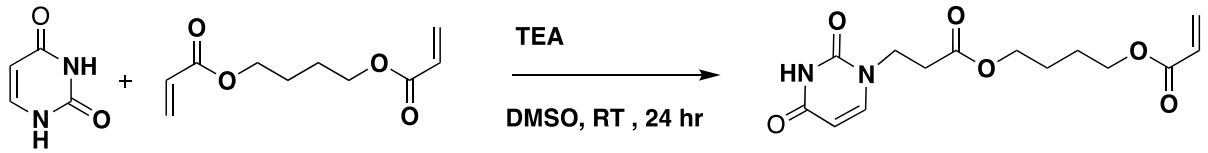
2H), 6.30 (dd, J = 17.3, 1.6 Hz, 1H), 6.16(dd, J = 17.3, 10.3 Hz, 1H), 5.93 (dd, J = 10.3, 1.6 Hz, 1H), 5.63 (d, J = 7.2 Hz,1H), 4.11–4.04 (m, 4H), 3.83 (t, J = 6.7 Hz, 2H), 2.67 (t, J = 6.7 Hz, 2H), 1.61 (m, 4H).



Scheme S3*: Reaction scheme for the synthesis of CAM*

3.4. Synthesis of uracil acrylate monomer

Uracil acrylate monomer was synthesized according to an adapted literature procedure. [2]Uracil (6.00 g, 1 eq.), TEA (1.50 mL, 0.20 eq.) and BHT (0.40 g,0.03 eq.) were suspended in 120 mL DMSO. The mixture was stirred for 1 h at room temperature. BDDA (20.00 mL, 2 eq.) was added, and the reaction mixture was stirred for 24 h. Then, the mixture was diluted with water (900 mL) and washed with hexane (150 mL) to remove excess of BDDA followed by extraction with DCM (4 x 100 mL). The organic phase was dried with MgSO4, filtered, and concentrated under reduced pressure. The residual mixture was purified by column chromatography using a $CHCl_3$/MeOH mixture (97/3 vol%). After removing of the solvent and drying under vacuum 8.63 g of the pure product was obtained as a white solid (52% yield). 1H-NMR (600 MHz, DMSO-d6, ppm) δ 11.26(broad singlet, 1H), 7.58 (d, J = 7.9 Hz, 1H), 6.30 (dd, J = 16.8, 1.6 Hz, 1H),6.16 (dd, J = 17.3, 10.2 Hz, 1H), 5.93 (dd, J = 10.3, 1.6 Hz, 1H), 5.52 (d, J =7.8 Hz, 1H), 4.11–4.04 (m, 4H), 3.88 (t, J = 6.8 Hz, 2H), 2.69 (t, J = 6.7 Hz, 2H),1.63 (p, J = 2.6 Hz, 4H).

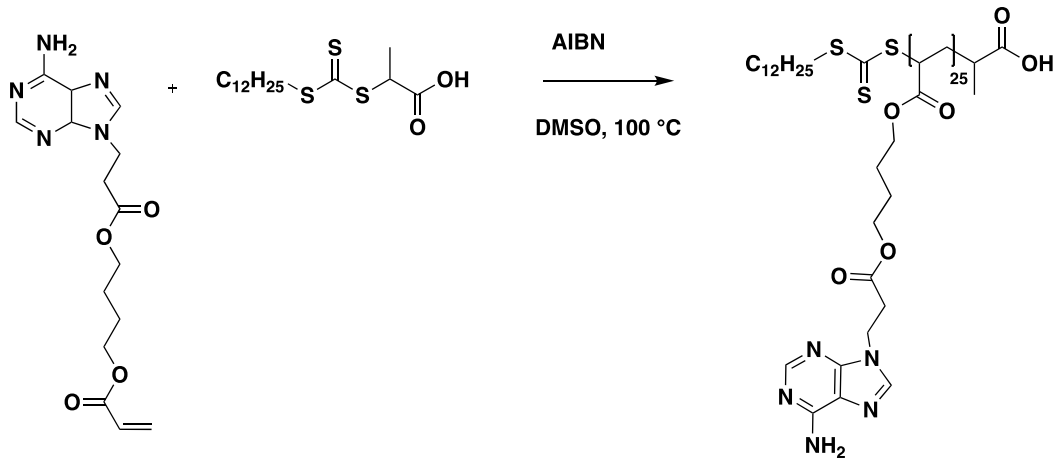


Scheme S4*: Reaction scheme for the synthesis of UAM*

3.5. Synthesis of poly(adenine acrylate)

For the synthesis of poly (adenine acrylate) (pAA) 3.0 mmol (1.00 g, 25 eq.) of the monomer AAM, 0.01 mmol (1.0 mg, 0.05 eq.) of AIBN,0.12 mmol (0.042 g, 1 eq.) of DoPAT RAFT agent

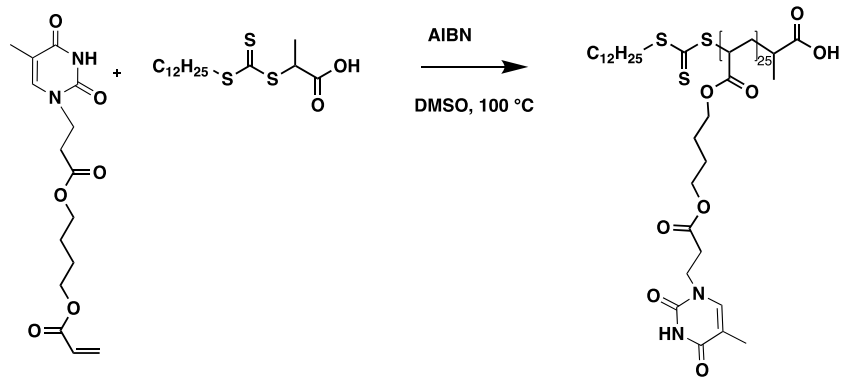
and 6 mL of DMSO were added into a glass vial with a magnetic stirrer. The glass vial was sealed with a rubber septum. The solution was degassed for 10 min with Ar and the mixture was subsequently reacted in for 45 min at 100 °C. Next, the polymerisation was quenched by exposure to ambient air after which the polymer was precipitated in water and centrifuged. The supernatant was discarded, and the polymer dried under high vacuum. The conversion was calculated by NMR to be 86%.



Scheme S5*: Reaction scheme for the synthesis of pAA25*

3.6. Synthesis of poly (thymine acrylate)

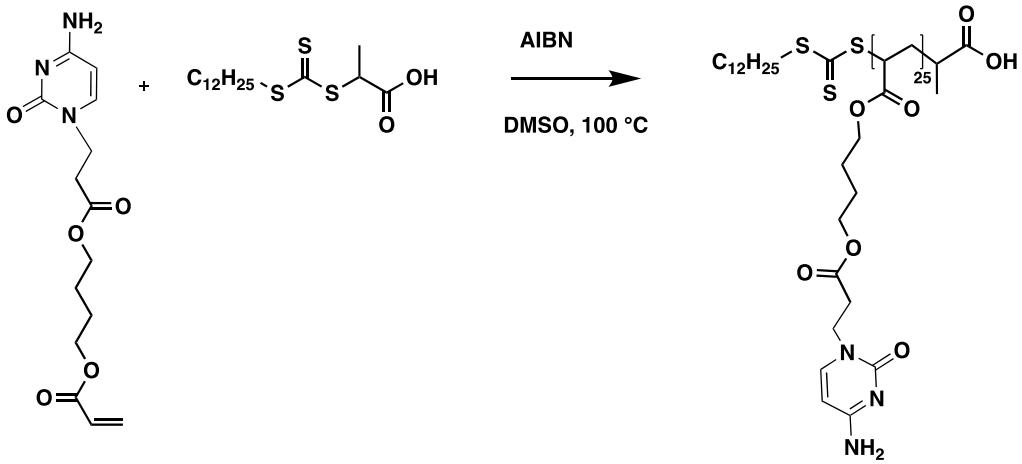
For the synthesis of poly (thymine acrylate) (pTA) 3.0 mmol (1.00 g, 25 eq.) of the monomer AAM, 0.01 mmol (1.0 mg, 0.05 eq.) of AIBN, 0.12 mmol (0.043 g, 1 eq.) of DoPAT RAFT agent and 6 mL of DMSO were added into a glass vial with a magnetic stirrer. The glass vial was sealed with a rubber septum. The solution was degassed for 10 min with Ar and the mixture was subsequently reacted for 45 min at 100 °C. Next, the polymerisation was quenched by exposure to ambient air after which the polymer was precipitated in water and centrifuged. The supernatant was discarded, and the polymer dried under high vacuum. The conversion calculated by NMR was 88%.



Scheme S6*: Reaction scheme for the synthesis of pTA25*

3.7. Synthesis of poly(cytosine acrylate)

For the production of poly (cytosine acrylate) (pCA) 3.2 mmol (1.00 g, 25 eq.) of the monomer CAM, 0.01 mmol (2.0 mg, 0.1 eq.) of AIBN,0.12 mmol (0.045 g, 1 eq.) of DoPAT RAFT agent and 6 mL of DMSO were added into a glass vial with a magnetic stirrer. The glass vial was sealed with a rubber septum. The solution was degassed for 10 min with Ar and the mixture was subsequently reacted in for 45 min at 100 °C. Next, the polymerisation was quenched by exposure to ambient air after which the polymer was precipitated in water and centrifuged. The supernatant was discarded, and the polymer dried under high vacuum. The conversion calculated by NMR was 93%.

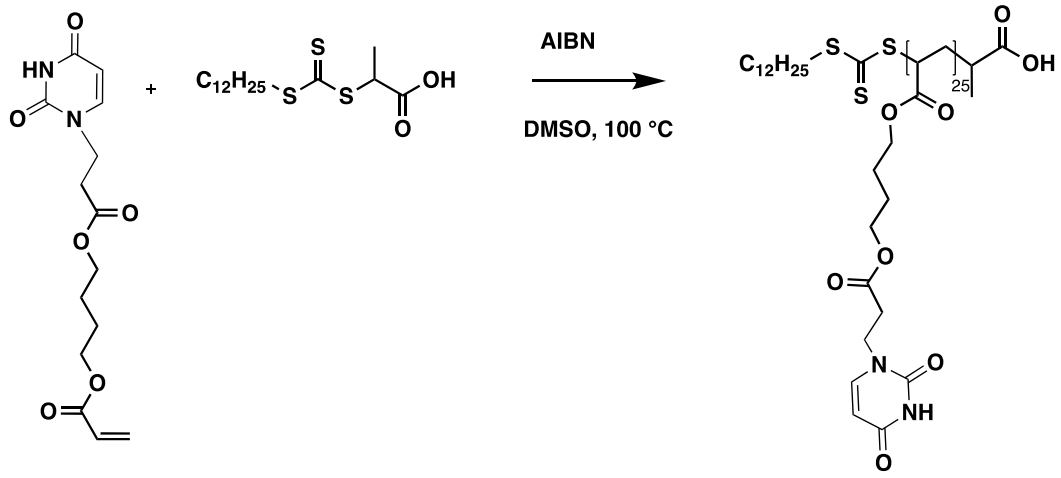


Scheme S7*: Reaction scheme for the synthesis of pCA25*

3.8. Synthesis of poly(uracil acrylate)

For the synthesis of poly (uracil acrylate) (pUA) 3.2 mmol (1.00 g, 25 eq.) of the monomer UAM, 0.01 mmol (2.0 mg, 0.1 eq.) of AIBN,0.12 mmol (0.045 g, 1 eq.) of DoPAT RAFT agent

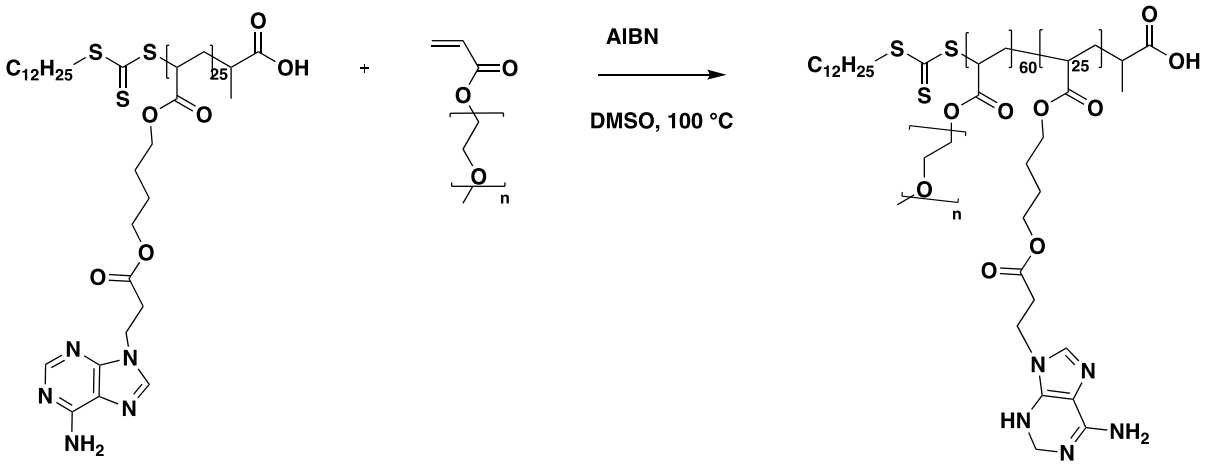
and 6 mL of DMSO were added into a glass vial with a magnetic stirrer. The glass vial was sealed with a rubber septum. The solution was degassed for 10 min with Ar and the mixture was reacted for 45 min at 100 °C. Next, the polymerisation was quenched by exposure to ambient air after which the polymer was precipitated in water and centrifuged. The supernatant was discarded, and the polymer dried under high vacuum. The conversion calculated by NMR was 85%.



***Scheme S8**: Reaction scheme for the synthesis of pUA25*

3.9. Synthesis of pAA-b-pPEGMEA

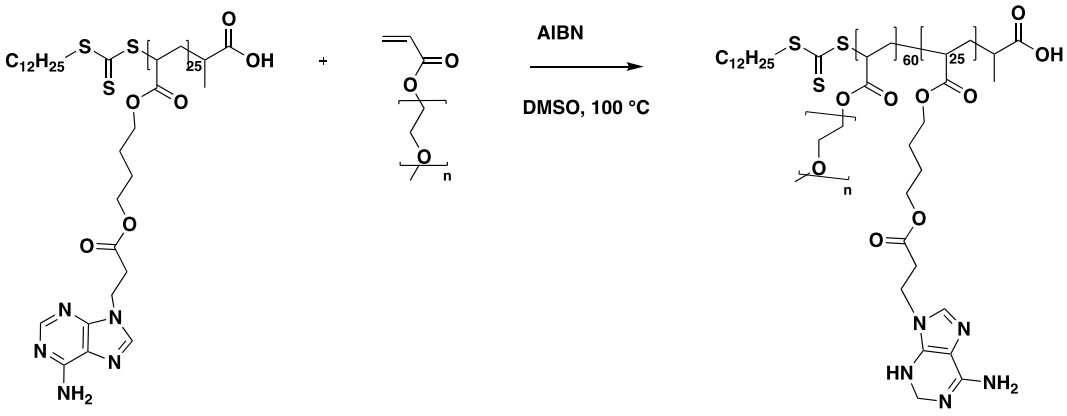
For the synthesis of pAA-b-pPEGMEA 0.04 mmol (0.250 g, 1 eq.) of the macro-RAFT agent pAA, 0.002 mmol (0.3 mg, 0.05 eq.) of AIBN, 2.63 mmol (1.2671 g, 60 eq.) of PEGMEA monomer and 5 mL of DMSO were added into a glass vial with a magnetic stirrer. The glass vial was sealed with a rubber septum. The solution was degassed for 10 min with argon and the mixture was subsequently reacted for 45 min at 100 °C. Next, the polymerisation was quenched by exposure to ambient air after which the polymer was dried under high vacuum. The conversion calculated by NMR was 87%.



***Scheme S9**: Reaction scheme for the synthesis of pAA25-b-PEG60*

3.10. Synthesis of pTA-b-pPEGMEA

For the synthesis of pTA-b-pPEGMEA 0.03 mmol (0.250 g, 1 eq.) of the macro-RAFT agent pTA, 0.0007 mmol (0.1 mg, 0.1 eq.) of AIBN, 1.76 mmol (0.847 g, 60 eq.) of PEGMEA monomer and 3.5 mL of DMSO were added into a glass vial with a magnetic stirrer. The glass vial was sealed with a rubber septum. The solution was degassed for 10 min with argon and the mixture was subsequently reacted for 45 min at 100 °C. Next, the polymerisation was quenched by exposure to ambient air after which the polymer was dried under high vacuum. The conversion calculated by NMR was 93%.

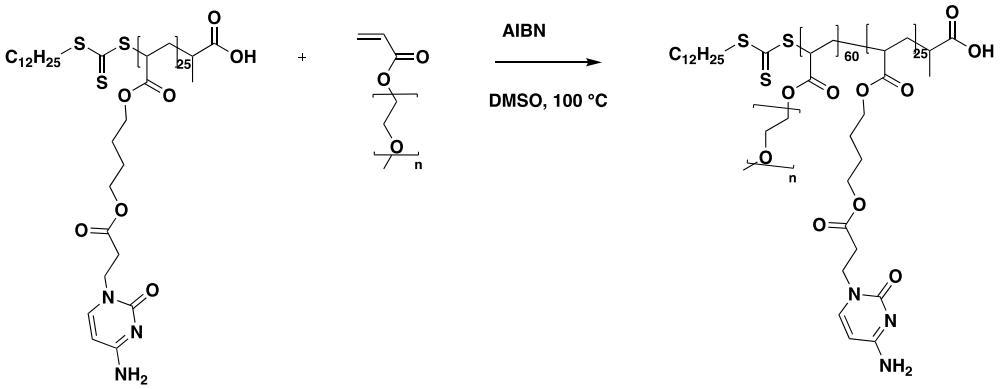


***Scheme S10**: Reaction scheme for the synthesis of pTA25-b-PEG60*

3.11. Synthesis of pCA-b-pPEGMEA

For the production of pCA-b-pPEGMEA 0.04 mmol (0.25 g, 1 eq.) of the macro-RAFT agent pCA, 0.0007 mmol (0.1 mg, 0.1 eq.) of AIBN, 2.53 mmol (1.21 g, 60 eq.) of PEGMEA monomer

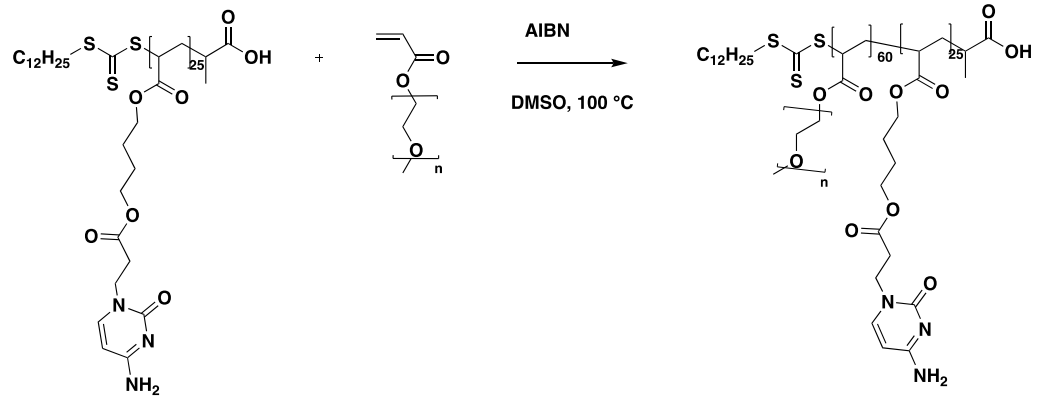
and 3.5 mL of DMSO were added into a glass vial with a magnetic stirrer. The glass vial was sealed with a rubber septum. The solution was degassed for 10 min with Ar and the mixture was subsequently reacted for 45 min at 100 °C. Next, the polymerisation was quenched by exposure to ambient air after which the polymer was dried under high vacuum. The conversion calculated by NMR was 91%.



Scheme S11*: Reaction scheme for the synthesis of pCA25-b-PEG60*

3.12. Synthesis of pUA-b-pPEGMEA

For the production of pUA-b-pPEGMEA 0.05 mmol (0.25 g, 1 eq.) of the macro-RAFT agent pUA, 0.0007 mmol (0.1 mg, 0.1 eq.) of AIBN, 3.2 mmol (1.53 g, 60 eq.) of PEGMEA monomer and 3.2 mL of DMSO were added into a glass vial with a magnetic stirrer. The glass vial was sealed with a rubber septum. The solution was degassed for 10 min with argon and the mixture was subsequently reacted for 45 min at 100 °C. Next, the polymerisation was quenched by exposure to ambient air after which the polymer was dried under high vacuum. The conversion calculated by NMR was 92%.



Scheme S12*: Reaction scheme for the synthesis of pUA25-b-PEG60*

4. Micelle particle formation

Micelle formation was performed in a flow reactor.[3] The custom-build design consisted of PFA tubing (2 mL internal volume, 1 mm internal diameter) connected to a static mixing tee. Syringe pumps were used to pump 2 reactor solutions. One syringe contained 10 $mg \cdot mL^{-1}$ block copolymer in DMF operated at 0.2 $mL \cdot min^{-1}$ while the other held pure deionised water that was pumped at a flow rate of 1.8 $mL \cdot min^{-1}$. The reactor was stabilized for 2 minutes before particle samples were collected over a 1-minute time period. Sample distributions were then measured on DLS.

The complementary and non-complementary polymer blends were prepared by mixing equimolar ratios of pAA25-b-PEG60 with either pTA25-b-PEG60 or pCA25-b-PEG60 and stirring overnight to allow H bonding formation. The final polymer concentration was kept at 10 $mg \cdot mL^{-1}$.

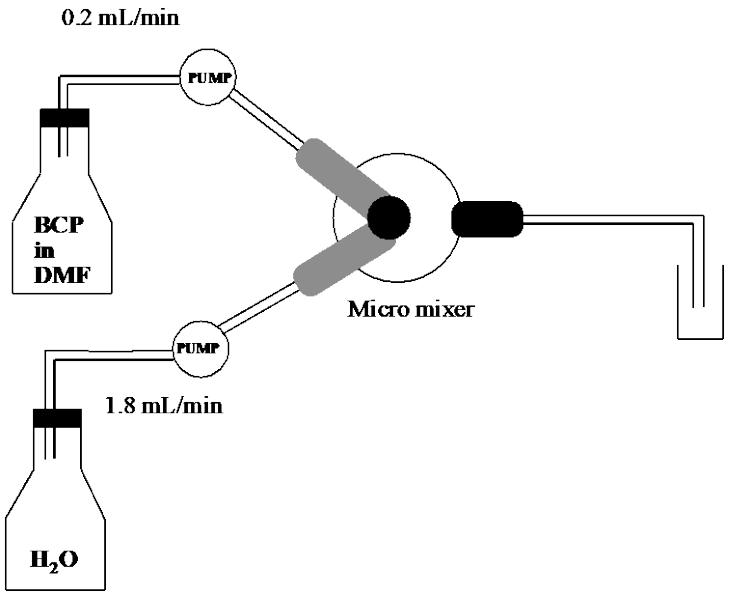


Figure S1*: Flow set up for micelle formation*

5. S4 Characterization

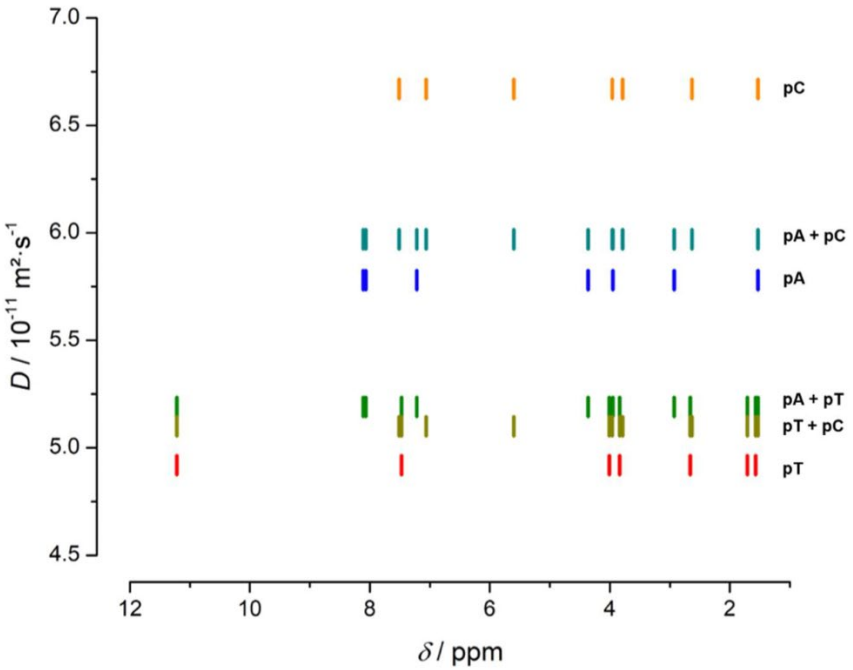


Figure S2:*Comparison of DOSY NMR spectra of nucleobase functionalized acrylates and their blends.*

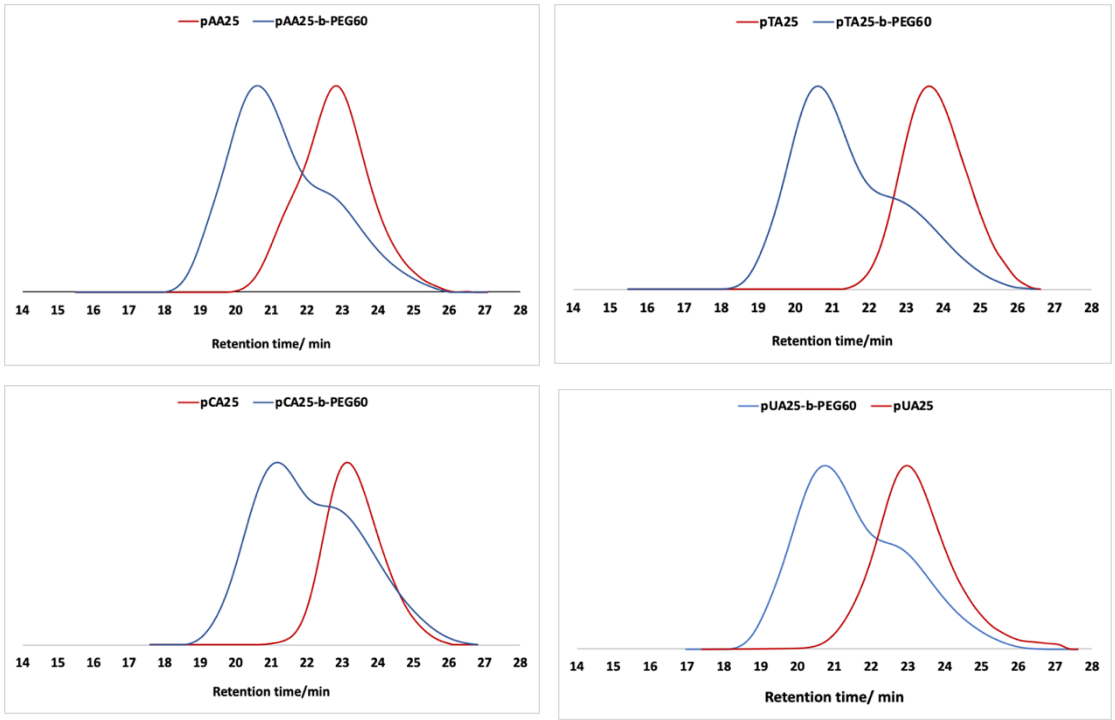


Figure S3*: SEC elugrams of pAA25, pTA25, pCA25 and pUA25 after chain extension with PEGMEA*

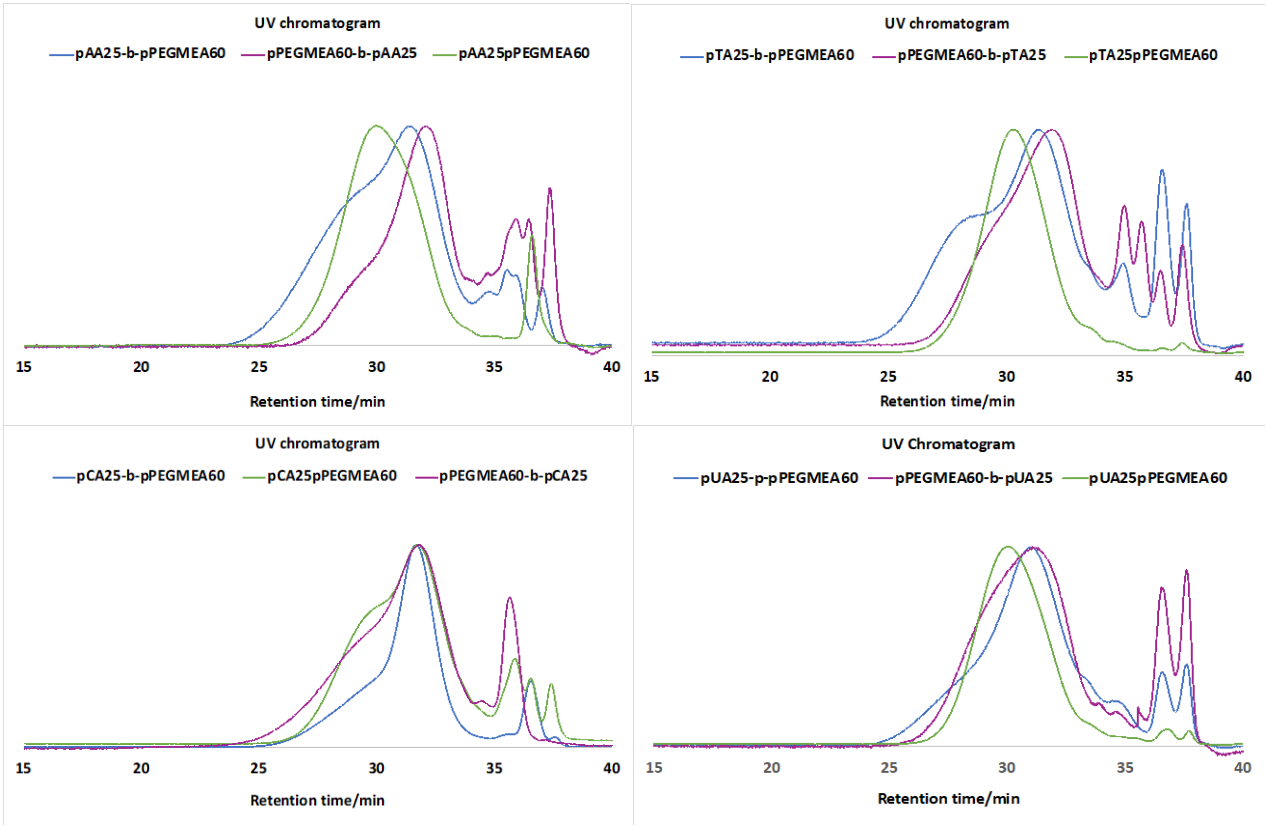


***Figure S4**: UV chromatograms for SEC for the block copolymers*

***Table S1**: Kinetic profile of the block copolymers synthesized in one pot*

Polymer	Time/min	Conversion (%)	MW SEC/ g/mol	PDI
pAA25	10	29.9	5443	1.12
	20	58.1	7362	1.11
	30	67.5	7741	1.10
	40	73.5	8086	1.11
pAA25-b-PEGMEA60	10	61.5	9909	1.23
	20	75.3	9152	1.24
	30	81.5	12303	1.23
	40	84.6	12459	1.30
pTA25	10	32.7	7678	1.16
	20	41.3	8097	1.18
	30	52.4	8399	1.18
	40	68.2	8680	1.17
pTA25-b-PEGMEA60	10	30.7	10797	1.24
	20	53.8	10986	1.27
	30	66.9	10981	1.27
	40	71.5	12615	1.29
pCA25	10	61.4	6388	1.10
	20	70.3	6576	1.11
	30	74.3	6444	1.14
	40	78.2	6761	1.14
pCA25-b-PEGMEA60	10	58.5	9863	1.31
	20	72.7	9794	1.31
	30	79.8	10027	1.32
	40	83.1	10277	1.38
pUA25	10	61.5	5458	1.13
	20	75.1	5818	1.13
	30	80.7	6000	1.13
	40	87.5	6293	1.14
pUA25-b-PEGMEA60	10	47.2	10620	1.25
	20	67.4	11154	1.27
	30	72.9	11306	1.29
	40	79.5	11057	1.32

***Table S2**: Summary of the DLS data obtained for different block copolymers*

Polymer	Intensity D_{avg}/nm	Volume D_{avg}/nm	Number D_{avg} /nm	Dispersity
1-pAA25-b-PEG60	72.45	49.41	38.93	0.19
2-pTA25-b-PEG60	86.07	103.98	74.31	0.13
3-pCA25-b-PEG60	271.14	315.12	220.9	0.25
4-pUA25-b-PEG60	153.33	122.22	100.38	0.21
5-pAA25-b-PEG60 + pTA25-b-PEG60	64.62	76.06	56.68	0.06
6-pAA25-b-PEG60 + pCA25-b-PEG60	114.91	101.97	91.78	0.12
7-PEG60-b-pAA25	59.82	42.94	35.09	0.23
8-PEG60-b-pTA25	104.04	85.67	73.75	0.15
9-PEG60-b-pCA25	353.82	245.00	232.72	0.26
10-PEG60-b-pUA25	125.37	114.67	105.42	0.12
11- PEG60-b-pAA25 + PEG60-b-pTA25	89.00	75.39	66.09	0.10
12-PEG60-b-pAA25 + PEG60-b-pCA25	127.11	107.20	93.11	0.12
13-pAA25-b-PEG60 one pot	95.22	51.45	34.85	0.23
14-pTA25-b-PEG60 one pot	71.63	61.00	53.58	0.15
15-pCA25-b-PEG60 one pot	241.07	179.31	199.51	0.18
16-pUA25-b-PEG60 one pot	121.95	105.66	93.67	0.17
17-pAA25-b-PEG60 + pTA25-b-PEG60 one pot	81.35	59.56	48.46	0.21
18-pAA25-b-PEG60 + pCA25-b-PEG60 one pot	133.03	108.86	92.72	0.21

***Table S3**: Overview of the number average diameter and dispersity for various nucleobase-PEGMEA block copolymer blends.*

Polymer	Mixing ratio	Number D_{avg} / nm	PDI
1+2	1:1	56.68	0.06
1+2	1:2	51.46	0.21
1+2	1:3	54.29	0.19
1+2	1:4	55.22	0.21
1+2	1:5	55.23	0.21
1+3	1:1	91.72	0.12
1+3	1:2	109.73	0.12
1+3	1:3	113.55	0.15
1+3	1:4	131.79	0.19
1+3	1:5	183.64	0.24

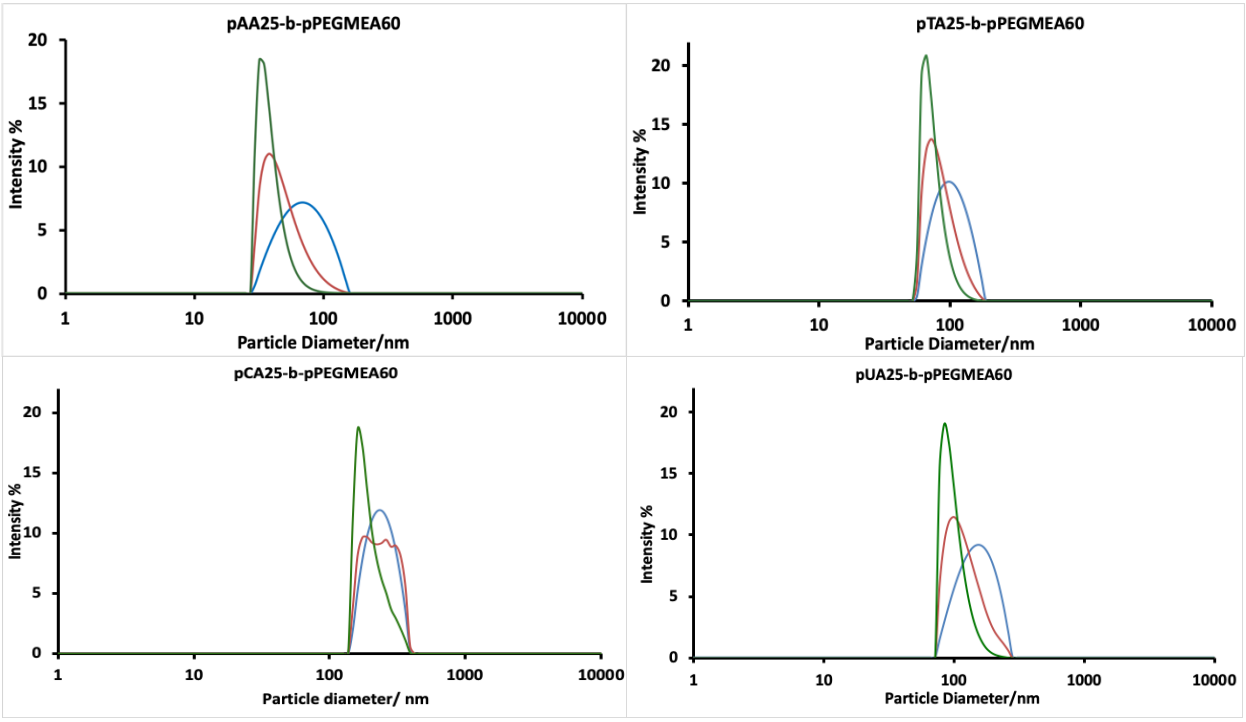


***Figure S5**: DLS results for BCPs where the nucleobase containing block is synthesized first. Showing the Intensity in (Blue), number (green) and volume (red)*

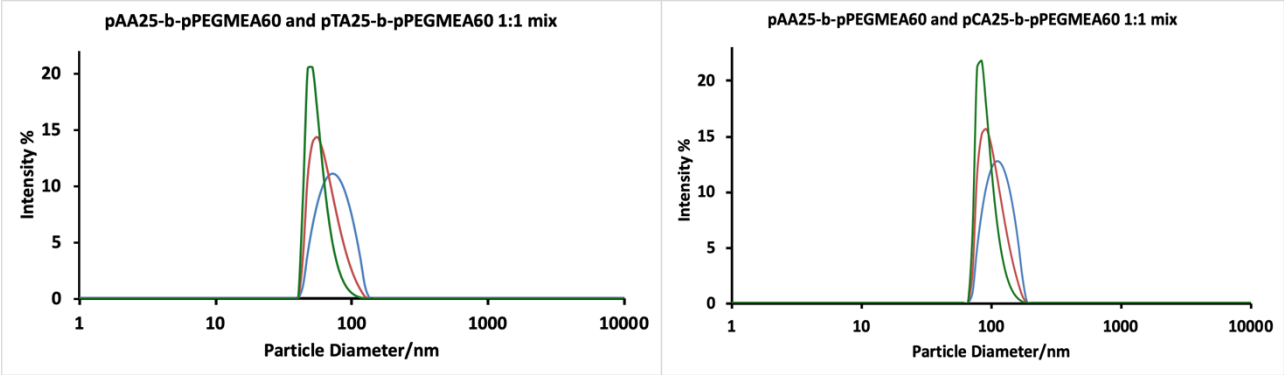


***Figure S6**: DLS results for 1:1 BCP blends of pAA25-b-pPEGMEA60 with pTA25-b-pPEGMEA60 and pCA25-b-pPEGMEA60 Showing the Intensity in (Blue), number (green) and volume (red)*

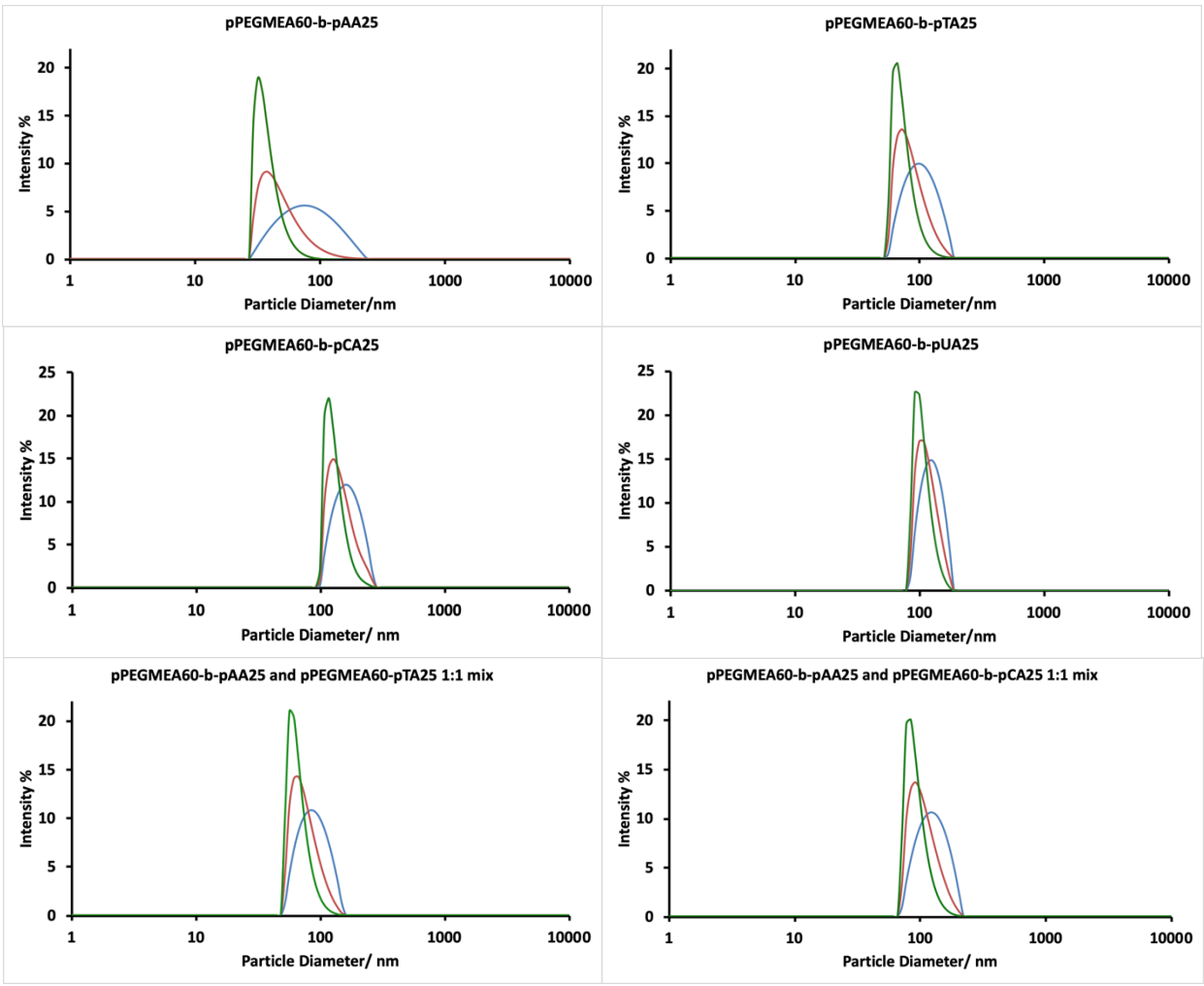


***Figure S7**: DLS results for BCPs where the PEGMEA block is synthesized first. Showing the Intensity in (Blue), number (green) and volume (red)*

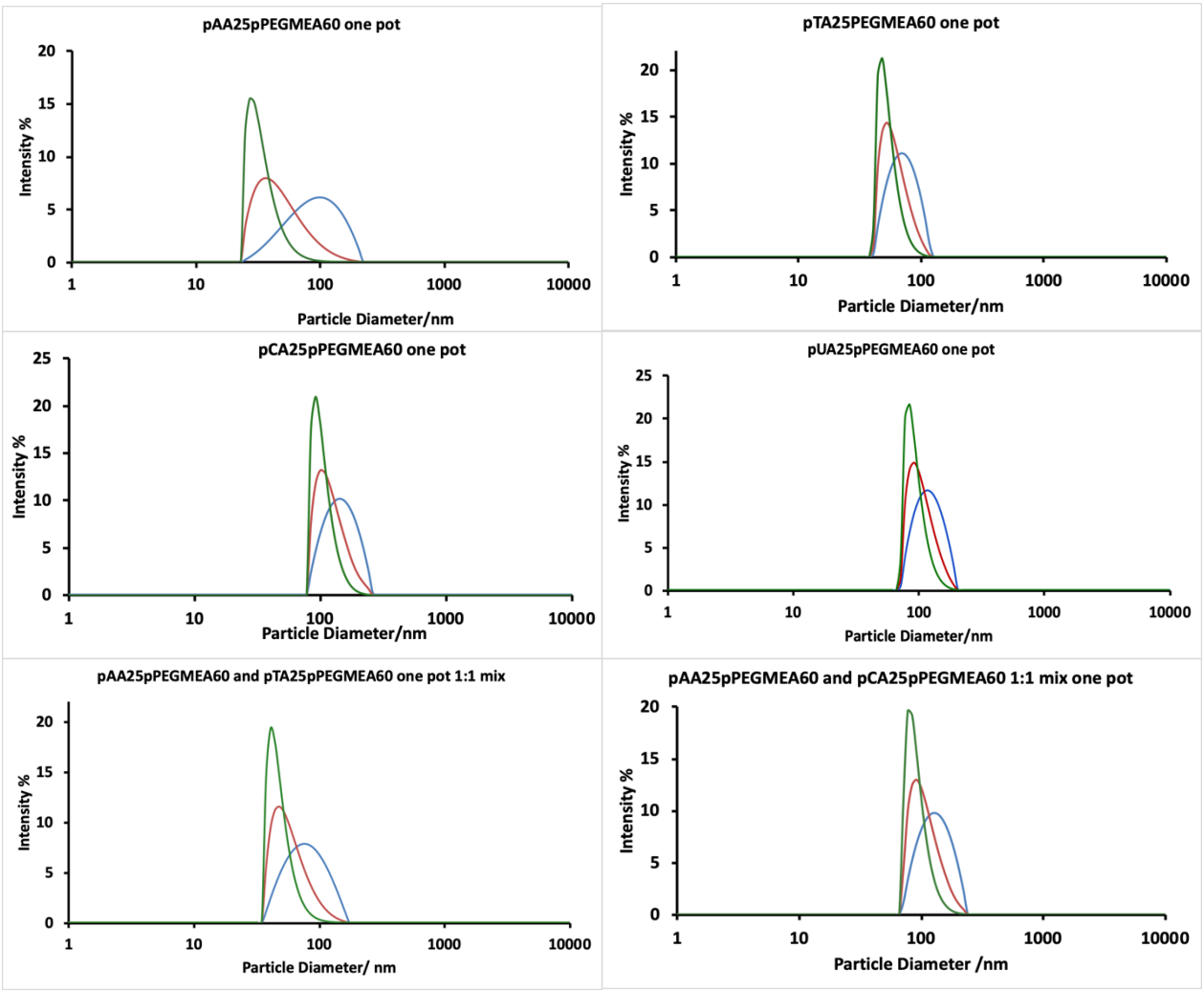


***Figure S8**: DLS results for BCPs synthesized in one pot. Showing the Intensity in (Blue), number (green) and volume (red)*

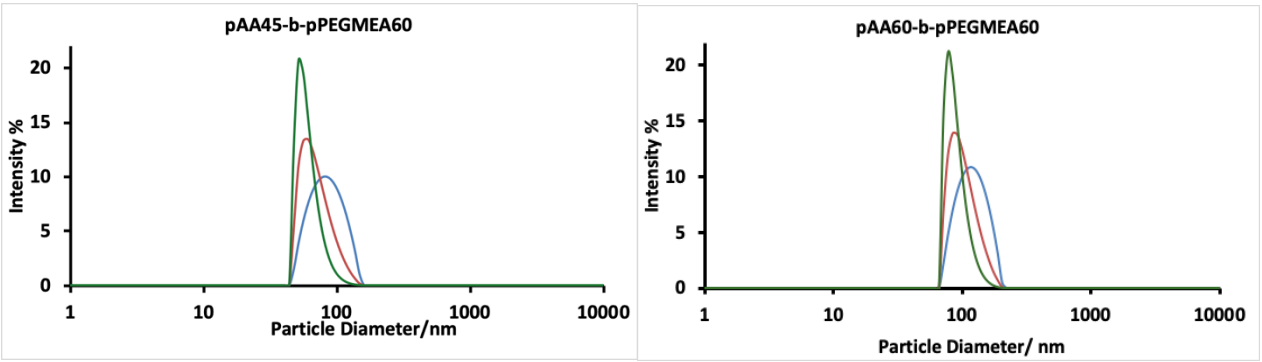


***Figure S9**: DLS results for pAA45-b-pPEGMEA60 and pAA60-b-pPEGMEA60 Showing the Intensity in (Blue), number (green) and volume (red)*

***Table S4**: DLS results of polymers at elevated temperatures*

Polymer	Diameter at 40 °C/ nm				Diameter at 80 °C/ nm			
	I	V	N	PDI	I	V	N	PDI
1	132.92	80.05	58.15	0.23	153.58	5.18	4.91	0.24
2	174.23	158.61	143.38	0.06	167.08	1.45	1.40	0.28
3	133.48	6.15	5.82	0.23	137.09	3.76	3.5	0.23
4	137.74	122.21	110	0.07	158.73	8.42	6.89	0.28
5	117.64	95.03	80.34	0.15	164.40	136.04	113.92	0.10
6	165.58	119.65	90.22	0.23	130.61	112.48	99.62	0.12

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