Electronic Supplementary Information

Unique Alternating Peptide-Peptoid Copolymers from Dipeptides via Ugi Reaction in Water.

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Experimental section.

Materials. Glycyl-glycine (gly-gly) (≥99%), glycyl-L-alanine (gly-ala) (≥99%), L-alanyl-glycine (ala-gly) , formaldehyde (HCHO) (37 wt. % in water), *tert*-butyl isocyanide (tBuNC) (98%), acrylic acid (99%), triethylamine (≥99%), succinic anhydride (≥99%), diethyl ether (Et_2O), AIBN (98%), methanol (MeOH), dimethylformamide (DMF) were purchased from Sigma-Aldrich (Belgium). Technical diethylether (Et_2O) was purchased from VWR. All chemicals and solvents were used without purification except for DMF which dried in the laboratory. Spectra/por® dialysis tubings (cut-off, 1 kDa, 8 kDa) were obtained from SpectrumLabs.

Characterizations.

Molar masses (M_n, M_w) and dispersity ($Đ$) of the copolymers were characterized by size exclusion chromatography (SEC) at 55 °C in dimethylformamide (DMF) containing LiBr (0.025 M) using a flow rate of 1 mL min^{-1} with a polystyrene calibration. SEC curves were recorded with a Waters chromatograph equipped with three columns (Waters Styragel pss gram 1000 Å (×2), 30 Å), a dual λ absorbance detector (Waters 2487) and a refractive index detector (Waters 2414).

^{1}H NMR and COSY spectra were recorded at 298 K with a Bruker spectrometer (400 MHz) and treated with MestraNova software. Termogravimetric analyses were performed with Hi-Res TGA Q500 from TA Instruments at the heating rate of 20 °C/min under nitrogen. Differential scanning calorimetry was performed using a DSC Q500 (TA Instruments) calibrated with indium. The sample is introduced in the calorimeter at 20 °C and is cooled down to -60 °C. Then, a temperature ramp (10 K min^{-1}) is applied up to 120 °C. Then, the sample is cooled using a temperature ramp (10 K min^{-1}) till –60 °C and heated again with a temperature ramp (10 K min^{-1}) to 200 °C. Dynamic light scattering (DLS) was used to obtain the average size of the particles by using a Delsa NanoC instrument with 165° scattering angle at 5 °C to 45

°C. 10 mg of bloc copolymer was dissolved in 1 mL of water. Three measurements were conducted and averaged for all the samples. Turbidity was determined by measuring the absorbance at 260 nm on a un Jasco v-630 spectrophotometer. The copolymer (P_1 and P_7) were dissolved in water milliQ (10 or 2 mg/mL) and subjected to heating-cooling cycles in a quartz cuvette at a constant rate of 2 °C/min in a temperature controlled multicell holder.

The Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-ToF) mass spectrum was recorded using a Waters QToF Premier mass spectrometer equipped with a Nd:YAG laser using the 3rd harmonic with a wave length of 355 nm. In the context of this study, a maximum output of ~65 J is delivered to the sample in 2.2 ns pulses at 50 Hz repeating rate. Time-of-flight mass analyses were performed in the reflection mode at a resolution of about 10 000. The matrix, α-cyano-4-hydroxycinnamic acid (α-cyano), was prepared as a saturated solution in acetone. The matrix solution (1 µL) was applied to a stainless steel target and air-dried. Polymer samples were dissolved in Methanol:Acetonitrile (1:9) to obtain 1 mg/mL solutions and 20 µL of NaI solution (2 mg/mL in acetonitrile) are added as source of cationization agent. Then, 1 µL aliquots of these solutions were applied onto the target area (already bearing the matrix crystals) and then air-dried.

General procedure for the synthesis of the poly(peptoid-*alt*-peptide) copolymer ($P_{1\text{-}3}$). In a typical experiment, a dipeptide (gly-gly, 132 mg, 1.0 mmol; gly-ala, 146 mg, 1.0 mmol, or ala-gly, 146 mg, 1.0 mmol) was dissolved in 0.62 mL of water milliQ. Formaldehyde (89 µL of a formaldehyde 37 wt% in water, 33 mg, 1.1 mmol) was added to the stirred solution at room temperature followed by addition of *tert*-butylisocyanide (150 µl, 120 mg, 1.3 mmol). During the polymerization carried out at r.t., the copolymer precipitates at the bottom of the flask and around the magnetic stirrer. After 24 h of reaction, the supernatant aqueous solution was removed and the polymer was washed with fresh milliQ water (5mL). Then, the polymer was dissolved in 0.5 mL of MeOH, precipitated in 10 mL of cold Et_2O (-20 °C) and then dried under vacuum at 50 °C.

P_1 Gly-gly based copolymer: ^{1}H NMR (DMSO, 400 MHz): δ = 1.1-1.3 ppm (d, 9H, -C(C$\underline{H}_3$)$_3$); 3.7-4.2 ppm (t, 6H, C$\underline{H}_2$); 7.7-8.8 ppm (m, 2H, N$\underline{H}$). (142 mg recovered, recovery yield= 48 %)

P_2 Gly-ala based copolymer: ^{1}H NMR (DMSO, 400 MHz): δ = 1.0-1.3 ppm (m and d, 12H, -CH-C$\underline{H}_3$ and C(C$\underline{H}_3$)$_3$); 3.7-4.2 ppm (m, 3H, -N(CH_2)$_2$); 4.3-4.4 ppm (m, 2H, 1H of -N(C$\underline{H}_2$)$_2$ and 1H of C$\underline{H}$-CH_3), δ = 7.7-8.9 ppm (m, 2H, N$\underline{H}$).). (172 mg recovered, recovery yield= 54 %)

P_3 Ala-gly based copolymer: ^{1}H NMR (DMSO, 400 MHz): δ = 1.1-1.3 ppm (d, 12H, -C(C$\underline{H}_3$)$_3$ and –CH-C$\underline{H}_3$); 3.6-4.1 ppm (m, 4H, -N-C$\underline{H}_2$-CO- and NH-C$\underline{H}_2$-); 4.3-4.9 (m, 1H, -C$\underline{H}$-CH_3), 7.5-8.8 (m, 2H, N$\underline{H}$). (184 mg recovered, recovery yield = 58 %)

General procedure for the synthesis of the poly(peptide-*alt*-peptoid) terminated by an acrylamide function (P_{4b}). Typically, the glygly dipeptide (table S1, entry 2) (119 mg, 0.9 mmol) was dissolved in 0.62 mL of water milliQ. Acrylic acid (7.2 mg, 6.9 µL, 0.1 mmol) was added to the solution. Then, formaldehyde (89 µL of a formaldehyde 37 wt% in water, 33 mg, 1.1 mmol) was added to the stirred solution at room temperature followed by addition of *tert*-butyl isocyanide (150 µl, 120 mg, 1.3 mmol). During the polymerization carried out at r.t., the copolymer precipitates at the bottom of the flask and around the magnetic stirrer. After 3 h of reaction, the supernatant aqueous solution is removed and the polymer was washed with fresh milliQ water (5 mL). The polymer was then treated as the previous one by solubilization in 0.5 mL of MeOH, precipitation in 10 mL of cold Et_2O (-20 °C) and dried under vacuum. (146 mg recovered, recovery yield= 45 %).

^{1}H NMR (DMSO, 400 MHz): δ = 1.1-1.3 ppm (d, 9H, -C(C$\underline{H}_3$)$_3$); 3.7-4.2 ppm (t, 6H, C$\underline{H}_2$); 4.4 ppm (s, 2H, CH2 in α position of terminal COOH); 5.6-6.6 (m, 3H, -C$\underline{H}$=C$\underline{H}_2$); 7.7-8.8 ppm (m, 2H, N$\underline{H}$).

Similar procedures were used for the synthesis of acrylamide-terminated poly(peptide-*alt*-peptoid) (P_{4a} and P_{4c}) using different amounts of acrylic acid (see Table S1 entries 1 and 3, respectively). (Recovery yield of P_{4a} = 32%, recovery yield of P_{4c} = 47%).

Procedure for the copolymerization of N,N-dimethylacrylamide and acrylamide-terminated poly(peptide-alt-peptoid) (P_{4a}) to form (P_5). A mixture of peptide-*alt*-peptoid copolymer (250 mg, 1800 g/mol, 0.138 mmol), N,N-DMA (124 mg, 1.25 mmol) and AIBN (6.7 mg, 0.04 mmol) were put in a tube under nitrogen atmosphere. 0.5 mL of degassed and anhydrous DMF was added by syringe trough a septum. The polymerization reaction was performed in a heated oil bath at 65 °C overnight and under inert conditions. Then, the contents in the tube were dissolved in 2 mL of MeOH, precipitated into 20 mL of cold Et_2O (-20 °C) and dried under vacuum. The precipitate was treated again by dialysis against

MeOH using cellulose tubing (molecular weight cut-off, 8000 g/mol). The copolymer P_5 was precipitated again in cold Et_2O (-20 °C) and dried under vacuum at 50 °C.

^{1}H NMR (MeOD, 400 MHz): δ = 1.2-1.4 ppm (d, 9H, -C(C$\underline{H_3}$)$_3$); 1.5-1.9 ppm (m, 2H, CH-C$\underline{H_2}$), 2.5-2.8 ppm (m, 1H, C$\underline{H}$-CH$_2$), 2.85-3.22 ppm (m, 6H, N(C$\underline{H_3}$)$_2$); 3.9-4.3 ppm (t, 6H, NC$\underline{H_2}$ and NHC$\underline{H_2}$); 4.5 ppm (s, 2H, CH2 in α position of terminal COOH).

Procedure for the synthesis of MeO-PEG$_{2000}$-CH$_2$CH$_2$OCH$_2$CH$_2$COOH (P_6). MeO-PEG$_{2000}$-OH (1g, 0.5 mmol) was dissolved in 5 mL of DMF. Et_3N (101.19 mg, 1 mmol) and succinic acid (100.07 mg, 1 mmol) were added and the mixture was stirred and heated at 45 °C overnight. The solution was concentrated by evaporation of DMF under vacuum and the copolymer was recovered by precipitation in cold diethylether (-20 °C). The polymer (P_6) was dissolved in water and filtered through a 0.2 µm Millipore filter before lyophilization.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 3.4 ppm (s, 3H, C$\underline{H_3}$O-); 3.6-3.75 ppm (s, 4H, C$\underline{H_2}$C$\underline{H_2}$O); 3.9 ppm (t, 2H, O-C$\underline{H_2}$–CH$_2$-CO$_2$H); 4.25 ppm (t, 2H, O-CH$_2$–C$\underline{H_2}$-CO$_2$H).

Procedure for the synthesis of PEG-*b*-(peptoid-*alt*-peptide) block copolymer (P_7). Glygly (119 mg, 0.9 mmol) and MeO-PEG$_{2000}$-CH$_2$CH$_2$OCH$_2$CH$_2$COOH (200 mg, 0.1 mmol) were dissolved in 1 mL of water milliQ. Formaldehyde (89 µL of a formaldehyde 37 wt% in water, 33 mg, 1.1 mmol) was added to the stirred solution at room temperature as previously followed by *tert*-butyl isocyanide (150 µl, 120 mg, 1.3 mmol). After 24h, the reaction medium was dialyzed against MeOH using cellulose tubing (molecular weight cut-off, 1000 g/mol). The copolymer was precipitated in cold Et_2O (-20 °C) and dried under vacuum at 50 °C.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.1-1.5 ppm (s, 9H, -C(C$\underline{H_3}$)$_3$); 3.4 ppm (s, 3H, C$\underline{H_3}$O-); 3.5-3.7 ppm (s, 4H, OC$\underline{H_2}$C$\underline{H_2}$O); 3.8-4.3 ppm (m, 6H, N-C$\underline{H_2}$ and NH-C$\underline{H_2}$ and 2H COCH$_2$C$\underline{H_2}$CO$_2$); 4.5 ppm (s, 2H, C$\underline{H_2}$ in α position of terminal COOH).

Table S1. Single step synthesis of functional poly(peptoid-*alt*-peptide)s and PEO-*b*-poly(peptoid-*alt*-peptide)s block copolymer.

Entry	Polymer	Gly-Gly /HCHO/ *t*BuNC/AA (mmol)	Time (h) / H_2O (mL)	M_w [a] (g.mol^{-1})	M_n [a] (g.mol^{-1})	$Đ$ [a]	M_n [b] (g.mol^{-1})
1	P_{4a}	0.80/1.1/1.3/0.2	3 / 0.62	3600	2700	1.32	2000
2	P_{4b}	0.90/1.1/1.3/0.1	3 / 0.62	4900	3200	1.53	3700
3	P_{4c}	0.95/1.1/1.3/0.05	3 / 0.62	6600	3900	1.70	4200
4	P_7	0.90/1.1/1.3/0.1	24 / 0.80	5400	4100	1.31	4400

Conditions. Reactions were carried out at room temperature in water. [a] Measured by SEC in DMF with a PS calibration. [b] Measured by NMR.

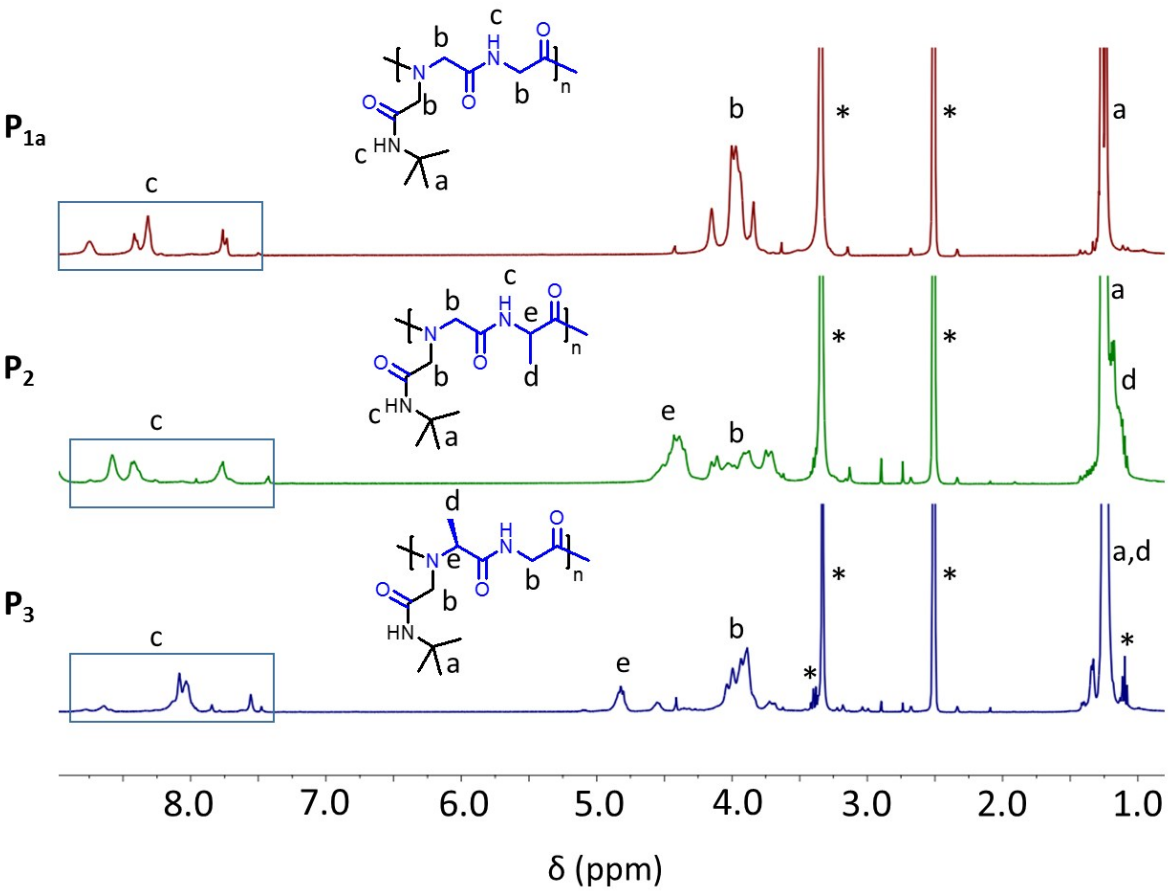

Figure S1. ^{1}H NMR spectra of alternating poly(peptide-*alt*-peptoid) copolymers **$P_{1\text{-}3}$** in DMSO-d_6. *corresponds to residual pics of water (3.3 ppm), DMSO (2.5 ppm) and Et_2O (3.4 and 1.1 ppm) .

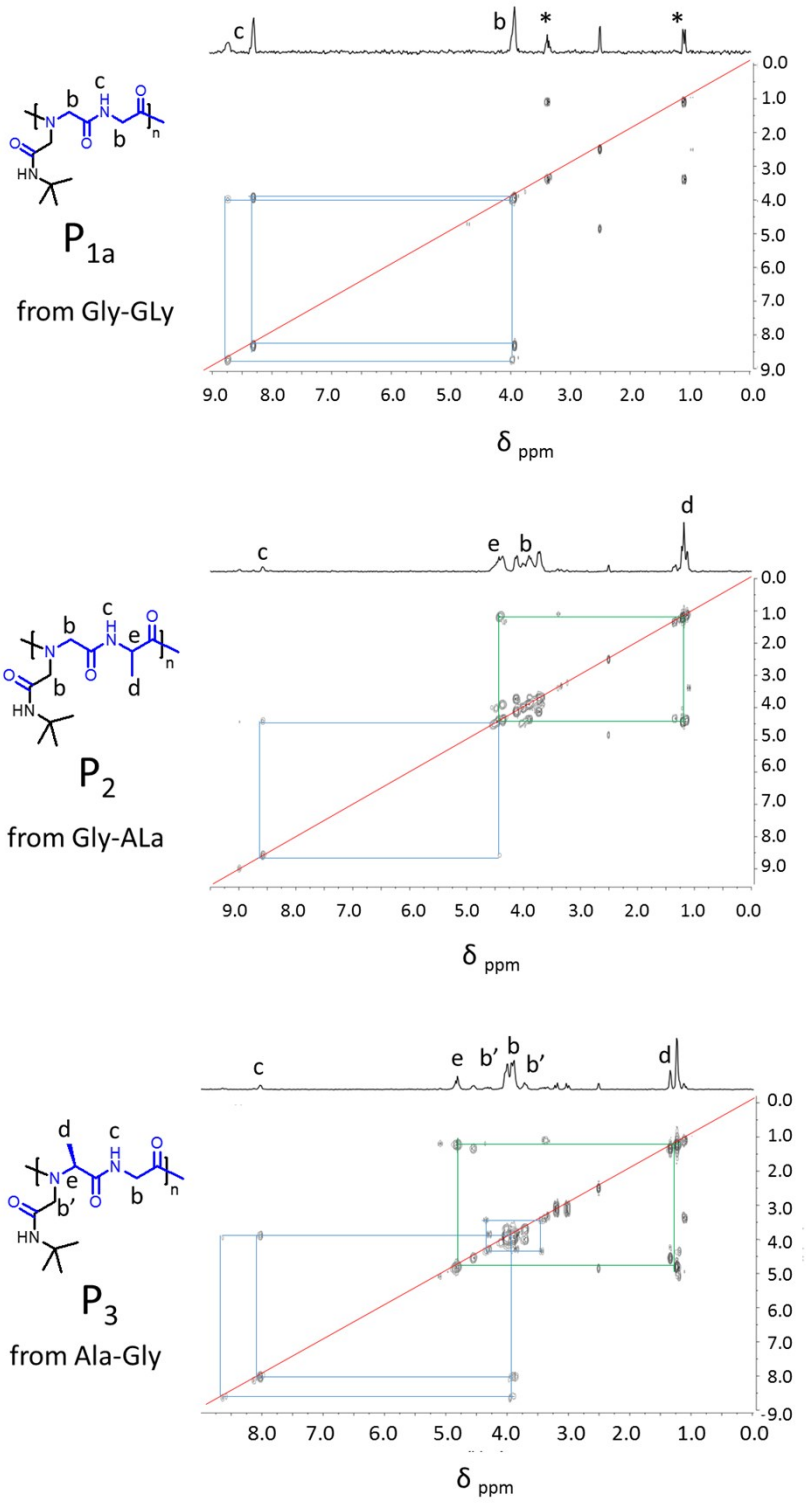

Figure S2. COSY NMR of alternating poly(peptide-*alt*-peptoid) copolymers **P_{1-3}** in DMSO-d_6. *corresponds to residual pics of Et_2O at 3.4 and 1.1 ppm.

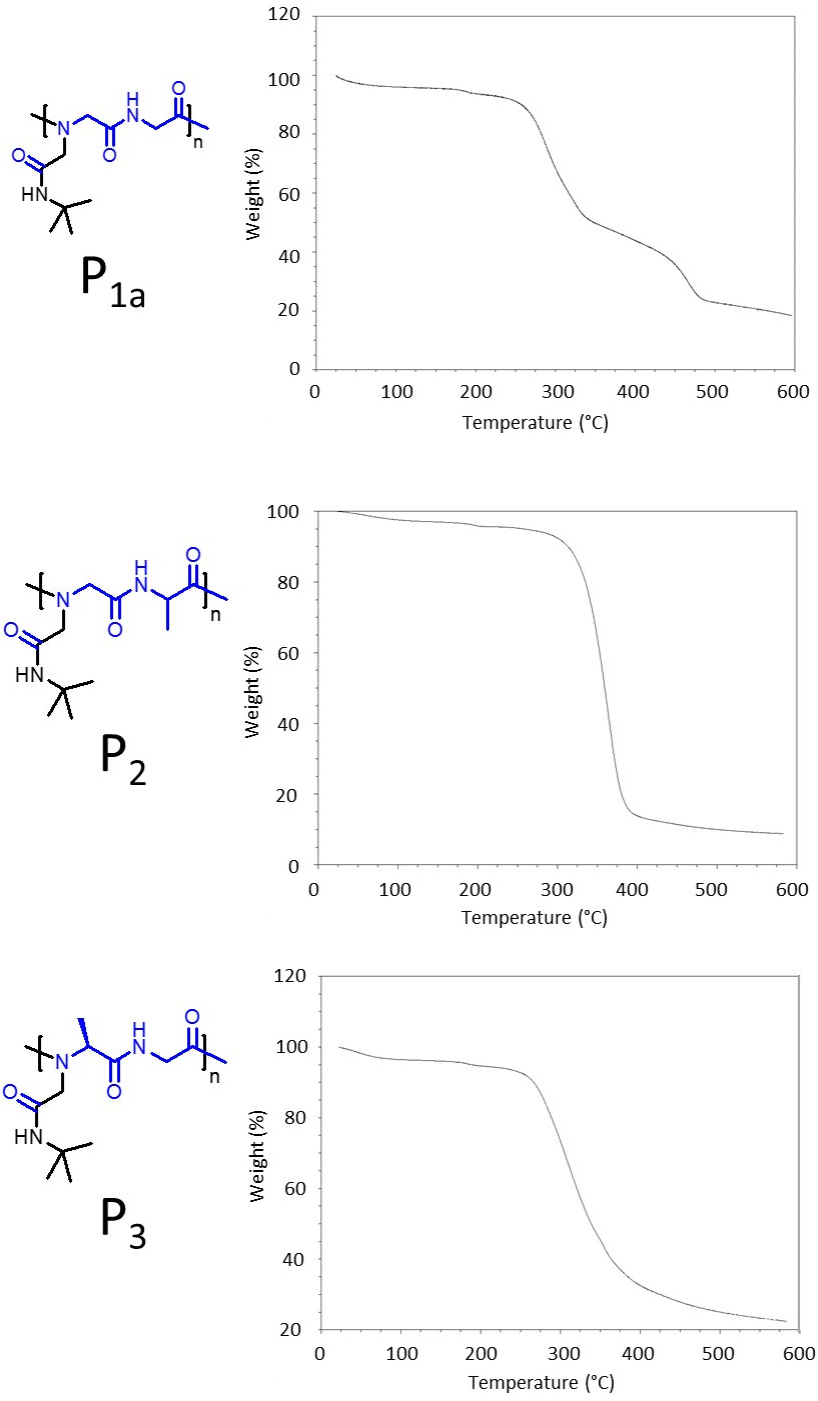

Figure S3. Thermogravimetric analysis of alternating poly(peptide-*alt*-peptoid) copolymers $\mathbf{P_{1\text{-}3}}$.

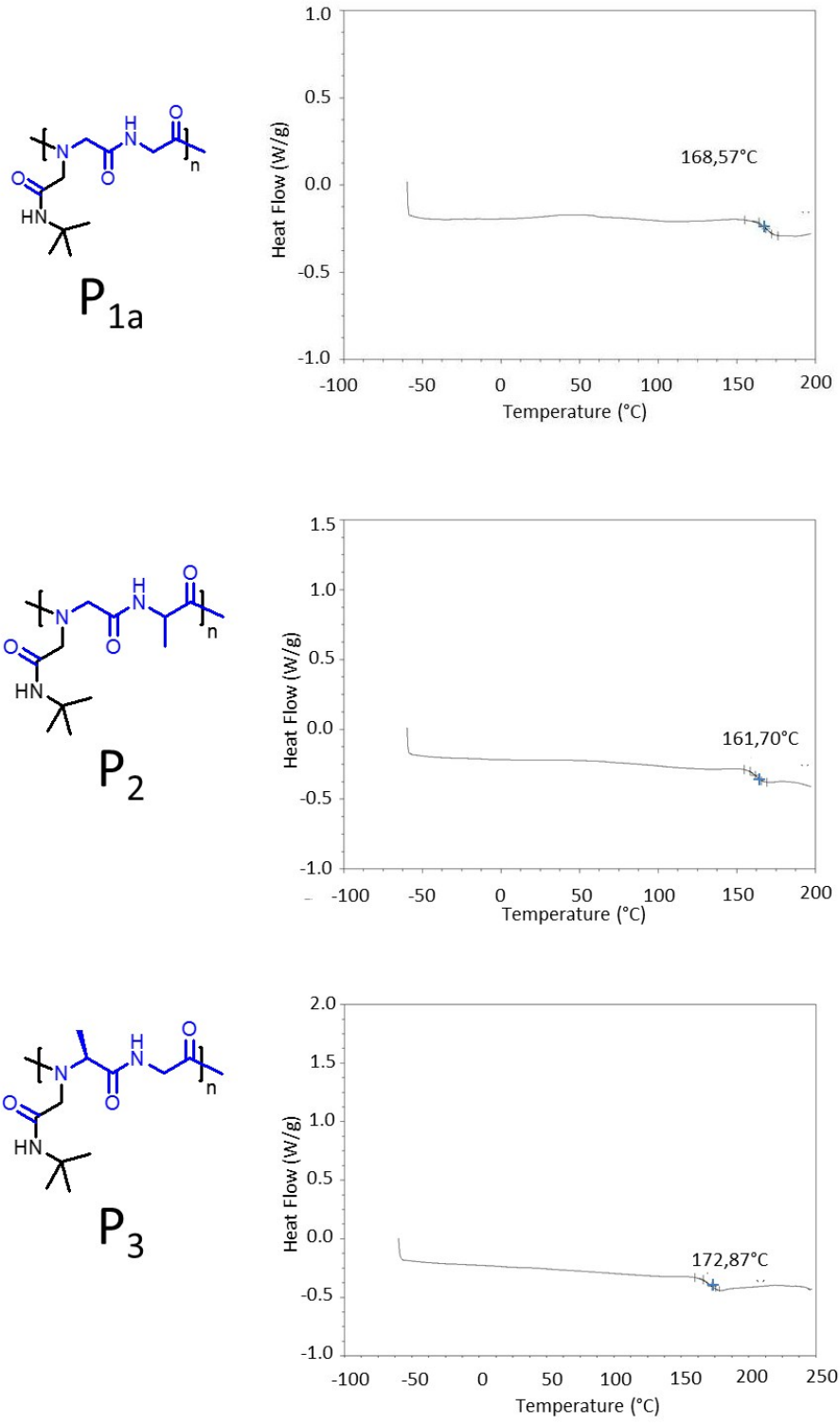

Figure S4. DSC traces of alternating poly(peptide-*alt*-peptoid) copolymers $\mathbf{P_{1\text{-}3}}$.

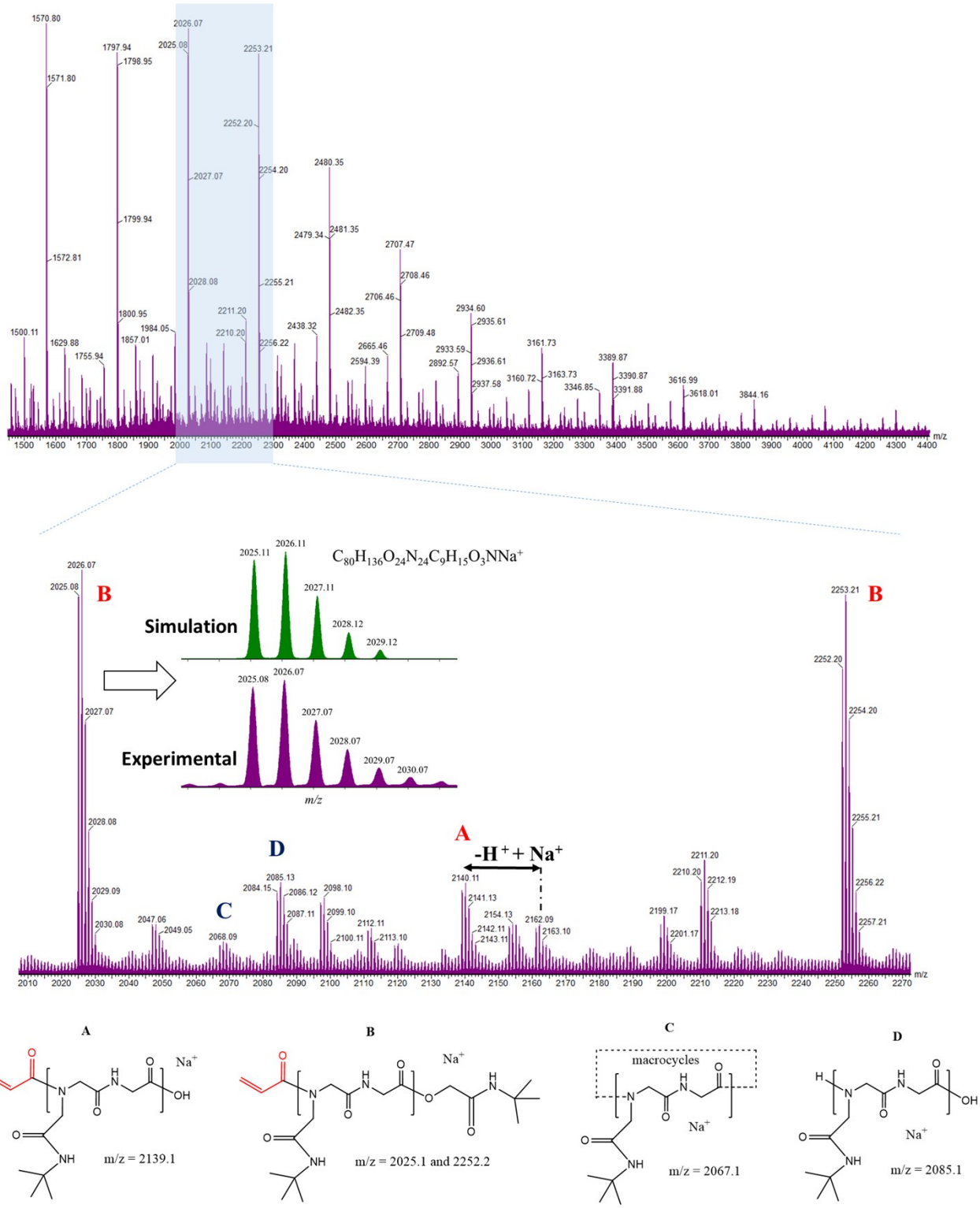

Figure S5. MALDI-ToF spectrum of the acrylamide-terminated poly(peptide-peptoid) **P_{4a}** and magnification between *m/z* 2010 – 2280. The inset corresponds to the comparison between the theoretical and experimental isotopic distribution for sodiated octamer.

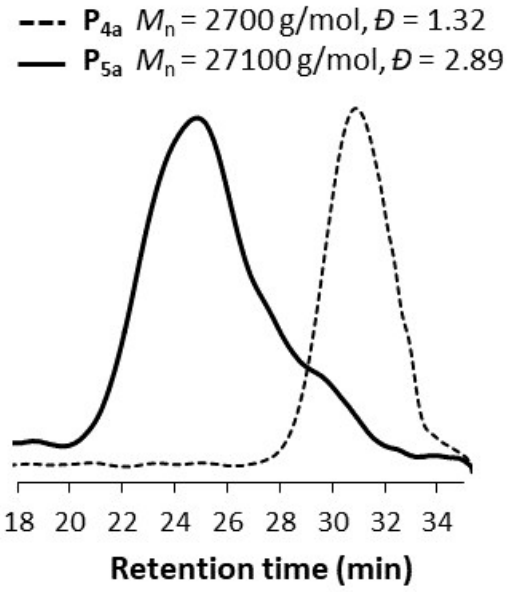

Figure S6. SEC chromatograms for the synthesis of the poly[DMA-graft-(peptoid-alt-peptide)]copolymer P5.

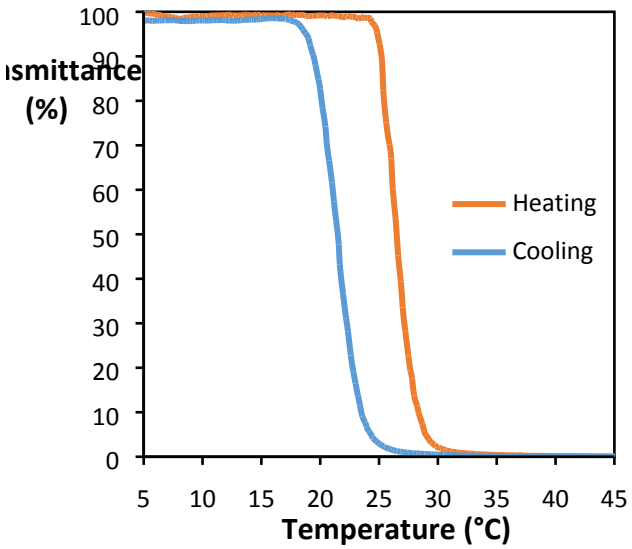

Figure S7. Turbidimetry measurements of aqueous solution of $\mathbf{P_1}$ at a concentration of 2 mg/mL (heating and cooling rate = 2 °C/min). The cloud point temperature measured upon heating is equal to 27 °C (temperature corresponding to 50 % loss of transmittance).

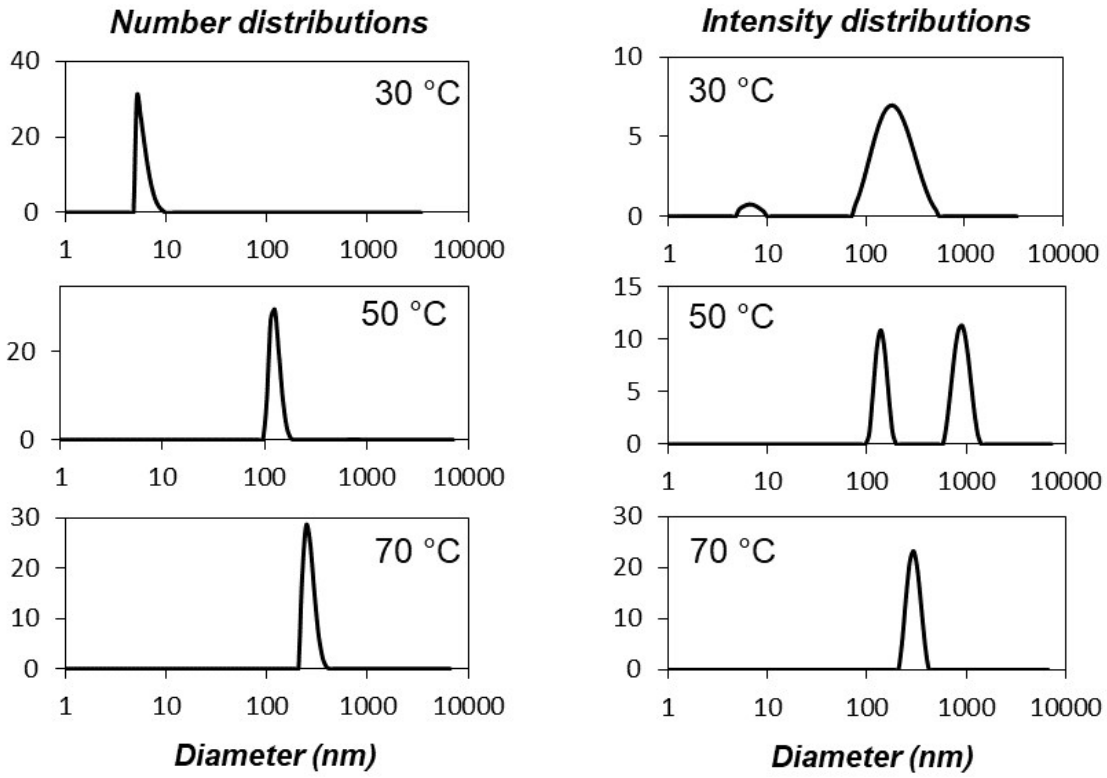

Figure S8. DLS measurements of aqueous solution of the block copolymer P7 at a concentration of 10 mg mL^{-1}.