Supporting Information to

Physical Gelation of α-Helical Copolypeptides

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Statistical Distribution of AG and BLG Units in P(BLG$_x$-*co*-AG$_{1-x}$)$_n$ Copolypeptides

Using ^{1}H NMR, the ratio of BLG to AG units was measured throughout the polymerization reaction of different P(BLG$_x$-*co*-AG$_{1-x}$)$_n$. For each polymerization reaction, a sample was taken out at regular intervals, precipitated and washed in methanol and measured by ^{1}H NMR in deuterated TFA (TFA-d_1). This ratio remained approximately constant during the polymerization reaction, which indicates that P(BLG$_x$-*co*-AG$_{1-x}$)$_n$ are statistical copolymers.

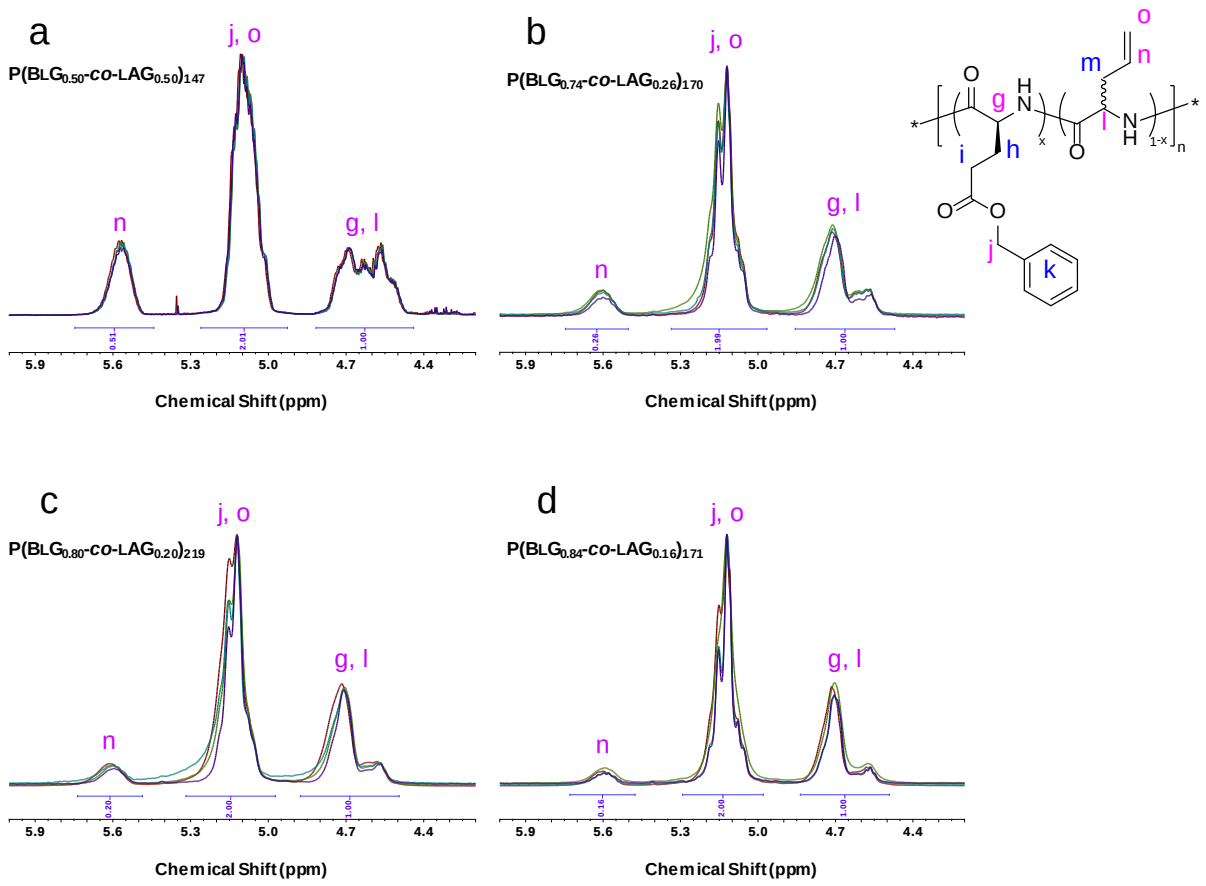

Figure S1. [4.2 - 6 ppm] sections of ^{1}H NMR spectra corresponding to polymerization samples taken at (a) (violet) 10 h, (blue) 24 h, (green) 32 h and (red) after termination; (b, c, d) (violet) 24 h, (blue) 48 h, (green) 72 h and (red) after termination; spectra were normalized to the 5.0-5.3 ppm peak

Homopolypeptide PBLG$_{51}$ (originally presented in a former study[1])

^{1}H NMR, 400 MHz, TFA-d_1, δ (ppm) 7.26-7.24 (br, 255H), 5.60-5.13 (br, 102H), 4.69-4.66 (br, 51H), 3.29 (br, 2H), 2.58-1.94 (br, 593H), 1.53-1.50 (br, 2H), 1.36-1.34 (br, 6H), 0.86-0.83 (br, 3H)

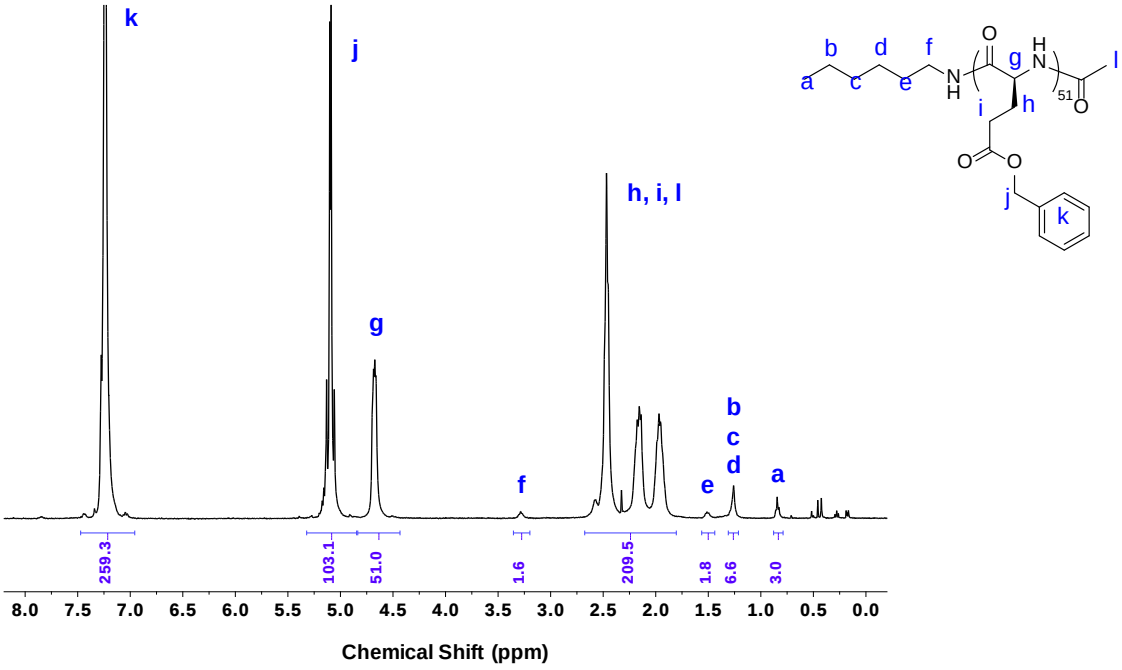

Figure S2. ^{1}H NMR spectrum (400 MHz) of PBLG$_{51}$ in TFA-d_1

Statistical Copolypeptide P(BLG$_{0.89}$-*co*-DLAG$_{0.11}$)$_{53}$ (originally presented in a former study[1])

^{1}H NMR, 400 MHz, TFA-d_1, δ (ppm) 7.25-7.23 (br, 235H), 5.59 (br, 6H), 5.16-5.02 (br, 106H), 4.71-4.53 (br, 53H), 3.27 (br, 2H), 2.53-1.94 (br, 203H), 1.51-1.48 (br, 2H), 1.33-1.25 (br, 6H), 0.85-0.82 (br, 3H)

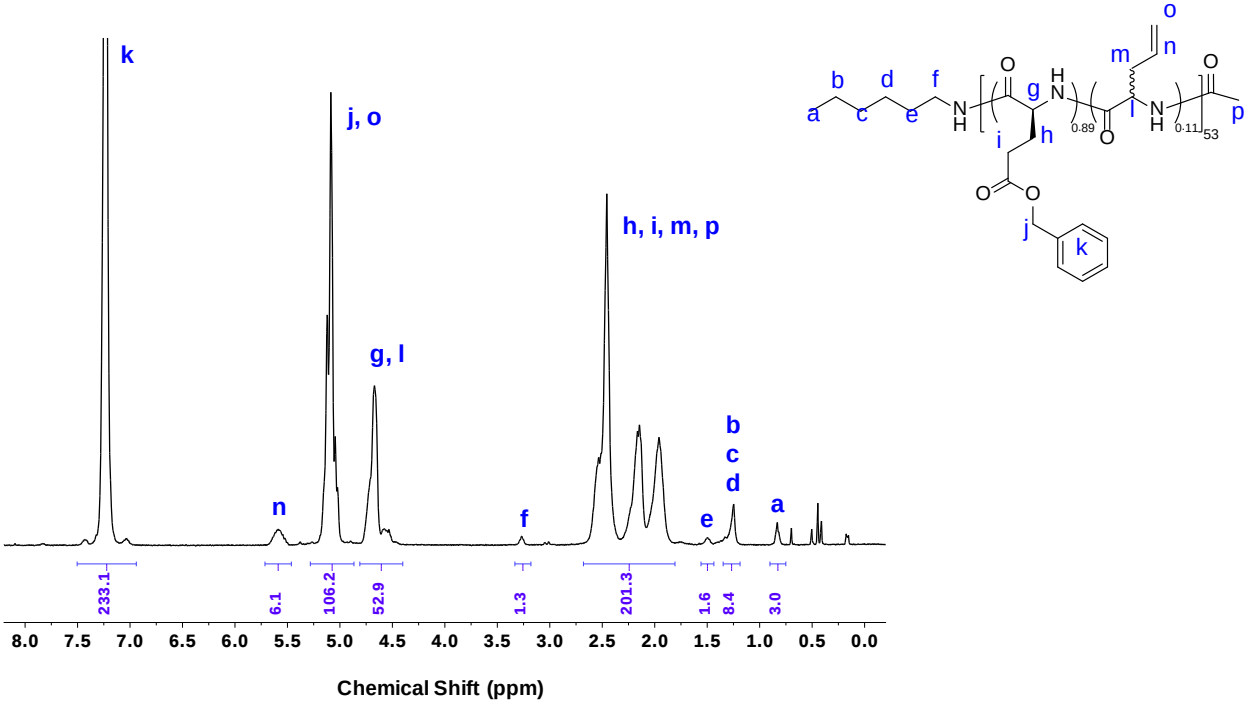

Figure S3. ^{1}H NMR spectrum (400 MHz) of P(BLG$_{0.89}$-*co*-DLAG$_{0.11}$)$_{53}$ in TFA-d_1

Statistical Copolypeptide P(BLG$_{0.76}$-*co*-DLAG$_{0.24}$)$_{59}$ (originally presented in a former study[1])

^{1}H NMR, 400 MHz, TFA-d_1, δ (ppm) 7.28-7.26 (br, 225H), 5.62 (br, 14H), 5.19-5.06 (br, 118H), 4.74-4.57 (br, 59H), 3.29 (br, 2H), 2.57-1.98 (br, 211H), 1.54-1.51 (br, 2H), 1.36-1.28 (br, 6H), 0.88-0.85 (br, 3H)

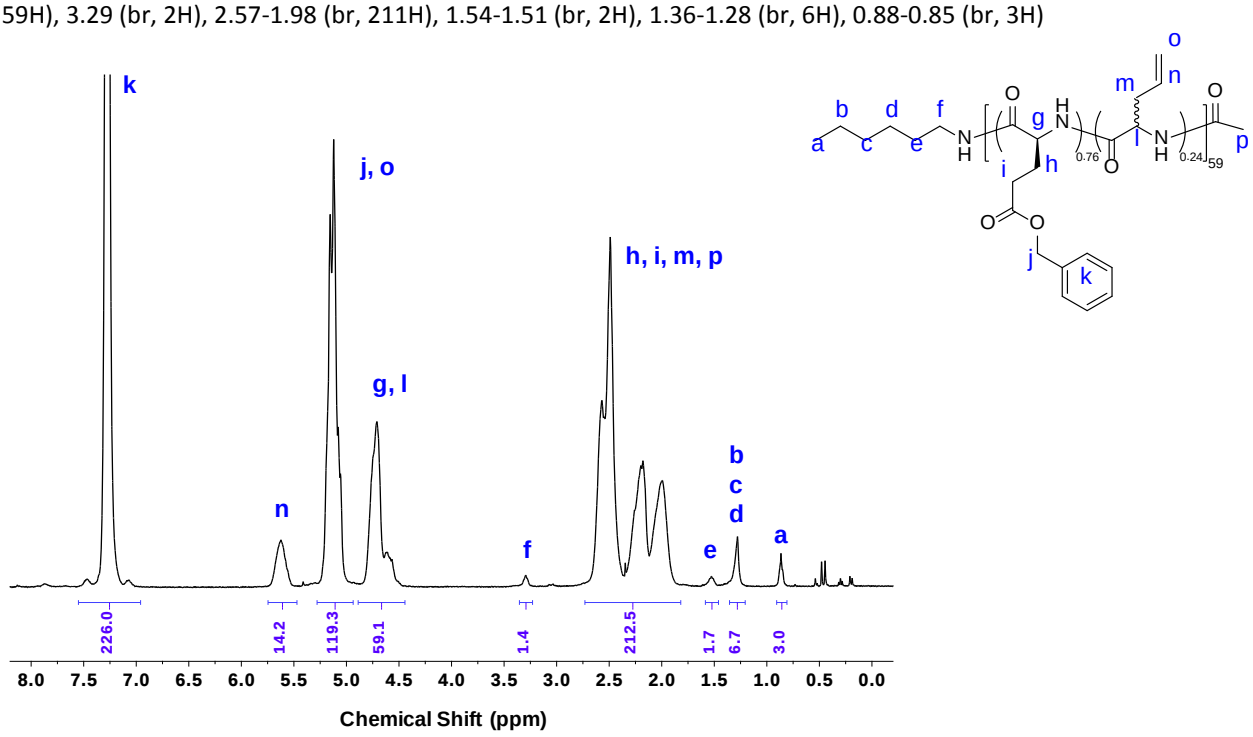

Figure S4. ^{1}H NMR spectrum (400 MHz) of P(BLG$_{0.76}$-*co*-DLAG$_{0.24}$)$_{59}$ in TFA-d_1

Statistical Copolypeptide P(BLG$_{0.77}$-*co*-LAG$_{0.23}$)$_{57}$ (originally presented in a former study[1])

^{1}H NMR, 400 MHz, TFA-d_1, δ (ppm) 7.29-7.27 (br, 220H), 5.60 (br, 13H), 5.19-5.06 (br, 114H), 4.74-4.57 (br, 57H), 3.30 (br, 2H), 2.59-1.99 (br, 205H), 1.55-1.51 (br, 2H), 1.37-1.28 (br, 6H), 0.88-0.85 (br, 3H)

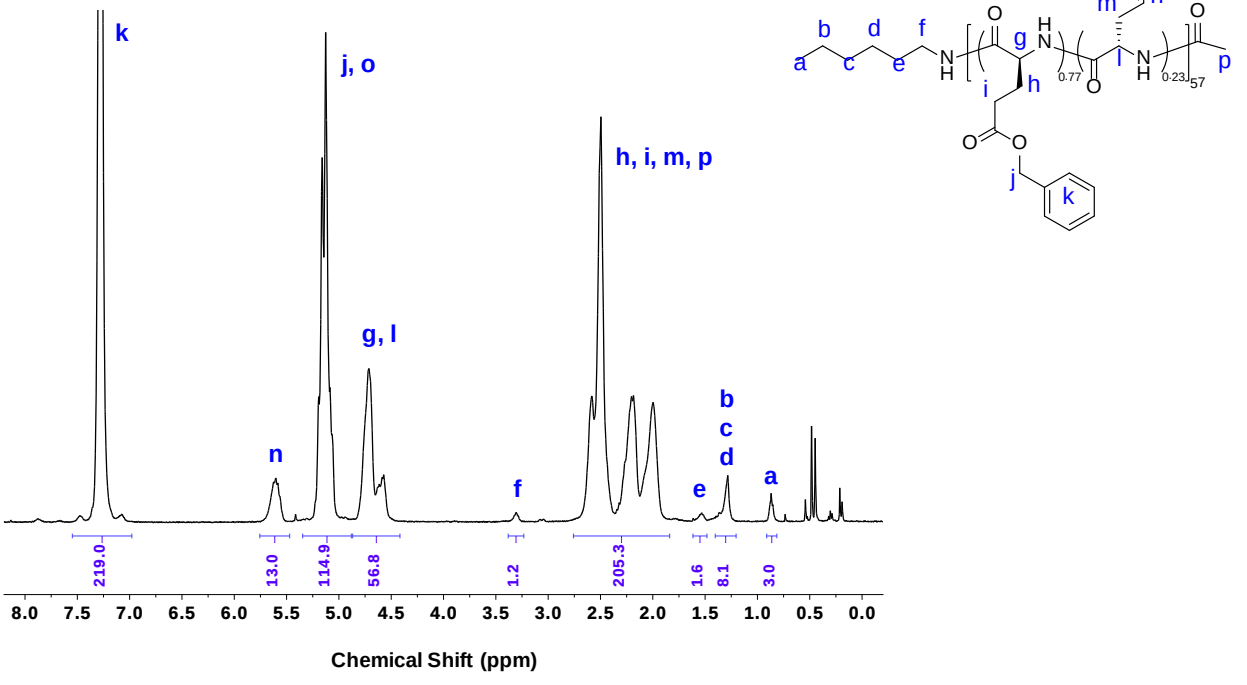

Figure S5. ^{1}H NMR spectrum (400 MHz) of P(BLG$_{0.77}$-*co*-LAG$_{0.23}$)$_{57}$ in TFA-d_1

Statistical Copolypeptide P(BLG$_{0.74}$-*co*-LAG$_{0.26}$)$_{91}$ (originally presented in a former study)[1]

^{1}H NMR, 400 MHz, TFA-d_1, δ (ppm) 7.22 (br, 335H), 5.56 (br, 24H), 5.15-5.02 (br, 182H), 4.73-4.52 (br, 91H), 3.23 (br, 2H), 2.55-1.94 (br, 319H), 1.54-1.48 (br, 2H), 1.33-1.23 (br, 6H), 0.81 (br, 3H)

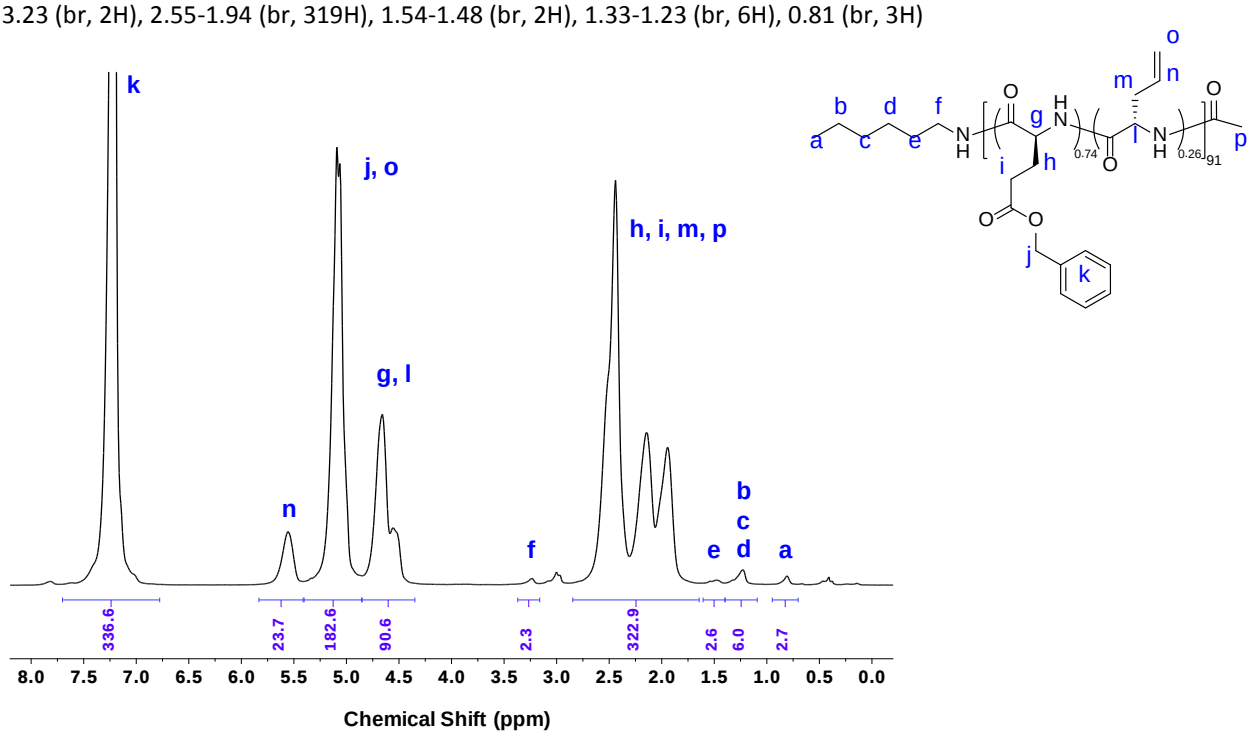

Figure S6. ^{1}H NMR spectrum (400 MHz) of P(BLG$_{0.74}$-*co*-LAG$_{0.26}$)$_{91}$ in TFA-d_1

Statistical Copolypeptide P(BLG$_{0.78}$-*co*-LAG$_{0.22}$)$_{96}$

^{1}H NMR, 400 MHz, TFA-d_1, δ (ppm) 7.20 (br, 375H), 5.54 (br, 21H), 5.14-5.00 (br, 192H), 4.70-4.50 (br, 96H), 3.23 (br, 2H), 2.51-1.93 (br, 345H), 1.53-1.47 (br, 2H), 1.34-1.22 (br, 6H), 0.80 (br, 3H)

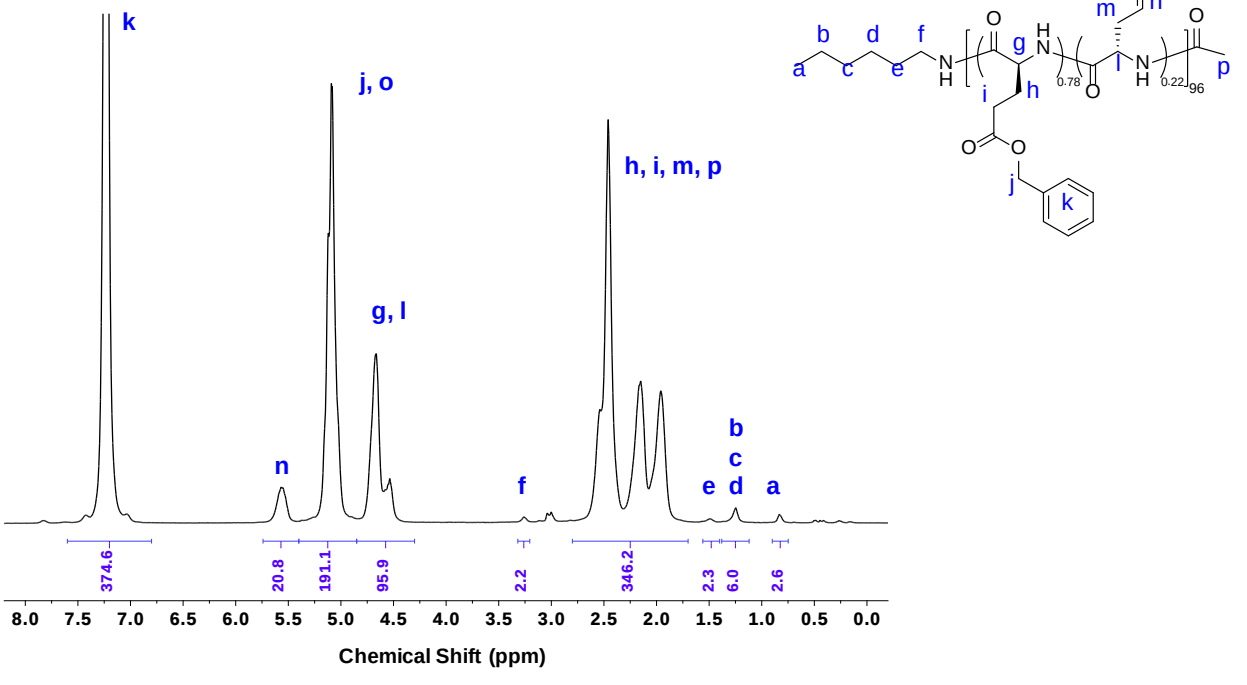

Figure S7. ^{1}H NMR spectrum (400 MHz) of P(BLG$_{0.78}$-*co*-LAG$_{0.22}$)$_{96}$ in TFA-d_1

Statistical Copolypeptide P(BLG$_{0.74}$-*co*-LAG$_{0.26}$)$_{170}$ (originally presented in a former study)[1]

^{1}H NMR, 400 MHz, TFA-d_1, δ (ppm) 7.20 (br, 625H), 5.54 (br, 45H), 5.14-5.00 (br, 340H), 4.70-4.50 (br, 170H), 3.23 (br, 2H), 2.51-1.93 (br, 593H), 1.53-1.47 (br, 2H), 1.34-1.22 (br, 6H), 0.80 (br, 3H)

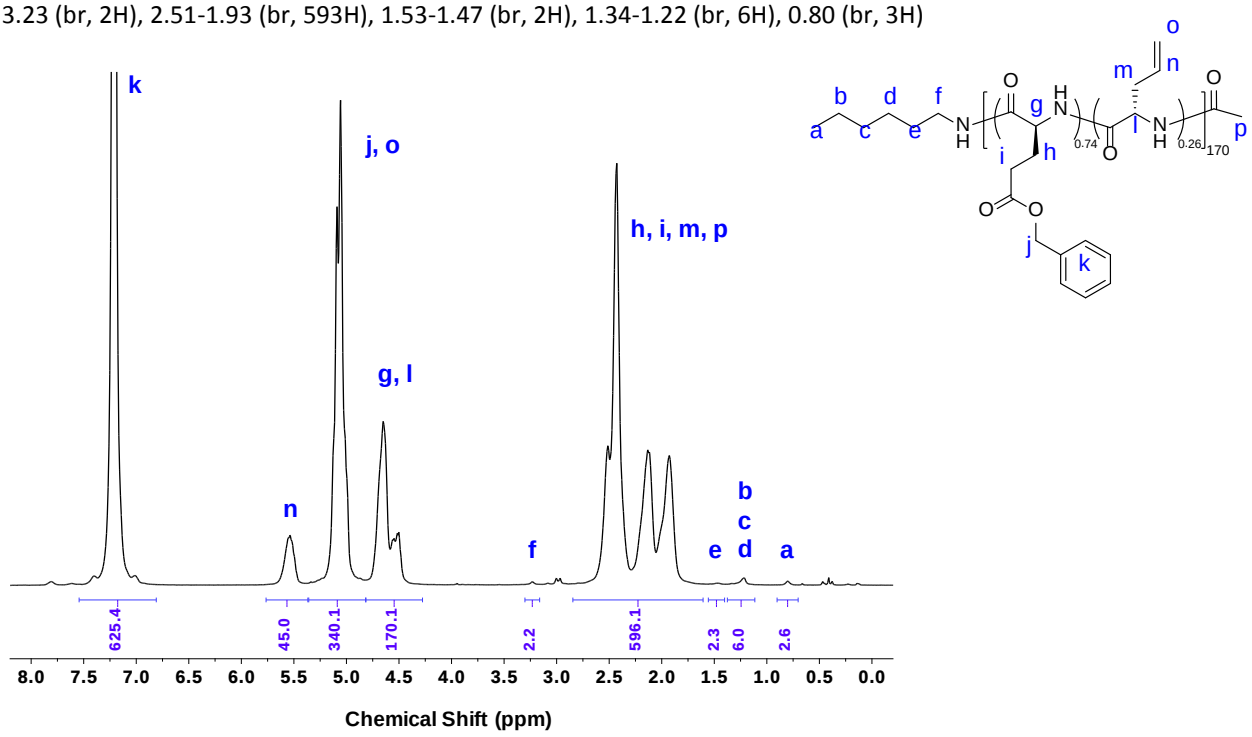

Figure S8. ^{1}H NMR spectrum (400 MHz) of P(BLG$_{0.74}$-*co*-LAG$_{0.26}$)$_{170}$ in TFA-d_1

Statistical Copolypeptide P(BLG$_{0.80}$-*co*-LAG$_{0.20}$)$_{219}$

^{1}H NMR, 400 MHz, TFA-d_1, δ (ppm) 7.20 (br, 875H), 5.54 (br, 44H), 5.14-5.00 (br, 438H), 4.70-4.50 (br, 219H), 3.23 (br, 2H), 2.51-1.93 (br, 791H), 1.53-1.47 (br, 2H), 1.34-1.22 (br, 6H), 0.80 (br, 3H)

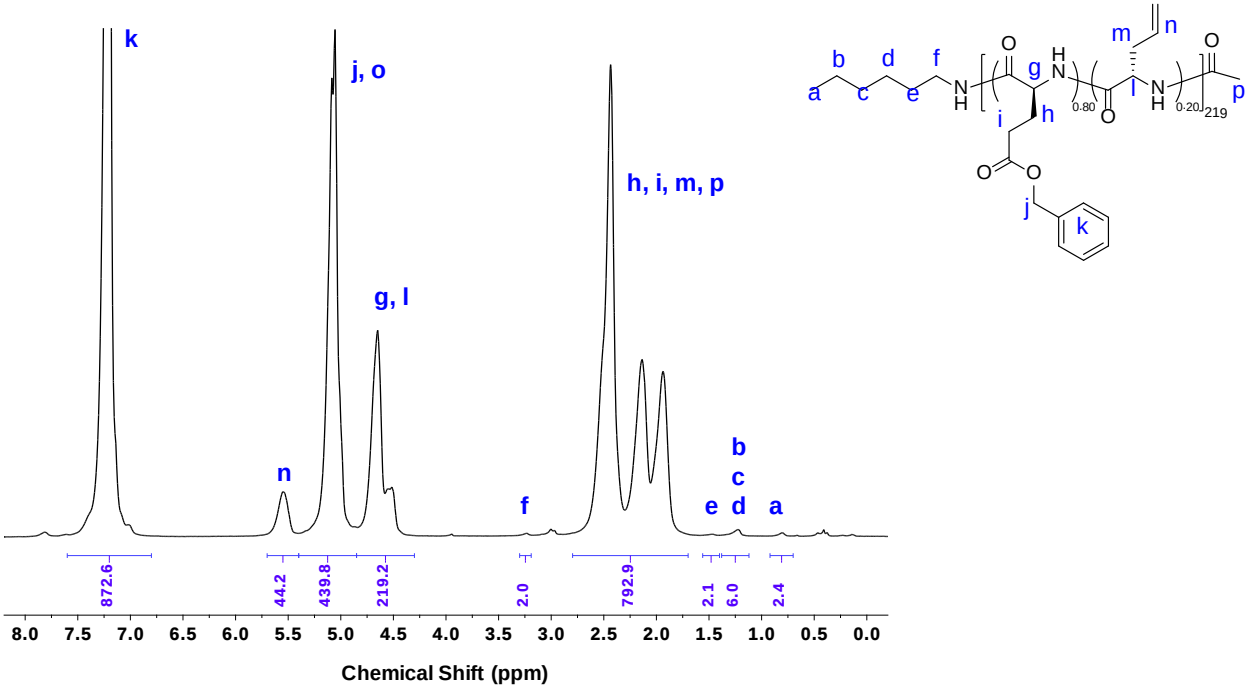

Figure S9. ^{1}H NMR spectrum (400 MHz) of P(BLG$_{0.80}$-*co*-LAG$_{0.20}$)$_{219}$ in TFA-d_1

Statistical Copolypeptide P(BLG$_{0.84}$-*co*-LAG$_{0.16}$)$_{171}$

^{1}H NMR, 400 MHz, TFA-d_1, δ (ppm) 7.20 (br, 715H), 5.54 (br, 28H), 5.14-5.00 (br, 342H), 4.70-4.50 (br, 171H), 3.23 (br, 2H), 2.51-1.93 (br, 631H), 1.53-1.47 (br, 2H), 1.34-1.22 (br, 6H), 0.80 (br, 3H)

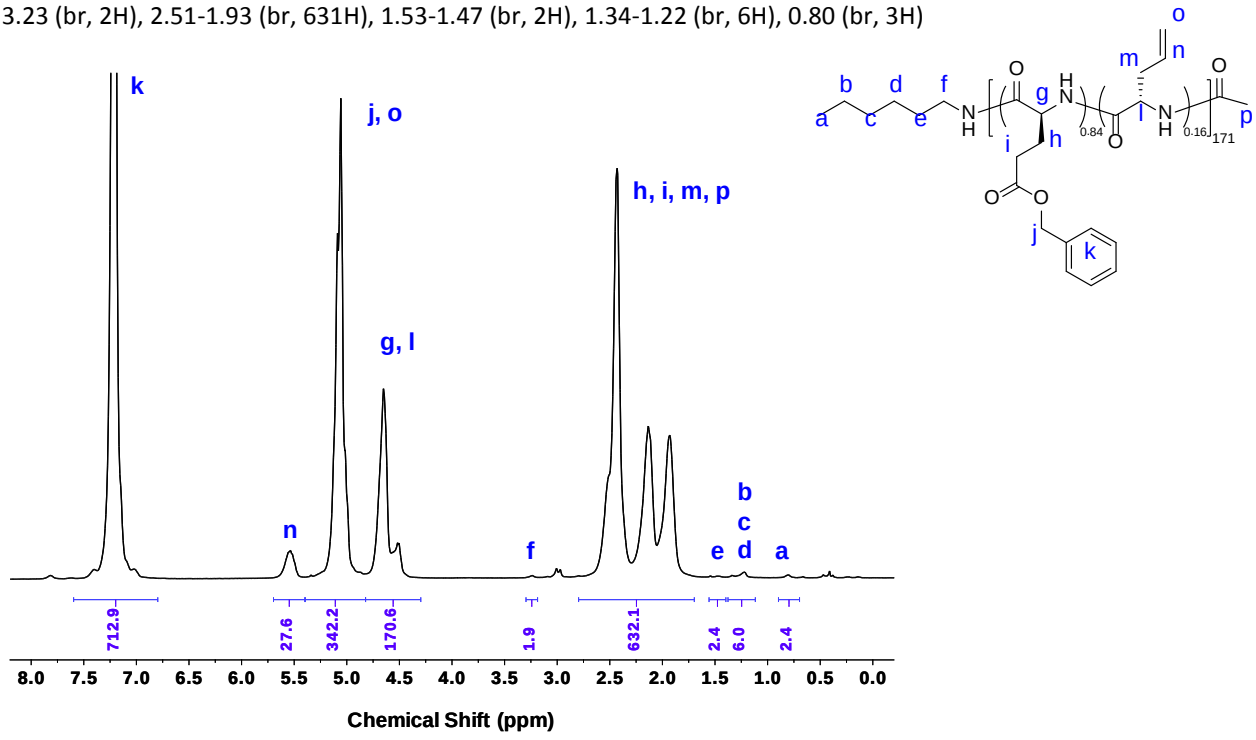

Figure S10. ^{1}H NMR spectrum (400 MHz) of P(BLG$_{0.84}$-*co*-LAG$_{0.16}$)$_{171}$ in TFA-d_1

Size Exclusion Chromatography (SEC)

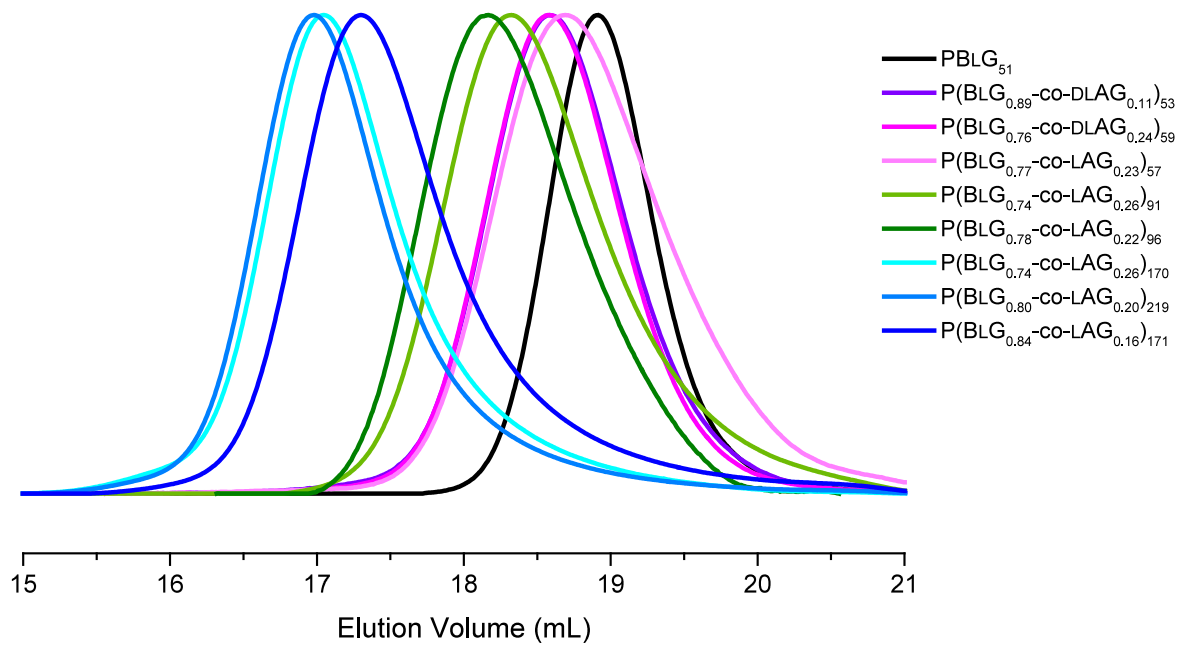

Figure S11. SEC-RI traces (eluent: NMP+LiBr) of PBLG and P(BLG$_x$-*co*-AG$_{1-x}$)$_n$ copolypeptides

FTIR Spectroscopy

Like the Raman spectra, the FTIR spectra of P(BLG$_x$-*co*-AG$_{1-x}$)$_n$ copolypeptides show an Amide I band composed of two peaks: a main α-helical peak at 1650 cm^{-1} (FTIR) and at 1653 cm^{-1} (Raman), and a secondary peak (noted α') at 1640 cm^{-1} (FTIR) and at 1643 cm^{-1} (Raman).

Relative to the α peaks, the α' peaks have a greater intensity in Raman than in FTIR spectroscopy. This can be explained by the different quantum mechanical selection rules for IR and Raman processes.[2] As a result of the deconvolution process performed on FTIR and Raman spectra, smaller peaks are likely to be tinged with a greater margin of error compared with larger peaks. This was a major driver for the choice of Raman spectra to help interpret rheological results.

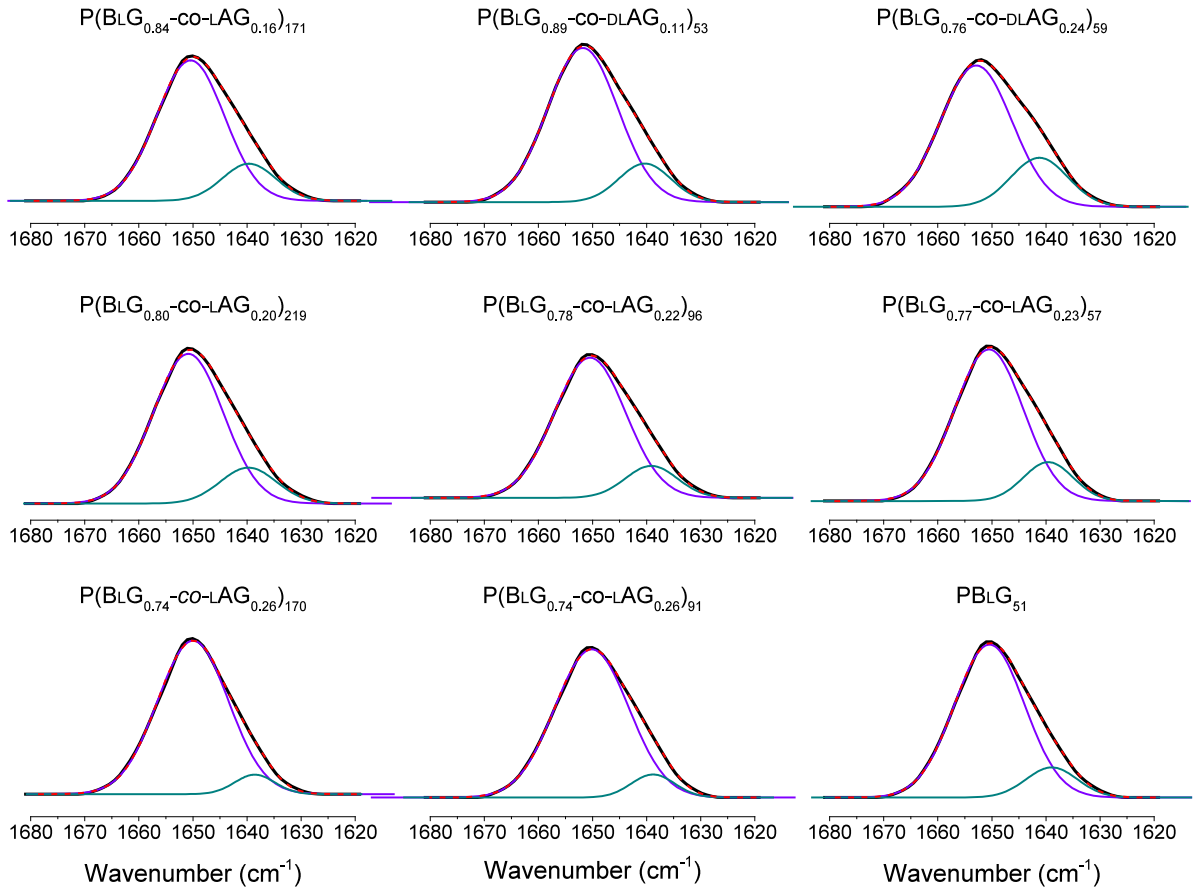

Figure S12. Amide I bands from FTIR spectra of PBLG and P(BLG$_x$-*co*-AG$_{1-x}$)$_n$ copolypeptides, composed of a main peak (α peak, purple) at approximately 1650 cm^{-1} that corresponds to an α-helical conformation, and a secondary peak (α' peak, green) at approximately 1640 cm^{-1}, whose exact nature is discussed in this study

Copolypeptide Solution Properties

Table S1. Properties of PBLG and P(BLG$_x$-*co*-AG$_{1-x}$)$_n$ copolypeptides studied in toluene (rheology) or toluene-d_8 (^{1}H NMR) at 20 g·L^{-1}

Copoylpeptide	Crossover Modulus (Stiffness)[a] (*Pa*)	Crossover Temperature (T_{gel})[a] (*°C*)	Complex Viscosity (η*) at 20 °C[a] (*mPa s*)	Temperature at which benzyl signal disappears in ^{1}H NMR (± 5 °C)[b] (*°C*)
PBLG$_{51}$	5	39	5900	10
P(BLG$_{0.89}$-*co*-DLAG$_{0.11}$)$_{53}$	26	-26	520	-40
P(BLG$_{0.76}$-*co*-DLAG$_{0.24}$)$_{59}$	27	-38	630	< -40
P(BLG$_{0.77}$-*co*-LAG$_{0.23}$)$_{57}$	30	-27	150	-40
P(BLG$_{0.74}$-*co*-LAG$_{0.26}$)$_{91}$	96	-35	780	< -40
P(BLG$_{0.78}$-*co*-LAG$_{0.22}$)$_{96}$	54	-23	620	-40
P(BLG$_{0.74}$-*co*-LAG$_{0.26}$)$_{170}$	158	-23	2270	-35
P(BLG$_{0.80}$-*co*-LAG$_{0.20}$)$_{219}$	444	-12	3000	-20
P(BLG$_{0.84}$-*co*-LAG$_{0.16}$)$_{171}$	331	-8	1450	-20

[a] Determined by rheometry. [b] Determined by ^{1}H NMR.

Temperature-sweep ^{1}H NMR Spectroscopy

During a sol-gel transition, gelators are incorporated in a supramolecular solid-like network, which causes line broadening, loss of spectral resolution, and signal disappearance in ^{1}H NMR measurements.[3–5] This phenomenon was observed in a temperature-sweep ^{1}H NMR experiment for dilute solutions of our polypeptides in toluene-d_8 (Figure S13 and S14). A good correlation was found between the disappearance of the benzene peak, noted k, and the gelation temperature measured by rheology (Table S1).

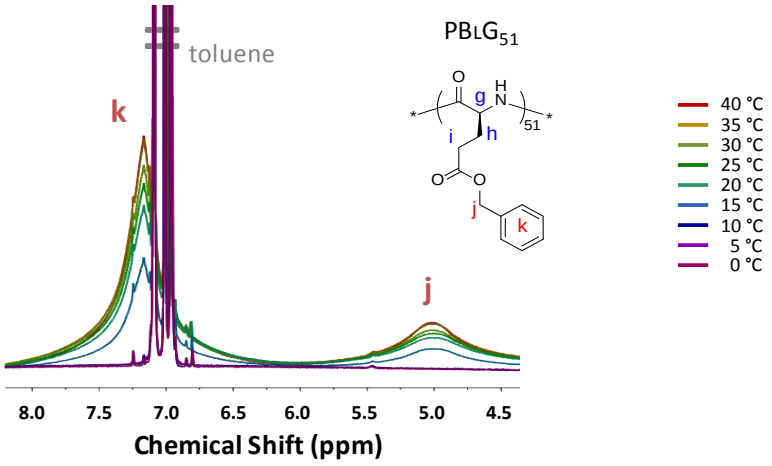

Figure S13. [4.5 - 8 ppm] sections of ^{1}H NMR spectra of a 20 g·L^{-1} solution of PBLG$_{51}$ in toluene-d_8, taken at incrementally decreasing temperatures, from 40 °C to 0 °C; all spectra were normalized to the toluene signal at 7.01 ppm

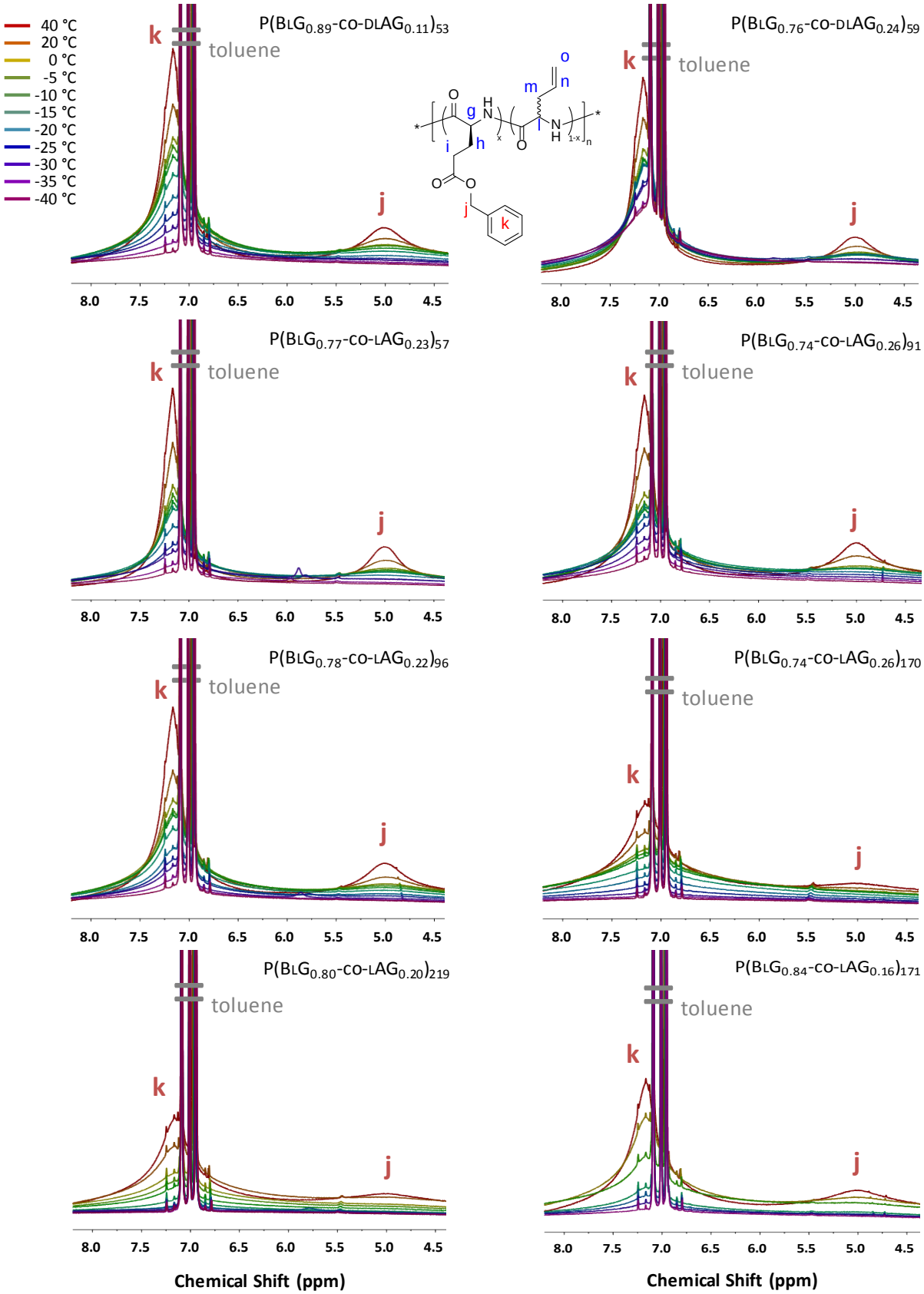

Figure S14. [4.5 - 8 ppm] sections of ^{1}H NMR spectra of 20 g·L^{-1} solution of P(BLG$_x$-*co*-AG$_{1-x}$)$_n$ copolypeptides in toluene-d_8, taken at incrementally decreasing temperatures, from 40 °C to -40 °C; all spectra were normalized to the toluene signal at 7.01 ppm

Circular Dichroism (CD) Spectroscopy

1,1,1,3,3,3-Hexafluoroisopropanol (HFIP) is known to be a helicogenic solvent for PBLG and was therefore used for CD measurements.[6] CD spectra of three selected P(BLG$_x$-*co*-AG$_{1-x}$)$_n$ copolypeptides and homopolymer PBLG$_{51}$ showed two minima at 208 nm and 222 nm, which are indicative of an α-helical conformation.

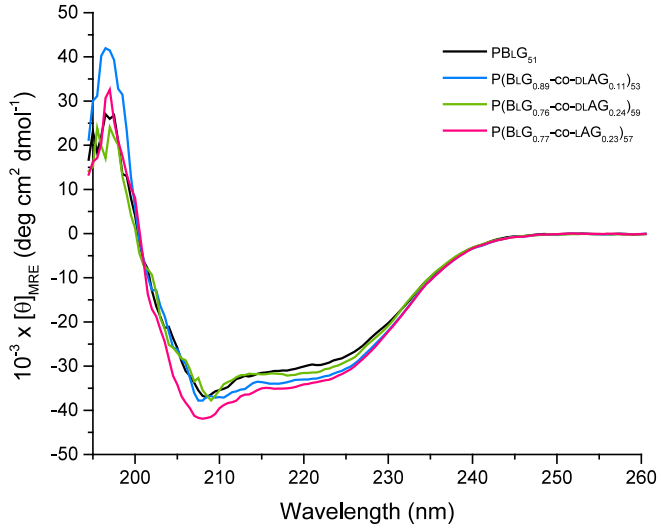

Figure S15. CD spectra of PBLG and P(BLG$_x$-*co*-AG$_{1-x}$)$_n$ copolypeptides in HFIP at 0.2 g·L^{-1}

Raman Spectroscopy

The following spectra (Figure S16 and S17) were normalized to the 1610 cm^{-1} peak, which corresponds to the ν(C-C) of the phenyl group of the BLG moiety.[7] Since the Amide I band (1620 - 1680 cm^{-1}) of our copolypeptides comprises of the ν(C=O) of both BLG and AG residues, its area, relative to the 1610 cm^{-1} peak, increases with increasing AG content.

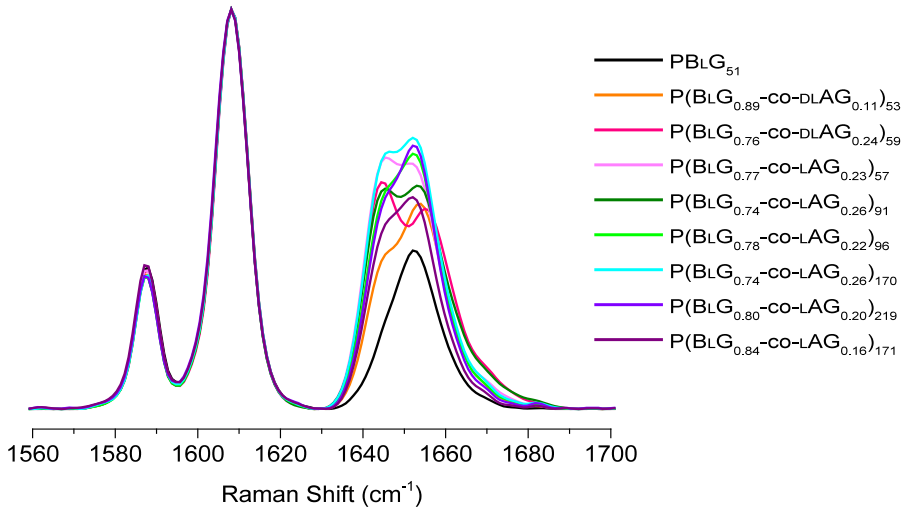

Figure S16. Amide I band of dry PBLG and P(BLG$_x$-*co*-AG$_{1-x}$)$_n$ copolypeptides after baseline correction and normalization

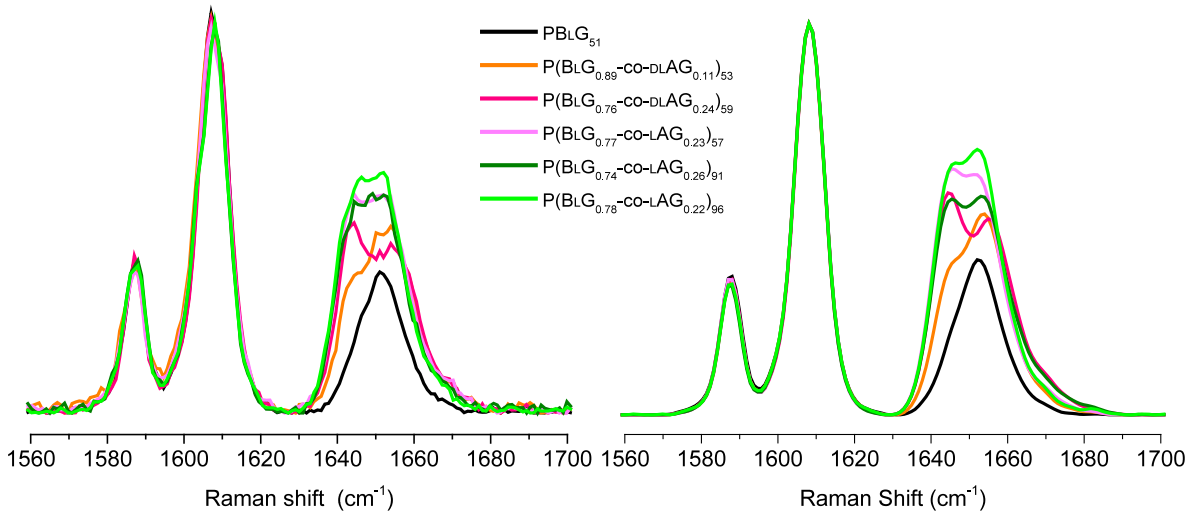

Figure S17. Amide I band of dry PBLG and P(BLG$_x$-*co*-AG$_{1-x}$)$_n$ copolypeptides (left) non compacted (originally presented in a former study[1]), (right) compacted and showing an improved signal-to-noise ratio (present study)

Wide Angle X-Ray Scattering (WAXS)

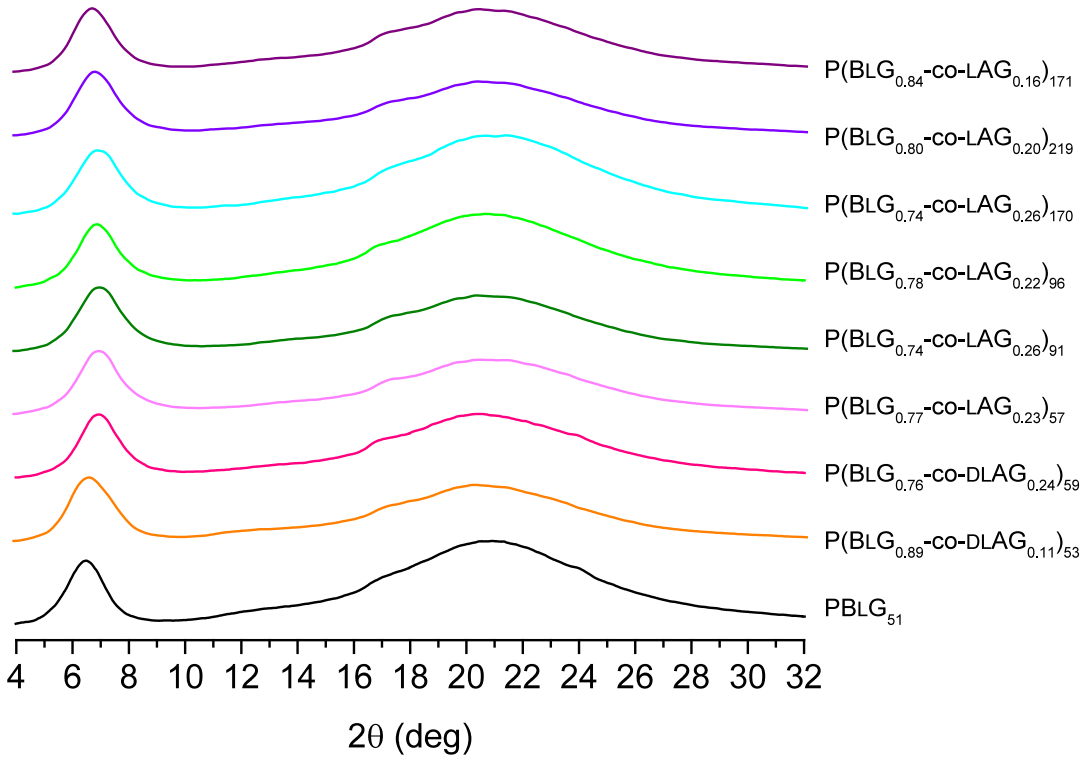

Figure S18. Full WAXS spectra of PBLG and P(BLG$_x$-*co*-AG$_{1-x}$)$_n$ copolypeptides, freeze-dried in dioxane; the peak at 6–7° corresponds to the d-spacing of ca. 1.3 nm from the pseudo-hexagonal arrangement of α-helical polymers; the shoulder at 17° corresponds to the pitch of α-helices (ca. 0.5 nm); the broad peak centered around 20–21° is the amorphous halo, resulting from the glass capillary (sample holder) and the measured polymers

References

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(7) Koenig, J. L.; Sutton, P. L. *Biopolymers* **1971**, *10*, 89–106.