Supporting Information

The influence of polymer topology and side chain functionality on the Schiff-base reactivity of biocompatible polypeptide hydrogels

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1. Experimental

1.1. Materials. All chemicals were obtained from Sigma Aldrich unless otherwise noted. H-Glu(Bzl)-OH (γ-benzyl-L-glutamate) and triphosgene were purchased from Fluorochem. The dialysis SnakeSkin® membrane (3.5 kDa MWCO) were purchased from Thermo Fisher Scientific.

1.2. Methods. ^{1}H-NMR spectra were recorded on a Bruker Avance 400 (400 MHz) spectrometer at room temperature using $CDCl_3$, TFA-d and DMSO-d as solvents. Attenuated total reflection (ATR) FTIR was recorded using a Thermo Scientific iS10 spectrometer in the region of 4000–600 cm^{-1}. Initially, a background measurement was performed before analysing the sample, with 16 scans completed using a resolution of 2 cm^{-1}. Size exclusion chromatography (SEC) was performed in hexafluoroisopropanol (HFIP) using an PSS SECurity GPC system equipped with a PFG 7 µm 8 × 50 mm pre-column, a PSS 100 Å, 7 µm 8 × 300 mm and a PSS 1000 Å, 7 µm 8 × 300 mm column in series and a differential refractive index (RI) detector at a flow rate of 1.0 mL min^{-1}.The system was calibrated against Agilent Easi-Vial linear poly(methyl methacrylate) (PMMA) standards and analysed by the software package PSS winGPC UniChrom.

1.3. Swelling ratio.

For swelling studies, hydrogels were prepared by separately dissolving the l-PBzAld (10.0 wt%) and l-PAHz2 (5.0 wt%) polypeptides in phosphate buffered saline (PBS) solution, at a BzAld/AHz molar ratio of 0.33, to form viscous solutions. The two solutions were then blended together to form hydrogels at room temperature. Hydrogels were then immersed in deionised water for 72 h at room temperature, followed by lyophilisation. After the dry mass was determined (*Wd*), the hydrogels were then swollen in excess water for 72 h to ensure equilibration of the networks. The hydrogel was then removed from water, patted dry to remove excess water on the surface and the mass was recorded again (*Ws*). The procedure was conducted in triplicate. Equilibrium swelling ratio (Q) can be calculated based on equation S1:

$$Q = \frac{Ws - Wd}{Wd}$$

Where *Ws*=mass of swollen hydrogel and *Wd*=mass of initial dry weight of the hydrogel.

1.4. Gel fraction.

For measuring gel fraction, hydrogels were prepared by separately dissolving the l-PBzAld (10.0 wt%) and l-PAHz2 (5.0 wt%) polypeptides in phosphate buffered saline (PBS) solution, at a BzAld/AHz molar ratio of 0.33, to form viscous solutions. The two solutions were then blended together to form hydrogels at room temperature, followed by lyophilisation. The dry mass (*Wd*) was determined, followed by the immersion into deionised water for 72 h at room temperature to remove unreacted polymer. After is period, the materials were once again lyophilised, and the final dry weight was recorded (*Wfd*). The procedure was conducted in triplicate. Gel fraction (G) was determined according to the following equation S2:

$$\mathrm{G} = \frac{w_{fd}}{w_d} \cdot 100$$

Where *Wfd*=mass of final dry weight and *Wd*=mass of initial dry weight of the hydrogel.

1.5. Self-healing demonstration.

Stained cylindrical hydrogels were prepared by separately dissolving the l-PBzAld (10.0 wt%) and l-PAHz2 (5.0 wt%) polypeptides in food-grade dye (red or blue) phosphate buffered saline (PBS) solution, at a BzAld/AHz molar ratio of 0.33. The viscous solutions were then mixed and transferred into a 1 mL syringe for hydrogel formation. Red and blue hydrogels were cut into smaller pieces with approximately 0.5 cm in length. Hydrogel pieces of different colour were then assembled into different configurations (column, semicircle and circle), and left for 30 min at room temperature to evaluate their self-healing capability.

1.6. Cell studies.

Human mesenchymal stem cells (hMSCs) were encapsulated in l-PBzAld/l-PAHz2 hydrogel, with a BzAld/AHz molar ratio of 0.33 and cultured in growth medium consisting of DMEM (Gibco) supplemented with 1 % (v/v) Penicillin/Streptomycin, 10 % (v/v) FBS for both assays. For metabolic activity, cell-laden hydrogels were cultured for a total of 14 days. At each evaluated time point, medium was removed from evaluated sample's well and replaced by growth medium containing 10% (v/v) alamarBlue (Invitrogen) before being incubated for 2 hours at 37°C. Media was then transferred to a black 96-well plate, and fluorescence was measured using a VarioSkan Flash plate reader (ThermoScientific, Germany). Metabolic activity was calculated as the

percentage (%) reduction in alamarBlue compared control group (empty well in the culture plate). For the viability assay, Live/Dead Cell Imaging Kit (Invitrogen) was used as instructed by manufacturer. Cell-laden hydrogels were prepared and cultured for 7 days in growth medium. Medium was removed from cell-laden hydrogel wells, hydrogels were washed with PBS once. Kit contents were mixed as specified in manufacturer's protocol and then added to wells, followed by incubation at room temperature for 20 minutes and immediate imaging by confocal microscopy (Zeiss).

2 Synthetic protocols

2.1. γ-benzyl-L-glutamate *N*-carboxyanhydride. Triphosgene (37.52 g, 126.45 mmol) and epichlorohydrin (96.62 g, 859.81 mmol) were initially dissolved in 800 mL of THF in a 1 L round-bottomed flask with stirring. γ-benzyl-L-glutamate (60.00 g, 252.89 mmol) was then added in one portion and the reaction suspension was heated under reflux (68 °C). The reaction was continued until all solids disappeared and the solution became clear (1.5 h). The solution was then cooled using N_2, any unreacted γ-benzyl-L-glutamate was filtered off and the solution was reduced to 1/3 of its original volume *in vacuo*. The NCA was precipitated by addition of 1 L of hexane and stored overnight at -18 °C to fully precipitate. The solid NCA was dried in vacuo and then re-dissolved in 400 mL ethyl acetate. This solution was then precipitated into excess hexane (1.8 L), filtered and dried (this was completed two to three times). It was then dried in vacuo to afford a white fluffy solid (yield: 54.35 g, 83%).

3. Additional Figures

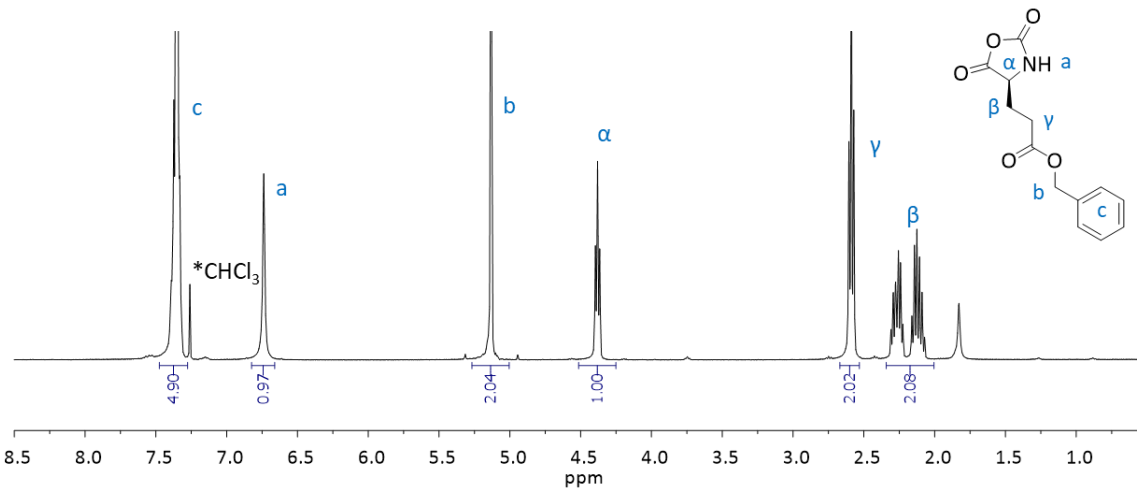

Figure S1. ^{1}H-NMR spectrum of BLG NCA in $CDCl_3$/d-TFA.

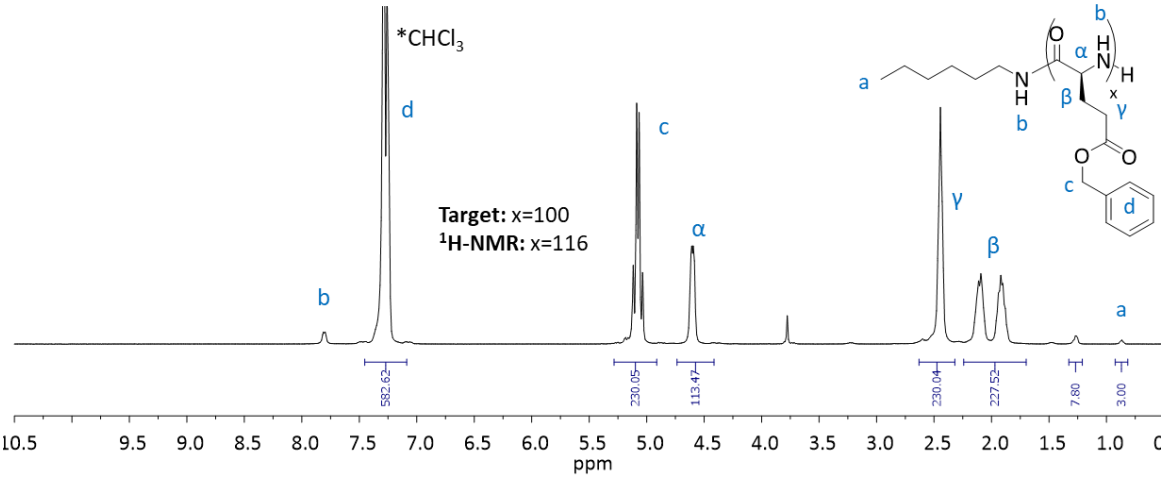

Figure S2. ^{1}H-NMR spectrum of l-PBLG$_{116}$ in $CDCl_3$/d-TFA.

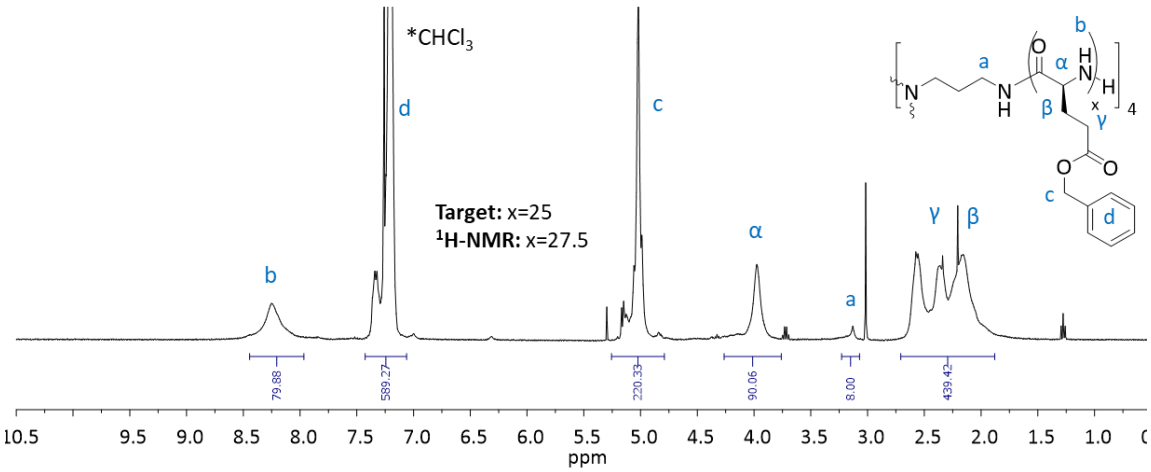

Figure S3. ^{1}H-NMR spectrum of s-PBLG$_{110}$ in $CDCl_3$/d-TFA.

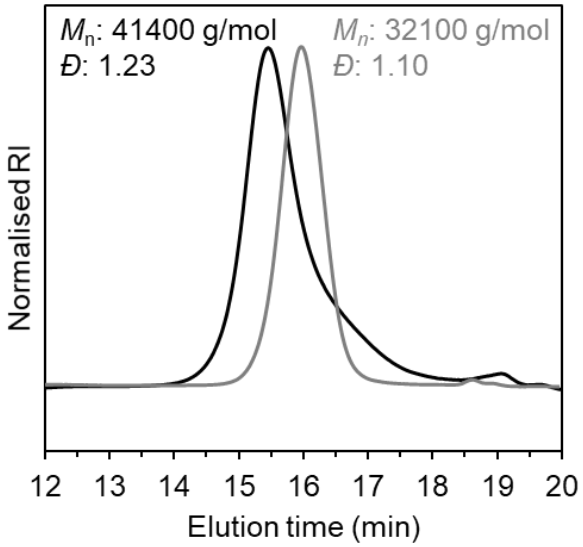

Figure S4. Normalised size exclusion chromatography (SEC) traces of l-PBLG$_{116}$ (black) and s-PBLG$_{110}$ (grey) polypeptides.

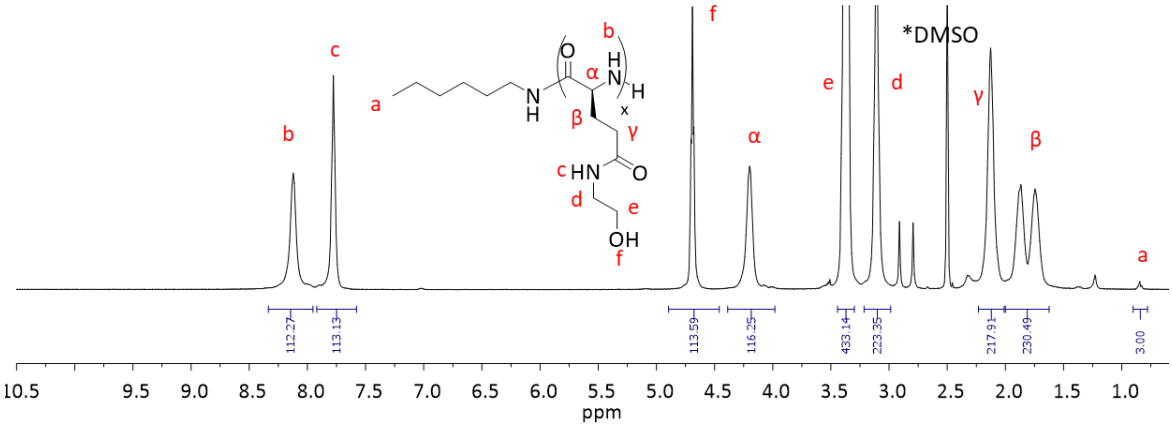

Figure S5. ^{1}H-NMR spectrum of l-PHELG$_{116}$ in DMSO-d.

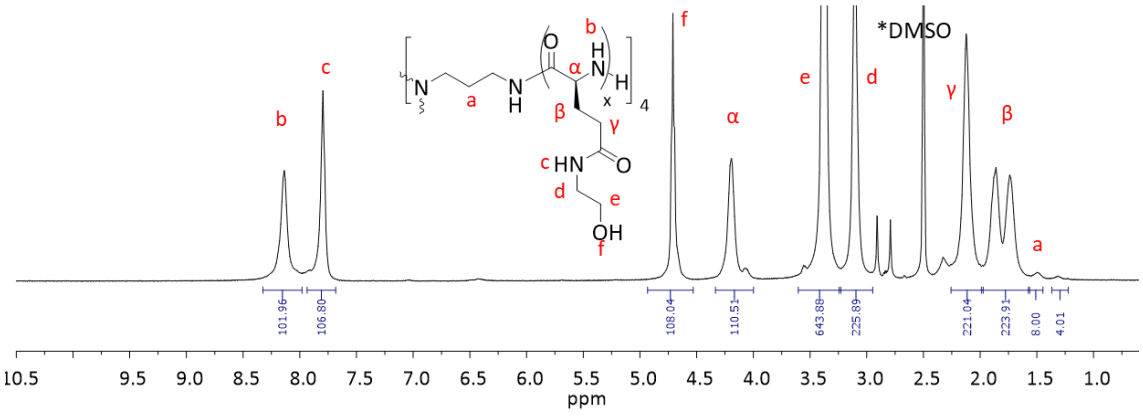

Figure S6. ^{1}H-NMR spectrum of s-PHELG$_{110}$ in DMSO-d.

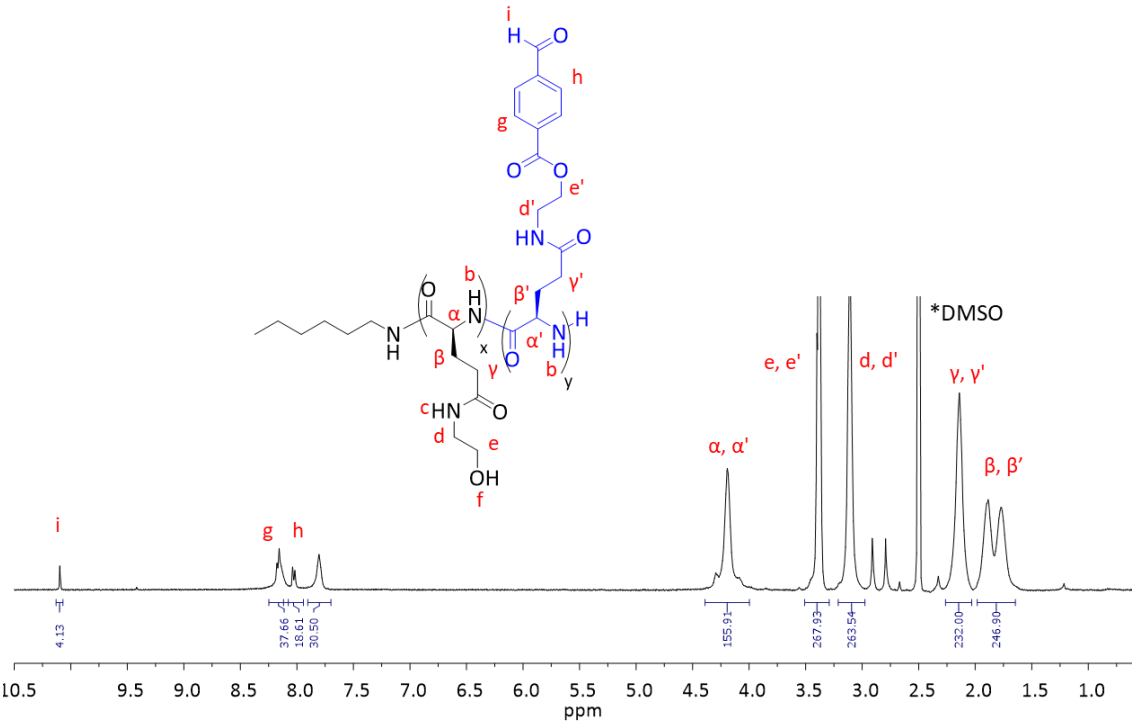

Figure S7. ^{1}H-NMR spectra of l-PHELG$_{105}$-co-PFEELG$_{9}$ (l-PAld) in DMSO-d.

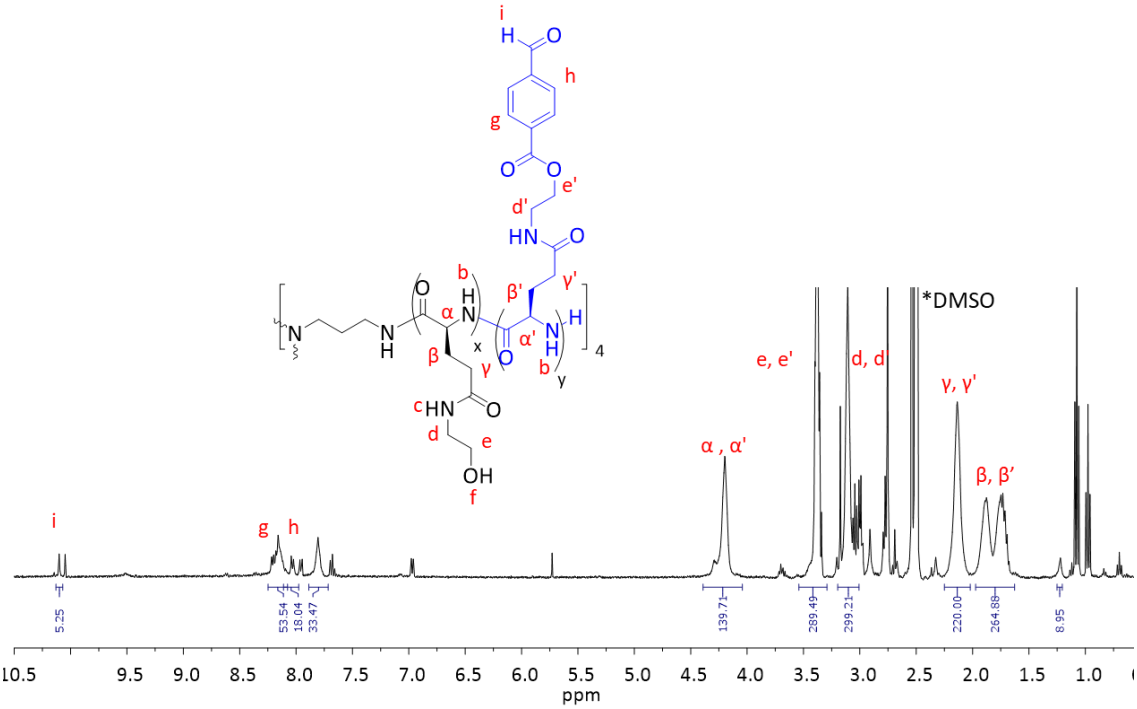

Figure S8. ^{1}H-NMR spectra of s-PHELG$_{101}$-co-PFEELG$_{9}$ (s-PAld) in DMSO-d.

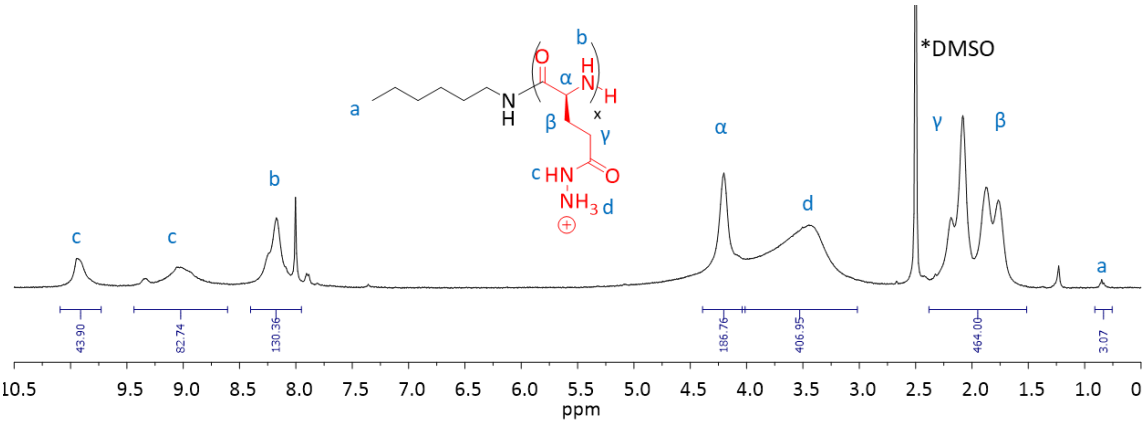

Figure S9. ^{1}H-NMR spectra of l-PAHLG$_{116}$ (l-PAHz1) in DMSO-d.

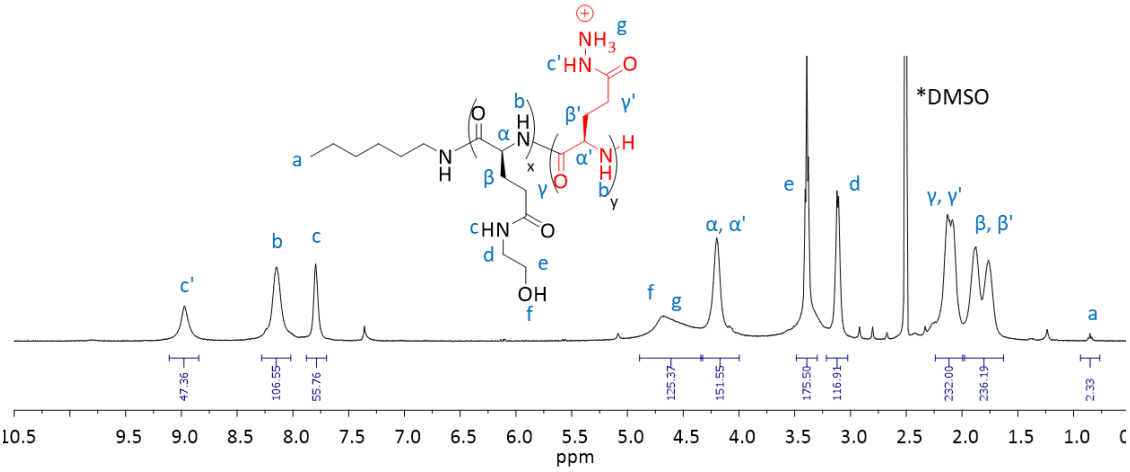

Figure S10. ^{1}H-NMR spectra of l-PHELG$_{58}$-co-PAHLG$_{57}$ (l-PAHz2) in DMSO-d.

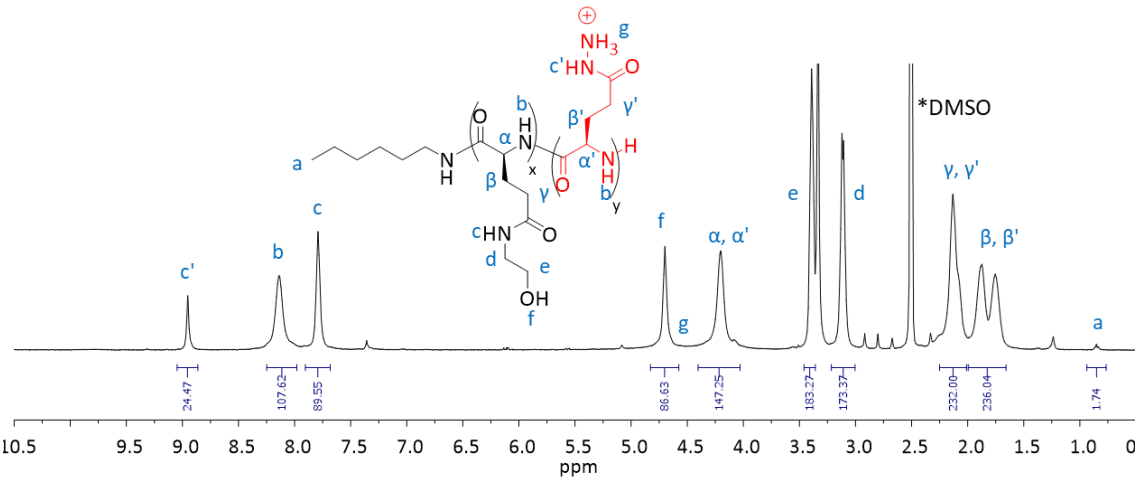

Figure S11. ^{1}H-NMR spectra of l-PHELG$_{87}$-co-PAHLG$_{29}$ (l-PAHz3) in DMSO-d.

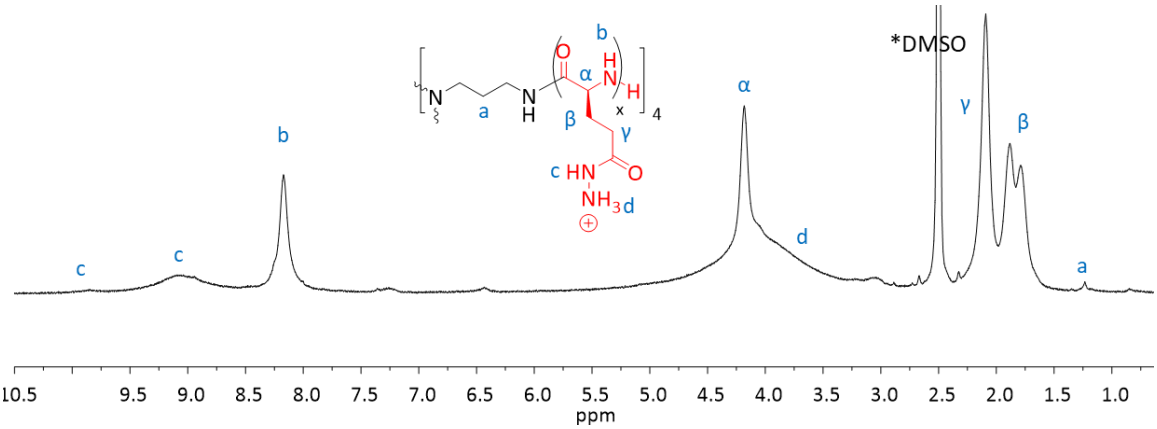

Figure S12. ^{1}H-NMR spectra of s-PAHLG$_{110}$ (s-PAHz1) in DMSO-d.

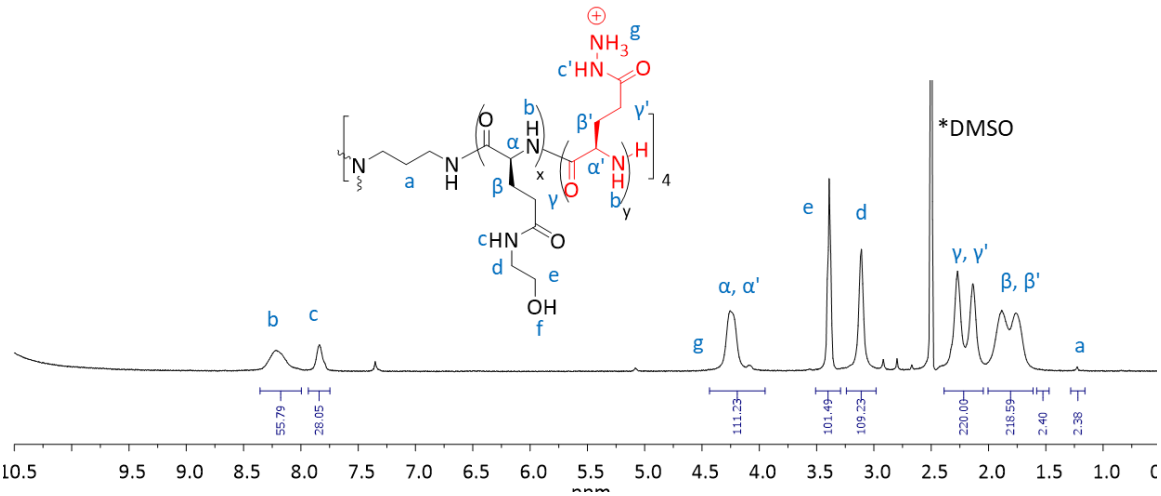

Figure S13. ^{1}H-NMR spectra of s-PHELG$_{55}$-co-PAHLG$_{55}$ (s-PAHz2) in DMSO-d.

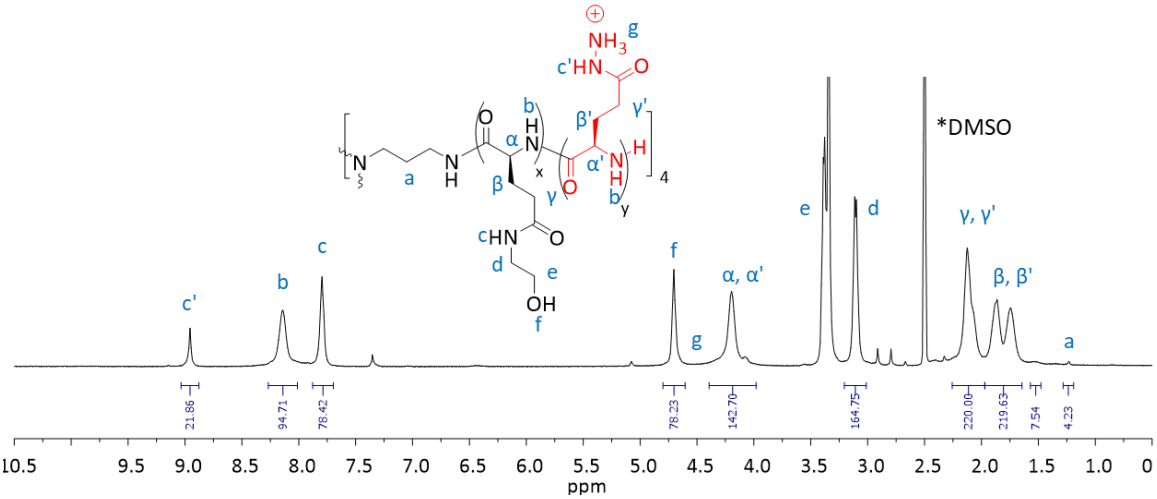

Figure S14. ^{1}H-NMR spectra of s-PHELG$_{82}$-co-PAHLG$_{28}$ (s-PAHz3) in DMSO-d.

Table S1. Analysis of modifications of polypeptide library using ^{1}H NMR spectrosopy.

Polymer	Total repeat units	Target BzAld DF & DF%	Actual BzAld DF & DF%	Target AHz DF & DF%	Actual AHz DF & DF%
l-PBzAld	116	35, 30%	9, 8%	-	-
s-PBzAld	110	33, 30%	9, 8%	-	-
l-PAHz1	116	-	-	116, 100%	116, 100%
l-PAHz2	116	-	-	29, 25%	57, 49%
l-PAHz3	116	-	-	12, 10%	29, 25%
s-PAHz1	110	-	-	110, 100%	110, 100%
s-PAHz2	110	-	-	22, 20%	55, 50%
s-PAHz3	110	-	-	11, 10%	28, 25%

Table S2. Secondary structures observed for library of polypeptides using BestSel.

Polymer	Helix	Sheet	Turn	Other
l-PBzAld	0	34	23	43
l-PAHz1	83	0	0	17
l-PAHz2	1	38	20	41
l-PAHz3	1	30	22	47
s-PBzAld	0	28	21	51
s-PAHz1	76	6	0	18
s-PAHz2	0	37	22	41
s-PAHz3	0	27	24	49

Table S3. Hydrogel properties observed for library of polypeptides via rheology.

Composition	Gel point (s)	Gelation time (s)	Modulus (Pa)	BzAld/AHz molar ratio
l-PBzAld + l-PAHz1	N/A	260	530	0.08
l-PBzAld + l-PAHz2	30	1200	2030	0.16
l-PBzAld + l-PAHz3	360	1720	110	0.35
l-PBzAld + s-PAHz1	N/A	200	240	0.08
l-PBzAld + s-PAHz2	100	1800	460	0.17
l-PBzAld + s-PAHz3	2980	5500	260	0.36
s-PBzAld + s-PAHz1	N/A	100	10	0.08
s-PBzAld + s-PAHz2	550	1490	90	0.16
s-PBzAld + s-PAHz3	N/A	N/A	N/A	0.28
s-PBzAld + l-PAHz1	N/A	620	120	0.08
s-PBzAld + l-PAHz2	N/A	1730	410	0.15
s-PBzAld + l-PAHz3	1740	2340	60	0.26

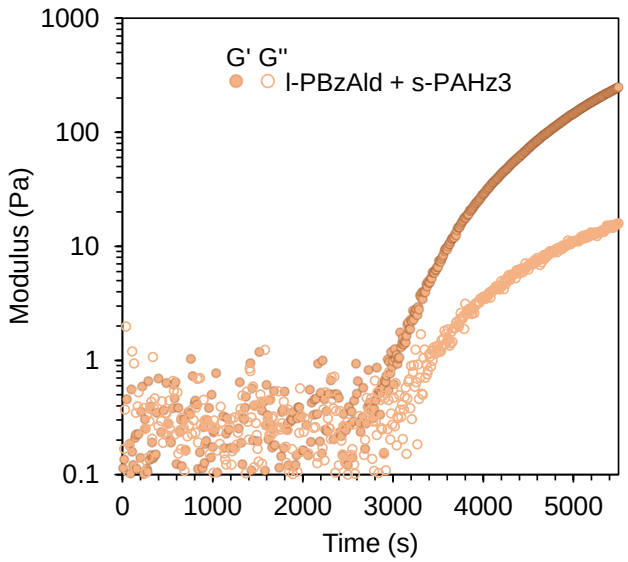

Figure S15. Rheological time sweep showing gelation point and viscoelastic regimes of hydrogels from blends of of l-PBzAld and s-PAHz3 polypeptides in PBS (strain = 0.1%, frequency = 0.1 rad/s).

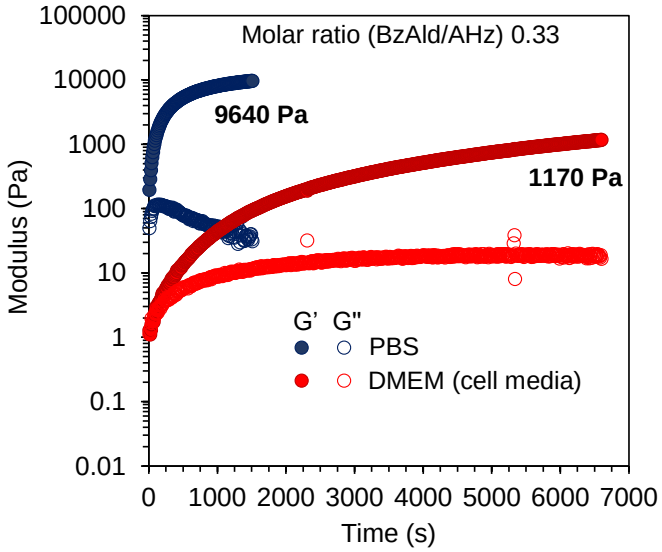

Figure S16. Rheological time sweep showing gelation point and viscoelastic regimes of hydrogels from blends of l-PBzAld and l-PAHz2 polypeptides in PBS and DMEM (strain = 0.1%, frequency = 0.1 rad/s).

Table S4. Hydrogel properties observed for library of polypeptides via rheology.

Composition	Modulus (Pa)	Gel fraction (G)	Swelling ratio (Q)	BzAld/AHz molar ratio
l-PBzAld + l-PAHz2	2030	87.60±4.20	7.90±0.60	0.33