Electronic Supplementary Material (ESI) for Soft Matter.

Supporting Information

Peptide-Polyurea Hybrids: A Platform for Tunable, Thermally-stable, and Injectable Hydrogels

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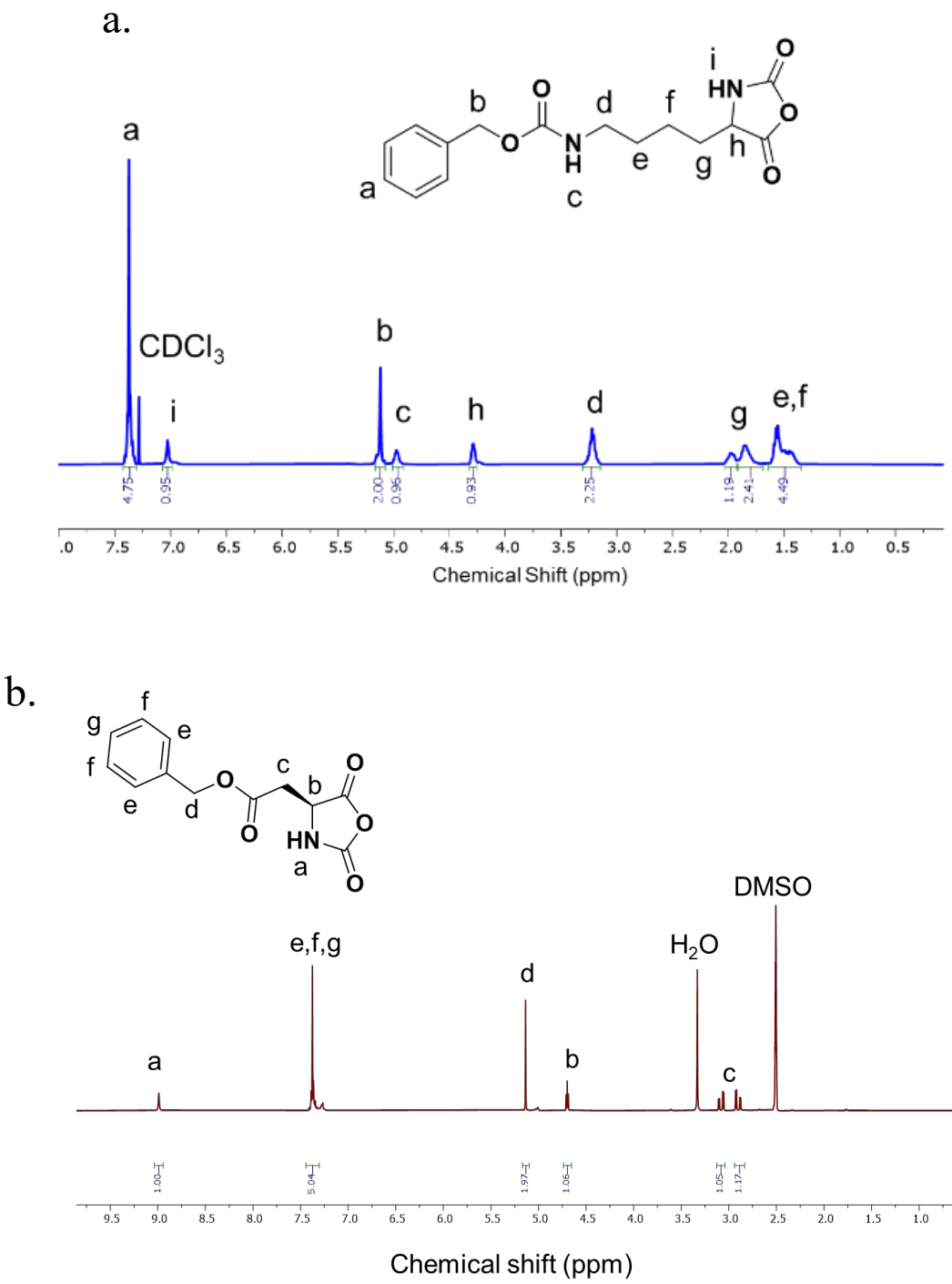
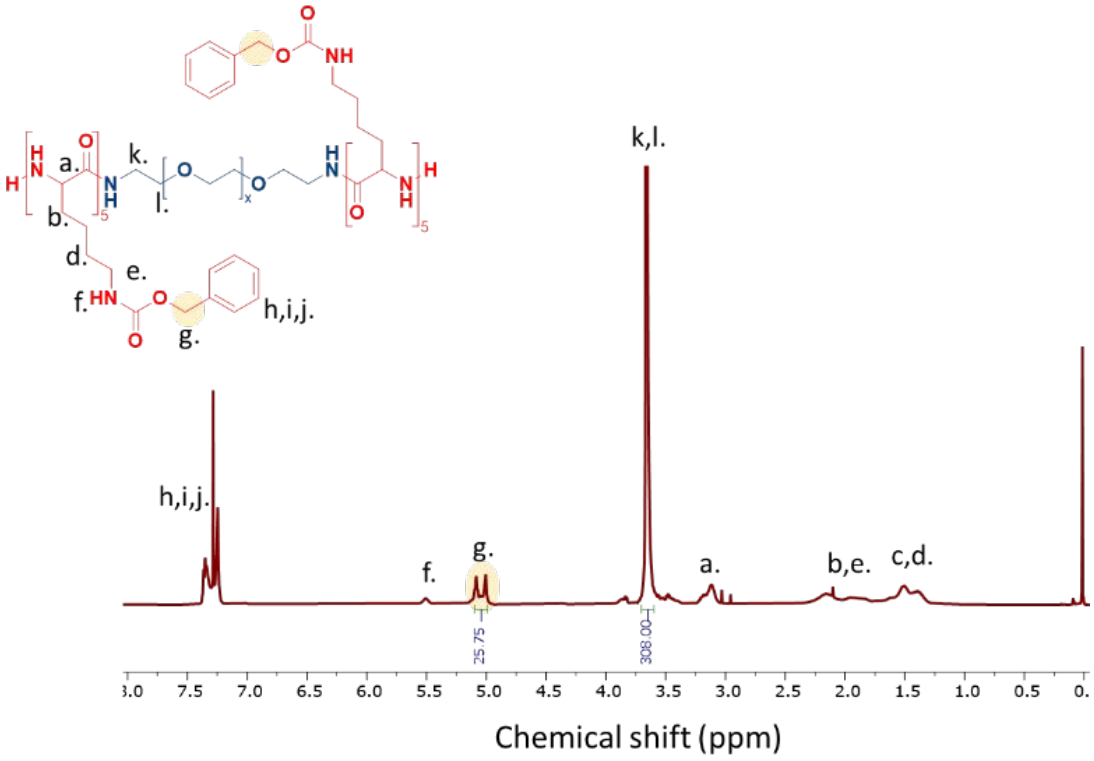
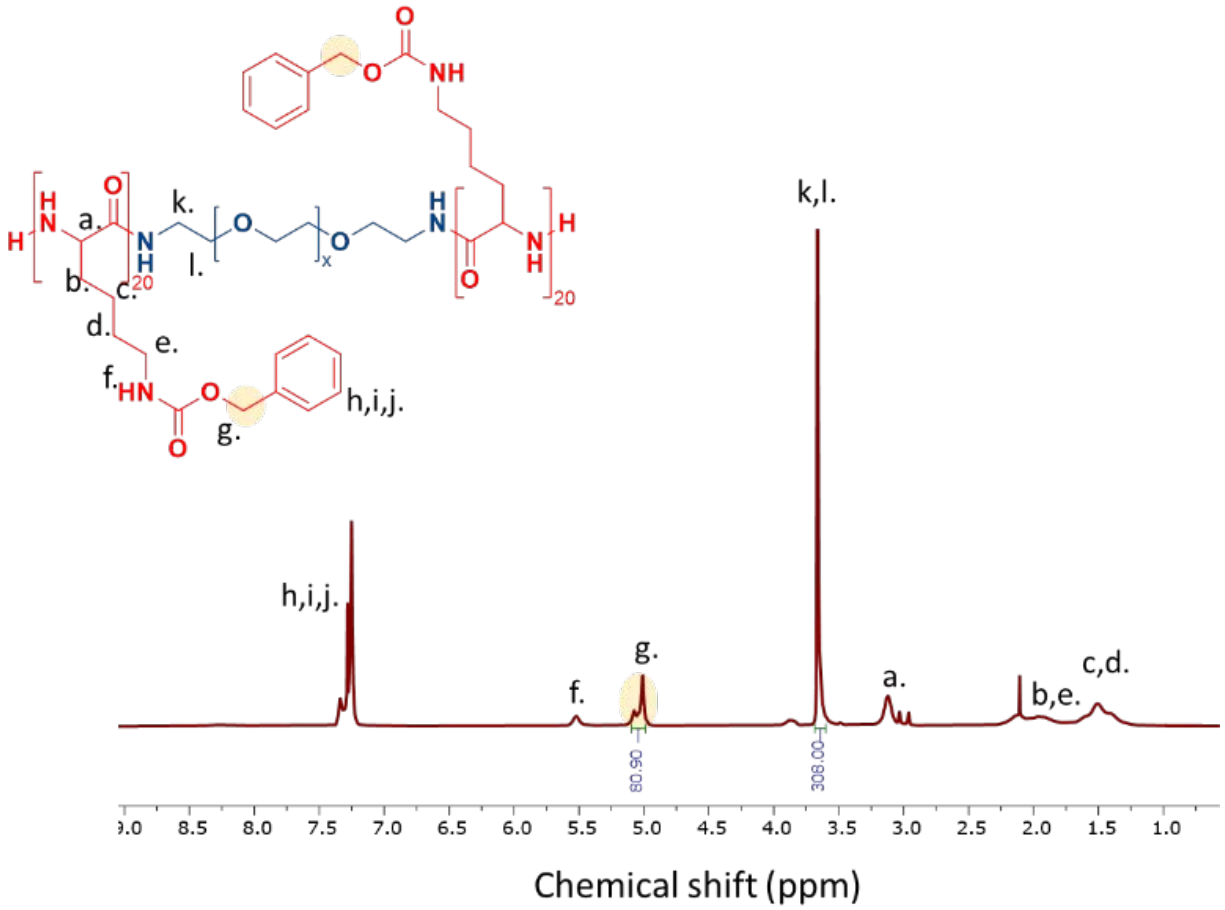


Figure S1: a. ^{1}H NMR of ZLY-NCA in $CDCl_3$: δ= 7.26 ppm (s, $CDCl_3$), δ= 7.48 ppm (m, 5H, Ph), δ= 7.02 ppm (NH) δ= 5.12 ppm (s, 2H, $PhCH_2O$), δ= 4.92 ppm (NH), δ= 4.28 ppm(t, 1H, CH), δ= 3.22 ppm (q, 2H, CH_2CH_2NH), δ= 1.90 ppm (m, 2H, CH_2CH_2CH), δ= 1.51 ppm (m, 4H, $CH_2CH_2CH_2$) b. ^{1}H NMR of BLA-NCA in D_6DMSO: δ= 9.0 ppm (NH), δ= 7.37 ppm (m, 5H, Ph), δ= 5.13 ppm (s, 2H, $PhCH_2O$) δ= 4.70 ppm (t, 1H, CH), δ= 3.08-2.90 ppm (m, 2H, CH_2), δ= 3.33 ppm (s, H_2O), δ= 2.5 ppm (s, D_6DMSO)

a.



b.


k,l.
h,i,j.
g.
f.
a.
c,d.
b,e.
80.90
308.00
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
Chemical shift (ppm)

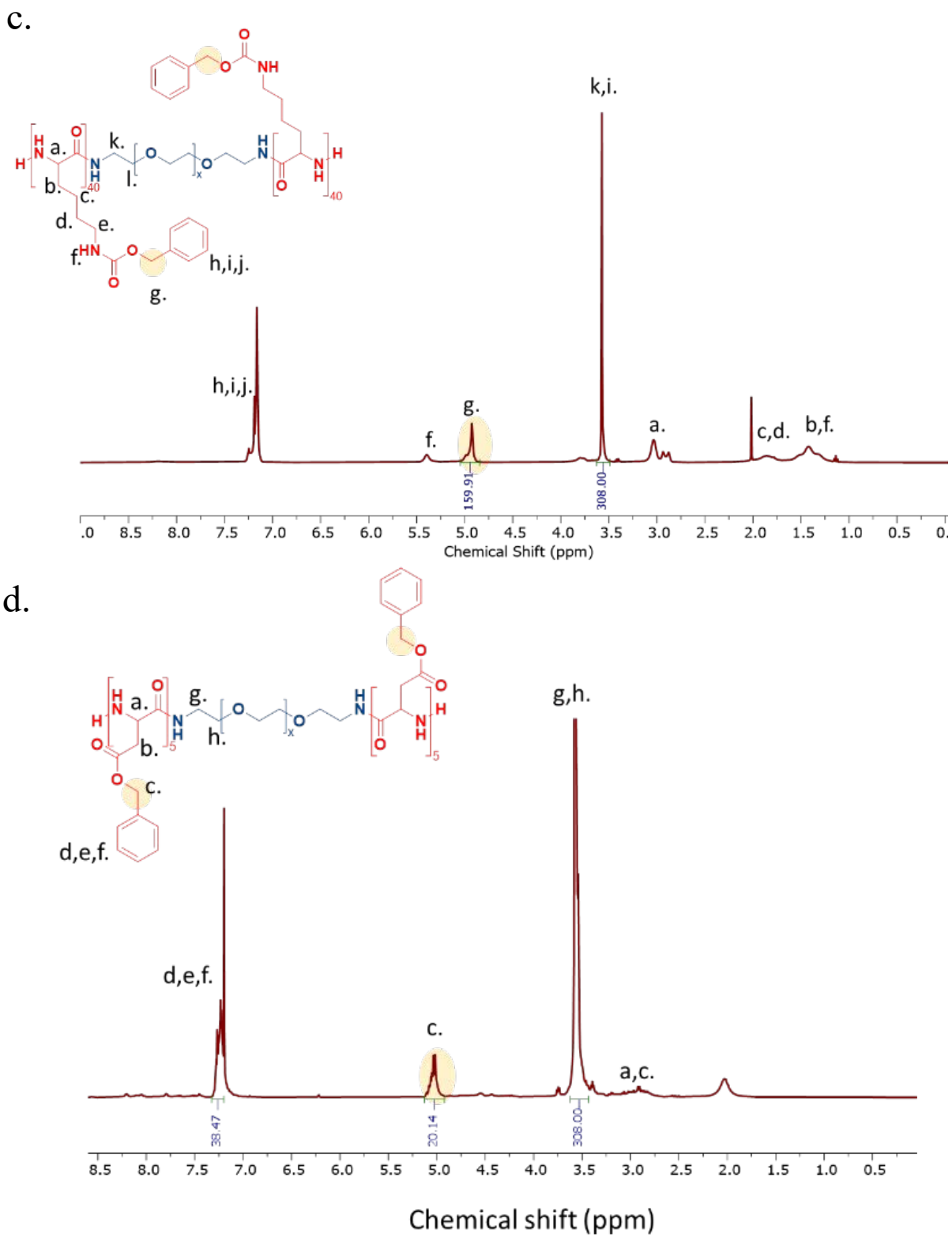


Figure S2: [1]H NMR of triblock copolymers; a. Z5-10 b. Z20-10, c. Z40-10, and d. A5-10. Peptide repeat length was confirmed via end group analysis of the carbobenzyloxy group,

specifically the benzyl-adjacent protons (peak at ~5.0 ppm) compared to the PEG backbone signal (3.64 ppm).

a. Z5-10, b. Z20-10, and c. Z40-10: δ= 7.26 ppm ($CDCl_3$), δ= 7.34 ppm (m, Ph), δ= 5.5 ppm (NH), δ= 5.04 ppm (broad s, $PhCH_2O$), δ= 3.64 ppm (CH_2CH_2O), δ= 3.12 ppm (m, CH_2CH_2CH), δ= 2.15 ppm (m, CH_2CH_2CH), δ= 2.0 (DMAc), δ= 1.48 ppm (m, CH_2CH_2CH)

d. A5-10 δ= 7.23 ppm (m, Ph), δ= 7.2 ppm ($CDCl_3$), δ= 5.03 ppm (broad s, $PhCH_2O$), δ= 2.9 ppm (m, CH), δ= 2.0 (s, DMAc)

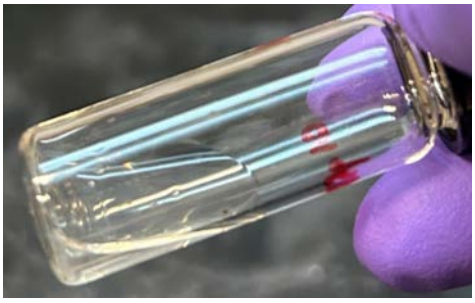


Figure S3: A5-10 10 wt% in PBS buffer. Solution fails the vial inversion test (*i.e.,* flows under gravity).

$$wt\%(peptide) = 100\left(\frac{xM_{PZLY}}{xM_{PZLY}+yM_{PEG}+zM_{HDI}}\right)$$

Equation S1: calculated weight fraction of peptide in peptide polyurea hybrids (PPUs), where x, y and z are the molar quantities of the PZLY triblock, PEG and HDI, respectively, and M_{ZLY}, M_{PEG} and M_{HDI} are the molecular weights of PZLY, PEG and HDI, respectively.

Table S1: Weight average molecular weight, number average molecular weight, and dispersity of PPU hybrids and non-peptidic control calculated from gel permeation chromatography (GPC) using 0.5 wt% LiBr in N,N-dimethyl acetamide (DMAc) used at the mobile phase. Distributions were generated using the calibration curve constructed for six poly(methyl methacrylate) standards (Agilent) in the range of 4.76 to 675.5 kg/mol.

Polymer	Weight-Average Molecular Weight, M_w (kg mol^{-1})	Number-Average Molecular Weight, M_n (kg mol^{-1})	Dispersity (M_w/M_n)
Z5-10	190	81.5	2.3
Z20-10	192	73.9	2.6
Z40-10	428	105	4.1
A5-10	88.4	42.2	2.0

PEG-PU	215	77.3	2.8

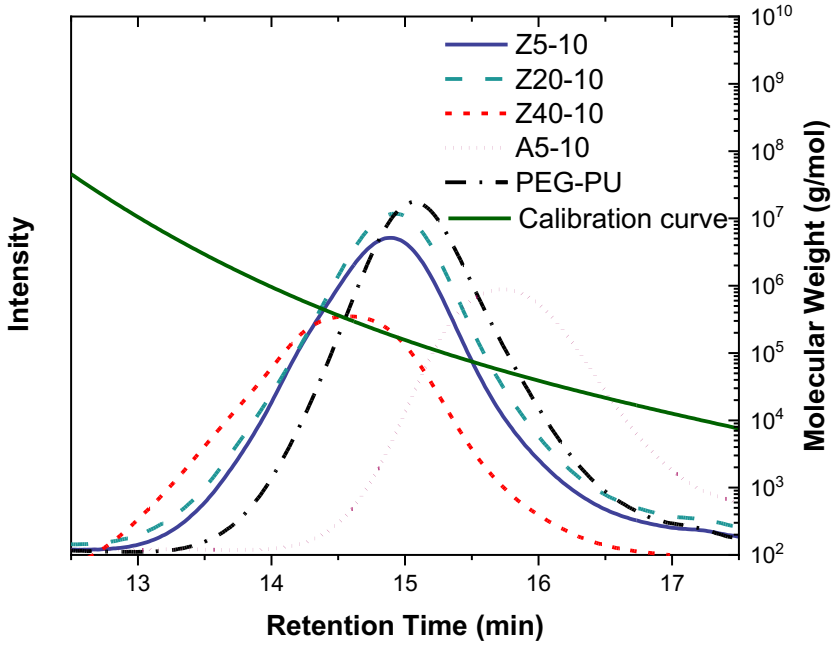


Figure S4: GPC traces of synthesized PPUs and PEG-PU control.

Rheological Characterization

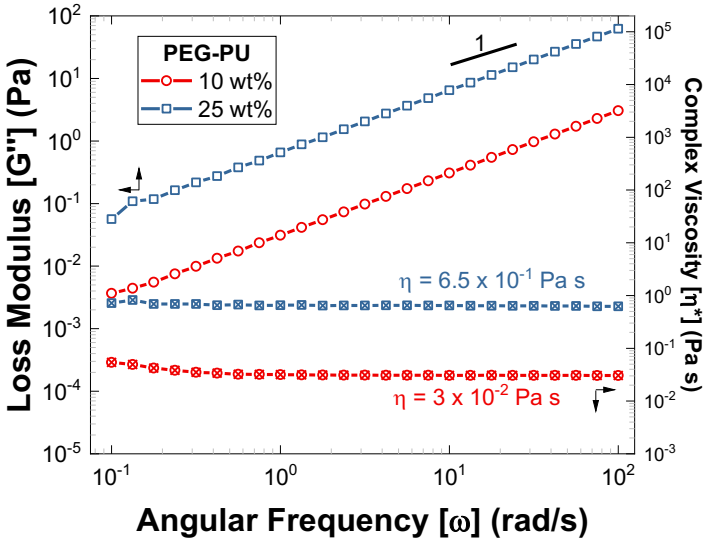


Figure S5: Frequency sweep of PEG-PU control sample at 10 and 25 wt% polymer in PBS buffer confirming liquid-like behavior. Storage moduli are omitted because of the measurements' low signal-to-noise ratio.

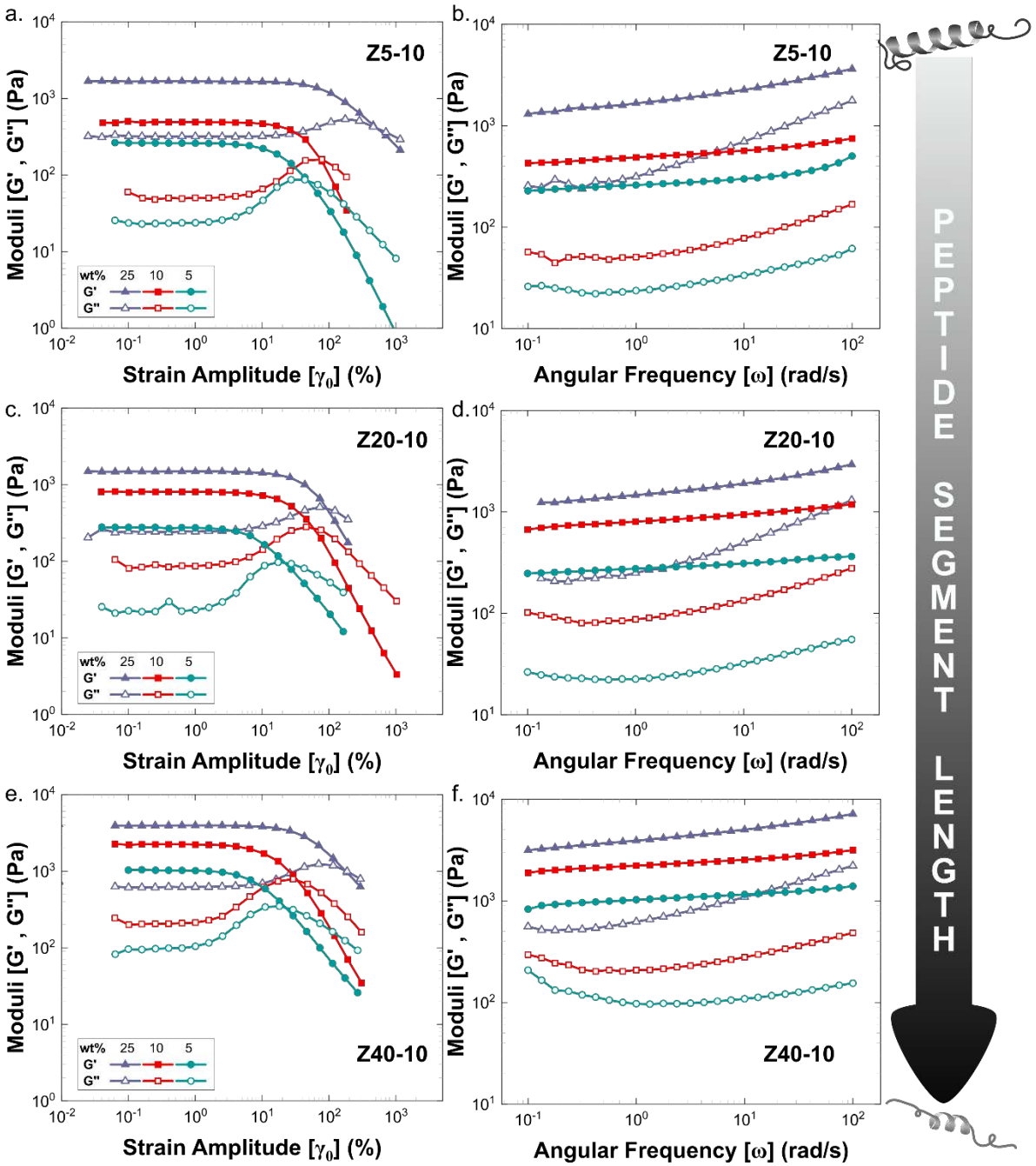


Figure S6. Frequency and amplitude sweeps for PPU hydrogels of varying peptide segment lengths and polymer concentration. Strain sweeps and frequency sweeps were performed at 1 rad/s and 0.5% strain, respectively. All measurements were made at 37 °C.

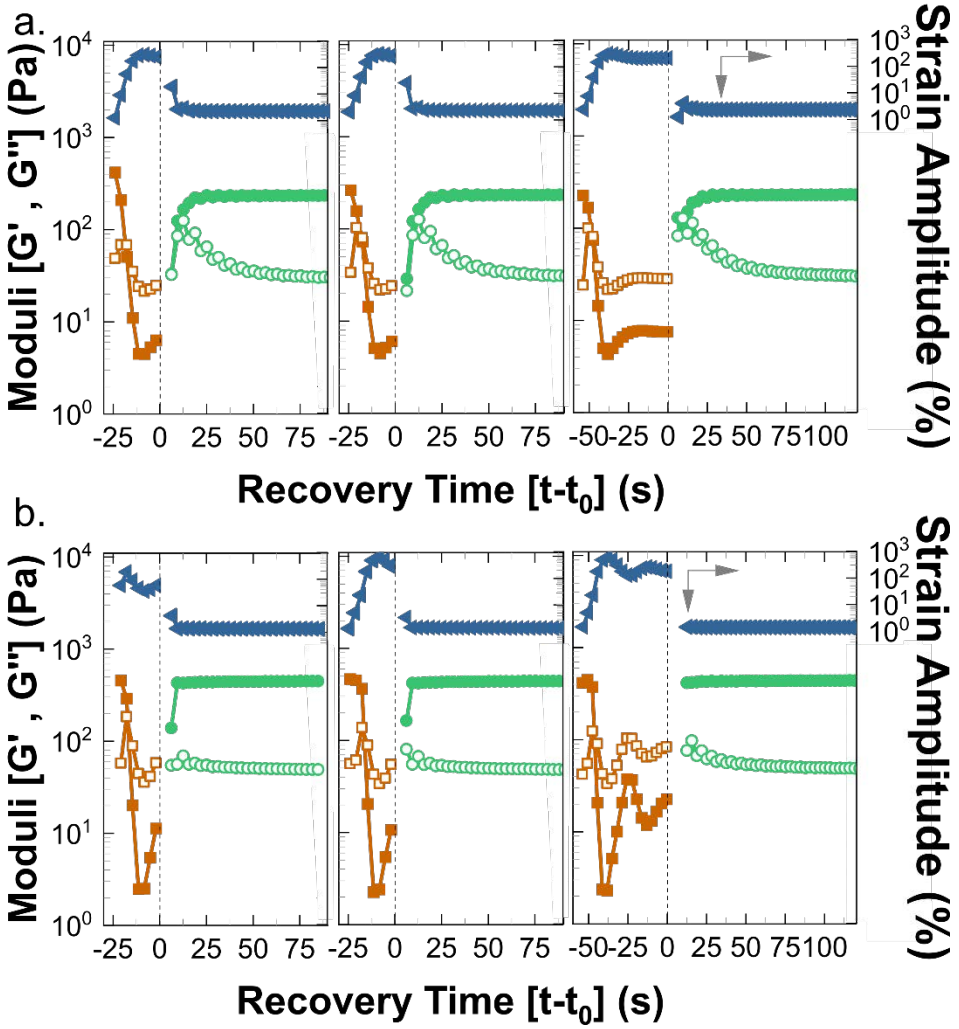


Figure S7. Simulated injection experiments on Z5-10 hydrogels at a. 5 wt% and b. 10 wt% in PBS. All experiments were performed at 37 °C. Filled and open symbols indicate storage (G') and loss (G'') modulus, respectively.

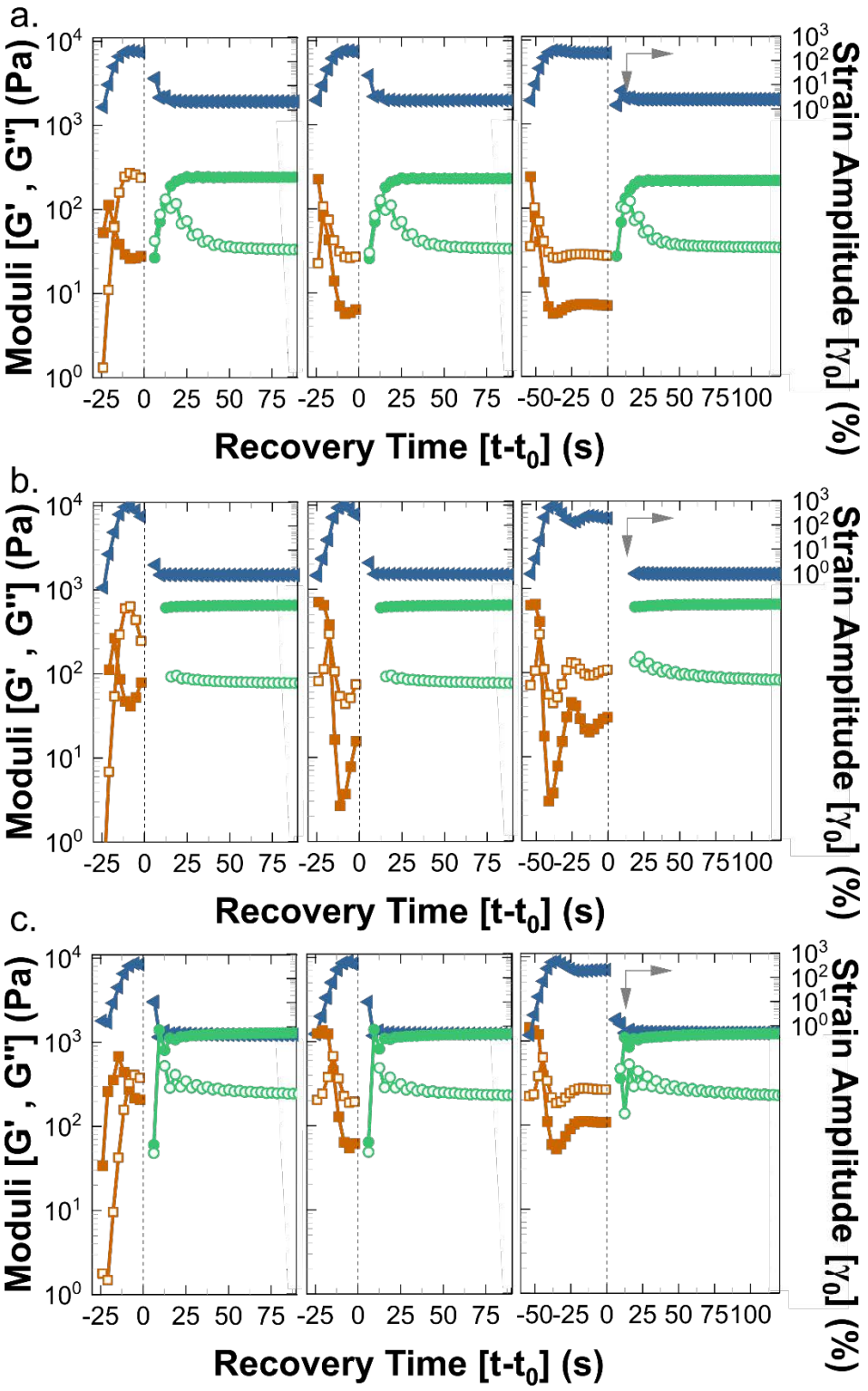


Figure S8. Simulated injection experiments on Z20-10 hydrogels at a. 5 wt%, b. 10 wt%, and c. 25 wt% in PBS. All experiments were performed at 37 °C. Filled and open symbols indicate G' and G'', respectively.

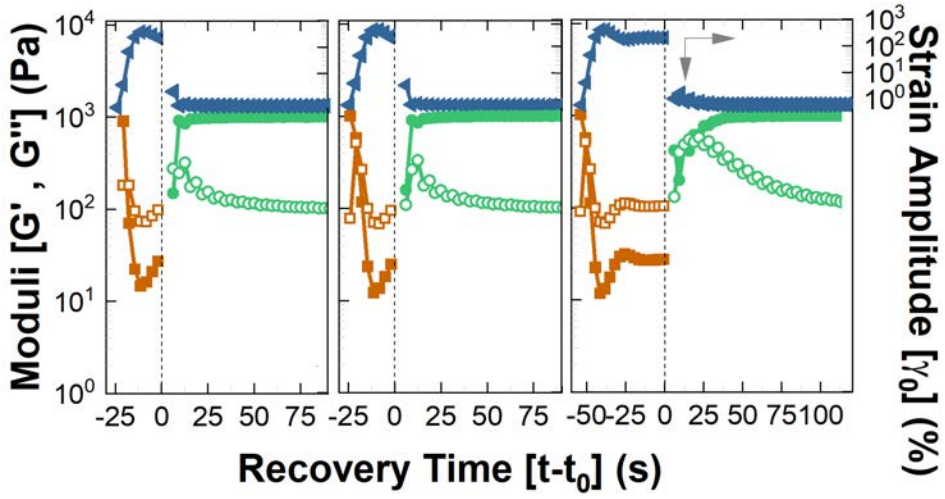


Figure S9. Simulated injection experiments on Z40-10 hydrogels at 5 wt% in PBS. The experiment was performed at 37 °C. Filled and open symbols indicate G' and G'', respectively.

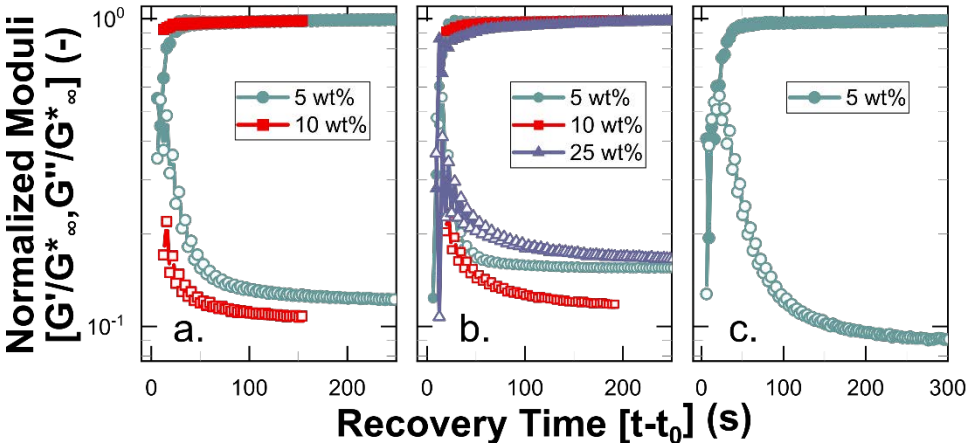


Figure S10. Recovery after simulated injection of a. Z5-10, b. Z20-10, and c. Z40-10 hydrogels at different concentrations in PBS. Experiment was performed at 37 °C. Moduli have been normalized by the value of the complex modulus at equilibrium (G^*_∞). Filled and open symbols indicate G'/G^*_∞ and G''/G^*_∞, respectively.

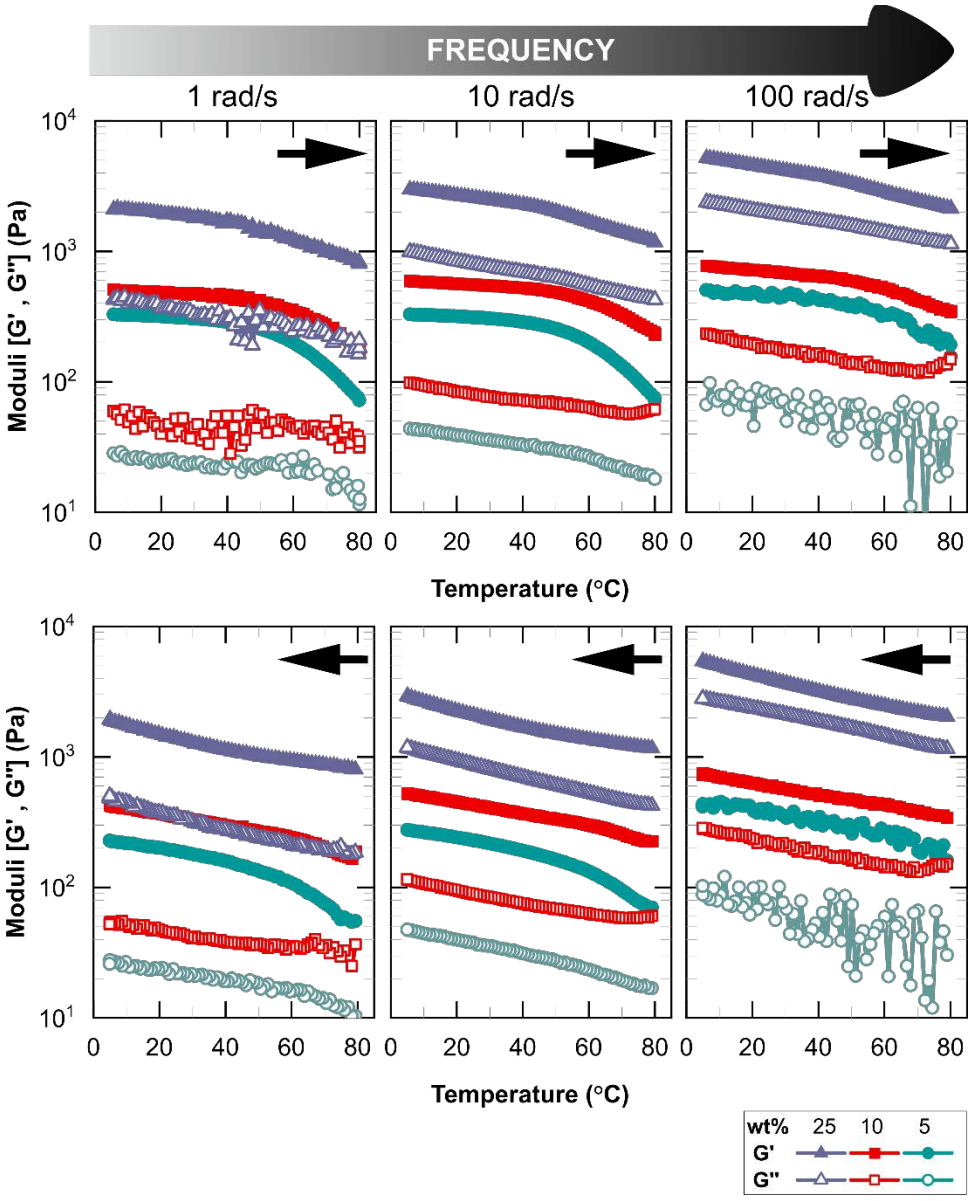


Figure S11. Temperature sweeps of Z5-10 PPU hydrogels of varying concentrations and measurement frequencies. Panels in the top and bottom rows are for heating and cooling cycles, respectively. Measurements were made at a fixed strain amplitude of 0.1%.

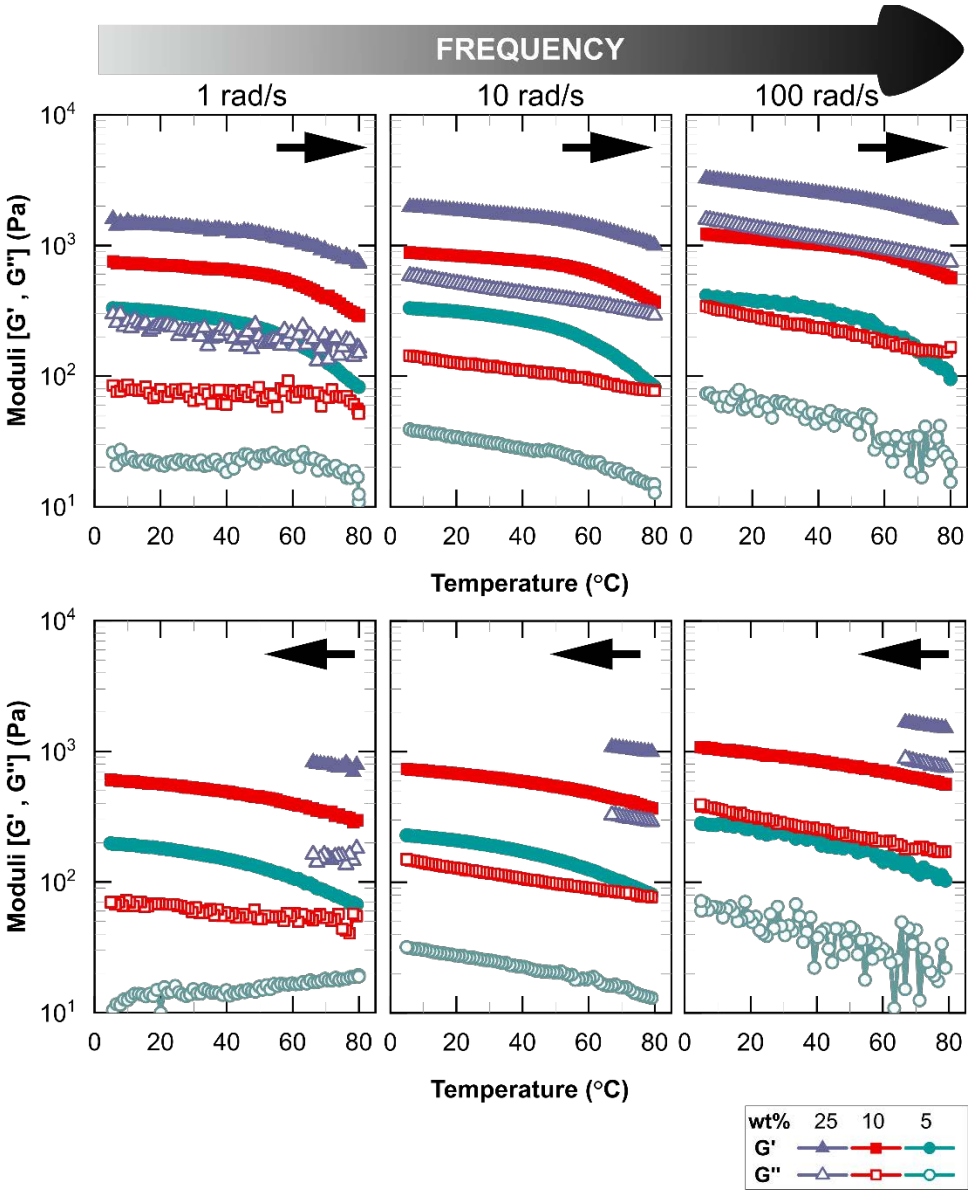


Figure S12. Temperature sweeps of Z20-10 hydrogels of varying concentrations and measurement frequencies. Panels in the top and bottom rows are for heating and cooling cycles, respectively. Measurements were made at a fixed strain amplitude of 0.1%. The cooling cycle for 25 wt% was stopped prematurely because of an experimental error.

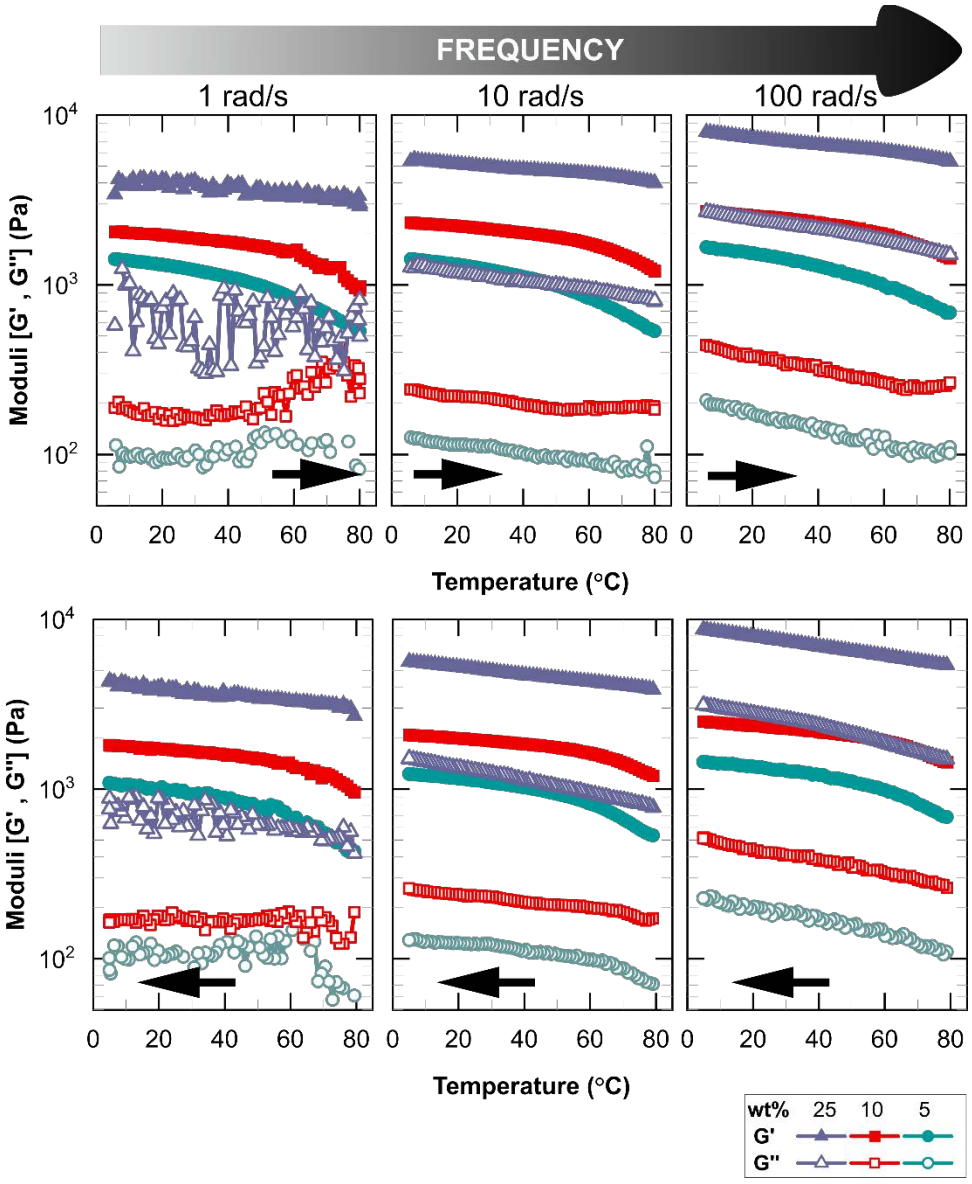


Figure S13. Temperature sweeps of Z40-10 hydrogels of varying concentrations and measurement frequencies. Panels in the top and bottom rows are for heating and cooling cycles, respectively. Measurements were made at a fixed strain amplitude of 0.1%.

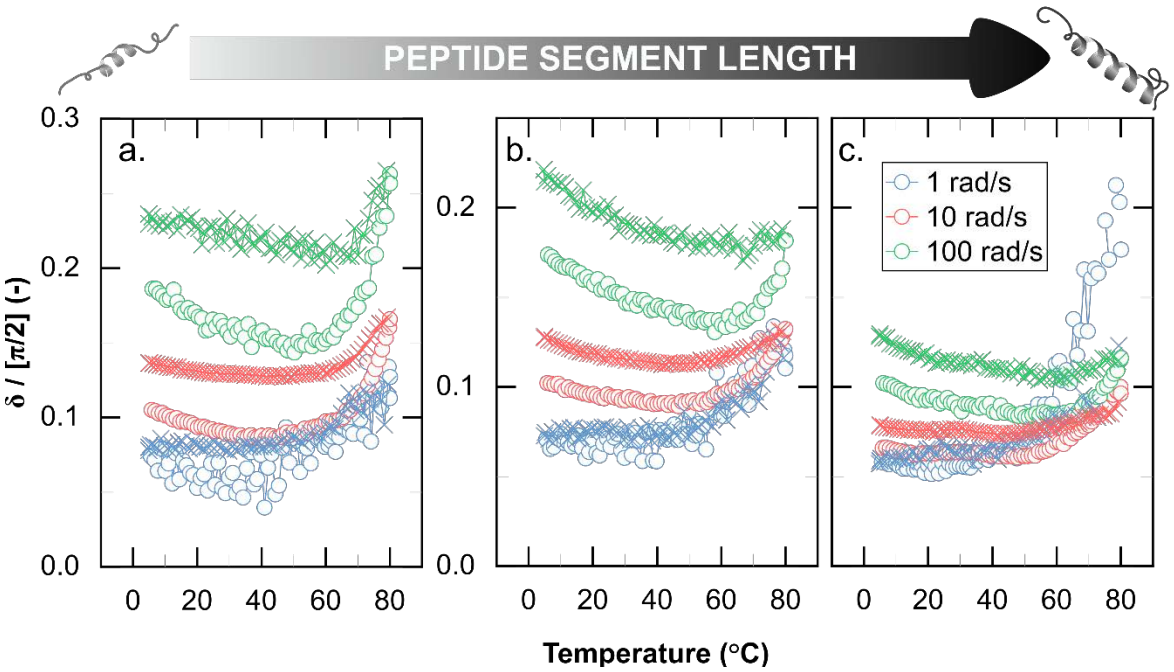


Figure S14. Quasi gel-point curves for 10 wt% a. Z5-10, b. Z20-10, and c. Z40-10 hydrogels undergoing heating (circles) and cooling (crosses) cycles of a temperature ramp depicting the phase angle (δ). Each curve was measured at a single frequency.

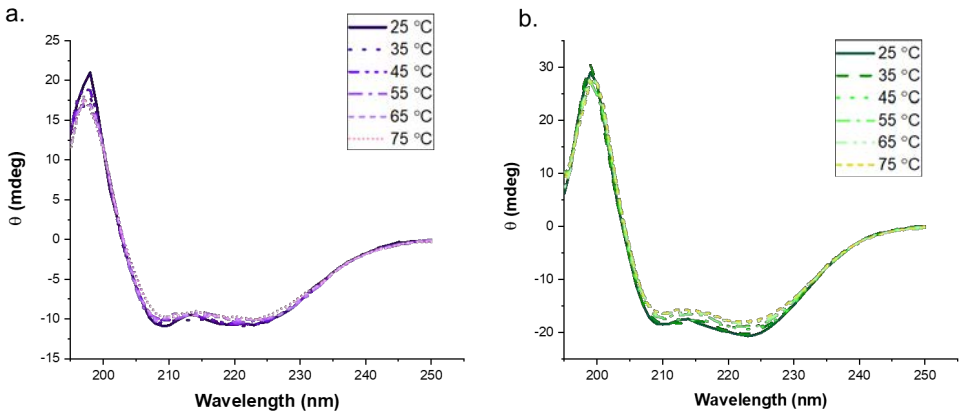


Figure S15: CD melting curves of a. Z5-10 and b. Z40-10 PPU hybrids. As in the Z20-10 sample shown in the main text, only slight melting of the secondary structures is observed, with the α-helices remaining largely stable up to 80 °C.

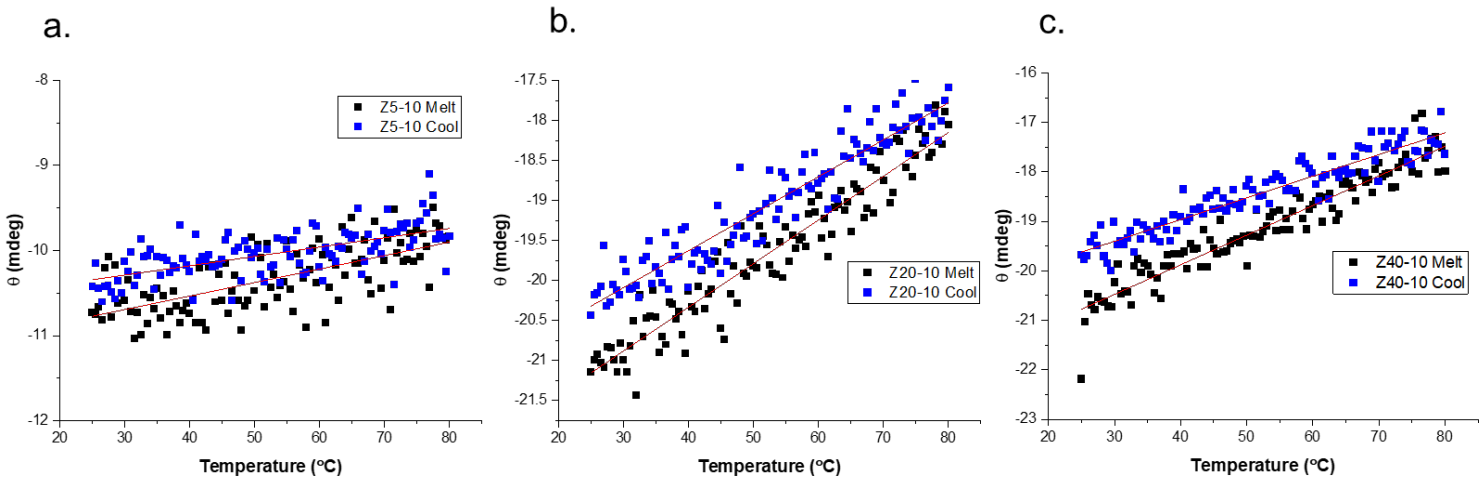


Figure S16: CD heating and cooling curves of PPUs monitored at 222 nm showing that slight melting of α-helices in PPUs is reversible, with some hysteresis being present in the cooling cycle for all samples.

Abbreviations:

BLA-NCA	β-Benzyl-L-aspartate N-carboxyanhydride
CD	Circular dichroism
$CDCl_3$	Deuterated chloroform
D_6DMSO	Deuterated dimethyl sulfoxide
GPC	Gel permeation chromatography
HDI	Hexamethylene diisocyanate
NMR	Nuclear magnetic resonance
PBLA	Poly(β-benzyl-L- aspartate)
PEG	Poly(ethylene glycol)
PPU	Peptide-polyurea
PZLY	Poly(ε-carbobenzyloxy-L-lysine)
SEM	Scanning electron microscopy
Cryo-TEM	Cryogenic-transmission electron microscopy
ZLA-NCA	Carbobenzyloxy-L-lysine N-carboxyanhydride

Final PPU samples are labeled with the nomenclature Z**N-X**, or A**N-X** where Z or A refer to PZLY or PBLA, respectively, **N** denotes the peptide repeat length, and **X** represents peptide weight percent in the polyurea.