Conformation directed formation of self-healing diblock copolypeptide hydrogels via polyion complexation

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Supporting Information

Materials and instrumentation Tetrahydrofuran (THF), hexanes, and methylene chloride were dried by purging with nitrogen and passage through activated alumina columns prior to use. $Co(PMe_3)_4$ and amino acid N-carboxyanhydride (NCA) monomers were prepared according to literature procedures.[1] All other chemicals were purchased from commercial suppliers and used without further purification unless otherwise noted. Selecto silica gel 60 (particle size 0.032– 0.063 mm) was used for flash column chromatography. Fourier Transform Infrared (FTIR) measurements were taken on a Perkin Elmer RX1 FTIR spectrophotometer calibrated using polystyrene film, and attenuated total reflectance (ATR-IR) data were collected using a PerkinElmer Spectrum 100 FTIR spectrometer equipped with a universal ATR sample accessory. ^{1}H NMR spectra were acquired on a Bruker ARX 400 spectrometer. Tandem gel permeation chromatography/light scattering (GPC/LS) was performed at 25 ^{o}C using an SSI Accuflow Series III pump equipped with Wyatt DAWN EOS light scattering and Optilab REX refractive index detectors. Separations were achieved using 100 Å and 1000 Å PSS-PFG 7 μm columns at 30 ºC with 0.5% (w/w) KTFA in 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) as eluent and sample concentrations of 10 mg/ml. Pyrogen free deionized water (DI) was obtained from a Millipore Milli-Q Biocel A10 purification unit. Circular Dichroism spectra were recorded in quartz cuvettes of 0.1 cm path length with samples prepared at concentrations between 0.10 to 0.17 mg/mL using Millipore deionized water. The spectra are reported in units of molar ellipticity [θ] (deg·cm^2·dmol^{-1}), using the formula, [θ] =

$(\theta \times 100 \times M_W)/(c \times l)$, where θ is the measured ellipticity in millidegrees, M_W, is the average residue molecular mass in g/mol, c is the peptide concentration in mg/mL; and l is the cuvette path length in cm.

General procedure for copolypeptide preparation

All polymerization reactions were performed in an N_2 filled glove box using anhydrous solvents. To a solution of L-methionine NCA (Met NCA) and L-alanine NCA (Ala NCA) in THF (50 mg/ml), a solution of $Co(PMe_3)_4$ in THF (20 mg/ml) was added.[1] The reaction was let to stir at ambient temperature (*ca*. 22 °C) for 60 min. Complete consumption of NCA was confirmed by FTIR spectroscopy, and then the desired amount of γ-benzyl-L-glutamate NCA (Bn-Glu NCA) or ε-TFA-L-lysine NCA (TFA-Lys NCA) in THF (50 mg/ml) was added to the reaction mixture, which was let to stir for an additional 60 min. FTIR was used to confirm complete consumption of all NCAs. Outside the glove box, the block copolypeptide solutions were precipitated into 10 mM HCl (20 ml), and then washed with 10 mM aqueous HCl (2x 20 ml) to remove residual cobalt ions. The white precipitates were then washed with DI water (3x 20 ml) and freeze-dried.[1]

Table S1. Copolymerization data for diblock copolypeptide synthesis.

Sample	M_w/M_n[a]	Composition[b]	Yield (%)[c]
$\mathbf{(M^OA)_{155}E_{30}}$	1.35	$(M^OA)_{156}E_{27}$	94
$\mathbf{(M^OA)_{155}E_{60}}$	1.41	$(M^OA)_{156}E_{59}$	96
$\mathbf{(M^OA)_{155}E_{90}}$	1.45	$(M^OA)_{156}E_{88}$	92
$\mathbf{(M^OA)_{155}E_{120}}$	1.42	$(M^OA)_{156}E_{117}$	97
$\mathbf{(M^OA)_{155}(\textit{rac}\text{-}E)_{60}}$	1.45	$(M^OA)_{156}(\textit{rac}\text{-}E)_{56}$	92
$\mathbf{(M^OA)_{155}K_{30}}$	1.38	$(M^OA)_{156}K_{28}$	97
$\mathbf{(M^OA)_{155}K_{60}}$	1.41	$(M^OA)_{156}K_{62}$	95
$\mathbf{(M^OA)_{155}K_{90}}$	1.40	$(M^OA)_{156}K_{88}$	95
$\mathbf{(M^OA)_{155}K_{120}}$	1.37	$(M^OA)_{156}K_{119}$	96

[a] Dispersity of oxidized, protected block copolypeptides were determined by GPC/LS. [b] Relative amino acid compositions of oxidized, deprotected block copolypeptides were determined by ^{1}H NMR

integrations. Degree of polymerization of initial $\mathbf{MA_x}$ segment was determined by end-group analysis using ^{1}H NMR. [c] Total isolated yield of purified block copolypeptides following deprotection.

Example synthesis of poly(L-methionine$_{0.88}$-*stat*-L-alanine$_{0.12}$)$_{155}$-*block*-poly(ε-trifluoroacetyl-L-lysine)$_{60}$, (MA)$_{155}$(TFA-K)$_{55}$ and poly(L-methionine$_{0.88}$-*stat*-L-alanine$_{0.12}$)$_{155}$-*block*-poly(γ-benzyl-L-glutamate)$_{60}$, (MA)$_{155}$(Bn-E)$_{60}$

Met NCA (120 mg, 0.71 mmol) and Ala NCA (11 mg, 0.097 mmol) were dissolved together in THF (2.7 ml) and placed in a 20 ml scintillation vial containing a stir bar. To the vial, $(PMe_3)_4Co$ initiator solution (260 µl of a 20 mg/ml solution in THF) was added via syringe. The vial was sealed and allowed to stir in the glove box for 1 h. An aliquot (20 µl) was removed and analyzed by FTIR to confirm that all the NCA was consumed. In the glove box, α-methoxy-ω-isocyanoethyl-poly(ethylene glycol)$_{45}$ (mPEG$_{23}$-NCO)[1] (20 mg) was dissolved in THF (1 ml) in a 20 ml scintillation vial. An aliquot (350 µl) of the polymerization solution containing active chain ends was removed and added to the solution of mPEG$_{23}$-NCO. The PEG end-capped sample ($\mathbf{MA_x}$-**mPEG$_{23}$**) was sealed, allowed to stir for 24 h, and then used for chain length determination (*vide infra*). Separately, aliquots of the polymerization solution containing active chains (1.2 ml each) were added to vials containing either Bn-Glu NCA (32 mg, 0.12 mmol) or TFA-Lys NCA (33 mg, 0.12 mmol) dissolved in THF (64 µl or 65 µl, respectively). The vials were sealed and allowed to stir in the glove box for 1 h to give the diblock copolypeptides, **(MA)$_{155}$(TFA-K)$_{60}$** and **(MA)$_{155}$(Bn-E)$_{60}$**. FTIR was used to confirm complete consumption of NCAs in both reactions. Outside the glove box, the block copolypeptide solutions were precipitated into 10 mM HCl (20 ml), and then washed with 10 mM aqueous HCl (2 x 20 ml) to remove residual cobalt ions. The white precipitates were then washed with DI water (3 x 20 ml) and freeze-dried (average yield = 98%).

Analytical data: (MA)$_{155}$(Bn-E)$_{60}$

^{1}H NMR (400 MHz, d-TFA, 25 ^{o}C): δ 7.38 (br m, 2.3H), 5.24 (br m, 0.93H), 4.97 (br s, 1H), 4.81 (br m, 0.54H), 2.81 (br m, 2H), 2.6 (br m, 1.06 H), 2.40 - 2.05 (br m, 6.37H), 1.61 (br s, 0.42H). FTIR (THF,

25 ^{o}C): 1738 cm^{-1} (benzyl ester), 1652 cm^{-1} (amide I), 1550 cm^{-1} (amide II).

Analytical data: $(MA)_{155}(TFA\text{-}K)_{60}$

^{1}H NMR (400 MHz, d-TFA, 25 ^{o}C): δ 4.86 (br s, 0.94 H), 4.60 (br m, 0.54H), 3.46 (br m, 1.23 H), 2.69 (br m, 2H), 2.17 (br m, 5H), 1.9 (br m, 1.42 H), 1.69 (br m, 1.34 H), 1.50 (br m, 1.32 H), 1.31 (br m, 0.68 H). FTIR (THF, 25 ^{o}C): 1726 cm^{-1} (TFA amide), 1652 cm^{-1} (amide I), 1550 cm^{-1} (amide II).

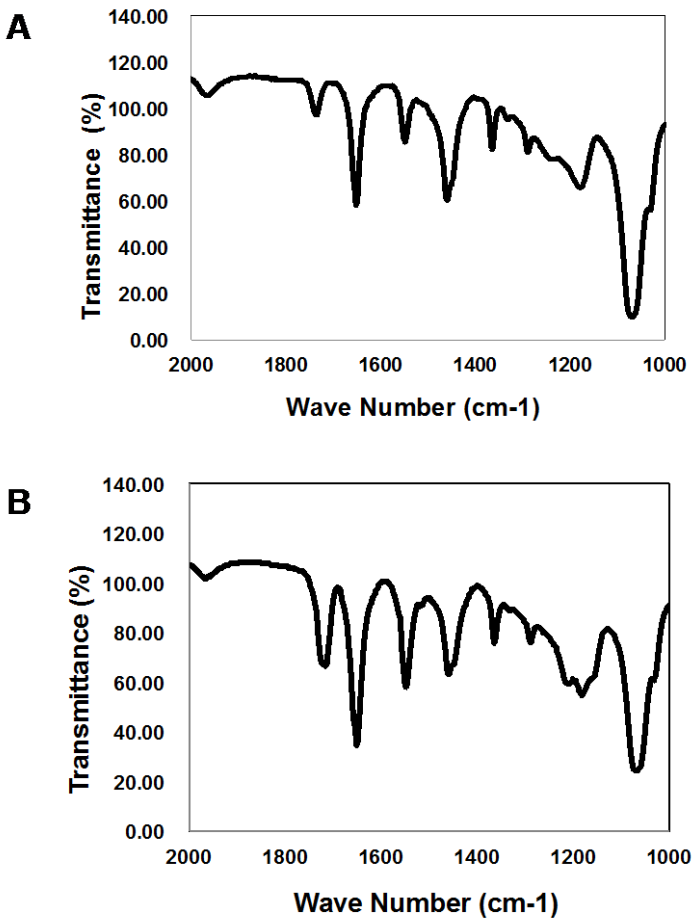


Figure S1. Sample FTIR spectra of (A) **$(MA)_{155}(TFA\text{-}K)_{120}$** and (B) **$(MA)_{155}(Bn\text{-}E)_{120}$** in THF.

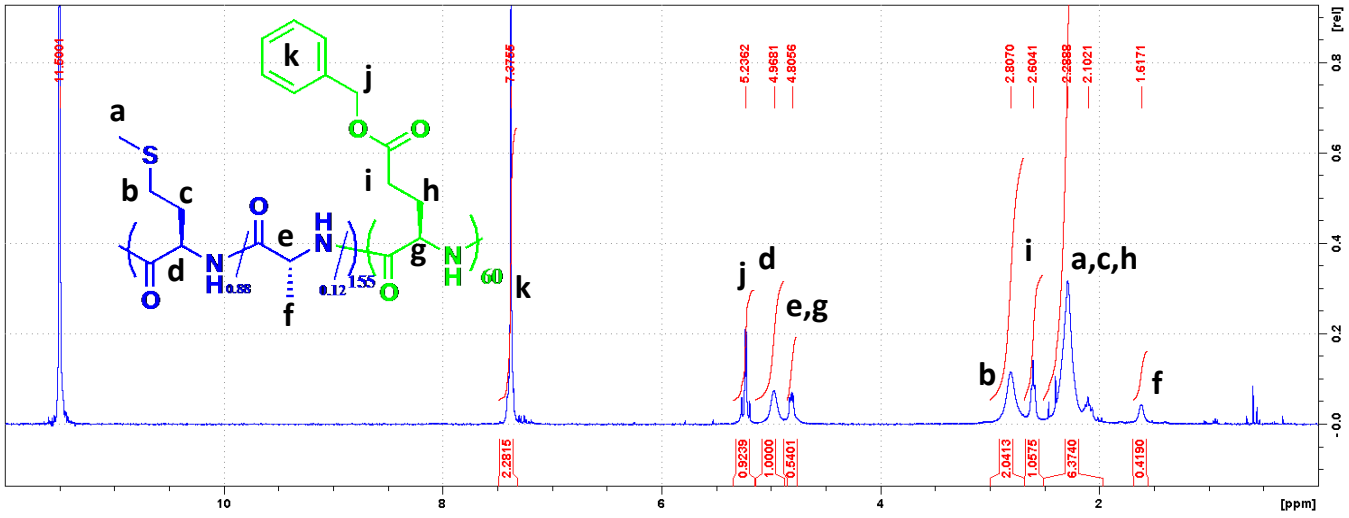


Figure S2. ^{1}H NMR spectrum of **(MA)$_{155}$(Bn-E)$_{60}$** in d-TFA.

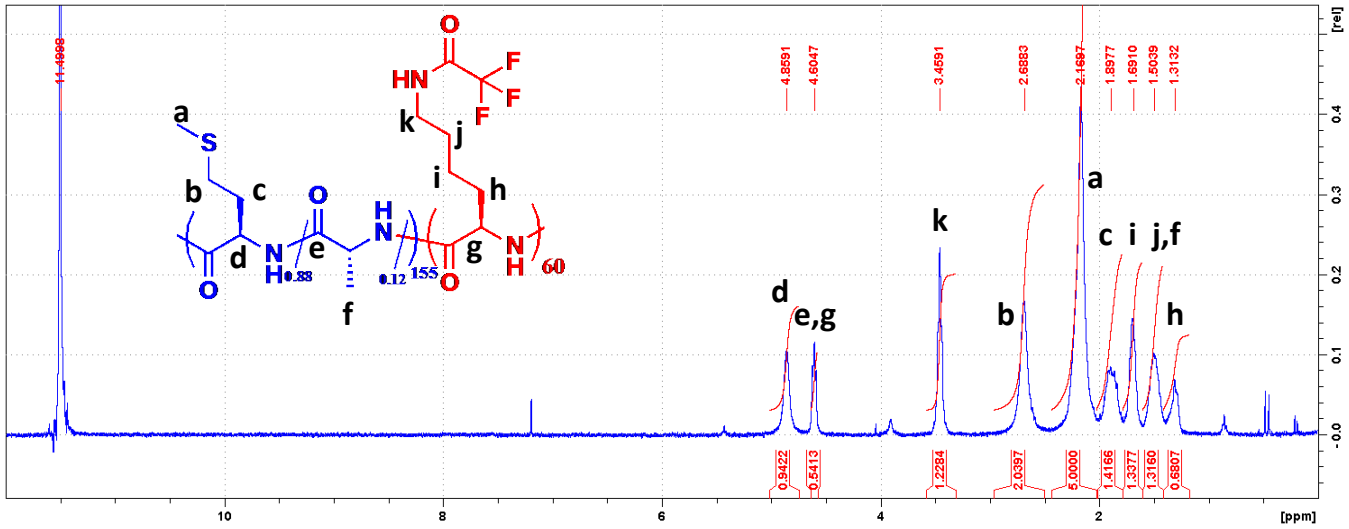


Figure S3. ^{1}H NMR spectrum of **(MA)$_{155}$(TFA-K)$_{60}$** in d-TFA.

Sample procedure for MA$_x$ chain length determination using end-group analysis

Outside of the glove box, the PEG end-capped sample (**MA$_x$-mPEG$_{23}$**) from above was washed with 10 mM aqueous HCl (2x). After stirring for 1 h, **MA$_x$-mPEG$_{23}$** was collected by centrifugation and washed with DI water (3 x 20 ml) to remove all non-conjugated mPEG$_{23}$-NCO. The remaining **MA$_x$-mPEG$_{23}$** was then freeze-dried to remove residual H_2O. To determine **MA$_x$** molecular weights (M_n), ^{1}H NMR spectra were obtained. Since it has been shown that end-capping is quantitative for $(PMe_3)_4Co$ initiated NCA polymerizations when excess isocyanate is used,[2] integrations of methionine (δ 2.70) and alanine

(δ 1.52) resonances versus the polyethylene glycol resonance at δ 3.92 could be used to obtain both **M** to **A** ratios and $\mathbf{MA}_x$ lengths (found: x = 156, designated as $\mathbf{MA}_{155}$). ^{1}H NMR (400 MHz, d-TFA, 25 ^{o}C): 4.87 (br s, 1H), 4.68 (br s, 0.167H), 3.92 (br m, 0.71H), 2.70 (br m, 2.03 H), 2.30 - 2.05 (br m, 5.16H), 1.52 (br s, 0.43H).

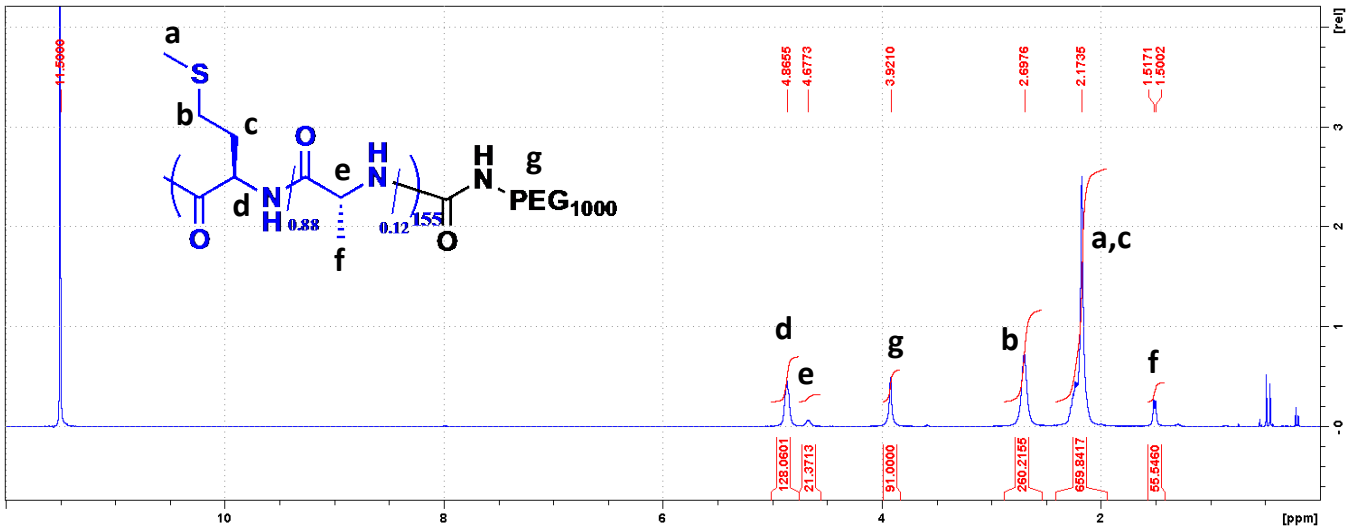


Figure S4. ^{1}H NMR spectrum of **$(MA)_{155}$-$mPEG_{23}$ (1000 Da)** in d-TFA.

Preparation of poly(L-methionine sulfoxide$_{0.88}$-*stat*-L-alanine$_{0.12}$)$_{155}$-*block*-poly(ε-trifluoroacetyl-L-lysine)$_{60}$, (M^OA)$_{155}$(TFA-K)$_{60}$, and poly(L-methionine sulfoxide$_{0.88}$-*stat*-L-alanine$_{0.12}$)$_{155}$-*block*-poly(γ-benzyl-L-glutamate)$_{60}$, (M^OA)$_{155}$(Bn-E)$_{60}$

In separate scintillation vials (5 ml) containing stir bars, **$(MA)_{155}$(TFA-K)$_{60}$** and **$(MA)_{155}$(Bn-E)$_{60}$** were suspended in 80% *tert*-butyl hydroperoxide (TBHP) in water (16 eq TBHP per methionine residue). Camphorsulfonic acid (0.2 eq per methionine residue) was then added to each vial, and DI water was added to give final copolymer concentrations of *ca*. 20 mg/ml. These reactions were stirred for 16 h at ambient temperature (*ca*. 22 °C). Saturated sodium thiosulfate (0.5 ml) was then added dropwise to each vial in order to quench the reactions, and the samples were transferred to 2000 MWCO dialysis tubes and then dialyzed against DI water for 2 d with frequent water changes. The resulting solutions were freeze-dried to yield white fluffy solids (average yield =97%).[3]

Analytical Data: $(M^{O}A)_{155}(Bn\text{-}E)_{60}$

^{1}H NMR (400 MHz, d-TFA, 25 ^{o}C): δ 7.24 (br m, 2.2H), 5.10 (br m, 0.91H), 4.85 (br s, 1H), 4.69 (br m, 0.55H), 3.45 - 3.10 (br m, 2.06H), 2.90 (br m, 3 H), 2.62 (br m, 1.04 H), 2.47 (br m, 1.86 H), 2.18 (br m, 0.45H), 1.97 (br m, 0.45), 1.49 (br s, 0.40 H).

Analytical Data: $(M^{O}A)_{155}(TFA\text{-}K)_{60}$

^{1}H NMR (400 MHz, d-TFA, 25 ^{o}C): δ 4.91 (br s, 1H), 4.64 (br m, 0.52H), 3.52-3.10 (br m, 2.96 H), 2.96 (br m, 3.03H), 2.67 (br m, 1.04 H), 2.46 (br m, 1 H), 1.96 (br m, 0.86 H), 1.73 (br m, 0.88 H), 1.54 (br m, 1.27 H).

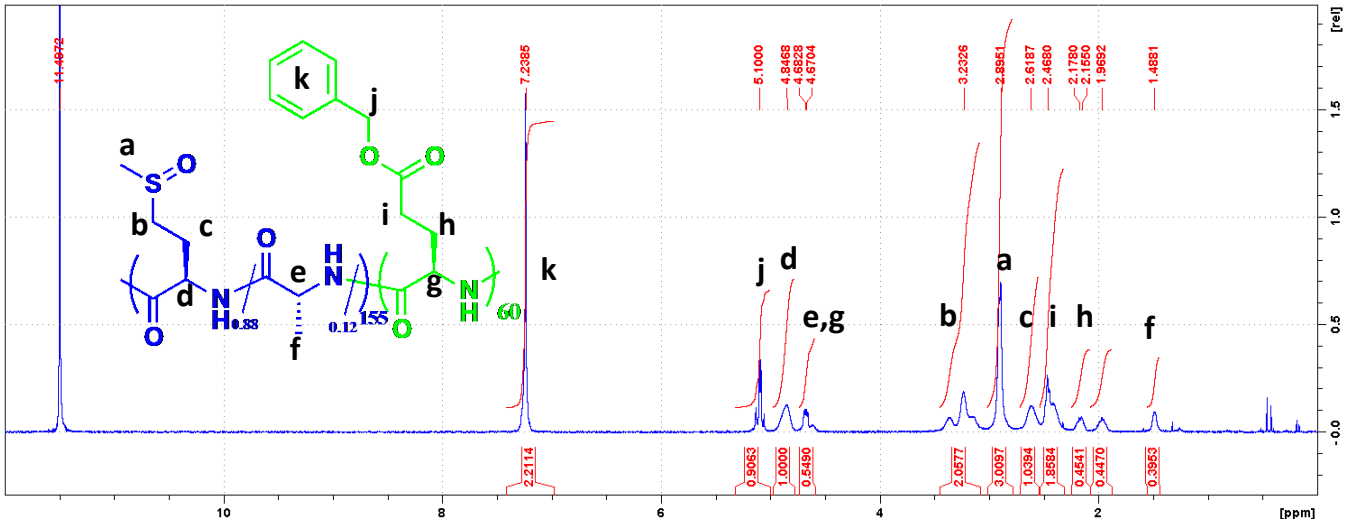


Figure S5. ^{1}H NMR spectrum of **$(M^{O}A)_{155}(Bn\text{-}E)_{60}$** in d-TFA.

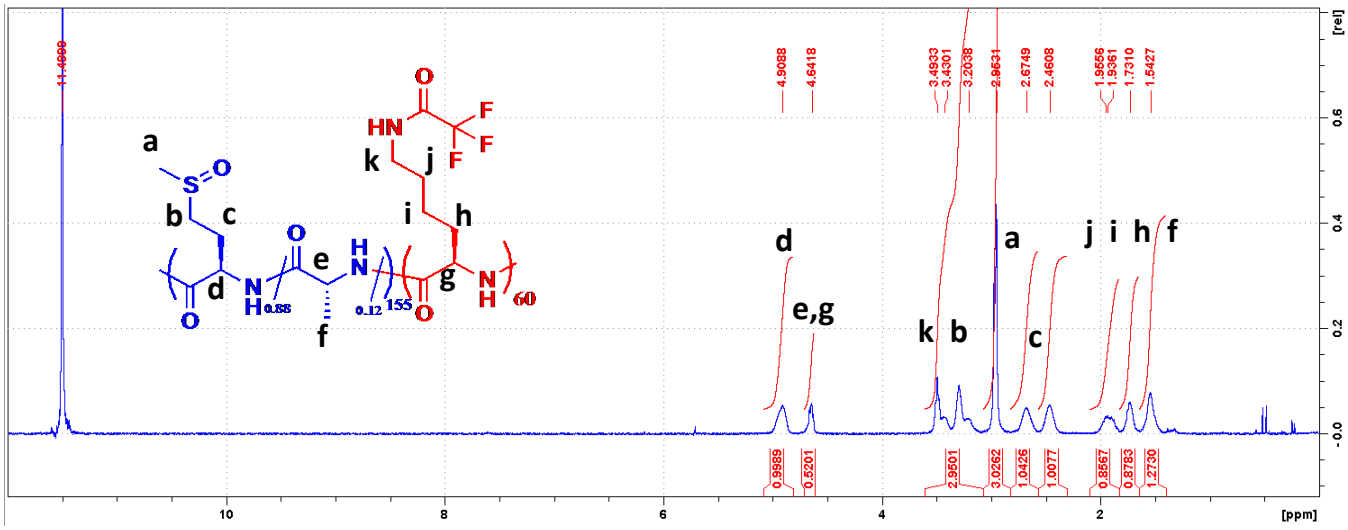


Figure S6. ^{1}H NMR spectrum of **$(M^{O}A)_{155}(TFA\text{-}K)_{60}$** in d-TFA.

Preparation of poly(L-methionine sulfoxide$_{0.88}$-*stat*-L-alanine$_{0.12}$)$_{155}$-*block*-poly(L-lysine)$_{60}$, (M^{O}A)$_{155}$K$_{60}$

A sample of **(M^{O}A)$_{155}$(TFA-K)$_{60}$** was dispersed in a 9:1 methanol:water mixture (20 mg/ml) and K_2CO_3 (2 eq per lysine residue) was added. The reaction was stirred for 8 h at 50 ^{o}C, and the majority of the methanol was then removed under vacuum. The resulting solution (*ca*. 10% of original volume) was then diluted with water (3 times the remaining volume), transferred to a 2000 MWCO dialysis bag, and then dialyzed against 0.10 M aqueous NaCl at pH 3 (HCl) for 24 h, followed by DI water for 48 hours with water changes twice per day. The contents of the dialysis bag were then lyophilized to dryness to give a white solid (yield = 93%).[1] ^{1}H NMR (400 MHz, D_2O, 25 ^{o}C): δ 4.52 (br s, 1H), 4.37 (br m, 0.52H), 3.2-2.8 (br m, 3.18 H), 2.75 (br m, 3.1 H), 2.40 - 2.20 (br m, 2.2 H), 1.73 (br m, 1.62H), 1.44 (br m, 1.32H). ATR-IR (25 ^{o}C): 1653 cm^{-1} (amide I), 1546 cm^{-1} (amide II).

Preparation of poly(L-methionine sulfoxide$_{0.88}$-*stat*-L-alanine$_{0.12}$)$_{155}$-*block*-poly(L-glutamate)$_{60}$, (M^{O}A)$_{155}$E$_{60}$

A sample of **(M^{O}A)$_{155}$(Bn-E)$_{60}$** was dissolved in trifluoroacetic acid (TFA, 30 eq per benzyl glutamate residue) in an ice bath. Methanesulfonic acid (MSA, 35 eq) and anisole (5 eq) were then added under vigorous stirring. The reaction mixture was stirred for 20 min in the ice bath, and then for an additional 90 min at ambient temperature. Next, the copolymer was precipitated using Et_2O (20 ml) and collected by centrifugation. The pellet was dissolved in 10% aqueous $NaHCO_3$ (3 ml), extensively dialyzed (2000 MWCO) against DI water for 2 d, and then freeze-dried to give a white solid (yield = 95%).[4] ^{1}H NMR (400 MHz, D_2O, 25 ^{o}C): δ 4.50 (br s, 1H), 4.40 (br m, 0.57H), 3.00 (br m, 2.03H), 2.75 (br m, 2.95 H), 2.40 - 2.10 (br m, 3 H), 2.10 - 1.80 (br m, 1H), 1.44 (br s, 0.4 H). ATR-IR (25 ^{o}C): 1653 cm^{-1} (amide I), 1546 cm^{-1} (amide II).

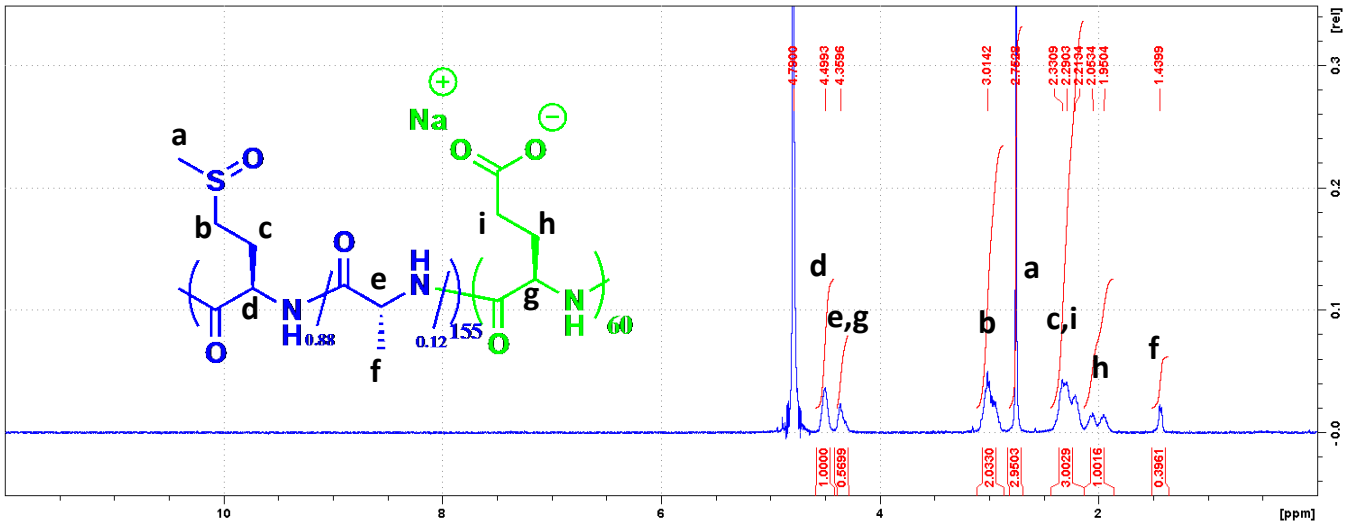


Figure S7. ^{1}H NMR spectrum of $\mathbf{(M^{O}A)_{155}E_{60}}$ in D_2O.

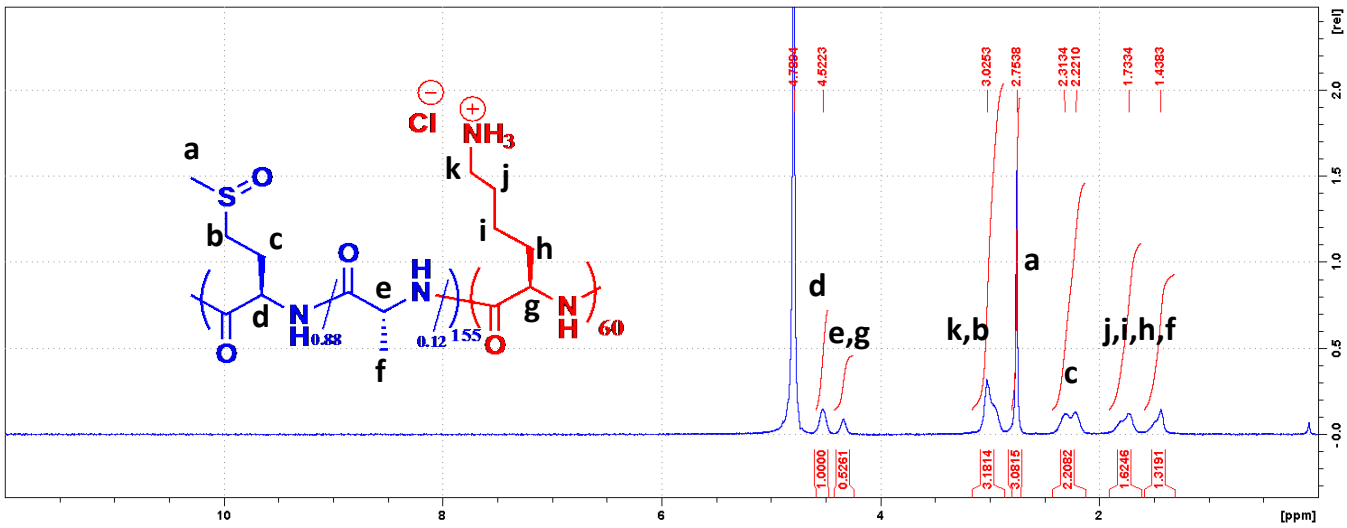


Figure S8. ^{1}H NMR spectrum of $\mathbf{(M^{O}A)_{155}K_{60}}$ in D_2O.

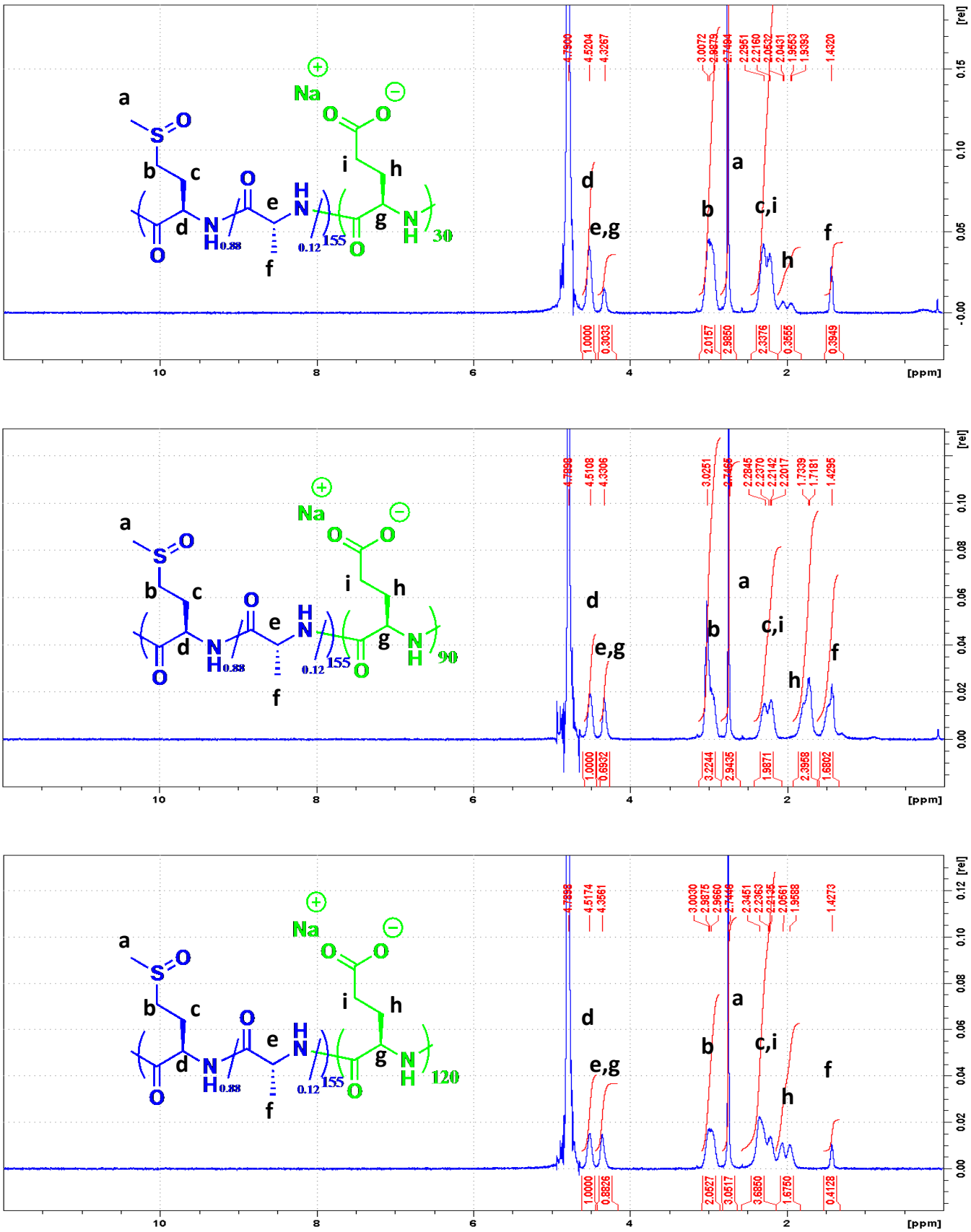


Figure S9. ^{1}H NMR spectra of $(M^{O}A)_{155}E_{30}$, $(M^{O}A)_{155}E_{90}$, and $(M^{O}A)_{155}E_{120}$ in D_2O.

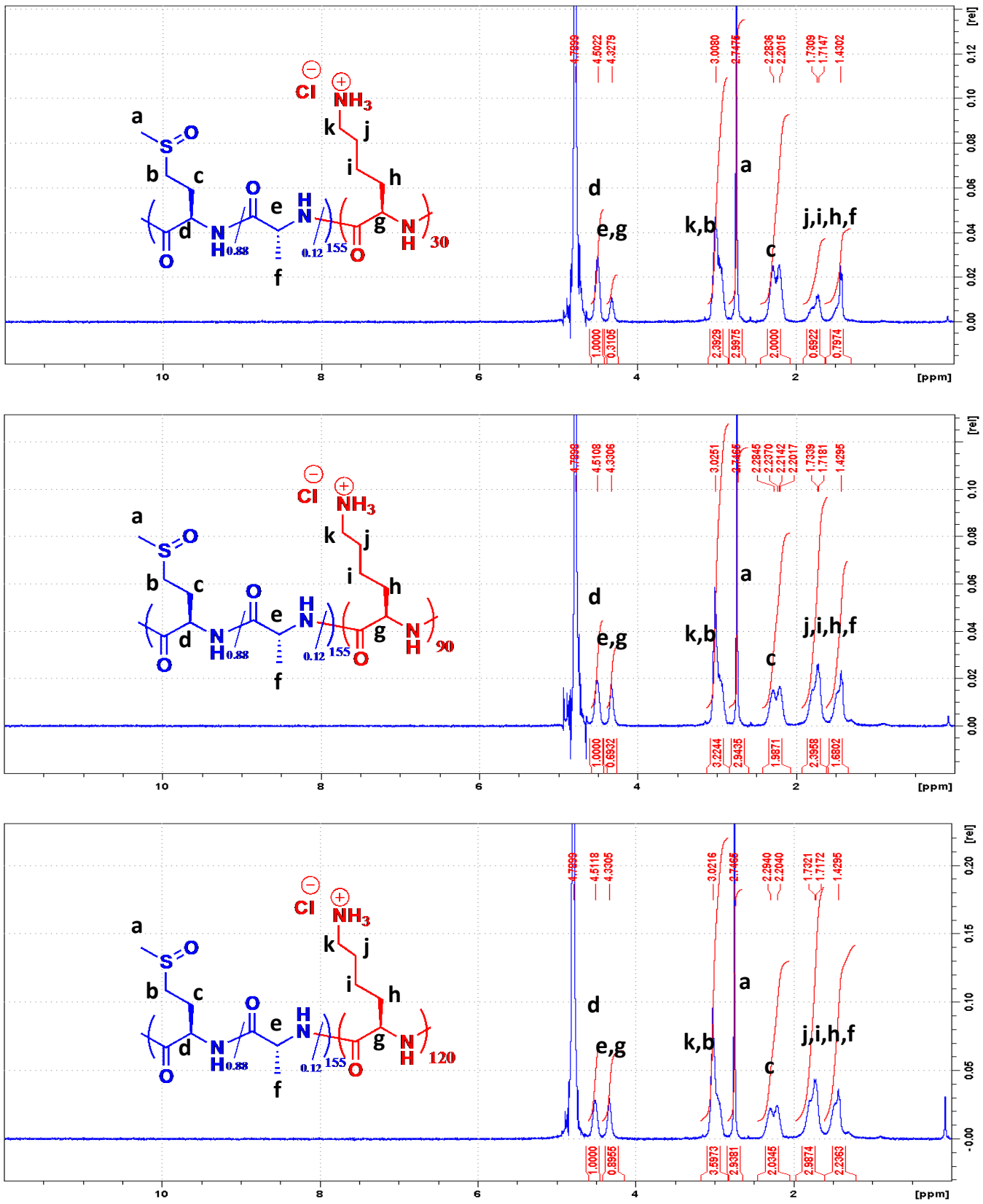


Figure S10. ^{1}H NMR spectra of $(M^{O}A)_{155}K_{30}$, $(M^{O}A)_{155}K_{90}$, and $(M^{O}A)_{155}K_{120}$ in D_2O.

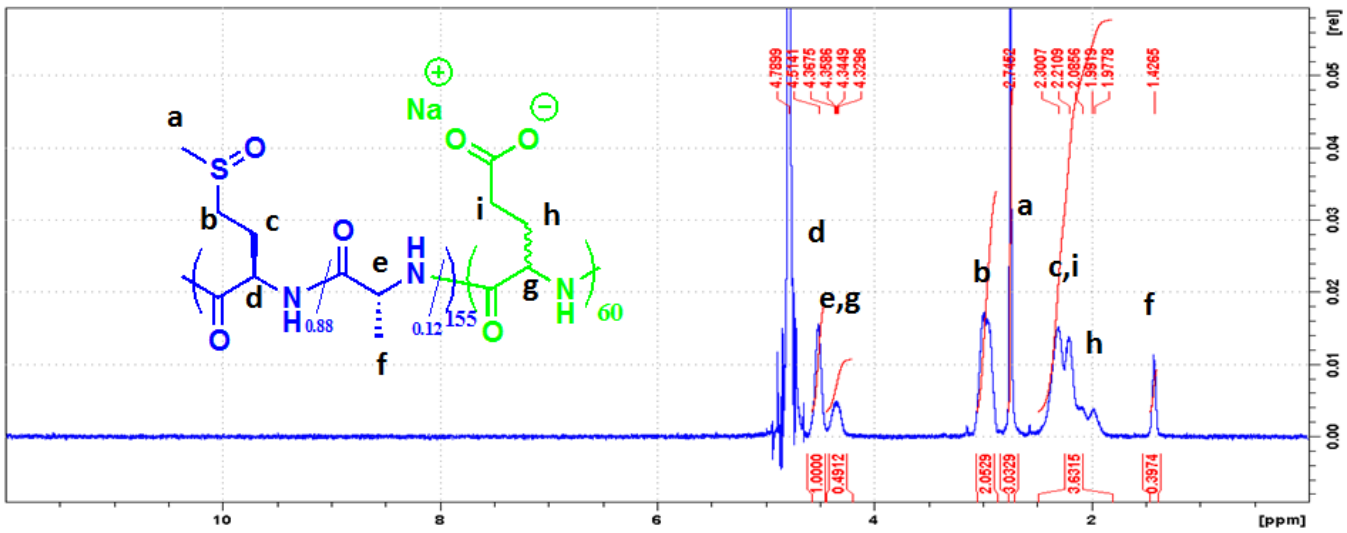


Figure S11. ^{1}H NMR spectrum of $\mathbf{(M^{O}A)_{155}(\textit{rac}\text{-}E)_{60}}$ in D_2O.

Example synthesis of poly(L-methionine sulfoxide$_{0.90}$-*stat*-L-alanine$_{0.10}$)$_{98}$, $\mathbf{(M^{O}_{/0.90}A_{/0.10})_{98}}$, test copolymer

Met NCA (50 mg, 0.29 mmol) and Ala NCA (3.3 mg, 0.029 mmol) were dissolved together in THF (50 mg/mL) and placed in a 20 ml scintillation vial containing a stir bar. To the vial, $(PMe_3)_4Co$ initiator solution (140 µl of a 20 mg/ml solution in THF) was added via syringe. The vial was sealed and allowed to stir in the glove box for 1 h. An aliquot (20 µl) was removed and analyzed by FTIR to confirm that all the NCA was consumed. In the glove box, mPEG$_{23}$-NCO[1] (20 mg) was dissolved in THF (1 ml) in a 20 ml scintillation vial. An aliquot (350 µl) of the polymerization solution containing active chain ends was removed and added to the solution of mPEG$_{23}$-NCO. The PEG end-capped sample was sealed, allowed to stir for 24 h, and oxidized to give the methionine sulfoxide derivative, $\mathbf{(M^{O}_{/0.90}A_{/0.10})_{98}}$**-mPEG$_{23}$**, which was then used for chain length determination as described above. The remainder of the polymerization mixture was isolated by precipitation, and then oxidized to the product methionine sulfoxide derivative, $\mathbf{(M^{O}_{/0.90}A_{/0.10})_{98}}$, following standard procedures described above. Copolymers with different **M** to **A** ratios were prepared following similar procedures.

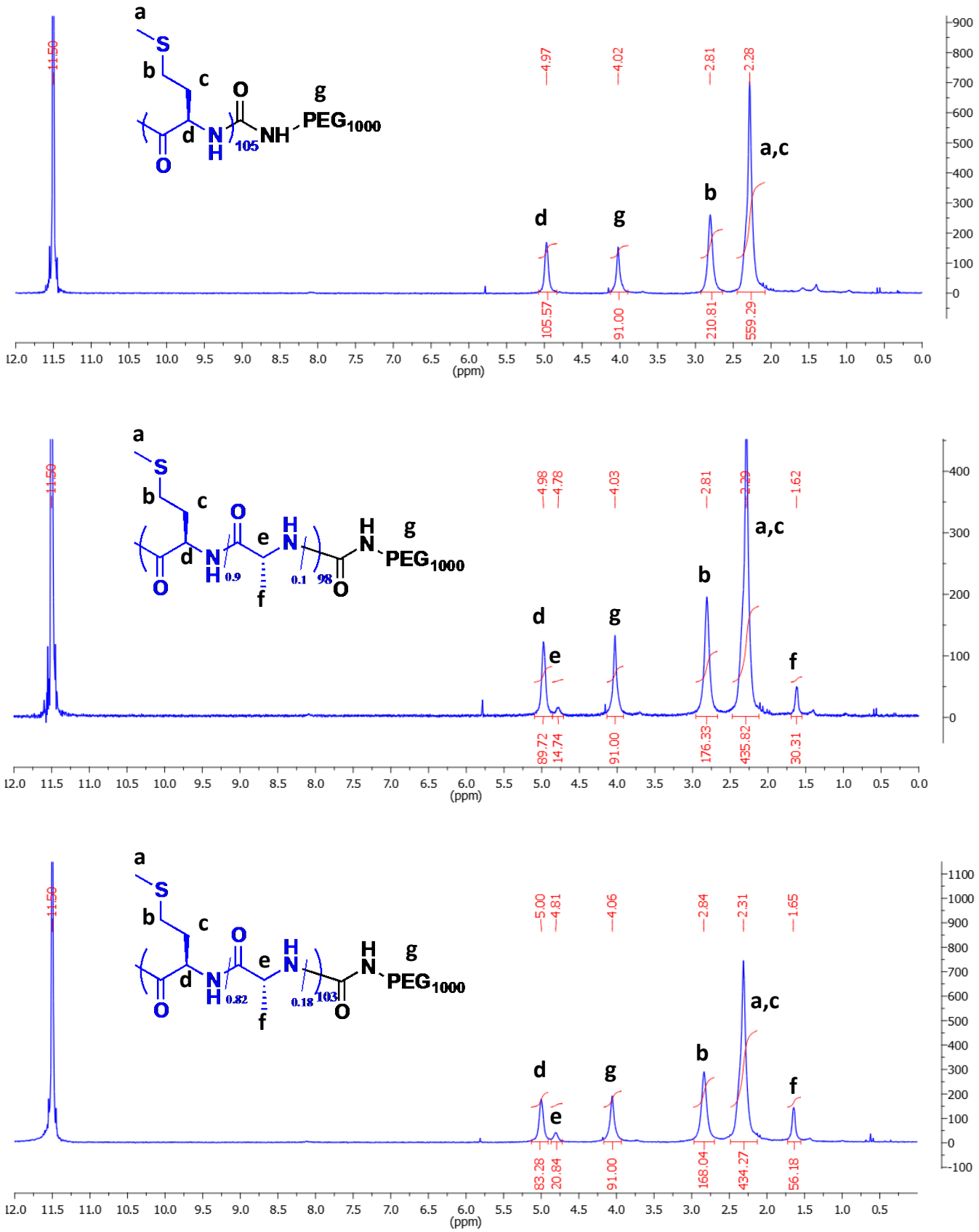


Figure S12. ^{1}H NMR spectra of $(M^{O})_{105}$-**mPEG**$_{23}$, $(M^{O}{}_{/0.90}A_{/0.10})_{98}$-**mPEG**$_{23}$, and $(M^{O}{}_{/0.82}A_{/0.18})_{103}$-**mPEG**$_{23}$ test polypeptides in d-TFA.

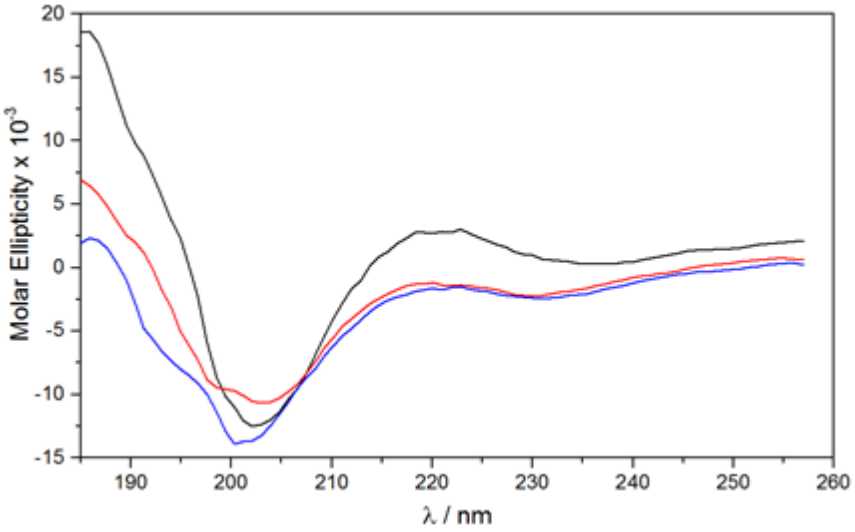


Figure S13. Circular dichroism spectra of $(M^{O})_{105}$ (black), $(M^{O}_{/0.90}A_{/0.10})_{98}$ (blue), and $(M^{O}_{/0.82}A_{/0.18})_{103}$ (red) test polypeptides in DI water at 20 °C.

Preparation of $(M^{O}A)_{155}E/K_x$ PIC hydrogels

Samples of $(M^{O}A)_{155}E_x$ and $(M^{O}A)_{155}K_x$ were separately dissolved in a desired aqueous medium (e.g. DI water, 1x PBS, etc.) at a desired concentration (e.g. 2.0, 3.0, or 5.0 wt%). Once each copolymer was fully dissolved, equal volumes of the copolymer solutions were combined in a scintillation vial (2 ml) and vortexed rigorously for 15 s using a Fisher Vortex Genie 2. The concentration of PIC hydrogel was defined as the sum of the concentrations of the two components after mixing, which is essentially the same as the starting concentrations of each component before mixing. The duration of time before gelation occurred (i.e. gelation time) was found to vary from seconds to minutes depending on sample concentration, the ionic strength, and copolymer composition. A "5 second inversion test" was used to initially confirm gel formation.[5]

Rheology measurements on $(M^{O}A)_{155}E/K_x$ PIC hydrogels

A TA Instruments AR 2000 rheometer with a 20 mm parallel plate geometry and solvent trap was used for all measurements. Frequency sweeps were measured at a constant strain amplitude of 0.05. Strain

sweeps were measured at a constant frequency of 5 rad/s. All measurements were repeated 3 times for each hydrogel sample and the results were averaged and plotted.

Table S2. Properties of diblock copolypeptide PIC hydrogels.

Sample	Concentration (wt%)	G' (Pa)	G'' (Pa)	Clarity
$(M^{O}A)_{155}E/K_{30}$	5.0	30	4	translucent
$(M^{O}A)_{155}E/K_{90}$	5.0	99	7	opaque
$(M^{O}A)_{155}E/K_{120}$	5.0	197	15	opaque
$(M^{O}A)_{155}E/K_{60}$	2.0	3	0.7	translucent
$(M^{O}A)_{155}E/K_{60}$	3.0	29	2	translucent
$(M^{O}A)_{155}E/K_{60}$	5.0	116	9	translucent
$(M^{O}A)_{155}E/K_{60}$	7.0	484	22	translucent
$(M^{O}A)_{155}E/K_{60}$	15	2280	181	translucent

Samples were prepared in PBS buffer, 20 °C. G' = storage modulus; G″ = loss modulus. Values are averages of triplicate runs at 5 rad/s and strain amplitude of 0.05. In general, the standard errors for frequency sweeps were less than 3.5%, while the standard errors for strain sweeps were less than 2.5%.

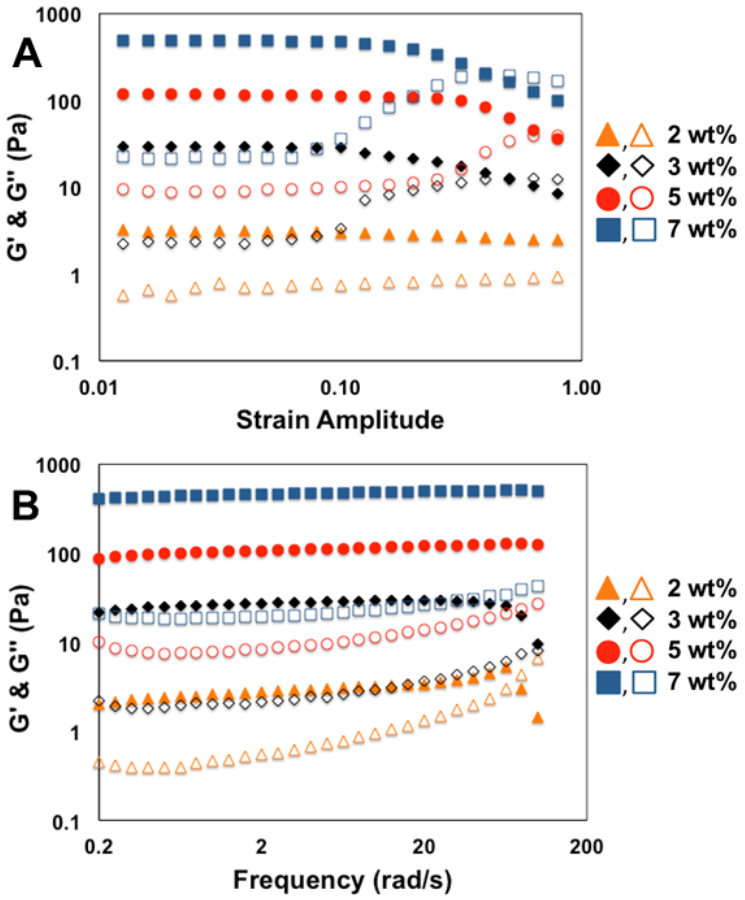


Figure S14. Rheology data for $\mathbf{(M^{O}A)_{155}E/K_{60}}$ hydrogels at different concentrations in PBS buffer at 20 °C. (A) Storage modulus, G' (Pa, solid symbols), and loss modulus, G" (Pa, open symbols), of $\mathbf{(M^{O}A)_{155}E/K_{60}}$ hydrogels as functions of strain amplitude. (B) G' (Pa, solid symbols) and G" (Pa, open symbols) of $\mathbf{(M^{O}A)_{155}E/K_{60}}$ hydrogels as functions of angular frequency. The crossover of G' and G" in the 2.0 and 3.0 wt% samples at high frequency is an artifact attributable to limitations of the measuring geometry (gap loading limit) and should not be considered to be a relaxation time.

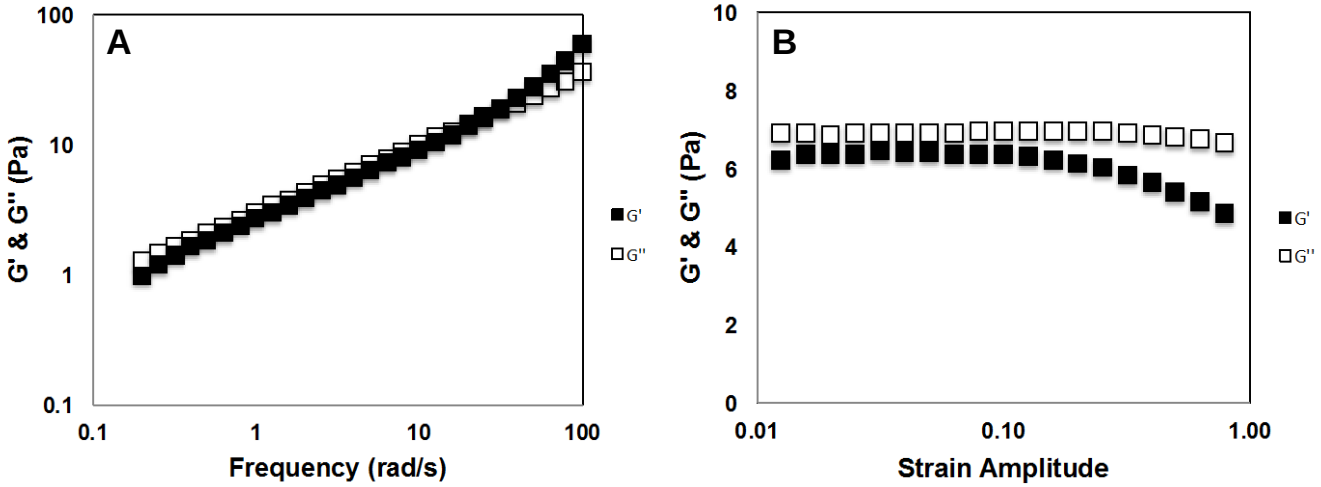
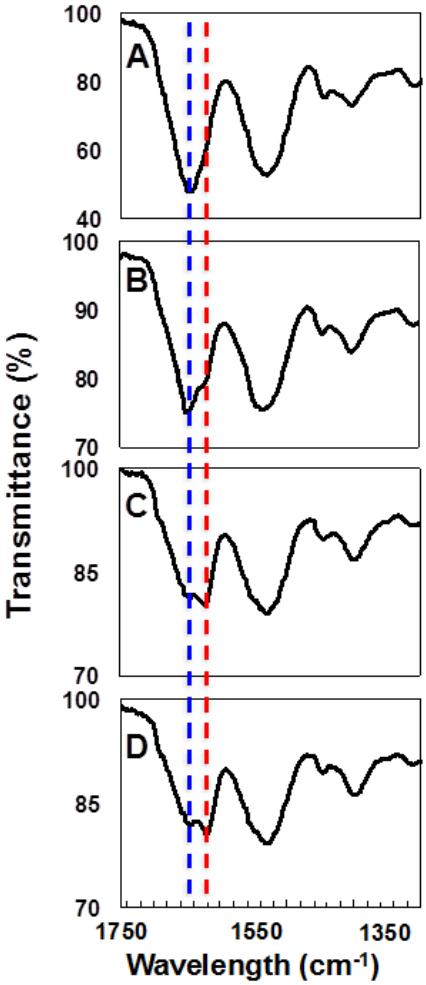


Figure S15. Rheology data for 5.0 wt% $\mathbf{(M^{O}A)_{155}(}$***rac*****-E)/K$_{60}$** in PBS buffer at 20 °C. (A) Storage modulus G' (solid symbols) and loss modulus G″ (open symbols) as a function of angular frequency (strain amplitude of 0.05). (B) Strain sweep at angular frequency of 5 rad/s.

Fluorescent probe conjugation to $\mathbf{(M^{O}A)_{155}E_{60}}$ and $\mathbf{(M^{O}A)_{155}K_{60}}$ copolypeptides

Tetramethylrhodamine isothiocyanate (TRITC) was conjugated to amine groups of lysine side chains. **$(M^{O}A)_{155}K_{60}$** (10 mg) was dissolved in pH 10 H_2O/NaOH (1 ml) in a scintillation vial (20 ml). TRITC was dissolved in DMSO (1 mg/ml) and added to the 1 % (w/v) copolypeptide solution at a 5:1 molar ratio of copolypeptide chains to fluorescent probes. The reaction was allowed to proceed for 24 h at ambient temperature. After TRITC modification, the resulting solution was dialyzed (2000 MWCO) against DI water for 2 d, and then freeze-dried to yield the product as an orange solid. Fluorescein isothiocyanate (FITC) was conjugated onto the N-terminal amine of **$(M^{O}A)_{155}E_{60}$** using a similar procedure.



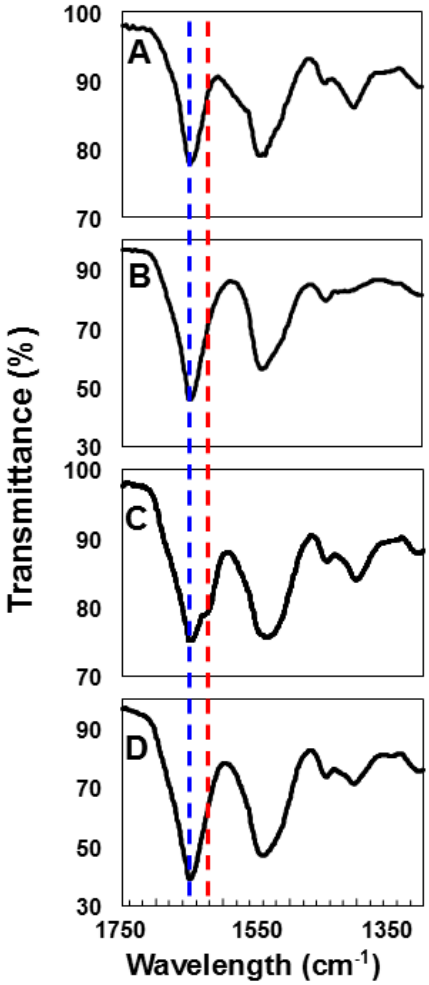


Figure S16. ATR-IR spectra of lyophilized **$(M^{O}A)_{155}E/K_x$** samples in the amide region. Blue line = 1653 cm^{-1} Amide I band characteristic of α-helical and disordered chain conformations. Red line = 1630 cm^{-1} Amide I band characteristic of β-sheet chain conformations. (A) **E/K_x**; x = 30. (B) **E/K_x**; x = 60. (C) **E/K_x**; x = 90. (D) **E/K_x**; x = 120. 1630 cm^{-1} β-sheet Amide I band increases with **E/K_x** content.

Figure S17. ATR-IR spectra of lyophilized samples in the amide region. Blue line = 1653 cm^{-1} Amide I band characteristic of α-helical and disordered chain conformations. Red line = 1630 cm^{-1} Amide I band characteristic of β-sheet chain conformations. (A) **$(M^{O}A)_{155}E_{60}$**. (B) **$(M^{O}A)_{155}K_{60}$**. (C) **$(M^{O}A)_{155}E/K_{60}$**. (D) **$(M^{O}A)_{155}$(*rac*-E)$/K_{60}$**. Only **$(M^{O}A)_{155}E/K_{60}$** shows presence of β-sheet content.

Laser scanning confocal microscopy (LSCM) of fluorescently labeled hydrogels

LSCM images of hydrogels (3.0 wt% in PBS) were taken on a Leica TCS-SP1 MP-Inverted Confocal and Multiphoton Microscope equipped with an argon laser (476 and 488 nm blue lines), a diode (DPSS) laser (561 nm yellow-green line), and a helium-neon laser (633 nm far red line). Fluorescently labeled hydrogel samples were visualized on glass slides with a spacer between the slide and the cover slip (double-sided tape) allowing the self-assembled structures to be minimally disturbed during focusing. A Z-slice thickness of 0.78 μm was used. Sample imaging was performed at the Advanced Light Microscopy/Spectroscopy Center (ALMS) at the UCLA California NanoSystems Institute (CNSI).

Cryoelectron microscopy (cryoEM) of hydrogels

25 μl of a 2.0 wt% $\mathbf{(M^{O}A)_{155}E/K_{60}}$ hydrogel in PBS buffer was applied on a glass coverslip to form a flat surface onto which a lacey carbon EM grid was gently placed using a pair of tweezers in order to acquire a thin layer of sample. The EM grid was then plunged into liquid nitrogen-cooled ethane to prepare the grid for cryoEM. The vitrified sample was examined in an FEI TF20 cryoelectron microscope at liquid nitrogen temperature. Low dose cryoEM images were recorded on a CCD camera at 4.4 Å/pixel on the specimen level and a defocus value of about -5 μm. Sample preparation and imaging was performed at the Electron Imaging Center for Nanomachines (EICN) at the UCLA California NanoSystems Institute (CNSI).

Viability of neural stem progenitor cells (NSPCs) encapsulated in hydrogels

NSPCs were harvested from the brain cortex of postnatal day 2 (P2) mice using procedures described in detail previously.[6] Tissues around the ventricles were excised, diced with a razor blade and placed in Accumax solution (Innovative Cell Technologies, San Diego, CA) for 1 hour to digest brain tissue extracellular matrix. Cells were dissociated and titrated to obtain a single cell suspension that was then cultured in suspension as neurospheres within neural basal media supplemented with B27 (Thermo

Fisher Scientific, Waltham, MA) and 20 ng/ml basic fibroblast growth factor (FGF-2) and epidermal growth factor (EGF) (Peprotech, Rocky Hill, NJ). Growth media was replaced every two days and neurospheres were passaged every four days or as needed. Cell encapsulation within hydrogels was performed by adding an equal volume of dissociated NSPC suspension in cell media (30,000 cells/μl) to a 10 wt% $\mathbf{(M^{O}A)_{155}E_{60}}$ solution in cell media to give a resulting copolymer concentration of 5.0 wt %. This mixture was rapidly combined with an equal volume of 5.0 wt% $\mathbf{(M^{O}A)_{155}K_{60}}$ solution in cell media to yield an overall 5.0 wt% cell containing $\mathbf{(M^{O}A)_{155}E/K_{60}}$ hydrogel. In a similar manner, a 4.0 wt% $\mathbf{K_{180}L_{20}}$ hydrogel control sample in cell media was diluted with an equal volume of cell suspension to yield a final hydrogel concentration of 2.0 wt%. A cell suspension alone in media (15,000 cells/μl) without any hydrogel was also used as a control and baseline. For each of these samples, a 20 μl aliquot was deposited on top of 1.0 wt% agarose gel in media within an Eppendorf tube. The samples were stored in an incubator (37 °C, 5% CO_2) and were removed after 1 day for analysis. The samples were diluted 50 fold with PBS, and the cells were pelleted using a microfuge. The Live/Dead® viability/cytotoxicity assay (Thermo Fisher Scientific, Waltham, MA) was employed to quantify the percentages of NSPCs both alive and dead after hydrogel encapsulation.[7] Samples were incubated with Live/Dead stain (2 μM calcein AM and 4 μM EthD-1 in PBS) for 30 min at room temperature. The samples were examined under a Zeiss fluorescence microscope (Carl Zeiss Inc) with filters separating light emission from calcein (live, green) and EthD-1 (dead, red). Finally, all the live/dead cells were counted using imageJ. The resulting counts were averaged (6 samples of $\mathbf{(M^{O}A)_{155}E/K_{60}}$ and 5 samples for both cell control and $\mathbf{K_{180}L_{20}}$) and normalized against the cell control.

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