Supporting Information

Self-sorting microscale compartmentalized block copolypeptide hydrogels

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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) spectra were acquired on a Perkin Elmer RX1 FTIR spectrophotometer calibrated using polystyrene film, and attenuated total reflectance infrared (ATR-IR) data were collected using a Perkin Elmer 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 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 (DI) water was obtained from a Millipore Milli-Q Biocel A10 purification unit.

Copolypeptide synthesis

Samples of poly(L-methionine sulfoxide$_{0.88}$-*stat*-L-alanine$_{0.12}$)$_{155}$-*block*-poly(L-lysine)$_{55}$, $(M^{O}A)_{155}K_{55}$, poly(L-methionine sulfoxide$_{0.88}$-*stat*-L-alanine$_{0.12}$)$_{155}$-*block*-poly(L-glutamate)$_{55}$, $(M^{O}A)_{155}E_{55}$, and poly(L-methionine sulfoxide$_{0.88}$-*stat*-L-alanine$_{0.12}$)$_{200}$-*block*-poly(L-leucine)$_{30}$ were prepared as previously described.[2,3] Briefly, 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) was added a solution of $Co(PMe_3)_4$ in THF (20 mg/ml).[2] Reactions were let stir at ambient temperature (*ca*. 22 ºC) 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), ε-trifluoroacetyl-L-lysine NCA (TFA-Lys NCA) or L-leucine NCA in THF (50 mg/ml) was added to the reaction mixtures, which were let stir for an additional 60 min. FTIR was used to confirm complete consumption of all NCAs. Once polymerizations were completed the block copolypeptide solutions were removed from the glove box, 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 to give products as white solids. Subsequent oxidation of samples, followed by deprotection of Bn-Glu or TFA-Lys groups were performed as previously described.[2,3]

Table S1. Characterization data for diblock copolypeptides.

Sample	M_w/M_n[a]	Composition[b]	Yield (%)[c]
$(M^OA)_{155}E_{55}$	1.37	$(M^OA)_{152}E_{52}$	92
$(M^OA)_{155}K_{55}$	1.38	$(M^OA)_{152}K_{54}$	94
$(M^OA)_{200}L_{30}$	1.40	$(M^OA)_{215}L_{31}$	91

[a] Dispersity of oxidized, protected block copolypeptides were determined by GPC/LS. [b] Actual amino acid compositions of oxidized, deprotected block copolypeptides were determined by ^{1}H NMR integrations. Degree of polymerization of initial $(MA)_x$ segments was determined by end-group analysis using ^{1}H NMR.[2,3] [c] Total isolated yield of deprotected, purified block copolypeptides.

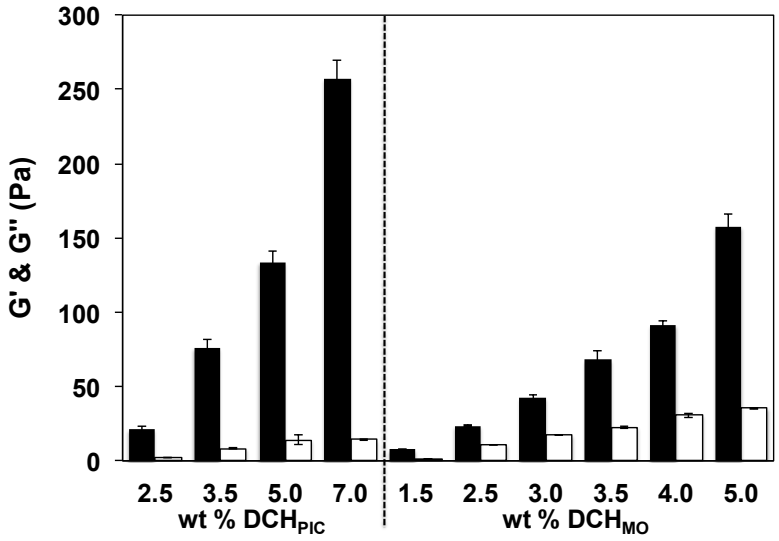

Figure S1. Mechanical properties of individual diblock copolypeptide hydrogels. (A) Storage modulus, G′ (Pa, black), and loss modulus, G″ (Pa, white), of individual DCH_{PIC} and DCH_{MO} hydrogel components at different concentrations in 1×PBS buffer at 25 °C. All G′ and G″ values were measured at an angular frequency of 5 rad/s and a strain amplitude of 0.01, and are averages of triplicate runs with bars indicating standard deviations.

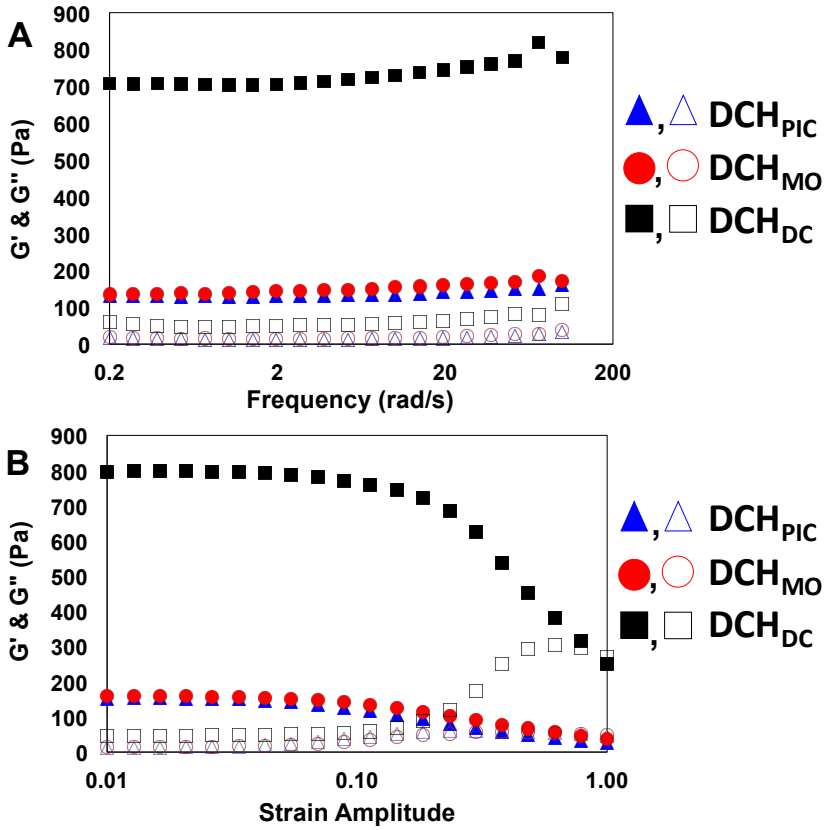

Figure S2. Rheology data for 5 wt% DCH_{PIC}, 5 wt% DCH_{MO}, and DCH_{DC} (3 wt% DCH_{MO} + 5 wt% DCH_{PIC}) hydrogels in 1×PBS buffer at 20 °C. (A) G' (Pa, solid symbols) and G" (Pa, open symbols) of hydrogel samples as functions of angular frequency at constant strain amplitude of 0.01. (B) Storage modulus, G' (Pa, solid symbols), and loss modulus, G" (Pa, open symbols), of hydrogel samples as functions of strain amplitude at a constant frequency of 5 rad/s.

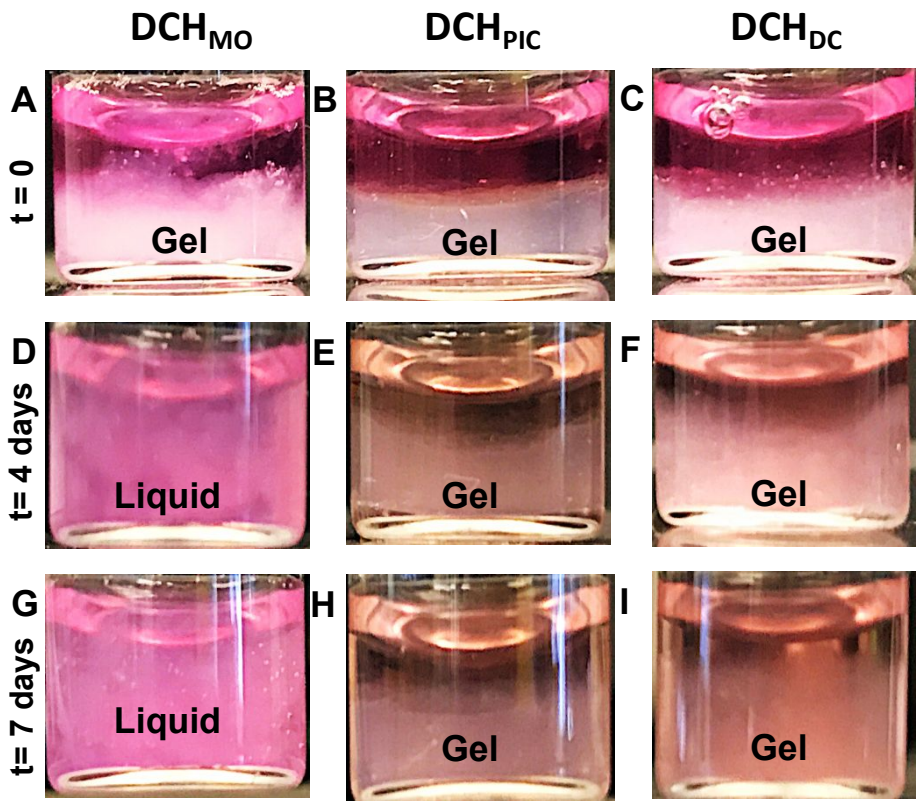

Figure S3. Stability of diblock copolypeptide hydrogels against dilution. Equal volume samples of DCH_{MO} (5.0 wt%), DCH_{PIC} (5.0 wt%), and DCH_{DC} (3 wt% DCH_{MO} and 5 wt% DCH_{PIC}) in 1×PBS were each diluted with an equal volume of DMEM cell culture media. (A-C) Layers of cell media formed over all hydrogels at the beginning of the experiment (t = 0). (D-F) After 4 days, the DCH_{PIC} and DCH_{DC} hydrogels remained intact while DCH_{MO} had dispersed into the full volume of media and was a liquid. (G-I) After 7 days, both DCH_{PIC} and DCH_{DC} remained intact as hydrogels underneath the media.

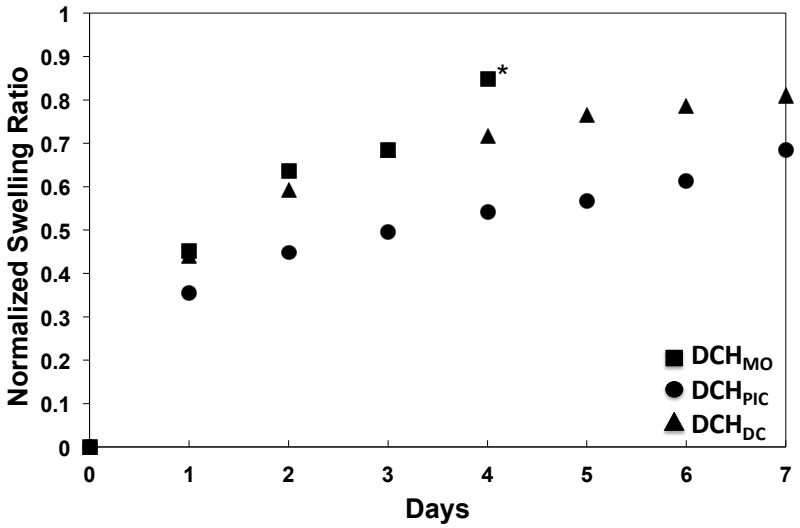

Figure S4. Normalized swelling ratio measurements. Equal volume samples of DCH_{MO} (5.0 wt%), DCH_{PIC} (5.0 wt%), and DCH_{DC} (3 wt% DCH_{MO} and 5 wt% DCH_{PIC}) in 1×PBS were each diluted with an equal volume of DMEM cell culture media. Hydrogel swelling was monitored by removal of all supernatant liquid above each hydrogel at different time points. Normalized swelling ratio was calculated as: (weight of sample after swelling - weight of initial sample) / weight of initial sample. *DCH_{MO} was no longer a hydrogel by day 4.

Preparation of $(M^OA)_{155}E/K_{55} + (M^OA)_{200}L_{30}$ double network hydrogels (DCH_{DC})

To prepare 1.0 mL of a target DCH_{DC} composition (e.g. 3 wt% DCH_{MO} and 5 wt% DCH_{PIC}), the cationic diblock copolypeptide, $M^OA_{155}K_{55,}$ was first dissolved at the desired concentration (e.g. 25 mg, 3.1 wt%) in 0.80 mL of 1× PBS. This solution was then used to dissolve the desired amount of $M^OA_{200}L_{30}$ (e.g. 30 mg, 3.8 wt%), resulting in a viscous weak hydrogel after 30 minutes. Separately, the anionic diblock copolypeptide, $M^OA_{155}E_{55,}$ was dissolved at the desired concentration (e.g. 25 mg, 13 wt%) in 0.20 mL of 1× PBS. The

anionic copolypeptide solution was then added to the viscous cationic copolypeptide solution, and the resulting mixture vortexed for 20 seconds leading to DCH_{DC} hydrogel formation within 5-30 seconds depending on copolypeptide concentrations and compositions. Note that concentrations of DCH_{PIC} are calculated as the combined mass of $M^{O}A_{150}K_{55}$ and $M^{O}A_{150}E_{55}$ in the sample.

Rheology measurements on copolypeptide hydrogels

An Anton Paar Instruments MCR 302 rheometer with a 25 mm diameter and 1^{o} cone plate geometry and solvent trap was used for all measurements. Frequency sweeps were measured at constant strain amplitude of 0.01 (i.e. 1%). Strain sweeps were measured at a constant frequency of 5 rad/s. All measurements were repeated 3 times for each hydrogel sample and are reported as averages of triplicate runs, in some cases with bars indicating standard deviations. To evaluate shear thinning and recovery behavior of DCH, the strain amplitude was stepped from 0.01 (i.e. 1%) to 10 (i.e. 1000%), maintained at 10 for 2 min and then returned to 0.01 to evaluate the recovery of mechanical properties at a fixed frequency of 5 rad/s.

Hydrogel swelling measurements

Hydrogels of 6 wt% DCH_{PIC}, 6 wt% DCH_{MO}, and DCH_{DC} (3 wt% DCH_{MO} and 5 wt% DCH_{PIC}) were prepared in 2 ml scintillation vials and allowed to stand for 1 hr. DMEM cell culture media was then placed on top of each hydrogel sample and samples were stored in a refrigerator

(0 ºC) for different periods of time. At each time point, the supernatant liquid was pipetted out of each sample without disturbing the gel at the bottom. The supernatant volumes were subtracted from the original media volume to determine swelling ratios. The hydrogel samples were also subjected to inversion tests to verify hydrogel integrity. Finally, the supernatant liquid was replaced on top of each hydrogel and incubation of samples allowed to continue.

Fluorescent probe conjugation to $(M^OA)_{155}E_{55}$ and $(M^OA)_{200}L_{30}$ copolypeptides

These conjugations were performed as previously reported.[2,3] Alexa Fluor 488 NHS ester (AF 488 NHS) was conjugated to the N-terminal amine groups of $(M^OA)_{155}E_{55}$.[2] $(M^OA)_{155}E_{55}$ (10 mg) was dissolved in DI water (pH 7) (1 ml) in a scintillation vial (20 ml). AF 488 NHS was dissolved in DI water (pH 7) (1 mg/ml) and added to the 1 % (w/v) copolypeptide solution at a 1.25:1 molar ratio of fluorescent probes per copolypeptide chain. The reaction was allowed to proceed for 24 h at ambient temperature. After AF 488 modification, the resulting solution was dialyzed (2000 MWCO) against DI water for 2 d, and then freeze-dried to yield the product as a yellow solid. Alexa Fluor 633 NHS ester (AF 633 NHS) was conjugated onto the N-terminal amine groups of $(M^OA)_{200}L_{30}$ using a similar procedure.[3] Each labeled polypeptide sample was found to possess adequate fluorescence at the appropriate wavelength, and was not further characterized since no experiments requiring fluorescence quantitation were performed.

Laser scanning confocal microscopy (LSCM) of fluorescently labeled hydrogels

Fluorescence imaging was performed by laser scanning confocal microscopy (LSCM) on a Leica TCS SP8 inverted microscope (Leica Microsystems, Wetzlar, Germany) equipped with

a White Light Laser continuum and a Leica HC PL APO 100x/1.40 oil objective. Fluorescently labeled hydrogel samples were mounted between a glass slide and a cover slip using double-sided tape as a spacer allowing the self-assembled structures to be minimally disturbed during focusing. An optical section of 0.896 μm was used. Scanning was performed at a line frequency of 400 Hz with an image format of 1024 x 1024 pixels. Each frame was averaged two times and the two emission signals (Alexa Fluor 488 and Alexa Fluor 633) were captured simultaneously onto two separate hybrid photodetectors (HyD SMD, Leica Microsystems). All images were analyzed and visualized in 3D using the LAS X software (Leica Microsystems, Wetzlar, Germany). The resulting 3D model was processed *via* histogram equalization. LSCM was performed at the Advanced Light Microscopy/Spectroscopy Laboratory and the Leica Microsystems Center of Excellence at the California NanoSystems Institute at UCLA.

References

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(2) Sun, Y.; Wollenberg, A. L.; O'Shea, T. M.; Cui, Y.; Zhou, H.; Sofroniew, M. V.; Deming, T. J. Conformation directed formation of self-healing diblock copolypeptide hydrogels via polyion complexation. *J. Am. Chem. Soc.* **2017**, *139*, 15114-15121.

(3) Wollenberg, A. L.; O'Shea, T. M; Kim, J. H.; Czechanski, A.; Reinholt, L. G.; Sofroniew, M. V.; Deming, T. J. Injectable polypeptide hydrogels via methionine modification for neural stem cell delivery. *Biomaterials* **2018**, *178*, 527-545.