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Supporting Information

Self-Assembly of α-Helical Polypeptides Driven by Complex Coacervation

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Instrumentation

^{1}H NMR spectra were recorded on a Varian U500 MHz spectrometer. Chemical shifts were reported in ppm and referenced to the solvent proton impurities. Gel permeation chromatography (GPC) experiments were performed on a system equipped with an isocratic pump (Model 1100, Agilent Technology, Santa Clara, CA, USA), a DAWN HELIOS multi-angle laser light scattering (MALLS) detector (Wyatt Technology, Santa Barbara, CA, USA), and an Optilab rEX refractive index detector (Wyatt Technology, Santa Barbara, CA, USA). The detection wavelength of HELIOS was set at 658 nm. Separations were performed using serially connected size exclusion columns (100 Å, 500 Å, 10^3Å, and 10^4 Å Phenogel columns, 5 μm, 300 × 7.8 mm, Phenomenex, Torrance, CA, USA) at 60°C using DMF containing 0.1 mol/L LiBr as the mobile phase. The MALLS detector was calibrated using pure toluene and can be used for the determination of the absolute molecular weights (MWs). The MWs of polymers were determined based on the dn/dc value of each polymer sample calculated offline by using the internal calibration system processed by the ASTRA V software (version 5.1.7.3, Wyatt Technology, Santa Barbara, CA, USA). Circular dichroism (CD) measurements were carried out on a JASCO J-815 CD spectrometer (JASCO, Easton, MD, USA). The polypeptide samples were prepared at a concentration of 0.2 mg/mL in aqueous solution at pH 7. The solution was placed in a quartz cell with 0.10 cm path length. For the ionic strength dependent experiments, polypeptides were dissolved in aqueous solution containing different concentrations of NaCl. Infrared spectra were recorded on a Perkin Elmer 100 serial FTIR spectrophotometer calibrated with polystyrene film (PerkinElmer, Santa Clara, CA, USA). Lyophilization was performed on a FreeZone lyophilizer (Labconco, Kansas City, MO, USA).

Materials

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as received unless otherwise specified. Anhydrous dimethylformamide (DMF) was dried by a column packed with 4Å molecular sieves and stored in a glovebox. Hexamethyldisilazane (HMDS) was dissolved in DMF in

a glovebox. Spectra/Por RC dialysis tubing with a molecular weight cut-off (MWCO) of 1 kDa was purchased from Spectrum Laboratories (Rancho Dominguez, CA, USA).

Synthesis of polypeptides

PPLGPG (poly(γ-3-(4-(guanidinomethyl)-1H-1,2,3-triazol-1-yl)propyl-L-glutamate)) was synthesized by following the literature procedure.[30] CP-L-Glu-NCA (100 mg, 0.401 mmol) was dissolved in DMF (1.5 mL) in a glovebox, followed by the addition of HMDS solution in DMF (80.1 μL, 0.1 mol/L, M/I = 50). The reaction mixture was stirred at room temperature for 48 h (monomer conversion > 99%, monitored by FTIR) to obtain the poly(γ-3-chloropropyl-L-glutamate) (PCPLG) homo-polymer. The polymerization mixture was taken out from the glovebox and DMF was removed under vacuum. PCPLG was then precipitated by cold methanol and collected by centrifugation as white solid (73% yield). ^{1}H NMR ($CDCl_3$:TFA-d, 85:15, v/v): δ 4.61 (s, 1H, α-H), 4.28 (m, 2H, -C*H*$_2$OOC-), 3.56 (t, 2H, -C*H*$_2$Cl), 2.53 (s, 2H, -CH$_2$C*H*$_2$COO-), 2.26-1.92 (m, 4H, -C*H*$_2$CH$_2$COO- and ClCH$_2$C*H*$_2$CH$_2$O-). PCPLG (50 mg, 0.243 mmol of chloro groups) was dissolved in DMF (2 mL) and NaN_3 (158 mg, 2.43 mmol) was added into the solution. The resulting mixture was stirred at 60 ^{o}C for 48 h followed by removal of the solvent under vacuum. The residue was then extracted with $CHCl_3$ three times and the remaining solid was discarded. The $CHCl_3$ solution was combined and concentrated under vacuum. The polymer poly(γ-3-azidopropyl-L-glutamate) (PAPLG) was precipitated by hexane/ether (1:1, v/v) and collected by centrifugation as yellow viscous solid (85% yield). ^{1}H NMR ($CDCl_3$:TFA-d, 85:15, v/v): δ 4.58 (q, 1H, α-H), 4.20 (m, 2H, -C*H*$_2$OOC-), 3.39 (t, 2H, -C*H*$_2$N$_3$), 2.51 (m, 2H, -CH$_2$C*H*$_2$COO-), 2.22-1.88 (m, 4H, -C*H*$_2$CH$_2$COO- and N$_3$CH$_2$C*H*$_2$CH$_2$O-).

In a glovebox, PAPLG (20 mg, 0.094 mmol of azido groups), *N*,*N*,*N*',*N*'',*N*''-Pentamethyldiethylenetriamine (PMDETA, 98 μL, 0.47 mmol) and *N*-pro-2-ynyl-guanidine solution in DMF (48 mg, 38 wt%, 0.19 mmol) were dissolved in DMF (1 mL). The resulting solution was transferred into a small vial charged with CuBr (27 mg, 0.19 mmol) and a magnetic stir bar. The mixture was stirred at room temperature for 24 h. The reaction was then quenched by transferring outside of glove-

box and exposing to air. The sample was dialyzed against DI water (MWCO = 1 kDa) for 3 days (DI water changed every 8 h), and the final polymer PPLG-PG was obtained as a light yellow powder after lyophilization (66% yield). ^{1}H NMR (TFA-d:D_2O, 80:20, v/v): δ 8.59 (s, 1H, triazole-H), 4.88 (t, 2H, -$COOCH_2CH_2CH_2$-), 4.80 (s, 1H, α-H), 4.50 (s, 2H, -$COOCH_2CH_2CH_2$-), 4.42 (s, 2H, -$CH_2NH(NH)NH_2$), 2.76 (s, 2H, -CH_2CH_2COO-), 2.58 (s, 2H, -$COOCH_2CH_2CH_2$-), 2.47-2.19 (m, 2H, -CH_2CH_2COO-). The un-assigned peaks in Figure S3A come from some impurities in deuterated tri-fluoroacetic acid (TFA-d) during storage. TFA-d is very active and thus corrosive to the container, and therefore some impurity peaks will show up after the container is open for some time (even stored in freezer). On the other hand, guanidine-based polypeptides have limited solubility in TFA-d due to the polar guanidine groups, therefore all polypeptide peaks have low intensity in Figure S3A compared with impurity peaks and solvent peak (8.2 ppm, come from un-deuterated TFA/water).

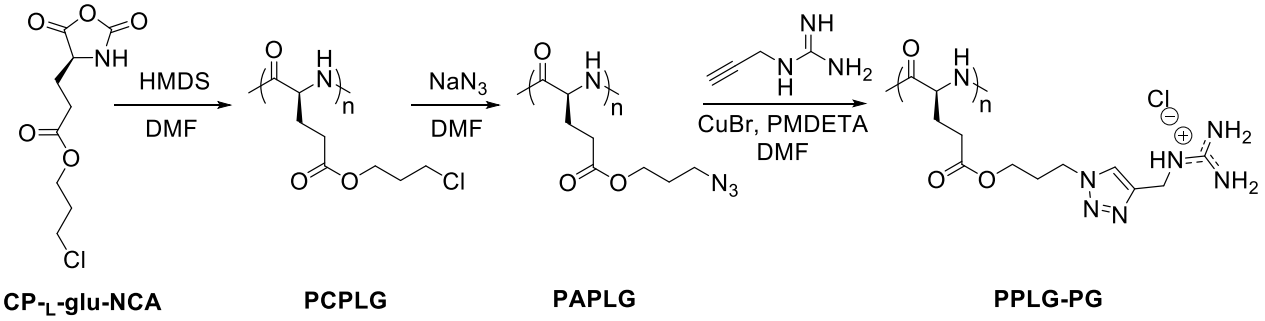

Figure S1. Synthetic route for the preparation of PPLGPG via NCA polymerization and post-modification.

PEG-*b*-PPLGPG diblock copolymers were synthesized using the same method with mPEG-NHTMS (10 kDa and 20 kDa) as initiator for CP-L-Glu-NCA polymerization.

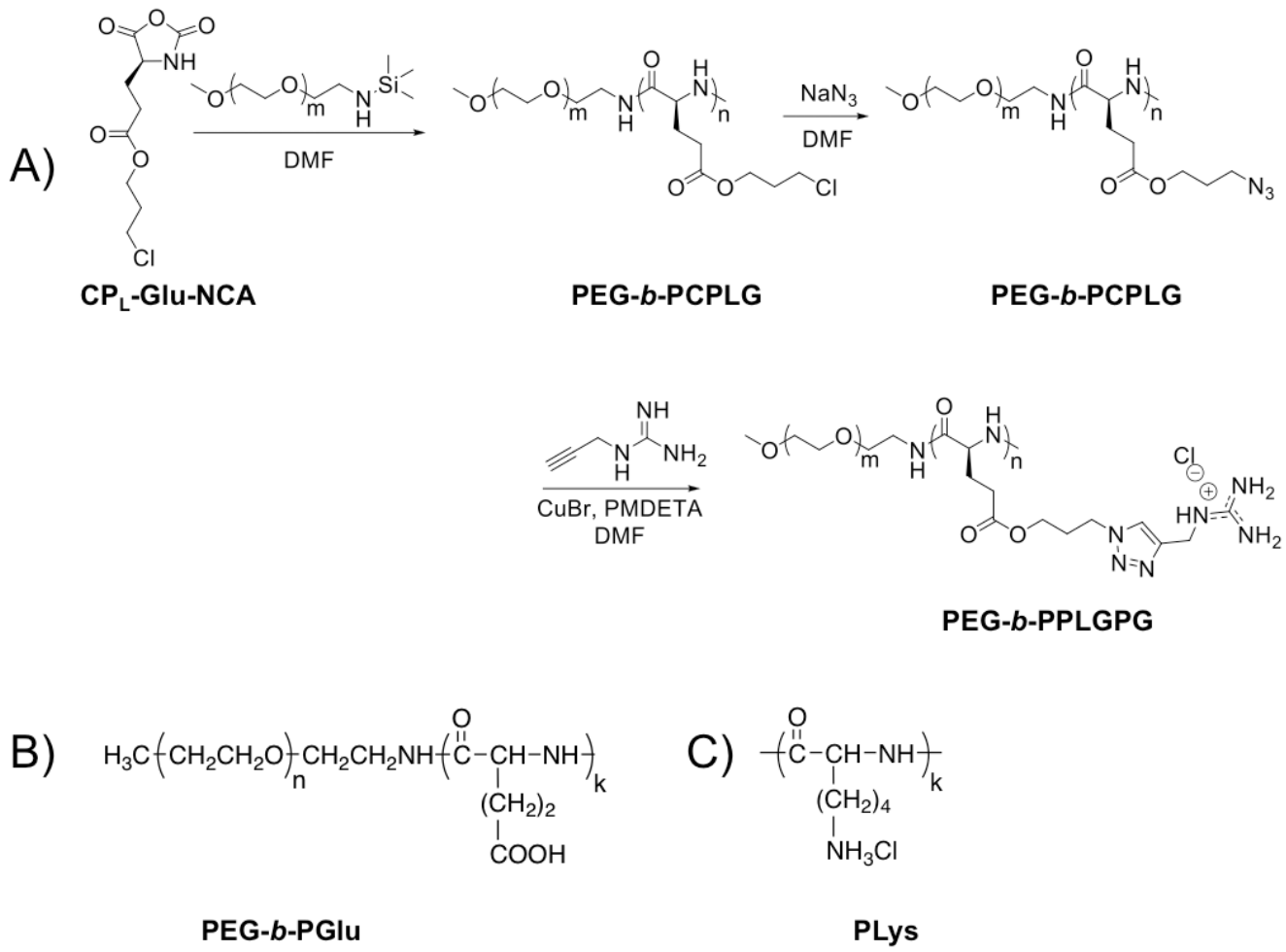

Figure S2. A) Synthetic route for the preparation of PEG-*b*-PPLGPG via NCA polymerization and post-modification. Chemical structures of B) Poly(ethylene glycol)-*b*-(polyglutamic acid) and C) Polylysine.

All polypeptides and co-polypeptides used in this study were purchased from Alamanda Polymers, Inc. (Huntsville, AL) and their molecular characterizes are presented in Table S1. All water was dispensed from a Milli-Q water purification system at a resistivity of 18.2 MΩ cm. Polypeptides were used as received without any further purification. Separate stock solutions of 10 mM by charge of each polypeptide were prepared in water. A stock solution of 5 M NaCl was prepared and adjusted to pH = 7.0.

Table S1. Molecular characteristics of the polypeptides used in this study.

Sample	Molecular Weight M_n (g/mol)	Polydispersity (M_w/M_n)	Degree of Polymerization (N)
P(L)Glu_{100}[2]	1.6×10^4	1.01	105
P(D)Glu_{100}[2]	1.5×10^4	1.04	102
$PGlu_{100}$[2]	1.5×10^4	1.02	98
$PGlu_{50}$[2]	0.8×10^4	1.03	51
P(L)Lys_{50}[2]	0.8×10^4	1.03	50
$PPLGPG_{50}$[1,3]	1.8×10^4	1.06	53
PEG_{10k}-*b*-$PPLGPG_{50}$[1,3]	2.8×10^4	1.06	53
PEG_{20k}-*b*-$PPLGPG_{50}$[1,3]	3.7×10^4	1.02	49
PEG_{5k}-*b*-$PGlu_{50}$[2]	1.2×10^4	1.04	47

[1)]The polymer was synthesized in-house via NCA polymerization. All other polymers were purchased from Alamanda Polymers Inc.

[2)]Reported from certificate of analysis.

[3)]M_n and degree of polymerization were determined by ^1H NMR. Polydispersity was determined by GPC and static light scattering.

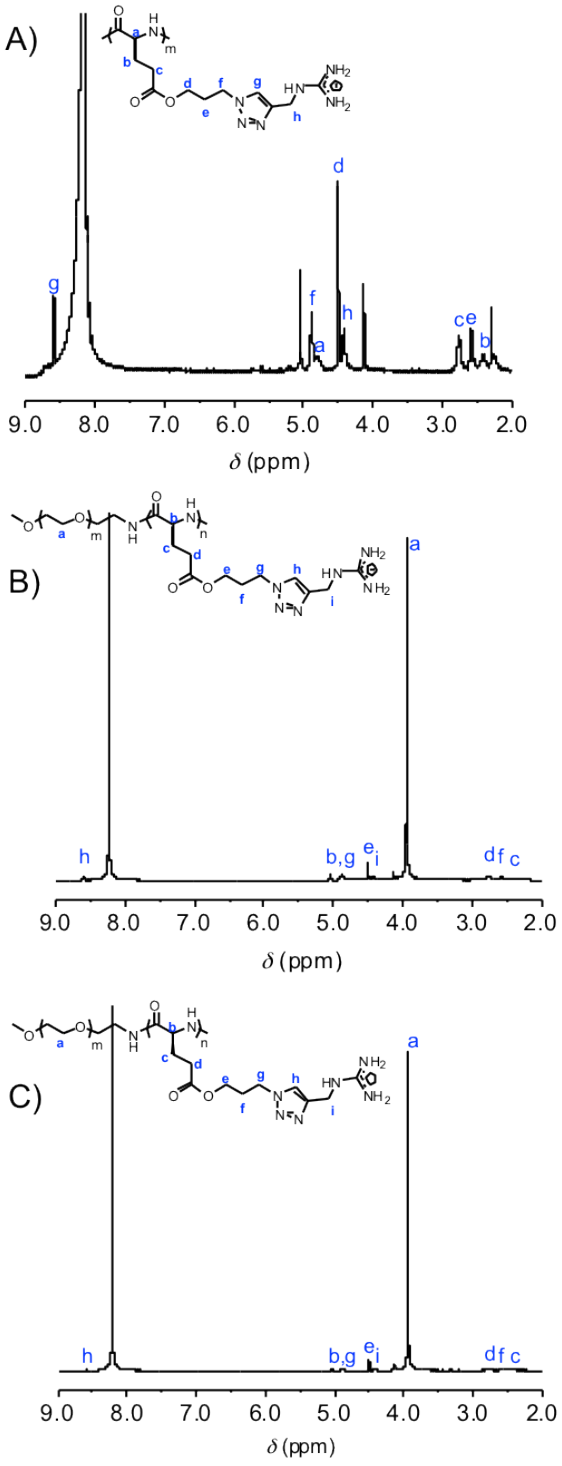

Figure S3. ^{1}H-NMR spectra of A) PPLGPG$_{50}$, B) PEG$_{10k}$-*b*-PPLGPG$_{50}$, and C) PEG$_{20k}$-*b*-PPLGPG$_{50}$ in TFA-d/D$_2$O (80:20, v/v). The molecular structures have been annotated to indicate the relevant proton signals in the ^{1}H NMR spectrum.

Circular Dichroism (CD)

CD was used to determine the secondary structure of both the individual polypeptides (**Figures 3 and S4**), and polypeptides incorporated into a coacervate domain. Samples were prepared as described previously, at a total polymer concentration of 0.25 mM and pH 7.0. Samples were measured on a Jasco J-815 Circular Dichroism Spectropolarimeter using a 1 mm quartz cuvette at 25ºC. Measurements were performed from 250 nm to 200 nm. An average of five scans is reported. Raw data was converted to units of mean residue ellipticity (MRE) by taking into account the concentration of individual (chiral) amino acids present in each sample. The helicity of the polypeptides was calculated by performing a linear interpolation between the measured spectra and known basis spectra for a α-helix, β-sheet and a random coil.

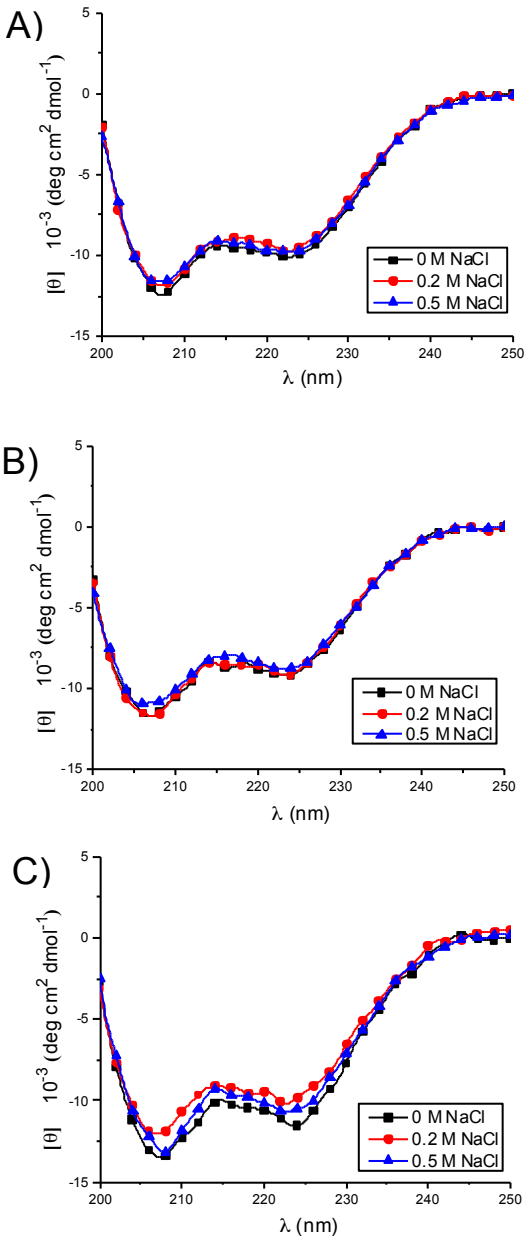

Figure S4. CD spectra of A) PPLGPG50, B) PEG10k-*b*-PPLGPG50, and C) PEG20k-*b*-PPLGPG50 at different salt concentrations, 0.25 mM total polymer concentration and pH 7.0, showing the helical character of the synthesized polypeptides.

Mixture preparation

Coacervation was achieved by mixing aqueous solutions of the polycation and the polyanion with concentrated NaCl solutions. First, separate stock solutions of 10 mM charge of each polyelectrolyte were prepared. Final mixtures (1.5 ml final volume) of 1:1 polyanion/polycation mixing ratios were then prepared by sequential addition of calculated amounts of the polycation, followed by the polyanion

stock solution, into NaCl solutions with pre-calculated concentrations to achieve a final salt concentration from 0 to 4 M NaCl. The polyelectrolyte complexes were prepared in microcentrifuge vials and were vortexed for five seconds after the addition of each component. The order of mixing was kept the same for all experiments. Experiments performed with different order of mixing showed no apparent effect on the size and properties of the structures. All complex coacervates were studied at room temperature (25 °C). An analogous procedure was used to prepare solutions of coacervate core micelles using stock solutions of the polypeptide and co-polypeptides.

Optical microscopy

An optical microscope (Leica DMI6000) using Leica AFC image acquisition software was used to obtain physical images of the polypeptide complexes prepared as described above. Imaging (Figure S5 and 1B) was performed using an ultra-low attachment 96-well plate (Costar, Corning Inc.).

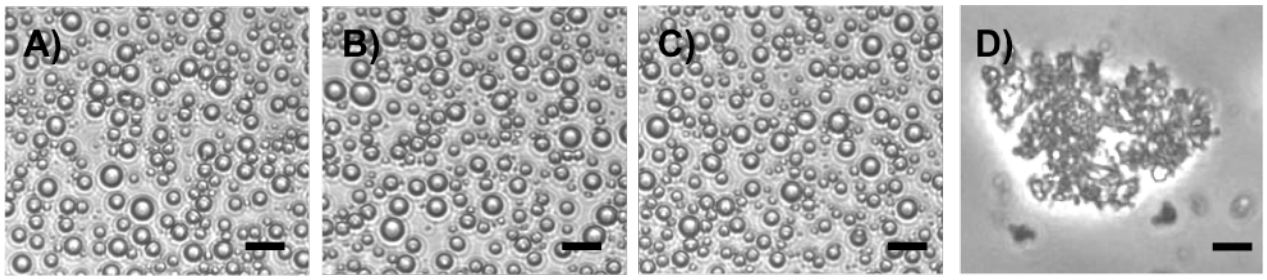

Figure S5. Optical micrographs showing the formation of liquid complex coacervates, regardless of the polypeptide chirality. Complexes formed from A) PPLGPG50/P(D)Glu100, B) PPLGPG50/P(L)Glu100 and PPLGPG50/ racemic PGlu100. D) Optical micrographs showing a solid polyelectrolyte complex formed from chiral (L) PLys50 and PGlu50 (all complexes were formed at a 1 mM total polymer concentration in polymer, 1:1 mol % mixing ratio and 100 mM salt concentration. Scale bar is 50 μm).

Rheological measurements

Rheological measurements were performed on a Bohlin Gemini HR nanorheometer (Malvern) fitted with a parallel plate geometry (8 mm in diameter). Polypeptide mixtures were centrifuged at 10,000 rpm for 15 min to expedite the coalescence of the coacervate droplets and the formation of the coacervate

phase (polymer rich) that exists in equilibrium with the aqueous (polymer poor) solution. After careful removal of the aqueous solution the coacervate phase was loaded onto the plate and was allowed to equilibrate for 10 min. Strain-sweep tests were carried out to determine the linear viscoelastic range (0−10%). Oscillatory tests were used to determine the storage modulus (G′) and loss modulus (G″) while varying the frequency from 0.1 to 100 rad/s with a constant strain of 10%, which was found to be in the linear regime. A steady shear rate sweep was performed to measure viscosity. Data presented in all figures are the average of at least three measurements (error bars were smaller than symbols and were therefore emitted).

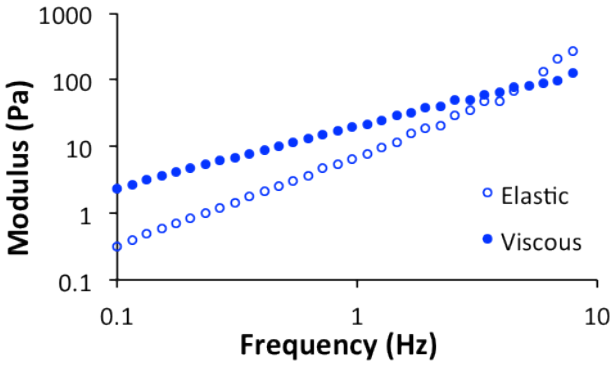

Figure S6. Frequency sweeps of storage (G') and loss modulus (G'') of coacervates formed from chiral $PPLGPG_{50}$ and $PGlu_{100}$ (10 mM total polymer concentration in polymer, 1:1 mixing ratio and 100 mM salt concentration, 10% strain for rheology experiments), showing their viscoelastic liquid behavior.

Turbidity measurements

A plate reader equipped with a UV spectrophotometer (Tecan, Infinite M200) was employed at a wavelength of 550 nm for the turbidity measurements. None of the polymers absorb light at this wavelength; thus, turbidity is due to light scattering from suspended coacervate droplets. The turbidity is defined by $-\ln(I/I_0)$, with I_0 = incident light intensity and I = intensity of light passed through the sample volume. Turbidity is measured in adsorption units (a.u.). Turbidity data presented in Figure 1A are an average of multiple (at least 3) measurements of 3 different samples prepared under the same conditions. Turbidity measurements for the study of the salt effect (Figure 1A) were performed in samples prepared at a 10 mM total polymer concentration in polymer and a 1:1 mol % mixing ratio and pH 7.0.

Dynamic Light Scattering (DLS)

Light scattering was measured at 90° using a BI-200SM goniometer containing a red laser diode with a wavelength of 637nm and TurboCorr digital correlator, all from Brookhaven Instruments (Holtsville, NY). Brookhaven Instruments Dynamic Light Scattering software was used to analyze inverse Laplace transforms of the intensity autocorrelation functions using the non-negatively constrained least-squares (NNLS) algorithm to obtain multimodal size distribution data. The hydrodynamic diameters reported are an average of 3 individual measurements from 3 samples, prepared at a total polymer concentration of 1 mM.

Table S2. DLS measurements of coacervate core micelles prepared in salt.

Polypeptide System	NaCl Concentration (M)	Hydrodynamic Diameter(nm)
PEG_{10k}-*b*-$PPLGPG_{50}/PGlu_{50}$	0.0	35.1
PEG_{10k}-*b*-$PPLGPG_{50}/PGlu_{100}$	0.0	35.7
PEG_{20k}-*b*-$PPLGPG_{50}/PGlu_{100}$	0.0	47.9
PEG_{20k}-*b*-$PPLGPG_{50}/PGlu_{100}$	1.0	46.6
PEG_{20k}-*b*-$PPLGPG_{50}/PGlu_{100}$	2.0	48.1
PEG_{20k}-*b*-$PPLGPG_{50}/PGlu_{100}$	3.0	46.4
PEG_{20k}-*b*-$PPLGPG_{50}/PGlu_{100}$	4.0	46.7

Table S3. DLS measurements of coacervate micelles with varying salt concentrations prepared by the addition of concentrated 5 M NaCl to a pre-mixed micelle sample.

Polypeptide System	NaCl Concentration (mM)	Hydrodynamic Diameter (nm)
PEG_{10k}-*b*-$PPLGPG_{50}/PGlu_{50}$	0.0	35.1
PEG_{10k}-*b*-$PPLGPG_{50}/PGlu_{50}$	50	35.9
PEG_{10k}-*b*-$PPLGPG_{50}/PGlu_{50}$	150	37.1
PEG_{10k}-*b*-$PPLGPG_{50}/PGlu_{50}$	300	33.1
PEG_{10k}-*b*-$PPLGPG_{50}/PGlu_{50}$	500	34.0
PEG_{10k}-*b*-$PPLGPG_{50}/PGlu_{50}$	1000	37.2

Small-Angle X-ray Scattering (SAXS)

Small-angle X-ray scattering (SAXS) experiments were performed at beamline 12ID-B of Advanced Photon Sources (APS) at Argonne National Laboratory. The wavelength, λ, of X-ray radiation was set as 0.866 Å. The beam size at the sample position is 20 μm (height) by 200 μm (width). Scattered X-ray intensities were measured using a Pilatus 2M detector (DECTRIS Ltd.). The sample-to-detector distance was set such that the detecting range of momentum transfer q [=4π sinθ/λ, where 2θ is the scattering angle] of SAXS experiments was 0.008-0.55 $Å^{-1}$. To reduce the radiation damage, a flow cell made of a cylindrical quartz capillary with a diameter of 1.5 mm and a wall of 10 μm was used, and the sample was flowed continuously during exposure. An exposure time of 1-2 seconds was used. In order to obtain good signal-to-noise ratios, twenty images were taken for each sample. The 2-D scattering images were converted to 1-D SAXS curves through azimuthally averaging after solid angle correction and then normalizing with the intensity of the transmitted X-ray beam, using the software package developed at the beamline 12ID-B. Data reduction involved averaging of n frames of data on-site, followed by subsequent analysis using the Irena small-angle scattering suite (SAS) suite.[39]

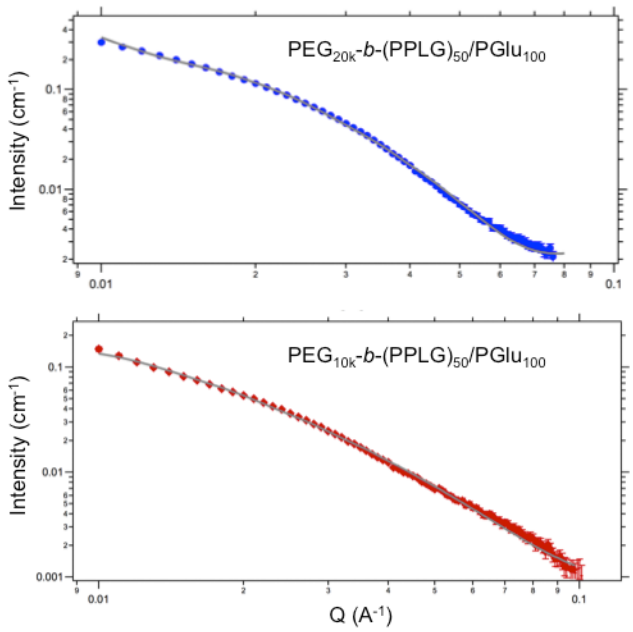

Figure S7. X-ray scattering data and fits (grey line) of PEG_{10k}-*b*-$PPLGPG_{50}/PGlu_{100}$ (red) and PEG_{20k}-*b*-$PPLGPG_{50}/PGlu_{100}$ (blue).

Transmission Electron Microscopy

Negatively stained TEM samples were prepared by placing micelles (prepared as described above) on 400 mesh lacey carbon grids (Ted Pella, Redding, CA, USA) and then staining with a 1 wt% uranyl acetate solution (98% solution, Sigma-Aldrich). Images were obtained using a FEI Tecnai F30 electron microscope operated at 300 kV (Figure S8).

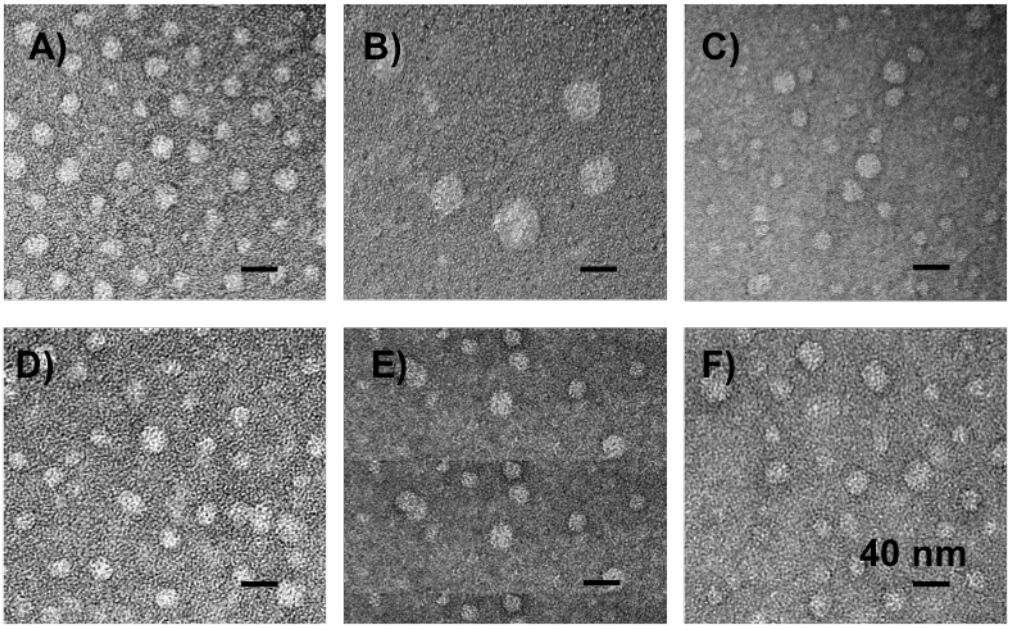

Figure S8. Negative-stain TEM images of micelles formed from A) PEG_{10k}-**b**-$PPLGPG_{50}$ and B) PEG_{20k}-**b**-$PPLGPG_{50}$ with $PGlu_{50}$, C) PEG_{5k}-*b*-$PGlu_{50}$ with $PPLGPG_{50}$, and D-F) PEG_{10k}-*b*-$PPLGPG_{50}$ with $PGlu_{50}$ showing the effect of PEG length and salt respectively (1 mM total polymer concentration, pH 7.0, A-C no salt, D at 50 mM, E at 150 mM, F at 300 mM).