Chirality-selected phase behaviour in ionic polypeptide complexes

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Polyelectrolyte complexes present new opportunities for self-assembled soft matter. Factors determining whether the phase of the complex is solid or liquid remain unclear. Ionic polypeptides enable examination of the effects of stereochemistry on complex formation. Here we demonstrate that chirality determines the state of polyelectrolyte complexes, formed from mixing dilute solutions of oppositely charged polypeptides, via a combination of electrostatic and hydrogen-bonding interactions. Fluid complexes occur when at least one of the polypeptides in the mixture is racemic, which disrupts backbone hydrogen-bonding networks. Pairs of purely chiral polypeptides, of any sense, form compact, fibrillar solids with a β-sheet structure. Analogous behaviour occurs in micelles formed from polypeptide block copolymers with polyethylene oxide, where assembly into aggregates with either solid or fluid cores, and eventually into ordered phases at high concentrations, is possible. Chirality is an exploitable tool for manipulating material properties in polyelectrolyte complexation.

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Polyelectrolyte complexation has a long history of utility in encapsulation applications\(^1\)\(^-\)\(^1\(^2\)\(^-\)\(^1\(^2\)\(^-\)\(^1\(^3\)\(^-\)\(^1\(^4\)\(^-\)\(^1\(^5\)\(^-\)\(^1\(^6\), produces compartmentalization that is implicated in development of certain biological assemblies\(^1\(^7\)\(^-\)\(^1\(^8\)\(^-\)\(^1\(^9\)\(^-\)\(^1\(^0\)\(^-\)\(^1\(^1\)\(^-\)\(^1\(^2\), and in origin of life scenarios\(^1\(^3\)\(^-\)\(^1\(^4\)\(^-\)\(^1\(^5\)\(^-\)\(^1\(^6\), and is accelerating new synthetic materials applications from surface coatings to self-assembled structures\(^3\(^-\)\(^1\(^2\)\(^-\)\(^1\(^3\). It is an entropically driven process, where the initial electrostatic attraction between oppositely charged polyelectrolytes is followed by the release of small, bound counter-ions and the restructuring of water molecules\(^3\(^-\)\(^1\(^6\)\(^-\)\(^1\(^7\). Studies on understanding and controlling polyelectrolyte complexation and self-assembly have focused almost exclusively on charge-driven phenomena, and controlling polyelectrolyte complexation and self-assembly is accelerated new synthetic materials applications from surface coatings to self-assembled structures.

The resulting complexes can form either solid precipitates or liquid complexes such as charge density, pH and ionic strength\(^4\(^,\(^5\)\(^-\)\(^1\(^8\)\(^-\)\(^1\(^9\)\(^-\)\(^2\(^3\). The resulting complexes can form either solid precipitates or liquid complex coacervates\(^4\). Wang and Schlenhoff\(^2\(^4\)\) give an excellent account of the progression from precipitate to coacervate to solution in a particular system, but the underlying causes and general understanding of phase selection between solid and fluid state complexes are obscure.

While precipitates result in dense, solid, hydrated materials, fluid coacervates retain significant amounts of water (up to 90% in some cases)\(^1\(^8\)\(^,\(^1\(^9\)\), display viscoelastic properties\(^1\(^6\)\(^,\(^2\(^2\)\(^,\(^2\(^5\)\(^,\(^2\(^6\) and have very low surface tension with water\(^2\(^0\)\(^,\(^2\(^3\). Complexation can be coupled with molecular design, linking polyelectrolyte domains to hydrophilic, neutral polymer blocks to stabilize microphase separation and drive the assembly of ordered phases associated with traditional block copolymers\(^5\)\(^,\(^-\)\(^6\)\(^,\(^1\(^0\)\(^,\(^1\(^2\)\(^,\(^1\(^3\)\(^,\(^2\(^7\)\(^,\(^2\(^8\). A delicate balance of forces determines whether complexation yields a liquid or a solid. This process occurs cooperatively, with the initial electrostatic interaction between the oppositely charged segments of two polymer chains nucleating phase formation\(^1\(^7\). The strength of these interactions can be mediated by charge screening and by parameters such as the acidity or basicity of the charged groups and the density of charges along the polymer\(^4\)\(^,\(^2\(^3\). Higher polyelectrolyte charge density provides an increased stability against salt-induced dissolution of the complexes and enables phase separation with shorter polyelectrolyte chains\(^1\(^8\)\(^,\(^2\(^2\)\(^,\(^2\(^3\). Higher charge density and strongly charged polyelectrolytes tend to favour solid precipitates over liquid coacervates\(^4\)\(^,\(^1\(^6\). The strength of electrostatic interaction appears to be the one recognized determinant of phase selection.

Various polyelectrolytes have been used in studies of complexation, ranging from charged particles and micelles\(^2\(^9\)\(^,\(^2\(^0\)\) to proteins\(^4\)\(^-\)\(^6\), biologically derived polymers such as gelatin, chitosan and heparin\(^5\)\(^,\(^3\(^1\)\(^,\(^3\(^2\)\) and synthetic polymers such as poly(acrylic acid) and poly(allylamine)\(^3\(^,\(^5\)\(^,\(^6\)\(^,\(^2\(^7\)\(^,\(^2\(^8\)\(^,\(^2\(^9\)\(^-\)\(^3\(^0\)\(^-\)\(^3\(^1\)\(^-\)\(^3\(^2\). Liquid coacervates are observed exclusively when at least one of the polyelectrolytes present was racemic (Fig. 1). Although the majority of the work done here utilized a 50/50 random copolymer of D and L monomers, we have also observed the formation of liquid coacervates when a sequence-controlled polymer of alternating D and L monomers was used (Supplementary Fig. 3).

**Results**

Characterization of chirality on bulk polyelectrolyte complexes. We present data here on the complexation of poly(lysine) (pK, using the single-letter abbreviation for amino acids,) with poly(glutamic acid) (pE) while systematically varying the polymer chirality (homochiral; pLK, pDK, pLE and pDE and racemic; p(D,L)K and p(D,L)E, see Supplementary Table 1 and Supplementary Figs 1 and 2 for details on characterization). Throughout this article, pK stands for polylysine and pE stands for polyglutamic acid; D and L indicate chirality, with D,L used to designate racemic polymers. Complexes were formed by mixing stoichiometric amounts of polyanion and polycation (that is, charge-matched conditions), in dilute solution in the presence of varying amounts of NaCl. Visual identification of liquid coacervates or solid precipitates was made using optical microscopy (Leica DMI6000 B). Liquid coacervates appear as small, spherical fluid droplets, while precipitates form amorphous solid clusters. Liquid coacervates were observed exclusively when at least one of the polypeptides present was racemic (Fig. 1). Although the strength of the interactions can be mediated by charge screening and by parameters such as the acidity or basicity of the charged groups and the density of charges along the polymer,\(^4\)\(^,\(^2\(^3\) Higher polyelectrolyte charge density provides an increased stability against salt-induced dissolution of the complexes and enables phase separation with shorter polyelectrolyte chains.\(^1\(^8\)\(^,\(^2\(^2\)\(^,\(^2\(^3\). Higher charge density and strongly charged polyelectrolytes tend to favour solid precipitates over liquid coacervates.\(^4\)\(^,\(^1\(^6\). The strength of electrostatic interaction appears to be the one recognized determinant of phase selection.

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In a broader sense, the stereochemistry of the polymer backbone can have a direct influence on the formation of semicrystalline or amorphous domains. However, these interactions are driven largely by stereoselective van der Waals interactions.\(^3\(^6\) Here we examine the chirality effects during polyelectrolyte complexation, taking advantage of not only electrostatic and stereospecific van der Waals forces, but also hydrogen-bonding interactions between polypeptide chains. This diverse set of orthogonal interactions enables the independent tuning of material properties without altering the chemical identity and demonstrates that chirality presents unique opportunities for the manipulation of physical properties in material systems built on polyelectrolyte complexation.

**Figure 1** | Optical micrographs of polyelectrolyte complexes. Bright-field optical micrographs showing the liquid coacervates or solid precipitates resulting from the stoichiometric electrostatic complexation of L, D, or racemic (D,L) poly(lysine) with L, D or racemic (D,L) poly(glutamic acid) at a total residue concentration of 6 and 100 mM NaCl. Complexes are formed from (a) pLK + pLE, (b) pDK + pLE, (c) p(D,L)K + pLE, (d) pLK + pDE, (e) pDK + pDE, (f) p(D,L)K + pDE, (g) pLK + p(D,L)E, (h) pDK + p(D,L)E, (i) p(D,L)K + p(D,L)E. Liquid coacervate droplets are only observed during complexation involving a racemic polymer. Scale bars, 25 μm.

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In contrast, complexes composed only of homochiral polymers (that is, pLK+pLE, pLK+pDE, pDK+pLE, pDK+pDE) formed solid precipitates, regardless of salt concentration.

To understand the secondary structure of the polypeptides within the resultant solid or fluid complexes, we employed transmission Fourier transform infrared spectroscopy (FTIR, Bruker Vertex 70 spectrometer). The location of the amide I carbonyl stretching vibration provides a characterization of polypeptide secondary structure. As can be seen in Fig. 2a, all samples (that is, individual polypeptides, liquid coacervates and solid precipitates) display a peak at 1,644 cm\(^{-1}\), characteristic of a random coil chain configuration (complete FTIR data and discussion are available in Supplementary Fig. 4 and Supplementary Note 1, respectively). This random coil structure was expected for the individual, charged polypeptides, and is consistent with previous characterization of the polymer structure in liquid coacervates closely approximating that of an ideal Gaussian chain. For the solid precipitates, we observe additional peaks that are characteristic of \(\beta\)-strands and amyloids. This signal was present at 1,611 cm\(^{-1}\) for solid precipitates formed from polypeptides with matching chirality (pLK+pLE, pDK+pDE) and at 1,613 cm\(^{-1}\) for complexes formed from polypeptides with opposite chirality (pLK+pDE, pDK+pLE). An additional, low-intensity peak near 1,680 cm\(^{-1}\) is also attributable to the presence of \(\beta\)-sheet structure. The blue-shift observed in the main amide I band has been attributed to a decrease in the number of peptide-water contacts, implying that complexes with opposite chirality exclude more water.

These results demonstrate the correlation between polypeptide chirality, polymer conformation and the fluid or solid nature of the resulting complex. While the random coil structure of an individual polypeptide homopolymer is a consequence of electrostatic repulsion from the charged side chains, neutralization of these charges allows attractive hydrogen-bonding interactions to dominate, resulting in the formation of close-packed polypeptide secondary structure (that is, \(\alpha\)-helix or \(\beta\)-sheet). For the case of homochiral polypeptides, the electrostatic interaction of oppositely charged side chains facilitates alignment of the peptide backbone and the formation of hydrogen-bonded \(\beta\)-strand structures where backbone hydrogen bonds replace surface-bound water that is released during the aggregation of the polymer. However, the presence of a racemic polypeptide prevents the formation of compact protein-like secondary structures and appears to limit both the extent of complexation and the expulsion of water from the polymer rich phase, while maintaining a Gaussian coil conformation.

**Stability of polyelectrolyte complexes against salt and urea.** We also investigated the stability of the various solid and fluid polyelectrolyte complexes with respect to salt. Turbidimetric measurements for liquid coacervates showed a characteristic increase in the amount of complex formation at low salt concentrations, followed by a decrease in complex formation up to a critical salt concentration, above which no phase separation is observed (Fig. 3a, Supplementary Fig. 5a and Supplementary Note 2). Taking into account the dependence of polymer chain length on the critical salt concentration (see Supplementary Table 2 and Supplementary Note 2), we observed a significant decrease in stability against salt (lower stability means that complexes dissociate and dissolve at lower salt concentrations) when fully racemic complexes were formed, as opposed to complexes with only a single racemic polymer (for example, p(D,L)K + p(D,L)E versus p(D,L)K + p(D,E)). However, coacervates were significantly less stable in salt as compared with solid precipitates, and solid complexes formed from polypeptides with opposite chirality showed even higher salt stability than those of matched chirality (for example, pLK + pLE, pLK + pDE) (Fig. 3a, Supplementary Fig. 5b and Supplementary Note 2). These variations as a function of polypeptide chirality suggest that electrostatic interactions are not solely responsible for the stability of the complex, but also van der Waals stereoregular interactions and hydrogen bonding.

In polypeptides, short-range hydrogen bonding plays a critical role in defining stable structural motifs. Therefore, we also investigated the stability of our complexes in urea, a denaturant that interacts preferentially with the peptide backbone and
disrupts hydrogen bonds\textsuperscript{39}. While urea did not affect the formation or stability of liquid coacervates, even at very high concentrations, a much more dramatic effect was observed for solid precipitates. The addition of sufficient quantities of urea resulted in the melting of solid precipitates into a coacervate-like liquid (Fig. 3b, Supplementary Fig. 6 and Supplementary Note 2), a transition that had only been previously observed in relation to charge-driven phenomena in polyelectrolyte complexes\textsuperscript{24}. Furthermore, the trends in stability observed as a function of urea matched those observed for salt (Supplementary Table 2), implying that the variations in salt stability are attributable to the strength of hydrogen-bonding effects. This conclusion is implying that the variations in salt stability are attributable to the presence of urea matched those observed for salt (Supplementary Table 2), suggesting that complexes with opposite chirality incorporate less water\textsuperscript{34,37,38}, and thus denaturant, from their surfaces, enhancing the stability of hydrogen-bonding interactions.

**Chirality effects in micellar polyelectrolyte complexes.** On the basis of experience with bulk complexes and previous reports on polyelectrolyte complex micelles formed using racemic polypeptides\textsuperscript{12,13}, we extended our investigation to include micellar complexes formed from the complexation of a homopolypeptide polypeptide with a diblock copolymer with one peptide block (Supplementary Fig. 2). In agreement with our results from bulk experiments, micelles formed from electrostatic complexation involving at least one racemic polypeptide (for example, PEG-p\textsubscript{L}K with p(D,L,E)) resulted in the formation of coacervate- or liquid-core micelles (LCMs), as described in the literature\textsuperscript{6,8,12}. These LCMs are highly monodisperse (R\textsubscript{h} = 27.1 nm, polydispersity = 0.074), as characterized by dynamic light scattering (DLS, Brookhaven Instruments BI-200SM, see Supplementary Table 3) and also similar in size to polypeptide-based polyelectrolyte complex micelles of comparable polymer chain lengths observed previously\textsuperscript{40}. Secondary structure analysis via both FTIR (Fig. 2b) and circular dichroism spectroscopy (CD, Fig. 4a) shows a random coil conformation, in agreement with the results obtained for bulk complexes. However, the structures formed from the complexation of homochiral polypeptides (for example, PEG-p\textsubscript{L}K with p\textsubscript{L}E) resulted in micelles that were larger, more polydisperse (R\textsubscript{h} = 32.8 nm, polydispersity = 0.117), and displayed \(\beta\)-sheet character (Figs 2b and 4a). We designate these complexes as solid-core micelles (SCMs) based on the behaviour of similar complexes in bulk. Interestingly, while the FTIR spectra for both LCMs and SCMs display the same features as the analogous bulk materials, the location of the \(\beta\)-strand peak for SCMs is slightly red-shifted compared with the bulk, from 1,611 to 1,610 cm\textsuperscript{-1}. This shift suggests that the confinement imposed by the micellar structure could enhance packing of the \(\beta\)-sheet network, compared with the bulk, because of forced chain alignment at the polyelectrolyte core-PEG corona interface\textsuperscript{37,38}.

CD enabled characterization of the urea-induced polypeptide structural changes in the micellar systems (Fig. 4b). While our
with an average polymer concentration using a polyethylene glycol-polyethyleneglycol (PEG-PE) block copolymer. Micelles were prepared at a polymer concentration of 0.01 mM total polymer concentration using a polyethylene glycol-polyethylene glycol (PEG-PE) block copolymer with an average N = 50 and either pLK with N = 100 for SCMs or p(D,L)KE with N = 100 for LCMs.

Molecular dynamics investigation of chirality effects. To further investigate the different intermolecular interactions that give rise to the various solid and liquid complexes, we performed molecular dynamics (MD) calculations. Multiple independent simulations were run for a 10-residue pLK interacting with a 10-residue p(D,L)KE, while one simulation was run for a 10-residue pLKE with a 10-residue pLE. All simulations were performed in 173 mM NaCl over the course of 1,000 ns. In all systems, the peptides formed an electrostatic complex in less than 10 ns, adopting a disordered structure. Around 150 ns, the pLKE + pLE system began to form a parallel β-sheet in the centre of the peptides, which was fully formed by 200 ns (Fig. 6). This structure remained stable throughout the duration of the simulation, with the exception of small fluctuations at the termini. The formation of a stable β-sheet structure allowed the peptides to bind more tightly, reducing the centre of mass distance to about 0.2 nm. However, the pLKE + p(D,L)KE system shown in Fig. 6 formed a less compact structure with a centre of mass distance varying between 0.3 and 0.9 nm. These peptides remained in mostly coil, bend and turn conformations, with only rapidly transient β-sheet formation observed for durations of less than 100 ns, and mostly in a region of the p(D,L)KE peptide where a sequence of three consecutive L amino acids was present (Fig. 6). Furthermore, the analysis of backbone hydrogen-bonding interactions indicated that the unstructured pLKE + p(D,L)KE system, corresponding to the liquid coacervate complexes, showed a preferential interaction with water and formed very few inter-peptide hydrogen bonds (Fig. 7a). In contrast, the dense β-sheet pLKE + pLE complex satisfied nearly all of its backbone hydrogen bonds through peptide-peptide interaction (Fig. 7b). Averaging multiple independent enactments of these 1,000-ns simulations with some variability of initial conditions, the pLKE + pLE system had an average β-sheet content over all residues of 61.3%, compared with 11.4% for the average of the pLKE + p(D,L)KE simulations. These results provide direct support of our experimental observations, confirming the structural modes of interaction between the various oppositely charged polypeptides and demonstrating the key role that chirality and hydrogen bonding play in determining the structural and physical state of poly-peptide-based polyelectrolyte complexes.

Discussion

In summary, the polypeptide chirality not only determines the physical state of the resulting polyelectrolyte complexes (that is, liquid or solid), but also defines the strength of intermolecular interactions, and thus the material properties. While electrostatic interactions act over long distances, the shorter-range nature of polar hydrogen-bonding forces, combined with steric packing and hydration, provide additional methods for controlling self-assembly. The effects we have presented here raise obvious follow-on questions about the effects of sequence distribution within globally achiral polymers or designed effects that can be created by tailoring sequences of chiral peptides, which we are now pursuing. While sequence specificity in biology controls the three-dimensional assembly of proteins, we propose that patterns of chirality could have significant implications for tailoring of material properties without otherwise altering the chemical composition of polypeptide-based materials. For instance, this type of control could be utilized to tailor the rheological response of bulk materials and formulate delivery systems with controlled water content. Furthermore, coupling this type of polar and electrostatically guided self-assembly with more complex molecular architectures, as in the block copolymer systems described here, enables the creation of interesting classes of new materials with novel self-assembling structures, functionality and...
responsiveness. We note, too, that while this work clarifies the role of chirality on phase selection in complexes of charged polypeptides, other polyelectrolyte complex systems also form both solid and fluid phases. We suggest that the causes of solid phase formation in polyelectrolyte complexes are to be found in short-range forces, which may be influenced by tacticity, hydration packing and other factors, acting in concert with longer range electrostatic forces.

Methods

Materials. Polypeptides were obtained either from Alamanda Polymers Inc. and used directly, without further purification, or were synthesized in house using N-carboxyanhydride polymerization. In a previously published article, we reported results on a polypeptide that we said was pE, after communication with the supplier (Alamanda Polymers Inc.) and performing further characterization, it came to our attention that the polymers in this previous work were not optically pure L but contained a number of D repeating units and should therefore have been referred to as p(L,D)E. A correction of this error has now been published. Identification of this issue led to the current work in which we have retested a wide range of conditions and have been unable to formulate liquid coacervates using homochiral polypeptides in any instance.

The degree of polymerization for the prepared polymers was obtained via 1H NMR. Gel permeation chromatography (Waters) coupled with refractive index (Optilab UT-rex, Wyatt Technologies) and light scattering detectors (miniDAWN Treos, Wyatt Technologies) was used to characterize the polydispersity of the samples (Supplementary Table 1). CD (Jasco J-815 CD Spectrometer) was used to confirm both the random coil structure and the homochiral or racemic composition of the individual polypeptides (Supplementary Figs 1 and 2).

Here we abbreviate poly(glutamic acid) as pE in general, or the different chiral polymers as pLE, pDE, p(L,D)E in specific, taking advantage of the single-letter abbreviation strategy for amino acids. Similarly, we refer to polyllysine as pK in general, or pL, pD, p(L,D)K in specific. This naming convention also allows referring to D-amino acids using a lower-case letter (that is, pk rather than pDK). This strategy will be useful for the investigation of sequence-controlled polymers of D and L amino acids (for example, (Kk)14W as in Supplementary Fig. 3), but for the current discussion of homochiral or random copolymers we will explicitly refer to D-amino acids using a lower-case letter (that is, pk rather than pDK).

Figure 6 | MD simulations of polyelectrolyte complexes. Visualization and residue maps indicating polypeptide secondary structure from representative MD simulations for two pairs of poly(lysine) and poly(glutamic acid) peptides, N = 10. Polypeptides are initially equilibrated in a random coil conformation and then allowed to complex for 1,000 ns. (a) A representative simulation of homochiral pLK complexing with racemic p(L,D)E indicates preservation of a mostly random coil structure, as would be expected for liquid coacervates, while (b) homochiral polypeptides pLK with pLE shows the evolution of β-strand structure expected for a solid precipitate. Map of secondary structure as a function of time for (c) pLK + p(L,D)E and (d) pLK + pLE.

Figure 7 | Hydrogen bonding in polyelectrolyte complexes. Quantification of the number of hydrogen bonds formed as a function of time during a 1,000 ns MD simulation of complex formation between (a) pLK + p(L,D)E and (b) pLK + pLE.
direct stoichiometric comparison of the number of positively and negatively charged units present in solution. Stock solutions of 2 M sodium chloride (NaCl, ACS reagent, ≥ 99%, Acros Organics) and 8 M urea (Bioreagent, Sigma) were prepared gravimetrically and adjusted to pH 7.0, as above.

Preparation of bulk polyelectrolyte complexes. Complexation was performed using stoichiometric quantities of positive and negatively charged polypeptides at a total residue concentration of 1 mM at pH 7.0, in the presence of varying concentrations of NaCl and urea. Under these conditions, it is a reasonable approximation to describe all of the residues on both polypeptides as charged. All polymers used for the preparation of bulk complexes have an approximate degree of polymerization N = 100. Samples were prepared by first mixing a concentrated solution of NaCl with MilliQ water in a microcentrifuge tube (1.5 mL, Eppendorf). Other additives such as concentrated urea were also added at this stage, unless otherwise indicated. A concentrated polyelectrolyte (pE) was then added to this mixture, followed by the polycation (pK) to a final volume of 500 μL. The mixture was vortexed for 5 s immediately after the addition of each component to ensure fast mixing. For all experiments, samples were prepared to a final concentration of 1 mM monomers (total cation and anion). Unless otherwise indicated, all bulk complexes (that is, liquid coacervates and solid precipitates) were prepared in 100 mM NaCl. The effect of salt was examined over the range of 0 to 1.5 M NaCl. The effect of urea was examined from 0 to 6.8 M. All samples were prepared immediately before analysis and studied at room temperature (25°C).

Preparation of micellar polyelectrolyte complexes. Micelles were formed by mixing either PEG-pK with pE or PEG-pL with pK at an equal charge molar ratio, in water, using the order of operations described above for the homopolymers. Micellar solutions for the DLS experiments were made using PEG-pL and pE, in which the average charged polypeptide segments of each molecule was N = 100. The micelle formed using PEG-pL and p(D,L)E was made at a total polymer concentration of 0.07 mM, which was subsequently diluted to 0.01 mM when measured using CD. The micelle formed using PEG-pK and p(D,L)E was made at a total polymer concentration of 0.05 mM, which was diluted to 0.0125 mM when measured using CD. The micelles were prepared by complexing PEG-pL with pK at a total polymer concentration of 0.04 mM, with all the charged segments containing an average N = 50. Micellar solutions for the FTIR experiments were made at a total polymer concentration of 0.19 mM in D2O using a PEG-pL block copolymer with a random average N = 50 and either pE or pL with N = 50 for SCMs or N = 100 for LCMs.

Visual characterization of complexes. The resulting complexes formed from homopolymers were imaged within 1 h of preparation. Bright-field and phase contrast optical microscopy (Leica DMI 6000B using Leica DIC phase image acquisition software) were used to identify the formation of liquid coacervates or solid precipitates, to identify the critical salt concentration, above which no phase separation occurs, and the minimum urea concentration necessary to trigger the transformation between solid precipitates and liquid coacervates. Liquid coacervates appear as small spherical droplets in solution, while precipitates form amorphous solid clusters (Figs 1 and 3 and Supplementary Figs 3 and 6). Imaging was performed using both ultra-low attachment 96-well plates (Costar, Corning Inc.) and glass slides (Fisherbrand).

Turbidimetry. Turbidity was used to qualitatively measure the extent of complex formation as a function of charge stoichiometry and salt concentration. Turbidity measurements were made using a plate reader equipped with a ultraviolet spectrophotometer (Tecan, Infinite M200 P80). Turbidity was measured at a wavelength of 550 nm and a temperature of 25°C. Turbidity is defined as the transmittance or absorbance at a specified wavelength (the baseline of the blank sample subtracted). Turbidity was measured at a wavelength of 550 nm for 10 min before data collection. One hundred and twenty-eight scans were collected from 4,000 to 800 cm⁻¹ at a spectral resolution of 2 cm⁻¹. Spectra were obtained using a D2O sample as a background and were dynamically corrected for atmospheric water by subtracting the signal obtained for the open chamber (exposed to air), taken with a NaO2 background and scaled by a factor of ~ 0.018. Data were then normalized such that the minimum baseline signal above the amide I signal (in the range of 1,755–1,700 cm⁻¹) was set to zero, and the height of the random coil peak near 1,644 cm⁻¹ was set to 1. The location of individual peaks was determined using non-linear least-squares fitting of Gaussian peaks to the features of the spectrum, with baseline correction where necessary. CD (Jasco J-815 CD Spectrometer) was used to confirm the random coil structure, the chiral nature of the individual polypeptide solutions (Supplementary Figs 1 and 2) and the secondary structure of micellar solutions (Supplementary Fig. 7). All the CD data presented are an average of five scans collected between 250 and 190 nm at room temperature, in a 0.1-cm cuvette. In experiments involving urea, the baseline measurements for LCMs were subtracted from the micellar spectra. The fitting of the CD data was done using a linear combination of (polylysine) basis spectra65 resulting in specific percentages of α-helix, β-sheet and random coil secondary structure.

Dynamic light scattering. DLS was measured at 90° using a BI-200SM goniometer containing a red laser diode with a wavelength of 637 nm and a TurbicoRR detector (Brookhaven Instruments, Holtsville, NY). Brookhaven Instruments DLS software was used to analyse the intensity autocorrelation function using the cumulant method67 to obtain characteristic decay rates from which apparent diffusion coefficients were calculated. The Stokes–Einstein equation was used to convert diffusion coefficients to hydrodynamic radii. Polydispersity was obtained from the second order cumulant term.

MD simulations. MD simulations were performed for 10-residue peptides of poly(glutamic acid) (pE) and poly(L-lysine) (pL). Two versions of pE were used: a homochiral L version (pLE) and a racemic version (p(D,L)E) with the sequence LDDDDQLLLD, which was chosen by shuffling a sequence with five L and five D chiral centres. The side chains were fully charged with the N terminus and C terminus consisting of a charged NH₂ and COO⁻ group, respectively. These were initialized with an unordered configuration. One pE chain and one pL chain were placed perpendicularly to each other at distances of 6 nm dodecahedral box. The system was then solvated with 4889 TIP3P waters and 16 Na⁺ and Cl⁻ ions (that is, 104 mM NaCl), modelled with the ion parameters from Joung et al.68 Molecular simulations were run using the GROMACS 4.6.3 simulation package69. The energy of the system was minimized for 500 steps without constraints and then for an additional 10,000 steps with all bonds constrained using the steepest descent algorithm. A MD simulation was then run using a step size of 2 fs and the CHARMM²² force field for 100 ps at constant pressure and a temperature of 298 K. This was continued for 1,000 ns at a constant pressure of 1 bar using the Nose–Hoover²² and Parrinello–Rahman²² coupling schemes to maintain the temperature and pressure, respectively. Electrostatic interactions were performed using a particle mesh Ewald method²⁰ with a cutoff of 0.9 nm and a Fourier spacing of 0.33 nm. Hydrogen bonds were constrained using the LINCS²⁵ algorithm. The secondary structure was determined using the DSSP (Define Secondary Structure of Proteins) criteria²⁵ over an average of four replicate simulations for p(D,L)E with pL and one simulation for pE with pL.

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