Expanding bio-functionality of peptide-based polyelectrolyte complexes through changes in chirality

Review Article

In the search for ways to handle soft materials at the nano-level, Polyelectrolyte Complexes (PECs) offer a lot of promise. They self-assemble and their enormous diversity of structure and chemical composition enable functionality to be fine-tuned. The biocompatibility and biodegradability of peptide polymer PECs means they are particularly useful in applications such as food additive encapsulation, micellar drug delivery, and scaffolding cell growth for tissue engineering. The physical properties of peptide polymer PECs can be modulated based on many factors. Naomi Pacalin, Lorraine Leon, and Matthew Tirrell at the University of Chicago, USA, have shown that changing the chirality of peptide polymer PECs alters the strength of polymer chain interactions, allowing chirality to be used to tailor polymers to have the precise properties needed for a particular application.

Putting a kink into the mix

PECs are formed by mixing solutions of oppositely charged polymer species that on mixing, assemble to form a dense polymer-rich phase and a dilute polymer-poor phase. The charged species in a PEC are held together by electrostatic, van der Waals and hydrogen bond interactions including secondary structure motifs such as β-sheets and α-helices. Forming PECs using peptide polymers offers a unique possibility of using chirality to enhance or disrupt these interactions and thereby modulate the amount of secondary structure formed.

In their previous work, the Tirrell research group had found that chiral peptides form solid complexes but adding just one racemic peptide results in a phase change from solid to fluid. They therefore decided to take this one step further and determine just how long a homochiral section needs to be to cause a phase change.

Synthesis of peptide polymers

The research group built their PECs using 30-mer polyglutamic acid (Ene) and polylysine (Knk) (where n = 1–9) peptides (Table 1). These peptides provided a continuous scale of chirality, from completely achiral (n=1) to 90% chiral (n=9). These peptides ranged from having 15 D- residues (n=1) to just 3 D-residues (n=9).

Table 1. *The chiral sequences of the peptide polymers used to form PECs.*

Ene/Knk (n)	Pattern
1	(*LD*)15
2	(*LLD*)10
3	(*LLLD*)7*LL*
4	(*LLLLD*)6
5	(*LLLLLD*)5
6	(*LLLLLLD*)4*LL*
7	(*LLLLLLLD*)3*LLLLLL*
8	(*LLLLLLLLD*)3*LLL*
9	(*LLLLLLLLLD*)3

The Ene and Knk peptides were synthesized by FMOC solid phase synthesis on a PS3 Automated Solid Phase Peptide Synthesizer using a low-loading Rink Amide resin as solid support. FMOC protected amino acids were added one by one to the resin. 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) was used as the amino acid activator with N-methylmorpholine base. The N-terminal FMOC was removed using 20% piperidine in DMF. After deprotection and activation, the amino acid and resin were mixed using nitrogen bubbling in a glass reaction vessel to enable coupling to the peptide chain. These steps were repeated for all 30 amino acids before removing the final FMOC group with 20% piperidine and cleaving the resin and amino acid side chain protecting groups with 95% trifluoroacetic acid (TFA), 2.5% triisopropylsilane (Tis), and 2.5% water for 2.5 h.

Confirming the chiral structures

The research group confirmed the chiral content of the chiral patterned species using circular dichroism (CD), which measures the absorbance difference of *L*- and *D*-polarized light (see example in Figure 1).

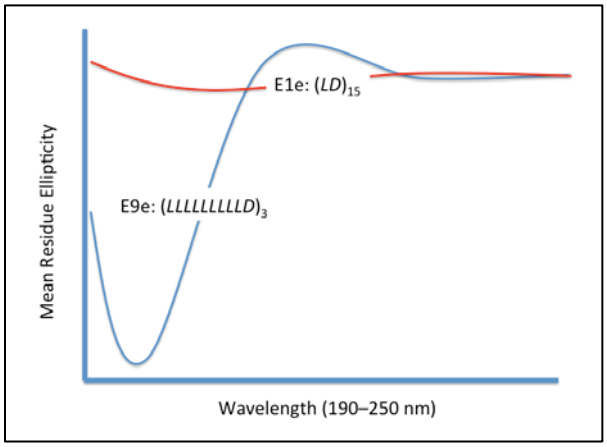

Figure 1. *Example of CD-analysis of two peptide polymers. Secondary structure was determined based on a linear interpolation between measured spectra and known spectra for α-helices, β-sheets, and random coil peptides. The sketch is based on data shown in Figure 3a, Pacalin et al., 2016.*

Finding the transition

When the team used optical imaging to look at the PEC mixtures, they could see clear transitions from fluid, spherical droplets with achiral PECs (n=1–7), to complexes that were amorphous and more polydisperse in size and shape when the level of homochirality was higher (n=8–9).

They examined the secondary structure of the polymers using FTIR in the Amide 1 region (1600–1700 cm^{-1}). Non-complexed polymers had random coil structure and the coacervate complexes (n=1–7) had spectra that were superpositions of the spectra from the non-complexed polymers. In contrast, the FTIR spectra for complexes with the highest level of homochirality (n=8–9) showed the characteristic β-strand peak at 1611 cm^{-1} that suggested the presence of β-sheets (see Figure 2).

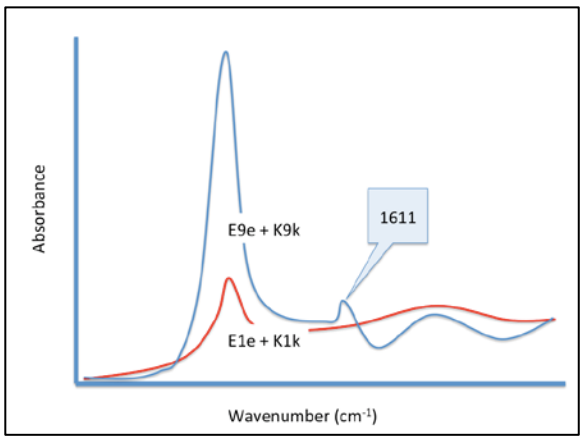

Figure 2. *FTIR analysis showed that the PECs formed with polymers with a high homochirality level (n= 8–9) showed a peak at 1611 cm^{-1} that is characteristic for β-strands. The sketch is based on data shown in Figure 5c, Pacalin et al., 2016.*

Fine-tuning function with chirality

Fine-tuning mechanical properties is key in many applications and modulation of chirality in PECs formed from peptide polymers is clearly an important addition to the toolbox. In conclusion, the authors state, *"For each PEC application, there is likely an optimal PEC mechanical strength required for the desired function. Chiral patterning may provide the capability to achieve phases of intermediate stability between coacervate and precipitate".* Chirality would also enable control over water content, and diffusion and release properties of embedded particles. A salient example for the utility of chiral patterning is for the modification of hydrogels, which are used for many applications such as cell scaffolding, drug delivery and medical adhesives.

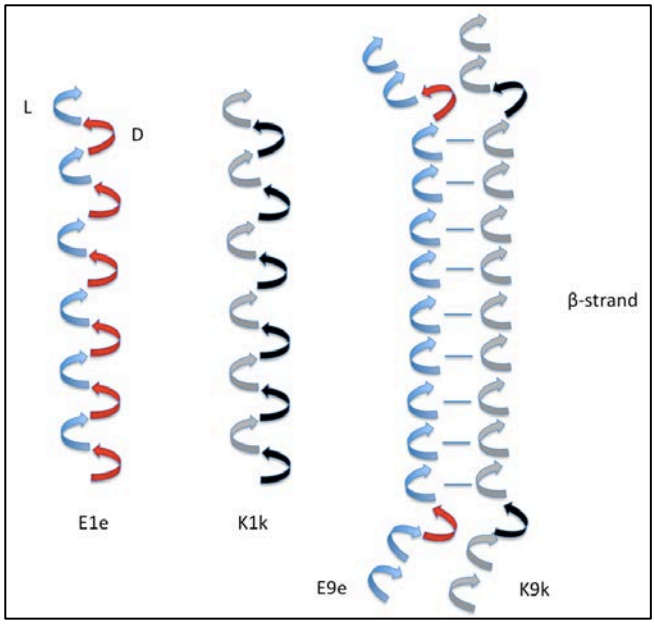

Figure 3. *Schematic showing that moving from achiral (E1e+K1k) to more homochiral mixtures of PECs (E9e+K9k) enables the formation of β-strands, thus changing the fundamental mechanical properties of the complexes.*

A peptide synthesizer that speeds up synthesis with overnight runs

Following up on this summary, we asked the authors about their experience with the PS3 Automated Solid Phase Peptide Synthesizer in their work on peptide polymer PECs:

"In this paper, we made eighteen different peptides, which would have been extremely time consuming to do by hand. The PS3 certainly helped speed up the production of all our different products by allowing us to quickly and easily set up our synthesis reagents and run from start to finish while doing other experiments," stated Naomi Pacalin who has since left the University of Chicago and will be starting graduate school at Stanford University in Bioengineering.

"Additionally, we were able to maximize our synthesis capabilities by setting up multiple reaction vessels and allowing them all to run overnight," added Lorraine Leon, a former postdoctoral researcher in the Tirrell group who, based on her positive experiences with the PS3, purchased the same instrument for her own lab at the University of Central Florida.

Reference

Directing the phase behavior of polyelectrolyte complexes using chiral patterned peptides. Pacalin, N., Leon, L. & Tirrell, M. Eur. Phys. J. Spec. Top. (2016) 225: 1805. doi:10.1140/epjst/e2016-60149-6