Published in final edited form as:

Biomacromolecules. 2013 September 9; 14(9): . doi:10.1021/bm4008309.

Split of Chiral Degeneracy in Mechanical and Structural Properties of Oligopeptide-Polysaccharide Biomaterials

Marc B. Taraban^{1,2}, Laura L. Hyland¹, and Y. Bruce Yu^{1,2,*}

Fischell Department of Bioengineering, University of Maryland, College Park, MD 20742 and Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, Baltimore, MD 21201

Abstract

Enantiomeric biomaterials which are mirror images of each other are characterized by chiral degeneracy—identical structural characteristics and bulk material properties. The addition of another chiral component, *D*-polysaccharide, has been shown to split such degeneracy and result in two distinct biomaterials. Dynamic oscillatory rheometry and small-angle X-ray scattering demonstrate that the natural biochirality combination of *L*-peptides and *D*-polysaccharides assembles faster, has higher elastic moduli (G'), and is structurally more beneficial as opposed to *D*-peptide and *D*-polysaccharide combination. Chemical modifications of the OH-groups in α -*D*-glucose units in *D*-polysaccharides weaken such splitting of chiral degeneracy. All these findings form a basis to design the approaches to novel biomaterials and provide additional insight on the opposite chirality of proteins and polysaccharides in biological world.

Keywords

oligopeptides; polysaccharides; biohomochirality; chiral degeneracy; dynamic rheometry; small-angle X-ray scattering

Introduction

In the human body, most of the soft tissues with the strictest requirements as to the viscoelastic characteristics and recoverability—cartilage, tendons, ligaments, etc.—are formed through interactions between proteins and polysaccharides. The large diversity in the properties of the soft tissues is defined by the capability of proteins and polysaccharides to assemble into constructs with various mechanical and structural characteristics. For example, the extracellular matrix—the supporting scaffold for cells and most connective tissues—is believed to form by the interlocking mesh of proteins and polysaccharides or glucosaminoglycans (GAGs) covalently or non-covalently (*e.g.*, in case of hyaluronic acid) bound to protein molecules (proteoglycans).¹

And like all biomacromolecules, proteins and polysaccharides are homochiral polymers. Moreover, the “monomers” of proteins and polysaccharides have the opposite chirality;

*To whom correspondence should be addressed. Current address of corresponding author: Department of Pharmaceutical Sciences, 20 Penn Street, Baltimore, MD 21201, USA; byu@rx.umaryland.edu; Phone: 410-706-7514; Fax 410-706-5017.

¹Fischell Department of Bioengineering, University of Maryland, College Park, USA.

²Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, Baltimore, USA

Supporting Information Available. Analytical HPLC and ESI-MS spectra of peptides; supplementary rheological data on all oligopeptide-polysaccharide hydrogels (frequency and strain sweeps); pictorial illustrations of the concepts of L_c and L_p . This material is available free of charge via the Internet at <http://pubs.acs.org>.

proteins are made only from left-handed *L*-amino acids while most polysaccharides are made from righted-handed *D*-sugars (nucleic acids also contain exclusively righted-handed *D*-sugars). This phenomenon is called biohomochirality and is considered a hallmark of life.^{2,3,4}

The “pros” and “cons” of homochirality and heterochirality in the protein realm have been recently discussed from the angle of the mechanical and structural properties of peptide-based biomaterials. To this end, hydrogels assembled from *L*- and *D*-peptides were investigated.⁵ Homochiral gels—*L*-gel made from two oppositely charged *L*-peptide modules and *D*-gel made from two oppositely charged *D*-peptide modules—demonstrated so-called *chiral degeneracy*,⁶ i.e., were mechanically and structurally very similar. In comparison, heterochiral peptide gels—made from oppositely charged *L*- and *D*-peptide modules were mechanically much weaker than, and structurally distinct from, the homochiral gels. This result suggests that homochirality confers mechanical advantages to peptide materials.

As to why amino acids and sugars in biopolymers have the opposite chirality, it is known that, at the molecular level, *L*-proteases hydrolyze *L*-peptides, but not *D*-peptides, while *D*-proteases hydrolyze only *D*-peptides, but not *L*-peptides.⁷ On the other hand, *L*-nucleases hydrolyze only *D*-nucleic acids, but not *L*-nucleic acids.⁸ Moreover, it has been shown that in the sugar-producing formose reaction, *L*-amino acids selectively catalyze the formation of the simplest sugar, *D*-glyceraldehyde.⁹ These observations suggest that under prebiotic conditions, the existence of chirality-mediated chemical link between *L*-proteins and *D*-polysaccharides played significant role in the origin of life.¹⁰ These observations highlight the *biochemical* consequences of biohomochirality. However, the *material* consequences of biohomochirality are unknown—it is unclear whether the combination of left-handed *L*-amino acid and right-handed *D*-sugars possesses certain structural distinctions which translate into material advantages such as mechanical strength.

In this work we explore whether materials made of *L*-amino acids and *D*-sugars are superior to those made of *D*-amino acids and *D*-sugars. This is accomplished by comparing biomaterials assembled from *L*-peptides and *D*-polysaccharides with those assembled from *D*-peptides and *D*-polysaccharides. To confirm the generality of the results, four *D*-polysaccharides with very different molecular characteristics were paired with *L*- and *D*-peptides to form hydrogels. Five pairs of materials, including one enantiomeric pair and four diastereomeric pairs, are characterized and compared:

1. Enantiomeric pair of homochiral peptide hydrogels. Such hydrogels are formed by pairing oppositely charged isotactic peptide modules, each comprised of either entirely *L*- or entirely *D*-amino acids. Such hydrogels act as model systems for the protein part of the extracellular matrix (ECM), creating mechanically sturdy and complex networks.^{11,12} Indeed, it has been shown recently that these peptide hydrogels can support the growth and proliferation of human mesenchymal stem cells.¹³
2. Hydrogels from (1) assembled in the presence of β -cyclodextrin (**β CD**, Figure 1A)—a cyclic neutral oligosaccharide comprised of 7 α -*D*-glucose units. Cyclodextrins have been widely used in pharmaceutical formulation and biomaterials engineering.^{14,15}
3. Hydrogels from (1) assembled in the presence of glycogen (**Glyn**, Figure 1B). **Glyn** is neutral, multi-branched polysaccharide, formed by the chains of α -*D*-glucose residues. **Glyn** is one of the main glucose storage depots in the body, and is ubiquitously present in muscle tissues.¹⁶

4. Hydrogels from (1) assembled in the presence of chondroitin sulfate (**Chd**, Figure 1C). **Chd** is a linear polysaccharide made of two alternating sugar units, *D*-glucuronic acid and *N*-acetyl-*D*-galactosamine and is negatively charged. **Chd** is naturally prevalent in soft tissues such as cartilage, ligaments, etc., and is extensively studied as a prospective scaffold for tissue regeneration.¹⁷
5. Hydrogels from (1) assembled in the presence of a chemically modified polysaccharide, hydroxyl-propyl cellulose (**HPC**, Figure 1D). **HPC** is a linear, uncharged amphiphilic polysaccharide which is a synthetic cellulose derivative. It is made of α -*D*-glucose units whose hydroxyl groups are partially or completely hydroxypropylated. The extent of hydroxypropylation is defined by the degree of substitution number or *DS*. For example, **HPC** with *DS* of 3 means that all OH-groups of α -*D*-glucose unit are substituted, and *DS* ~ 3.5 means that about half of the introduced hydroxypropyl groups (which also bear hydroxyl) are further substituted. Due to its high biocompatibility, **HPC** is widely used as thickener in food industry and as lubricant and binder in numerous pharmaceutical formulations. Its propensity to form strong hydrogels under elevated temperatures (~ 45 °C)¹⁸ makes **HPC** a prospective polysaccharide for soft and bone tissue engineering and regeneration.¹⁹

In this work, dynamic oscillatory rheology and small-angle X-ray scattering (SAXS) were used to respectively probe the mechanical and structural properties of hydrogels. SAXS was selected for structural studies because these hydrogels pose challenges for other structure characterization techniques. First, these hydrogels are rather opaque,⁵ and such opaqueness leads to high noise level in circular dichroism and fluorescence spectroscopy.²⁰ Second, in the gelled state, molecules that have been incorporated into the fiber matrix become invisible to solution state NMR due to line broadening.²¹ Finally, electron microscopy would require either drying or freezing of the hydrogel samples, procedures that are likely to distort the underlying fiber structure. But these issues do not arise with SAXS because the wavelength of neutron and X-ray is very short and therefore are not affected by the optical opaqueness of the samples. Also, SAXS is capable to investigate the samples *in situ* and do not require special preparations of the samples, *e.g.*, drying or freezing. Thus, the hydrogels are investigated under their natural state with no danger of structural distortion caused by drying or freezing. For these reasons, SAXS, along with small-angle neutron scattering (SANS), are ideally suited to explore hydrogel structure as discussed in a recent review.²²

From a standpoint of biology, we want to address the question whether the biohomochirality combination of *L*-amino acids and *D*-sugars leads to superior material properties. From the stand point of engineering, we want to use chirality as a means to control material properties. Of course, biology and engineering are related, particularly in the area of stem cells, where it is known that stem cell proliferation and differentiation are affected by the stiffness of the surrounding matrix. For example, for mesenchymal stem cells, soft matrices are neurogenic while stiff matrices are myo- and osteogenic.²³ Another example is that when cardiocytes mature from mesoderm to adult myocardium, their surrounding ECM experiences 9-fold stiffening.²⁴ Chirality provides a unique angle to make artificial ECMs with suitable stiffness and assembly kinetics for stem cell engineering.¹³

Experimental Section

Materials

β CD, M.W. 1.14 kDa, *Sigma-Aldrich*; **Glyn**, M.W. 100–1000 kDa, *Sigma-Aldrich*; **Chd** (bovine chondroitin sulfate sodium salt), M.W. ~ 20 kDa, *Pfaltz & Bauer*; and **HPC**, M.W. ~ 80 kDa (degree of substitution, *DS* ~ 3.5),²⁵ *Sigma-Aldrich*, were used as purchased.

Fmoc-protected amino acids for peptide synthesis, *aapptec, Inc.*, were used without further purification.

Peptide design and preparation

Oppositely charged undecapeptides were made using the approach described in our previous work.⁵ For each peptide sequence, both *D*- and *L*-versions were made (Table 1). Mixing positively charged (containing lysine, K) and negatively charged (containing glutamic acid, E) peptide modules triggers their co-assembly into a hydrogel.²⁶

Synthesis of all four peptides was performed on rink-amide MBHA resin (*Adaptec, Inc.*) using a Liberty microwave automated peptide synthesizer (*CEM, Co.*) based on standard solid-phase Fmoc-chemistry.²⁷ The crude peptides were cleaved off the resin using a cleavage cocktail (95% trifluoroacetic acid (TFA) with 2.5% triisopropyl silane (TIS), and 2.5 % water). After cleavage, the products were concentrated under nitrogen gas flow, precipitated and washed several times with cold ether. The crude products were then dissolved in water and lyophilized. The purity and the molecular weight of each purified peptide were verified by reverse-phase analytical HPLC and mass spectrometry, respectively (see, Supporting Information). Each purified peptide sample was then dissolved in ultrapure water and dialyzed at room temperature against water for 24 h using a dialysis membrane with a molecular weight cutoff of 100–500 Da. The concentration of each peptide sample was determined on the basis of the UV absorption of the tryptophan residues in each peptide, using a molar extinction of 5690 M⁻¹cm⁻¹ at 280 nm for each tryptophan residue.²⁸ In these UV measurements the peptide solutions were diluted by 6 M guanidine hydrochloride solution to ensure full unfolding of the peptides.

From the above listed oligopeptides and polysaccharides, five pairs of hydrogels were prepared, including (1) *L*⁺*L*⁻ vs. *D*⁺*D*⁻, which serve as the control; (2) *L*⁺*L*⁻ + **βCD** vs. *D*⁺*D*⁻ + **βCD**; (3) *L*⁺*L*⁻ + **Glyn** vs. *D*⁺*D*⁻ + **Glyn**; (4) *L*⁺*L*⁻ + **Chd** vs. *D*⁺*D*⁻ + **Chd**; (5) *L*⁺*L*⁻ + **HPC** vs. *D*⁺*D*⁻ + **HPC**. Note that pair (1) is enantiomeric while pairs (2–5) are diastereomeric. Samples were prepared at room temperature (*ca.* 23.5 °C) and characterized at 25°C by dynamic rheometry and SAXS. All solutions were made in PBS buffer (50 mM phosphate and 100 mM NaCl) at pH 7.4. The conductivity of each solution was adjusted to 16–17 mS·cm⁻¹, same as PBS, using a conductivity meter. This serves to control the ionic strength of the peptide solutions. Gravimetrically measured amount of the respective polysaccharide was added to the initial solution of the respective peptide at the target concentration. After complete dissolution of the polysaccharide, the pH of the resulting solution was again checked and adjusted to 7.4, and the final solution conductivity was again tuned to 16–17 mS·cm⁻¹. Hence sample consistency was ensured by controlling three parameters: concentration, pH and conductivity.

Dynamic Rheometry

Hydrogels were prepared by mixing two solutions of oppositely charged peptide modules. Neutral polysaccharides (**βCD**, **Glyn** and **HPC**) were added to both peptide solutions prior to mixing. The negatively charged polysaccharide **Chd** was added only to the solution of *L*⁻ or *D*⁻ prior to mixing (to prevent premature gelation between **Chd** and *L*⁺ or *D*⁺). Details of sample preparation procedure can be found in Supporting Information (see also Figure S3).

To make a gel, two solutions of oppositely charged peptide modules with respective polysaccharide were centrifuged separately for 10 min at 8,000 rpm. After degassing for 1 min using a house vacuum (21 Torr), 200 μL of each solution were mixed through a Y-shaped connector in the sealed cell of the rheometer, followed by time-sweep monitoring of the hydrogelation process.

Dynamic rheological measurements were performed using a sealed-cell NOVA rheometer (*ATS REOLOGICA Instruments Inc.*, Sweden) with a null-balance system for nano-torque measurements, a temperature-control unit, a custom-made humidifier to prevent sample dehydration, and a 25-mm diameter cone-and-plate stainless steel geometry (4° cone angle). All experiments were conducted at 25°C. The sequence of rheological experiments has been described in details in our previous paper.²⁹ Specifically, time-sweep measurements were conducted at 0.2% strain amplitude and 1 rad/s angular frequency, followed by frequency-sweep measurements, also at 0.2% strain amplitude within the angular frequency range from 0.01 to 100 rad/s with 18 data points per frequency decade (see, Supporting Information). Then, the stability of the hydrogel was additionally probed with time-sweep of 3 h, also at 0.2% strain amplitude and 1 rad/s angular frequency. This was followed by strain-sweep measurements at 1 rad/s angular frequency; the strain amplitude ranged from 0.1% to 100% with 23 data points per decade (see, Supporting Information).

Rheological monitoring of gelation for low concentration L^+L^- and D^+D^- peptide hydrogels (2.5 mM of each peptide) presented a number of challenges, since at the early stages of gelation, it was unable to hold water. Hence, in order not to disturb the gel prematurely, we mixed the L^+ and L^- (or D^+ and D^-) peptide solutions in the rheometer sealed cell, but started the measurements only after 48 h, to allow full maturation of the gel.

Small-Angle X-Ray Scattering (SAXS)

Initial peptide solutions containing respective polysaccharide were prepared in the same way as for rheological experiments. To prepare samples for SAXS experiments, 10–15 μL equal volumes of each solution were centrifuged for 20 s at 500 rpm into a cylindrical thin-walled (0.001 mm) 1.0-mm glass capillary (*Charles Supper Co.*). Scattering data were collected 72 h after mixing.

SAXS scattering profiles were collected using the pinhole geometry beamline 12ID-B of the Advanced Photon Sources (APS) at the Argonne National Laboratory. The spot of 0.07 mm $\times$ 0.20 mm of the monochromatic X-ray beam ($\lambda = 0.689 \text{ \AA}$) was passed through the center of the capillary for 0.2 s to avoid radiation damage to the samples. X-ray scattering intensities were collected using the 2D detector Pilatus 2M (*DECTRIS Ltd*). The 2D scattering images were converted into 1D scattering profiles of $I(Q)$ vs. Q in the Q -range from 0.007 $\text{\AA}^{-1}$ to 0.6 $\text{\AA}^{-1}$ by means of azimuthal averaging after solid-angle correction. The resulting scattering profile was normalized over the intensity of the transmitted X-ray beam, using the software package at the beamline 12ID-B. $I(Q)$ is the scattering intensity of the X-ray, and Q is the scattering vector amplitude which is related to the X-ray wavelength λ and the scattering angle 2θ by

$$Q = \frac{4\pi}{\lambda} \sin\theta \quad (1)$$

Subtraction of buffer scattering involved normalization based on the ratio of incident and transmitted X-ray photon counts to account for the slight differences in the thickness of different capillaries.

The Guinier plot for rod-like particles, $\ln I(Q)$ vs. Q^2 , is linear in all systems studied, which suggests that fibers of the hydrogels under study have one dimension (length) much longer than the other two. The length of the fibers exceeds the detection limit (Q_{min}) of our scattering data ($Q_{min} \sim 0.007 \text{ \AA}^{-1}$, thus maximum observable dimension is $\sim 500 \text{ \AA}$). Therefore, the analysis of the scattering data was performed in terms of fiber cross-sections. For this purpose, the scattering intensity $I(Q)$ was multiplied by the scattering vector Q , thus,

effectively removing the information about the longest dimension (length) of the scattering particle.³⁰ To restore the 2D fiber cross-section, we used a simulated annealing algorithm similar to one commonly employed to get low-resolution 3D shapes of scattering objects.³¹ 2D cross-sections of the fibers formed by the hydrogels under study were modeled using an algorithm in a purpose-written program that is described elsewhere.³² This 2D simulation annealing procedure used a flat grid of 20×20 close-packed spherical dummy atoms, each 3 Å in diameter. The program modeled the pair distance distribution function of the cross section, $P_c(r)$, composed of dummy atoms. $P_c(r)$ is the probability function which reflects the distribution of distances between two random scattering area elements in the cross-section, weighted by the scattering density at distance r . From $P_c(r)$, one could derive the radius of gyration of the cross-section R_c and the maximum cross-sectional dimension $d_{\max}$, both in Å, the cross-section area S_c in Å², and the zero-angle scattering intensity $I_c(0)$, which is proportional to the mass per unit length of the fiber (in arbitrary unit per Å). The fiber density ρ in arbitrary unit per Å³ (aU/Å³) was estimated as $I_c(0)/S_c$. The zeroth and second moments of $P_c(r)$ yield $I_c(0)$ and R_c , respectively, and the latter, R_c , is the contrast-weighted root mean square distance of all area elements in the cross-section from the center of scattering density. $d_{\max}$ is the r value at which $P_c(r)$ goes to 0. The restored shape of the cross-section is also visualized in the atomic coordinate files in the Protein Data Bank (PDB) format for their pictorial presentation.

The same 2D approach was used to compare cross-sectional correlation lengths of the hydrogels. IRENA software³³ for IGOR Pro 6.3 (*WaveMetrics, Inc.*) was used to process $QI(Q)$ vs. Q scattering data to calculate the cross-sectional mesh size of hydrogel networks based on the Debay-Bueche approximation:³⁴

$$I(Q) \propto \frac{L_c^3}{(1+Q^2L_c^2)^2} \quad (2)$$

here L_c is the cross sectional correlation length which reflects the average cross-sectional mesh size in the plane cross-cutting the hydrogel network (see Figure S9, Supporting Information). Larger L_c corresponds to larger spatial extent of the cross-linking fibrous regions.³⁵ L_c has been explored and successfully applied to understand the effects of ionic strength³⁶ and electrostatic interactions³⁷ on the structural characteristics of peptide-based hydrogels.

NIST NCNR model functions for small-angle scattering data analysis under IGOR Pro 6.3³⁸ were also used to estimate the persistence length L_p of the hydrogel fibers. The model function used describes the fiber as a semi-flexible chain of locally stiff cylinders of length L_p .³⁹ L_p reflects the length over which the cylinder could be considered a rigid rod (see Figure S10, Supporting Information).^{39,40}

In sum, parameters derived from SAXS data allow us to consider the relationships between structure and bulk material properties of the hydrogels under study. Specifically, R_c and $d_{\max}$ describe fiber cross-sectional dimensions, ρ and L_p represent the density and rigidity of individual fibers, and L_c reflects the mesh size of the whole fibrous network.

Results and Discussion

Chiral degeneracy in enantiomeric peptide hydrogels

One might reasonably expect that being mirror images of each other, L^+L^- and D^+D^- hydrogels will display identical mechanical and structural properties, i.e., chiral degeneracy. Indeed, the gelation time-sweep data for these two hydrogels are very similar. At 8 mM

concentration of each peptide module (after mixing), both pairs result in solid-like hydrogel networks with very close plateau elastic moduli (G') which fall within 10% difference interval (Figure 2B, $G'(L^+L^-) \sim 120 \pm 12$ kPa; $G'(D^+D^-) \sim 140 \pm 14$ kPa). The observed chiral degeneracy in mechanical properties appears to be concentration independent. Dilution down to 2.5 mM final concentration of each peptide also results in hydrogels with very similar elastic moduli ($G'(L^+L^-) \sim 6.6 \pm 0.7$ kPa; $G'(D^+D^-) \sim 8.3 \pm 0.9$ kPa, Figure S4, Supporting Information). The two hydrogels also have the same strain yield value, γ_{yield} , which is *ca.* 3–4% (Figure S4).

Evidently, this expected degeneracy in the bulk material properties of L^+L^- and D^+D^- hydrogels stems from the degeneracy in their structural properties as observed by SAXS. SAXS scattering profiles $I(Q)$ vs. Q of the two hydrogels almost superimpose with each other (Figure 2C). Further, their linear Guinier plots for rod-like particles $\ln I(Q)$ vs. Q^2 , which characterize the fiber cross-section, are also very close (Figure 2C, inset). This observation favors the conclusion that both L^+L^- and D^+D^- hydrogels are assembled from elongated fibers with very similar cross-sectional dimensions. Additional structural similarity between the two hydrogels is demonstrated by their almost identical cross-sectional pair distance distribution functions, $P_c(r)$ (Figure 2D).

Analysis of SAXS data reveals that structural chiral degeneracy persist throughout different levels of the hydrogel network hierarchy: at the fiber cross-section level, the radii of gyration of the cross-section, R_c , and the maximum dimensions of the cross-section, d_{max} , are similar in L^+L^- and D^+D^- ; at the individual fiber level, the fiber densities, ρ , and the persistent lengths, L_p , are similar; at the network level, the cross-sectional correlation lengths (mesh size) of the fiber network, L_c , are also similar.

In summary, the two enantiomeric peptide hydrogels, L^+L^- and D^+D^- , display both mechanical and structural chiral degeneracy. Such degeneracy is expected to split upon the addition of *D*-polysaccharides. This is because in the presence of a *D*-polysaccharide, the two hydrogels, $L^+L^- + D$ -polysaccharide and $D^+D^- + D$ -polysaccharide, are no longer enantiomeric, but instead diastereomeric. The question is whether such splitting will favor the L^+L^- gel, *i.e.*, whether $L^+L^- + D$ -polysaccharide is superior to $D^+D^- + D$ -polysaccharide in material properties.

Split of Chiral Degeneracy by β CD

Compared with pure peptide hydrogels, when β CD is added, it somewhat slows down gelation but has little effect on the plateau G' value and the strain yield value (Figure 3 and Figure S5). Within the $L^+L^- + \beta$ CD and $D^+D^- + \beta$ CD diastereomeric pair, the splitting of the chiral degeneracy is not obvious at 8 mM gelator concentration. A vague hint of possible splitting is that the gelation trace of $L^+L^- + \beta$ CD is slightly ahead of that of $D^+D^- + \beta$ CD.

Structurally, the cross-sectional, fiber and network parameters of $L^+L^- + \beta$ CD and $D^+D^- + \beta$ CD are very similar, consistent with no splitting in rheological parameters. Compared with pure peptide hydrogels, the presence of β CD makes individual fibers thinner (lower R_c and d_{max}), less dense (lower ρ) and stiffer (higher L_p), but has no impact on the fiber network's mesh size (L_c remains unchanged), see Table 2. Insignificant changes in the elastic moduli G' of these hydrogels as compared to pure L^+L^- and D^+D^- gels could be satisfactorily explained based on the MacKintosh theory:⁴¹

$$G' \propto \frac{L_p^n}{L_c^m} (a)^k \quad (3)$$

where L_p is the persistence length of a fiber, L_c is the mesh size of the network, and a reflects the dimensions (incl. cross-sectional) of the fiber segment (unit). Thus, in accordance with (3), when βCD is added to pure L^+L^- and D^+D^- gels, the decrease in cross-sectional fiber dimensions a is compensated by the increase in fiber persistence length L_p , and G' remains essentially the same (Table 2).

The structural results suggest that βCD , which is known to form inclusion complexes with tryptophan,⁴² is probably incorporated into the peptide fibers by latching onto the aromatic side chain of tryptophan residues. If so, the extent of incorporation must be very similar in L^+L^- and D^+D^- gels because there is no splitting of either structural or mechanical properties. The lack of detectable NMR signals in the gelled state precluded us to confirm such incorporation directly.

Not being able to verify the formation of such inclusion complexes directly, we tested this proposition indirectly, in two ways. First, we note that the dissociation constant of the inclusion complex between βCD and tryptophan is in the mM range.⁴² Hence the lack of splitting might be due to saturation, because the concentration of both the peptides and βCD is 8 mM in the experiments shown in Figure 3. As a result of saturation, βCD is incorporated into the two hydrogels to similar extent, and hence no splitting. If so, then lowering the concentrations of the peptides and βCD should induce splitting. This turns out to be the case. As shown in Figure 4, when the concentration is lowered from 8 to 4 mM for both the peptides and βCD , splitting indeed becomes obvious. $L^+L^- + \beta\text{CD}$ gels faster than $D^+D^- + \beta\text{CD}$ and has higher plateau elastic modulus (75 vs. 39 kPa).

The observed split of mechanical chiral degeneracy at 4 mM corresponds to split of structural chiral degeneracy as revealed by SAXS. Here, X-ray scattering profiles of $L^+L^- + \beta\text{CD}$ and $D^+D^- + \beta\text{CD}$ hydrogels show evident differences (Figure 4B). The same holds true for the linear Guinier plots for rod-like particles (inset of Figure 4B) and the cross-sectional pair distance distribution functions $P_c(r)$ (Figure 4C). The splitting of structural parameters persists throughout the hierarchy, at the fiber cross-section level, at the individual fiber level and at the network level. Within this diastereomeric pair, fibers in $L^+L^- + \beta\text{CD}$ are thinner (lower R_c and d_{max}) and less dense (lower ρ), which is consistent with faster gelation, but have higher persistent length L_p and lower network mesh size L_c , which is consistent with higher elastic modulus G' .

To further test, indirectly, on the possible formation of inclusion complex between βCD and tryptophan residues in the peptides, we note that αCD cannot form such complex with tryptophan due to its smaller size.⁴² The cavity of αCD comprises of 6 *D*-glucose units, 1 less than that of βCD . Nonetheless, αCD can still bind to tryptophan with a dissociation constant also in the mM range, very similar to that of βCD . However, such binding is due to superficial interaction at the rim of αCD ⁴² and is likely to be dislodged during gelation. The implication is that with αCD , no splitting should occur, even at lower concentration. This turns out to be indeed the case. At 4 mM concentration, $L^+L^- + \alpha\text{CD}$ and $D^+D^- + \alpha\text{CD}$ have the same gelation kinetic and the same elastic modulus (see Supporting Information, Figure S6), in sharp contrast with the obvious splitting between $L^+L^- + \beta\text{CD}$ and $D^+D^- + \beta\text{CD}$ at the same concentration.

While these two lines of indirect evidence provide some plausibility of inclusion complex formation between βCD and tryptophan residues in the peptides, more direct confirmation awaits further study. However, regardless of the molecular mechanism, it is clear that βCD indeed splits the chiral degeneracy between L^+L^- and D^+D^- , and the $L^+L^- + \beta\text{CD}$ combination is indeed superior to the $D^+D^- + \beta\text{CD}$ combination in terms of kinetics and mechanical strength. One might expect that similar effects could be observed with other *D*-

polysaccharides that are known to interact with proteins in soft tissues. To verify, we tested glycogen.

Split of Chiral Degeneracy by Glyn

Compared with pure peptide hydrogels, the addition of **Glyn** slowed down gelation and softened the hydrogel, resulting in lower plateau elastic modulus (Figure 5 and Table 2). Similar to **β CD** at the same concentration, the addition of **Glyn** also led to thinner and less dense fibers. While **β CD** has little effect on L_c , the mesh size of the network, **Glyn** increases L_c . All these structure features are consistent with softer gels, represented by lower plateau G' value relative to pure peptide hydrogels. However, the impact of **Glyn** is exerted unevenly on L^+L^- and D^+D^- hydrogels, leading to clear split of chiral degeneracy. L^+L^- + **Glyn** gels significantly faster and has much higher elastic modulus than D^+D^- + **Glyn** (110 vs. 70 kPa). Interestingly, the addition of bulky **Glyn** to L^+L^- hydrogel very slightly changes the plateau elastic modulus of the resulting hydrogel, L^+L^- + **Glyn**, as compared to pure peptide network, while significantly weakens the strength of D^+D^- + **Glyn** hydrogel with respect to pure D^+D^- (Table 2, cf. Figure 2A and Figure 5A). This is quite consistent with stronger interactions between L -peptides and D -glycogen—since in this case, stiffer branched fibrous network could be formed, while with weaker interacting D -peptides, the network is simply “diluted” by high molecular weight **Glyn**.

As one would expect, splitting of mechanical properties and gelation kinetics corresponds to splitting of structural properties (Figure 5B). Fibrous morphology of both L^+L^- + **Glyn** and D^+D^- + **Glyn** hydrogels is evident from the Guinier plots for rod-like particles, $\ln Q(Q)$ vs. Q^2 (Figure 5B, inset).

Within this diastereomeric pair, fibers in L^+L^- + **Glyn** are thinner (lower R_c and $d_{\max}$) and less dense (lower ρ), which is consistent with faster gelation kinetics, but stiffer (large L_p) and the network has smaller mesh size (smaller L_c), which is consistent with higher elastic modulus. The situation is very similar to the situation of **β CD** at 4 mM, where SAXS results provide a way to understand faster gelation and higher network stiffness. The order of the mesh size, $L_c(L^+L^-) \approx L_c(D^+D^-) > L_c(L^+L^- + \text{Glyn}) > L_c(D^+D^- + \text{Glyn})$, suggests that **Glyn** “dilutes” the peptide fibers, with the dilution effect more pronounced in D^+D^- , presumably due to weaker interaction between L -peptides and D -polysaccharides.

With **β CD** and **Glyn**, there is a clear material advantage for the biohomochirality combination of L -amino acids + D -sugars. What **β CD** and **Glyn** have in common is that they both contain unmodified α - D -glucose. Chiral interaction between peptides and polysaccharides is likely mediated by the hydroxyl group in D -sugars. If so, their chemical modification should weaken the observed chiral splitting. To verify this, we tested **Chd** and **HPC**, two D -polysaccharides whose hydroxyl groups have been chemically blocked/modified to different extents.

Chd and HPC Lead to Weaker Splitting of Chiral Degeneracy

As shown in Figure 1, 50% of the hydroxyl groups in **Chd** (3 out of 6 in the disaccharide unit) are blocked while 100% of those in **HPC** are blocked. Both are macromolecular D -polysaccharides, similar to **Glyn**. Hence, if the chiral interaction between peptides and polysaccharides are indeed mediated by the hydroxyl groups, **Chd** should result in weaker chiral splitting than **Glyn**, and **HPC** weaker yet still.

In the case of **Chd**, one would expect that the negatively charged **Chd** will interfere with gelation between oppositely charged peptides. Indeed, in the presence of 1% w/v **Chd**, gelation is slower than just the two peptides (Figure 6A vs. Figure 2B). However, this

slowdown is much more pronounced for $D^+D^- + \mathbf{Chd}$ than for $L^+L^- + \mathbf{Chd}$, leading to faster gelation for $L^+L^- + \mathbf{Chd}$. However, this splitting in gelation kinetics does not translate into splitting of elastic modulus, whose plateau values are very similar for the two gels— $G' (L^+L^- + \mathbf{Chd}) \sim 110 \pm 11$ kPa; $G' (D^+D^- + \mathbf{Chd}) \sim 115 \pm 12$ kPa (Figure 6A). Also, $L^+L^- + \mathbf{Chd}$ and $D^+D^- + \mathbf{Chd}$ hydrogels have very similar strain yield values ($\gamma_{\text{yield}} \sim 2\text{--}3\%$, see, Supporting Information).

Thus, in the case of naturally modified α -D-glucose units of $\mathbf{Chd}$ —through the introduction of carboxylic, sulfate and amide functional groups in D-glucuronic acid and N-acetyl-D-galactosamine units—the splitting of chiral degeneracy is restricted to gelation kinetics with no splitting of mechanical properties. Why kinetic splitting but not mechanical splitting is not clear at this time—this is also complicated due to negative charge of $\mathbf{Chd}$ which adds another level of complexity (electrostatic interaction) to the hydrogel assembly. To understand, one would need to follow the gelation process using SAXS so that the emergence and the evolution of fiber structures in these two gels can be observed and compared. Such studies are outside the scope of the present work and will be reported in the future.

While the addition of 1% w/v $\beta\mathbf{CD}$, $\mathbf{Glyn}$, and $\mathbf{Chd}$ slowed down gelation, the addition of 1% w/v $\mathbf{HPC}$ tremendously accelerated gelation. In the presence of 1% w/v $\mathbf{HPC}$, gelation was instantaneous (unpublished observation), making sample loading into the rheometer impossible. To slow down gelation, the concentration of $\mathbf{HPC}$ in the gel was lowered to 0.125% w/v, which still leads to significantly faster gelation than the pure peptides (cf. Figure 6A with Figure 2B). At 8 mM peptide concentration, the two gels have almost identical gelation trace and $D^+D^- + \mathbf{HPC}$ has actually slightly higher plateau elastic modulus than $L^+L^- + \mathbf{HPC}$ ($G' (L^+L^- + \mathbf{HPC}) \sim 90 \pm 9$ kPa; $G' (D^+D^- + \mathbf{HPC}) \sim 120 \pm 12$ kPa).

In an effort to slow down the gelation so that any chiral splitting can be amplified, we lowered the peptide concentration to from 8 to 2 mM while maintained the $\mathbf{HPC}$ concentration at 0.125% w/v. This indeed caused a discernible split in gelation kinetics, with $L^+L^- + \mathbf{HPC}$ gelling faster than $D^+D^- + \mathbf{HPC}$ (Figure 7A). After 50 h of gelation, $L^+L^- + \mathbf{HPC}$ has slightly higher elastic modulus than $D^+D^- + \mathbf{HPC}$ (11 vs. 8 kPa). Hence, although lower gelator concentration leads to splitting of mechanical chiral degeneracy in both $\beta\mathbf{CD}$ and $\mathbf{HPC}$, the splitting is extremely weak in the case of $\mathbf{HPC}$, whose hydroxyl groups are 100% blocked.

Corresponding to the very weak splitting in mechanical chiral degeneracy, $L^+L^- + \mathbf{HPC}$ and $D^+D^- + \mathbf{HPC}$ also have very similar X-ray scattering profiles, suggesting very similar structural features (Figure 7B). Cross-sectional dimensions (R_c , d_{max}) and the persistence length (L_p) of their fibers are almost identical (Table 2). However, the mass per unit length of $L^+L^- + \mathbf{HPC}$ (area under the corresponding pair distance distribution function of the cross-section, $P_c(r)$) is larger than that of $D^+D^- + \mathbf{HPC}$ (Figure 7C), which results in higher fiber density ρ of this gel (528 vs. 499 aU/Å³). $L^+L^- + \mathbf{HPC}$ also has lower cross-sectional mesh size of the network than for $D^+D^- + \mathbf{HPC}$ (36.1 Å vs. 41.7 Å). Such higher fiber density and lower mesh size provide a structural understanding on the slightly higher elastic modulus of the $L^+L^- + \mathbf{HPC}$ hydrogel at 2 mM final concentration of each peptide module (Figure 7A).

Thus thorough alkylation of the hydroxyl groups in $\mathbf{HPC}$ drastically weakens the splitting of the chiral degeneracy of the two homochiral peptide hydrogels. This suggests that the interaction between peptide and polysaccharides is indeed mediated by the hydroxyl groups in the α -D-glucose unit. Further, 100% modification of the hydroxyl groups (as in $\mathbf{HPC}$)

leads to even weaker splitting than 50% modification (as in **Chd**). This provides strong evidence that the chiral interaction between peptides and polysaccharides in these hydrogels is mediated by the hydroxyl groups in the polysaccharides.

Conclusions

It has been shown that chiral degeneracy in mechanical and structural properties of two enantiomeric peptide hydrogels could be broken by the addition of *D*-polysaccharides, resulting in a pair of diastereomeric hydrogels with distinctive structural organizations. Using four *D*-polysaccharides of very different molecular characteristics, it was found that in all cases the *L*-peptides + *D*-polysaccharide combination leads to superior material properties than the *D*-peptides + *D*-polysaccharide combination. This result suggests that there is a material advantage for natural biopolymers to be made of building blocks with opposite chirality, namely left-handed *L*-amino acids and righted-handed *D*-sugars. This advantage is weakened when the hydroxyl groups in *D*-sugars are chemically blocked, suggesting that the chiral interaction between peptides and polysaccharides is mediated by sugar hydroxyl groups.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Financial support provided by the NIH (EB004416) is gratefully acknowledged. Use of the Advanced Photon Source, an Office of Science User Facility operated for the U.S. Department of Energy (DOE) Office of Science by the Argonne National Laboratory, was supported by the U.S. DOE under Contract No. DE-AC02-06CH11357. Beamtime was awarded through the program of General User Proposals to GUP-24524. We also thank Drs. J. Ilavsky and Xiaobing Zuo (ANL) for assistance.

References

- Waldeck HM, Kao WJ. *Compr Biomater*. 2011; 2:113–126.
- Fitz D, Reiner H, Plankensteiner K, Rode BM. *Curr Chem Biol*. 2007; 1:41–52.
- Green MM, Jain V. *Orig Life Evol Biosph*. 2010; 40:111–118. [PubMed: 19911302]
- Joyce GF, Visser GM, Van Boeckel CAA, Van Boom JH, Orgel LE, Van Westrenen J. *Nature*. 1984; 310:602–604. [PubMed: 6462250]
- Taraban M, Feng Y, Hammouda B, Hyland LL, Yu YB. *Chem Mater*. 2012; 24:2299–2310. [PubMed: 23641124]
- Barron, LD. *Molecular Light Scattering and Optical Activity*. Cambridge University Press; New York: 2004.
- Milton RC, Milton SC, Kent SBH. *Science*. 1992; 256:1445–1448. [PubMed: 1604320]
- Urata H, Shinohara K, Ogura E, Akagi MJ. *Am Chem Soc*. 1991; 113:8174–8175.
- Breslow R, Cheng ZL. *Proc Natl Acad Sci USA*. 2010; 107:5723–5725. [PubMed: 20231487]
- Hein JE, Blackmond DG. *Acc Chem Res*. 2012; 45:2045–2054. [PubMed: 22353168]
- Mason PE, Lerbret A, Sabounji ML, Neilson GW, Dempsey CE, Brady JW. *Proteins*. 2011; 79:2224–2232. [PubMed: 21574187]
- Vásquez-Ibar JL, Guan L, Svrakic M, Kaback HR. *Proc Natl Acad Sci USA*. 2003; 100:12706–12711. [PubMed: 14566061]
- Hyland LL, Twomey JD, Vogel S, Hsieh A, Yu YB. *Biomacromolecules*. 2013; 14:406–412. [PubMed: 23256640]
- Martin Del Valle EM. *Process Biochem*. 2004; 39:1033–1046.
- Loftsson T, Brewster ME. *J Pharm Sci*. 1996; 85:1017–1025. [PubMed: 8897265]

16. Sullivan MA, O'Connor MJ, Umana F, Roura E, Jack K, Stapleton DI, Gilbert RG. *Biomacromolecules*. 2012; 13:3805–3813. [PubMed: 23004915]
17. Simson JA, Strehin IA, Lu Q, Uy MO, Elisseeff JH. *Biomacromolecules*. 2013; 14:637–643. [PubMed: 23320412]
18. Carotenito C, Grizzuti N. *Rheol Acta*. 2006; 45:468–473.
19. Yang J, Jiang J, Li Y, Li H, Jing Y, Wu P, Yu D, Chen S. *Int Orthop*. 2013; 37:515–521. [PubMed: 23180106]
20. Ramachandran S, Taraban MB, Trehella J, Gryczynski I, Gryczynski Z, Yu YB. *Biomacromolecules*. 2010; 11:1502–1506. [PubMed: 20481580]
21. Feng Y, Taraban MB, Yu YB. *Soft Matter*. 2011; 7:9890–9893. [PubMed: 22287979]
22. Hyland LL, Taraban MB, Yu YB. *Soft Matter*. 2013 revision submitted.
23. Engler AJ, Sen S, Sweeney HL, Discher DE. *Cell*. 2006; 126:677–689. [PubMed: 16923388]
24. Young JL, Engler AJ. *Biomaterials*. 2011; 32:1002–1009. [PubMed: 21071078]
25. Geng Y, Almeida PL, Fernandes SN, Cheng C, Palfy-Muhoray P, Godinho MH. *Sci Rep*. 2013; 3:Art. No. 1028.
26. Ramachandran S, Tseng Y, Yu YB. *Biomacromolecules*. 2005; 6:1316–1321. [PubMed: 15877347]
27. Chan, WC.; White, PD. *Fmoc Solid Phase Peptide Synthesis: A Practical Approach*. Oxford University Press; New York: 2000. p. 1-75.
28. Gill SC, von Hippel PH. *Anal Biochem*. 1989; 182:319–326. [PubMed: 2610349]
29. Hyland LL, Taraban MB, Feng Y, Hammouda B, Yu YB. *Biopolymers*. 2012; 97:177–188. [PubMed: 21994046]
30. Glatter, O.; Kratky, O., editors. *Small Angle X-Ray Scattering*. Academic Press; London: 1982.
31. Svergun DI. *Biophys J*. 1999; 76:2879–2886. [PubMed: 10354416]
32. Whitten AE, Jeffries CM, Harris SP, Trehella J. *Proc Natl Acad Sci USA*. 2008; 105:18360–18365. [PubMed: 19011110]
33. Ilavsky J, Jemian P. *J Appl Crystallogr*. 2009; 42:347–353.
34. Debye P, Bueche AM. *J Appl Phys*. 1949; 20:518–525.
35. Soni VK, Stein RS. *Macromolecules*. 1990; 23:5257–5265.
36. Feng Y, Taraban MB, Yu YB. *Soft Matter*. 2012; 8:11723–11731. [PubMed: 23185209]
37. Joyner K, Taraban MB, Feng Y, Yu YB. *Biopolymers (Pept Sci)*. 2013; 100:174–183.
38. Kline SR. *J Appl Crystallogr*. 2006; 39:895–900.
39. Pedersen JS, Shurtenberger P. *Macromolecules*. 1996; 29:7602–7612.
40. Chen W-R, Butler PD, Magid LJ. *Langmuir*. 2006; 22:6539–6548. [PubMed: 16830995]
41. MacKintosh FC, Käs J, Janmey PA. *Phys Rev Lett*. 1995; 75:4425–4428. [PubMed: 10059905]
42. Kahle C, Holzgrabe U. *Chirality*. 2004; 16:509–515. [PubMed: 15290685]

Figure 1. Structures of *D*-polysaccharides used to study chiral splitting peptide-based biomaterials. (A) β -cyclodextrin (**β CD**); (B) glycogen (**Glyn**); (C) chondroitin sulfate (**Chd**); (D) hydroxypropyl cellulose (**HPC**).

Figure 2.

Chiral degeneracy of mechanical and structural properties of L^+L^- and D^+D^- hydrogels which are mirror images of each other. (A) Pictorial presentation of L^+L^- and D^+D^- peptide modules assembly. (B) Time-sweep monitoring of gelation process of L^+L^- and D^+D^- hydrogels at 8 mM final concentration of each peptide module ($G'(L^+L^-) \sim 120 \pm 12$ kPa; $G'(D^+D^-) \sim 140 \pm 14$ kPa). (C) SAXS scattering profiles $I(Q)$ vs. Q of the L^+L^- and D^+D^- hydrogels at 8 mM final concentration of each peptide module, 72 h after mixing. Inset shows the corresponding linear Guinier plots for rod-like particles, $\ln QI(Q)$ vs. Q^2 . (D) Pair distance distribution functions of the cross-section, $P_c(r)$, of the L^+L^- and D^+D^- hydrogels from 2D data analysis (fit quality, $\chi^2 \sim 0.5-0.8$). Inset shows a pictorial presentation of the 2D average cross-section of the peptide fibers. Blue: L^+L^- ; orange: D^+D^- .

Figure 3.

No split in chiral degeneracy in $L^+L^- + \beta\text{CD}$ and $D^+D^- + \beta\text{CD}$ hydrogels at 8 mM final concentration of each peptide module and 8 mM βCD . (A) Time-sweep gelation monitoring, plateau elastic modulus: $G'(L^+L^- + \beta\text{CD}) \sim 135 \pm 14$ kPa; $G'(D^+D^- + \beta\text{CD}) \sim 145 \pm 15$ kPa. (B) Similarity in SAXS scattering profiles $I(Q)$ vs. Q , 72 h after mixing. Inset shows the closeness of linear Guinier plots for rod-like particles, $\ln I(Q)$ vs. Q^2 . (C) Identical pair distance distribution functions of the cross-section, $P_c(r)$, of the $L^+L^- + \beta\text{CD}$ and $D^+D^- + \beta\text{CD}$ hydrogels from 2D data analysis (fit quality, $\chi^2 \sim 0.6\text{--}0.9$). Inset shows a pictorial presentation of the identical 2D average cross-section of the peptide fibers. Blue: $L^+L^- + \beta\text{CD}$; orange: $D^+D^- + \beta\text{CD}$.

Figure 4.

Split in both mechanical and structural chiral degeneracy in $L^+L^- + \beta CD$ and $D^+D^- + \beta CD$ hydrogels assembly at 4 mM final concentration of each peptide module and 4 mM βCD .

(A) Time-sweep monitoring of the gelation process, plateau elastic moduli: ($G'(L^+L^- + \beta CD) \sim 75 \pm 8$ kPa; $G'(D^+D^- + \beta CD) \sim 39 \pm 4$ kPa. (B) SAXS scattering profiles $I(Q)$ vs. Q of the $L^+L^- + \beta CD$ and $D^+D^- + \beta CD$ hydrogels at 4 mM final concentration of each peptide module and 4 mM βCD , 72 h after mixing. Inset shows the corresponding linear Guinier plots for rod-like particles, $\ln QI(Q)$ vs. Q^2 . (C) Pair distance distribution functions of the cross-section, $P_c(r)$, of the $L^+L^- + \beta CD$ and $D^+D^- + \beta CD$ hydrogels from 2D data analysis (fit quality, $\chi^2 \sim 0.8-1.0$). Inset shows a pictorial presentation of the 2D average cross-section of the hydrogel fibers. Blue: $L^+L^- + \beta CD$; orange: $D^+D^- + \beta CD$.

Figure 5.

Splitting in both mechanical and structural chiral degeneracy in $L^+L^- + \text{Glyn}$ and $D^+D^- + \text{Glyn}$ hydrogels at 8 mM final concentration of each peptide module and 1% w/v **Glyn**. (A) Elastic modulus monitoring during gelation (plateau elastic modulus: $G'(L^+L^- + \text{Glyn}) \sim 110 \pm 11$ kPa; $G'(D^+D^- + \text{Glyn}) \sim 70 \pm 7$ kPa). (B) SAXS scattering profiles $I(Q)$ vs. Q , 72 hours after mixing. Inset shows the corresponding linear Guinier plots for rod-like particles, $\ln QI(Q)$ vs. Q^2 . (C) Pair distance distribution functions of the cross-section, $P_c(r)$, of the $L^+L^- + \text{Glyn}$ and $D^+D^- + \text{Glyn}$ hydrogels from 2D data analysis (fit quality, $\chi^2 \sim 0.4\text{--}0.6$). Inset shows a pictorial presentation of the 2D average cross-section of the hydrogel fibers. Blue: $L^+L^- + \text{Glyn}$; orange: $D^+D^- + \text{Glyn}$.

Figure 6.

Weaker splitting in mechanical chiral degeneracy in the presence of modified *D*-polysaccharides. (A) Split of mechanical chiral degeneracy seen from the gelation kinetics of $L^+L^- + \text{Chd}$ and $D^+D^- + \text{Chd}$ hydrogels at 8 mM final concentration of each peptide module and 1% w/v **Chd** ($G'(L^+L^- + \text{Chd}) \sim 110 \pm 11$ kPa; $G'(D^+D^- + \text{Chd}) \sim 115 \pm 12$ kPa). Blue: $L^+L^- + \text{Chd}$; orange: $D^+D^- + \text{Chd}$. (B) Lack of splitting between $L^+L^- + \text{HPC}$ and $D^+D^- + \text{HPC}$ hydrogels assembly at 8 mM final concentration of each peptide module and 0.125% w/v **HPC** ($G'(L^+L^- + \text{HPC}) \sim 90 \pm 9$ kPa; $G'(D^+D^- + \text{HPC}) \sim 120 \pm 12$ kPa). Blue: $L^+L^- + \text{HPC}$; orange: $D^+D^- + \text{HPC}$.

Figure 7.

(A) Weak splitting of mechanical chiral degeneracy between $L^+L^- + \text{HPC}$ and $D^+D^- + \text{HPC}$ hydrogels assembly at 2 mM final concentration of each peptide module and 0.125% w/v HPC ($G'(L^+L^- + \text{HPC}) \sim 11.0 \pm 1.1$; $G'(D^+D^- + \text{HPC}) \sim 8.0 \pm 0.8$ kPa). Inset highlights faster gelation kinetics of $L^+L^- + \text{HPC}$ within first 10 h of gelation. (B) SAXS scattering profiles $I(Q)$ vs. Q of the $L^+L^- + \text{HPC}$ and $D^+D^- + \text{HPC}$ hydrogels at 2 mM final concentration of each peptide module and 0.125% w/v HPC, 72 h after mixing. Inset shows the corresponding linear Guinier plots for rod-like particles, $\ln QI(Q)$ vs. Q^2 . (C) Pair distance distribution functions of the cross-section, $P_c(r)$, of $L^+L^- + \text{HPC}$ and $D^+D^- + \text{HPC}$ hydrogels from 2D data analysis (fit quality, $\chi^2 \sim 0.6\text{--}0.8$). Inset shows a pictorial presentation of the 2D average cross-section of the hydrogel fibers. Blue: $L^+L^- + \text{HPC}$; orange: $D^+D^- + \text{HPC}$.

Table 1Sequence, molecular weight, and charges of undecapeptide modules.^a

Peptides	Sequences	M.W. (Da)	Charges
<i>L</i> ⁺	<i>acetyl</i> - <i>L</i> K- <i>L</i> W- <i>L</i> K- <i>L</i> A- <i>L</i> K- <i>L</i> A- <i>L</i> K- <i>L</i> A- <i>L</i> W- <i>L</i> K- <i>amide</i>	1,413	+ 6
<i>D</i> ⁺	<i>acetyl</i> - <i>D</i> K- <i>D</i> W- <i>D</i> K- <i>D</i> A- <i>D</i> K- <i>D</i> A- <i>D</i> K- <i>D</i> A- <i>D</i> K- <i>D</i> W- <i>D</i> K- <i>amide</i>	1,413	+ 6
<i>L</i> ⁻	<i>acetyl</i> - <i>L</i> E- <i>L</i> W- <i>L</i> E- <i>L</i> A- <i>L</i> E- <i>L</i> A- <i>L</i> E- <i>L</i> A- <i>L</i> E- <i>L</i> W- <i>L</i> E- <i>amide</i>	1,419	- 6
<i>D</i> ⁻	<i>acetyl</i> - <i>D</i> E- <i>D</i> W- <i>D</i> E- <i>D</i> A- <i>D</i> E- <i>D</i> A- <i>D</i> E- <i>D</i> A- <i>D</i> E- <i>D</i> W- <i>D</i> E- <i>amide</i>	1,419	- 6

^a A, alanine; E, glutamic acid; K, lysine; W, tryptophan. The *N*-, *C*- terminal charges are blocked by acetylation (*acetyl*-) and amidation (*-amide*), respectively.

Table 2

Structural parameters and elastic moduli of oligopeptide-polysaccharide hydrogels.^a

Oligopeptide Pairs	Polysaccharides	Cross-Sectional Parameters			Fiber Parameters		Network parameters	
		R_c (Å)	d_{max} (Å)	ρ (aU/Å ³)	L_p (Å)	L_c (Å)	G' (kPa)	
Enantio meric Pair	8 mM ($L^+ + L^-$)	19.1	72.0	529	24.3±1.0	27.8±0.5	120±12	
	8 mM ($D^+ + D^-$)	19.0	73.0	525	24.7±2.5	27.5±0.4	140±14	
	8 mM ($L^+ + L^-$)	16.9	62.0	304	42.0±0.8	27.6±0.5	135±14	
	8 mM ($D^+ + D^-$)	17.0	62.0	312	44.3±0.6	27.5±0.5	145±15	
Diastereomeric Pairs	4 mM ($L^+ + L^-$)	10.2	38.0	174	60.2±0.5	23.2±2.1	75±8	
	4 mM ($D^+ + D^-$)	13.6	47.0	266	17.2±1.4	32.3±1.1	39±4	
	8 mM ($L^+ + L^-$)	12.4	44.0	253	34.6±0.5	39.5±0.1	110±11	
	8 mM ($D^+ + D^-$)	14.7	52.0	354	17.8±2.3	44.9±0.9	70±7	
	2 mM ($L^+ + L^-$)	17.1	61.0	528	11.8±1.5	36.1±0.7	11.0±1.1	
	2 mM ($D^+ + D^-$)	17.3	61.0	499	12.6±1.6	41.7±0.8	8±0.8	

^aConcentrations refer to the final concentration of oligopeptides and polysaccharide in the hydrogel; R_c , radius of gyration of the fiber cross-section in Å; d_{max} , maximum dimension of the fiber cross-section in Å; ρ , fiber density in arbitrary unit per Å³ (aU/Å³), $\rho = I_c(0)/S_c$, where $I_c(0)$, is the mass per unit length of the fiber in arbitrary unit per Å; and S_c , the area of the fiber cross-section in Å²; L_p , persistence length of hydrogel fibers; L_c , correlation length of fiber cross-section; β CD, β -cyclodextrin; **Gly**n, glycogen; **HPC**, hydroxypropyl cellulose.