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Review

Peptide and Protein Stereocomplexes

Ravi Saklani and Abraham J. Domb*

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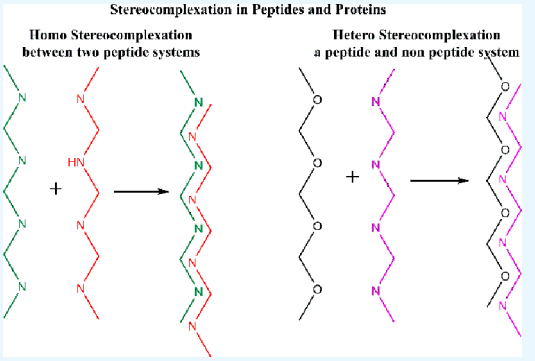
ABSTRACT: Stereocomplexation in peptides and proteins is a fascinating phenomenon arising from their inherent stereoisomerism. Peptides and proteins, with their three-dimensional helical structures, exhibit stereoselectivity and form intertwined complexes when complementary left- and right-handed structures are mixed together. Stereocomplexation provides an unprecedented opportunity to impart some valuable biological, chemical, and physical properties in peptide and protein polymeric platforms that can be employed in various applications such as catalysis and drug delivery and to improve the stability of these therapeutics. However, exploration of stereocomplexation in peptides and proteins remains limited. We report on a comprehensive understanding of stereocomplexation in peptides and proteins, compiling existing reports, discussing its implications, and highlighting its role in different applications, aiming to inspire further research and advancements in this direction.



1. INTRODUCTION

Stereoisomerism is a ubiquitous property that is found in many biological macromolecules. Stereoisomerism, also known as spatial isomerism, refers to a form of isomerism in which molecules have the same molecular formula and sequence of bonded atoms but differ in the three-dimensional orientations of their atoms in space. At the molecular level, biomolecules such as amino acids, sugars, and their polymers, i.e., polysaccharides, nucleic acids, polypeptides, and proteins, exhibit preferred handedness, adopting specific structures. These structures further assemble into supramolecular complexes that decide their specific functions in various biological systems.[1−4]

Specifically, peptide and protein moieties are characterized by stereoselectivity, which is a hallmark feature determining their biomolecular processes, ranging from catalysis to self-assembly. Peptides and proteins are composed of amino acids as monomer units having the same backbone structure with a basic amino group and an acidic carboxyl group, but they differ in their side chains. More importantly, all natural amino acids, except glycine, have a chiral carbon atom, making them optically active and existing as an L-enantiomer. So, peptides possess a three-dimensional helical structure that naturally folds into a left-handed helix ("L-configuration").[5] The mirror image enantiomers of these chiral biomolecules can be biologically inert or even toxic to us due to the inability of our peptide-based stereoselective enzymes to metabolize them. In nature, stereoselectivity is best exemplified by the stereoselective interaction between L-Lactic acid and peptide enzyme monocarboxylate transporter-1 (MCT1). MCT1 is responsible for stereoselective transport of L-lactic acid across the plasma membrane of muscle fibers, erythrocytes, and other cells. For lactate, MCT1 is stereospecific with the K_m for the L-isomer (5−10 mM) being an order of magnitude lower than that for the D-isomer.[6]

This stereoisomerism in chiral polymers gives rise to another intriguing phenomenon, called **Stereocomplexation**. Stereocomplexation is a phenomenon in which a set of complementary left-handed and right-handed structures forms an intertwined complex on mixing together. It occurs when the stereoselective interaction between two complementary stereoregular polymers of opposite chirality surpasses the interaction between polymers of the same stereoregularity or chirality. These complex assemblies are known as stereocomplexes.[7]

The force for complexation may come from electrostatic interactions, hydrogen-bonding formation, or stereospecific van der Waals interactions.[8] Interlocking of the chains gives rise to a supramolecular composite differing in crystal structure and physical properties from the parent polymers.[9] Stereocomplexation has garnered significant attention as an important design tool, enabling precise control over the material structure and properties. This phenomenon has transformative effects on mechanics, morphology, and

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Table 1. Stereocomplexation between Enantiomeric Poly(lactic acids) (PLAs) and Their Derivative Polymers

Stereocomplex pair	Example	Applications
Homostereocomplexation between Unsubstituted or Substituted PLAs with Opposite Configurations but Similar Chemical Structures		
Stereocomplexation between unsubstituted PLA moieties with similar chemical structure and opposite configuration	D-Polylactic acid and L-Polylactic acid[11,14,23,24,15−22]	Crystallization, stereocomplex as a nucleating agent, stereocomplex polymer with improved thermal and physical properties compared to enantiopure parent polymers, stereocomplex biomaterial for controlled drug release
Stereocomplexation between substituted PLA moieties with similar chemical structure and opposite configuration	Poly(L-2-hydroxybutanoic acid) and Poly(D-2-hydroxybutanoic acid)[25−28]	Crystallization, stereocomplex polymer with improved thermal and physical properties compared to enantiopure parent polymers
	Poly(L-2-hydroxy-3-butanoic acid) and Poly(D-2-hydroxy-3-butanoic acid)[29,30]	Crystallization, stereocomplex polymer with improved thermal and physical properties compared to enantiopure parent polymers
	Poly(L-lactide-*stat*-3-ethylglycolide) and Poly(D-lactide-*stat*- 3-ethylglycolide)[31]	Tuning the melting point and crystallinity of the polymer system
Heterostereocomplexation of PLA with Different Structured PLA Block and Graft Polymers		
Stereocomplexation of PLA with PLA block copolymers of opposite configuration	PLA and PLA Block copolymers with PEG[32−39]	Crystallization, Stereocomplex polymer with improved thermal and mechanical properties, Preparation of controlled drug delivery hydrogel and particle systems
	PLA and PLA Block copolymers with poly(ε-caprolactone)[40,41]	Stereocomplex polymer with improved thermal, mechanical, and biodegradation properties
	PLA and PLA block copolymers with poly(butylene succinate)[42]	Preparation of electrospun nonwoven mats with high thermal stability.
	PLA and PLA Block copolymers with aliphatic polycarbonate[43]	Crystallization, Stereocomplex polymer with improved thermal and mechanical properties
	PLA and PLA Block copolymers with polystyrene[44]	Crystallization, stereocomplex polymer with improved thermal and physical properties compared to enantiopure parent polymers.
	PLA and PLA Block copolymers with ricinoleic acid[45]	Stereocomplex polymer with improved thermal properties compared to enantiopure parent polymers.
Stereocomplexation between PLA graft copolymers of opposite configuration	PLA and Dextran-based graft copolymers[46,47]	Synthesis of biodegradable nanogels and implantable biomaterials with favorable mechanical properties
	PLA and Polyrotaxane-based graft copolymers[48]	Synthesis of biomaterials with favorable mechanical, thermal, and biodegradation properties
	PLA and Poly(γ-glutamic acid)-based graft copolymers[49−51]	Synthesis of nanomaterials for controlled drug delivery applications
	PLA and Cellulose-based graft copolymers[52]	Crystallization, stereocomplex polymer with improved thermal and physical properties compared to enantiopure parent polymers
Heterostereocomplexation between PLA and Polymers with Opposite Configurations and Different Chemical Structures		
Stereocomplexation between unsubstituted PLA and substituted PLA moieties with different chemical structures and opposite configurations	Stereocomplexation between Polylactic acid and poly-2-hydroxybutanoic acid[53−55]	Stereocomplex polymer with improved thermal, mechanical, and degradation properties
	Stereocomplexation between poly(D-2-hydroxy-3-methylbutanoic acid) and poly(L-2-hydroxybutanoic acid)[56]	Stereocomplex polymer with improved thermal, mechanical, and degradation properties
Stereocomplexation between unsubstituted or substituted PLA and peptide structures with opposite configurations	Stereocomplexation between D-Polylactic acid and Leuprolide[57−62]	Synthesis of polymeric nanoparticles for the controlled release of leuprolide
	Stereocomplexation between D-Polylactic acid and insulin[63]	Synthesis of polymeric nanoparticles for the controlled release of insulin
	Stereocomplexation between PEG-D-Polylactic acid and insulin[64]	Synthesis of polymeric nanoparticles for the controlled release of insulin

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degradation, offering opportunities to fine-tune these properties in biomaterials.[7] The emphasis on stereocomplexes arises from their crucial role in fundamental and biological processes, as well as their promising biomedical applications.[10] The phenomenon of stereocomplexation has great implications in materials science too, as the stereocomplexes have improved mechanical and thermal properties compared to their homochiral polymers.[8,11,12]

One well-studied example is the stereocomplexation between poly(L-lactide) (PLLA) and poly(D-lactide) (PDLA). Blending the enantiomers, PLLA and PDLA, leads to the formation of a stereocomplex, a unique structure distinct from the individual homochiral polymers.[13] In PLA, helices of the same absolute configuration pack differently compared with pairs of opposite handed β-helices. The formation of racemic crystals involves the side by side, alternating packing of β-form 3_1-helices with opposite configurations. Single crystals of the stereocomplex adapt a triangular shape, providing an advantageous configuration for the polymer loops during their growth in hexagonal packing.[14]

The van der Waals forces between the β-helices in the stereocomplex drive specific energetic interactions, resulting in a higher melting point. The resulting stereocomplex of PLA has higher heat resistance (higher melting temperature), improved mechanical properties, and higher hydrolysis-resistance compared to the corresponding enantiomerically pure PLA.[11] Due to these properties, the PLA stereocomplex has been extensively studied for its role in different biomedical, materials science industry, and environmental applications. In biomedical science, it is widely studied for its role as a controlled drug release biomaterial. Stereocomplexation enhances the barrier properties of PLA-based hydrogels and micro- or nanodrug carriers, thereby providing sustained drug release. In materials science, it serves as an excellent nucleating or crystallization accelerating agent for various stereocomplementary biopolymers, e.g., poly(menthide), poly(butylene succinate), poly(ε-caprolactone), poly(hexamethylene/pentamethylene carbonate), poly(acrylic acid), poly(*N*,*N*-dimethylamino-2-ethyl methacrylate), poly(isoglycerol methacrylate), polystyrene, etc., yielding polymers with improved mechanical and thermal properties. Stereocomplexation in PLA strictly relies on the ester backbone of PLA; thus, a wide variety of stereocomplexes can be formed even with substituted PLA moieties with different functional groups, as long as the chiral ester backbone is intact.[11] Moreover, it has been recently found that poly(lactic acid) can form heterostereocomplexes with polymer moieties having opposite configurations but a different chemical structure from that of Poly(lactic acid). This is exemplified by the heterostereocomplexation of Poly(lactic acid) with poly-2-hydroxybutanoic acid as well as with peptide and protein polymers. Table 1 summarizes the examples of stereocomplexation in PLA and their applications.

Extensive studies have also been conducted on stereocomplexation between syndiotactic and isotactic poly(methyl methacrylate),[65−69] as well as in poly(2-hydroxybutyrate)[70] and poly(propiolactones).[71−73] While over 2000 papers have been published on stereocomplexation (Scifinder search), only 50 of these publications include references to "peptide" or "protein". Of these, a majority of them concentrated on the homostereocomplexation between peptides themselves. Several papers reported on the use of stereocomplexation between D-PLA and L-PLA for the delivery of peptide and protein therapeutics, and the remaining very few reported on heterostereocomplexation between peptide and nonpeptide moieties. So, this article mainly focuses on stereocomplexation in peptides and proteins. It aims to offer a comprehensive understanding of stereocomplexation in peptides and proteins, identifying the gaps, discussing its implications, and highlighting the role of stereocomplexation in various applications, aiming to inspire further research and advancements in this direction. Stereocomplexation in other polymers is outside the scope of this report, since it already has been extensively covered elsewhere.[11,65−73] It is briefly covered here. Interested readers are referred to the cited literature on the same topic. The focus of this report is to critically discuss stereocomplexation observed in peptides and proteins and its implications in different biomaterial applications.

2. STEREOCOMPLEXATION IN PEPTIDE AND PROTEINS

Chirality has long been a guiding force in shaping nature's intricate designs. It is evident in remarkable structures like the protein molecules and the double helix of DNA. Polypeptides were the first molecules reported to form stereocomplexes (Figure 1).[28,74] Peptide and protein moieties, owing to their

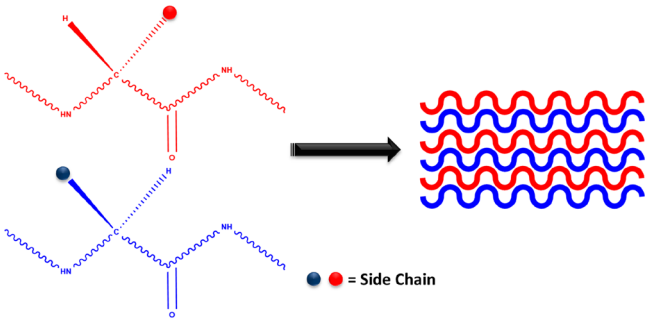

Figure 1. Stereocomplexation between D- and L-Polypeptide chains.

intrinsic helical structure twisting in a right-handed ("D-configured") or left-handed direction ("L-configured"), can form stereocomplexes among themselves (**Homostereocomplexation**) and also with nonpeptide stereocompatible moieties like esters (**Heterostereocomplexation**). These stereocomplexes exhibit intriguing properties that are not observed in the enantiomerically pure parent strands.

2.1. Homostereocomplexation between Peptides Themselves. One of the earliest accounts of stereocomplexation was reported by Pauling and Corey in their seminal report in 1953, where they report the formation of "racemate species" from a racemic mixture of polypeptides, describing what is known today as stereocomplexes.[75,76] In their work, the Pauling and Corey group unveiled the structural configuration in homochiral and racemic mixture of polypeptides, where adjacent peptide chains show edge-to-edge interaction through hydrogen bonds. In homochiral assemblies, peptides are composed of enantiopure peptides either D or L, arranged in a pleated β-sheet structure. However, in heterochiral assemblies, peptides are composed of alternating L and D enantiomeric peptides, adopting a rippled appearance and termed as rippled β-sheets. Distinct from the homochiral pleated β-sheet assembly, in heterochiral rippled β-sheet assemblies, alternating L-and D-peptide chains are arranged in such a manner that α-carbons between two adjacent peptide chains are oriented opposite each other. The arrangements of

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bonds around the α carbon atoms allow for the inclusion of a side chain that projects almost perpendicular to the sheet. This occurs when alternate chains are composed of D-amino acid residues and L-amino acid residues, respectively. Consequently, when racemic polypeptides (peptide strands with opposing chirality, i.e., racemic D- and L-enantiomers) are mixed together, they form layered structure termed rippled sheets.[74−77]

While the pleated β-sheet structure was accepted and established immediately, the rippled β-sheet assembly was accepted and validated much later, since heterochiral assembly is not very common in nature. Homochiral Pleated β-sheet assembly between L-peptides is ubiquitous in nature, but rippled β-sheets are absent in nature, because ribosomal peptide protein moieties are comprised exclusively of L-amino acids[78,79] Subsequent to the seminal report of Pauling and Corey, numerous more studies followed and validated this complex interaction between racemic mixture of polypeptides. Raskatov JA and co-workers give a detailed account, defining the evolution of a Pauling-Corey Rippled Sheet model in their recent review article.[77] For an in-depth exploration of the evolution of the Pauling-Corey Rippled Sheet model, readers are encouraged to refer to the cited article.

Stereocomplexation has been thoroughly investigated, particularly in enantiomeric polymers of glutamate, to elucidate the stereocomplexation model between peptides. The infrared absorption spectrum of an equimolar mixture of D- and L-enantiomers of poly-Υ-benzyl-glutamate shows characteristic difference from the spectrum of enantiomerical pure L- or D-poly-Υ-benzyl-glutamate.[80] While the positions, intensities, and dichroic properties of the NH stretching and amide I, amide II, amide III, and amide V bands are somewhat similar, differences are primarily observed in the relative intensities and dichroic properties of the side-chain bands. These observations suggest that there is a significant interaction between the side chains of adjacent polypeptide chains, and this interaction differs between D- and L-chains compared to interactions between two L-chains or two D-chains. Further X-ray diffraction studies reveal a meridional reflection of 1.5 Å in both systems, indicating an α-helix conformation in both systems. However, in either pure L or D polypeptide form, a strong meridional arc of 5.3 Å is observed, while it is significantly less prominent in the equimolar complex of D- and L-polypeptide. Instead, in the mixture of D- and L-polypeptides, a robust meridional arc of 10.4 Å is evident, which is nearly absent in the enantiomerically pure D- or L-polypeptide forms. The 5.3 Å meridional reflection corresponds to approximately one pitch of the α-helix, and the 10.4 Å reflection corresponds to roughly two pitches. This implies a unique side chain-side chain interaction between L- and D-chains running antiparallel to each other, forming a pair with a center of symmetry.[80]

When the helical homopolypeptides of Υ-methyl-D-glutamate and Υ-methyl-L-glutamate are mixed together, they form a distinct racemic compound both in solution and in the solid state. The behavior of either the D- or L-enantiomer shows marked differences from that of an equimolar mixture of the two enantiomers (D and L) in the same solution.[81] When a chloroform-dioxane solution containing the two enantiomers is mixed at a temperature of 37 °C, the solution transitions into a rigid gel system, accompanied by a slight phase separation. Furthermore, when a solution of the two enantiomers in dimethylformamide is blended together at 100 °C, a white polymeric precipitate is produced, accompanied by a loss of optical activity. Similar to the parent enantiomorphs, the transformed stereocomplex precipitate displays infrared absorption bands at 1650 and 1545 cm^{-1}, corresponding to the amide I and amide II bonds characteristic of the α-helix. Collectively, these findings indicate that the precipitated material is a 1:1 mixture of D- and L-enantiomers of poly-Υ-methyl-glutamate, and the polypeptide modification is more than a mere stoichiometric mixture of the two enantiomorphs; rather, it forms a racemic compound.[81]

An X-ray diffraction pattern of oriented fibers prepared from either enantiomerically pure poly-Υ-benzyl-glutamate or an equimolar racemic mixture of L- and D-poly-Υ-benzyl-glutamate also shows marked differences observed in Bragg reflection patterns from layer-line streaks.[82] Both fibers display distorted α-helices and exhibit a nearly hexagonal packing without regularity, indicating a procrystalline form. However, the enantiomerically pure form shows a α-helix with small distortions and a repeat length of 27 Å. In contrast, the racemic mixture shows an α-helix with a repeat length of 106 Å and strong layer lines, indicating considerable distortion. The distortion is attributed chiefly to side chains, presumably not passed on to the helical polypeptide core, which is relatively free from the distortion. These differences are attributed to the difference between the interaction of left- and right-handed α-helices in the racemic mixture, compared to the interaction between α-helices of the same chirality.[82]

The X-ray diffraction pattern in poly-γ-benzyl glutamate shows that the benzyl group at the end of the side-chain is far more uniform in the fibers prepared from the equimolar racemic mixture than in enantiomerically pure poly-Υ-benzyl-glutamate.[83] In the fibers obtained from the racemic mixture, the main-chain forms an α-helix that has 3.58 residues per turn, while some benzyl groups align along a helix presenting different screw symmetry from the main-chain helix. This unique arrangement of benzyl groups is believed to result from interactions between side chains in both left- and right-handed α-helices within the racemic mixture, leading to additional layer lines.[84] These findings are also corroborated by X-ray diffraction analysis both of dry fibers of racemic poly-γ-benzyl glutamate and of liquid-crystalline solutions of racemic poly-γ-benzyl glutamate in dimethylformamide and acetophenone.[83]

The prediction of Pauling and Corey that enantiomeric β-sheet peptides coassemble into rippled β-sheets with an alternating L/D-peptide configuration is further validated by thermodynamic studies. Isothermal titration calorimetry demonstrates that the coassembly of racemic peptides mixtures into rippled β-sheets has a thermodynamic advantage over the self-assembly of single enantiomers into pleated β-sheets.[85]

Similar to enantiomeric polymers of glutamate, the chirally opposite aspartate polymers also show the stereocomplexation phenomenon. When the racemic D- and L-poly(β-benzyl aspartate) dissolved in chloroform are mixed together, a complex precipitate obtained is comprised of equal amount of L- and D-enantiomers. This complexation is accompanied by transformation of an α-helical backbone into a β-sheet-like conformation with L- and D-peptide strands linked by a hydrogen bond in an alternate and antiparallel manner.[86]

Interestingly, the stereocomplexation in the peptides relies on the chiral peptide backbone; thus, a wide variety of stereocomplexes can be formed even with the substituted peptide moieties with different functional groups, as long as the chiral peptide backbone is intact. Sakajiri K. and co-workers studied the stereocomplex formation in the racemic mixture of

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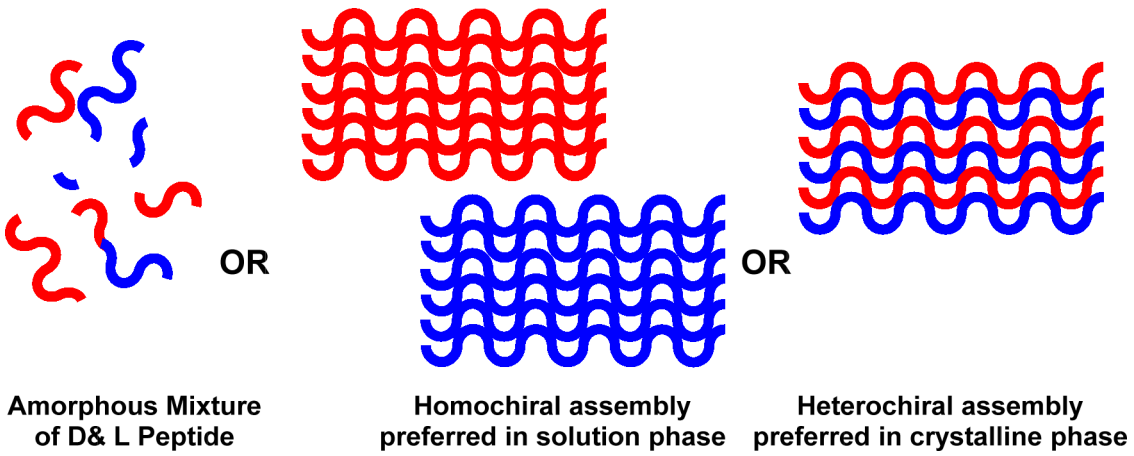

Figure 2. Homochiral and heterochiral assembly between enantiomeric peptides. Adapted from ref 89 (Zhang et al.) and ref 78 (Li, Rios, and Nowick).

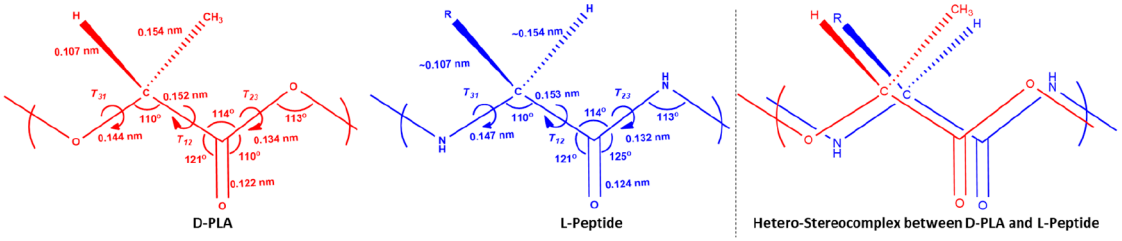

Figure 3. Heterostereocomplexation between D-PLA and L-Peptide, attributed to the similarity in their structure backbone in the context of torsion angles and bond length.

poly(ϒ-alkyl-glutamate) with short alkyl side-chains varying from 1 to 6 carbon lengths.[10] Stereocomplexation occurred on the basis of chiral recognition between two equimolar enantiomeric polygutamates, when the alkyl side chain was relatively short ($n \leq 3$). The stereocomplex was a fine precipitate or whitish gel with a distinct tetragonal packing structure of α-helices. This unusual tetragonal packing symmetry was attributed to the "knobs-into-holes" packing of side chains between two enantiomeric helices regularly arrayed in a lattice. In an equimolar racemic mixture of D- and L-polymers, the benzyl groups at the end of side chains were configured in such a way that they interlocked between the side chains of polymer helices of opposite chirality. In contrast, in enantiomerically pure polymer systems, the arrangement of side-chain benzyl groups was less regular.[10] These findings are in close agreement with the findings of the Mitsui, Y. research group.[84]

Xu et al. investigated the self-assembly dynamics of left- and right-handed molecular screws, specifically collagen peptide triple-helices composed of L- or D-proline with a cyclic aliphatic side chain. They propose a geometric ridges-in-grooves model to predict the preferential association of heterochiral helices. Geometric and experimental analyses demonstrate a preference for supramolecular interactions between opposite-handed helices over like-handed assemblies. The heterochiral assembly forms columnar associations between opposite-handed helices forming a well-ordered structure. When observed under a microscope, this arrangement exhibits a micrometer scale sheet-like morphological appearance, with an average single-layer thickness of approximately 10 nm. Atomic force microscopy and X-ray scattering measurements confirm the assembly of one peptide-length thick sheets, supporting the tight ridges-in-grooves packing of left- and right-handed triple helices. This study highlights the role of stereoselectivity and intermolecular interactions in the self-assembly of synthetic polypeptides and provides insights into the general rules governing stereoselectivity in such systems.[87]

The rippled β-sheet layer configuration between racemic peptide systems proposed by Paul and Corey has also been recently validated using crystal structure studies.[88] However, an intriguing contrast emerges in a report by Zhang, Y and co-workers, in which enantiopure D- and L-polyaspartates dissolved in chloroform, when mixed together, do not form a stereocomplex but rather a homocomplex.[89] This preference for homocomplex formation over stereocomplex formation aligns with "Wallach's rule" as elucidated by Li, X. and co-workers in their recent report, in which they studied the homochiral and heterochiral assembly between β-sheet peptides.[78] Their findings suggest a general preference for heterochiral packing over homochiral arrangements in crystalline structures, resulting in denser solid forms and a preference for racemic crystal formation, a phenomenon termed as "Wallach's rule" (Figure 2). Their observations suggest that the formation of heterochiral mixtures of β-sheet peptides and occasionally rippled β-sheets is driven by solid-state packing forces. However, in solution phases, in which the influence of crystal packing is absent, the formation of rippled β-sheets is not favored.[78]

2.2. Heterostereocomplexation; Stereocomplexation of Peptides with Nonpeptide Polymers. The number of polymer combinations having the ability to form heterostereocomplexes is very limited compared to the number of polymers forming homostereocomplexes. In addition, the formation of heterostereocomplexes can be more difficult to

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determine.[60] So while homostereocomplexation between polymers with identical chemical structures has been extensively reported, examples of heterostereocomplexation involving polymers with nonidentical chemical compositions are very limited. Notably, our group was the first to report heterostereocomplexation between polymers with ester and peptide backbones.[90] This finding introduces a novel approach for the controlled delivery of peptides and protein therapeutics. There are very few examples of heterostereocomplexation of peptide and protein moieties with other nonpeptide moieties. They are as follows.

2.2.1. Stereocomplexation of Peptides with PLA. Previous reports reveal that the L-configured backbone of peptide moieties such as leuprolide, insulin, and LHRH shows complementarity with D-configured poly-α-hydroxyesters such as D-PLA, in terms of torsion angles and bond lengths.[8,12,91,57−59,61−64,90] PLA is composed of repeating lactic acid units and a α-hydroxy acid with a chiral center. Peptides are composed of α-amino acids with a chiral center (Figure 3). PLA adopts a 3-D structure that is twisted either in a clockwise configuration (D-configured) or counterclockwise configuration (L-configured).[11] Accordingly, when the right-handed helix D-PLA is mixed with the left-handed helix peptide/protein, a stable stereocomplex is formed owing to the convex-concave fitness between their surfaces (Figure 3). Stereocomplexation occurs between a peptidyl chain and D-PLA chain. Thus, for linear peptides or peptidyl chains extending from a protein, stereocomplexation takes place. For more complicated protein structures, stereocomplexation may disrupt the protein 3D structure and thus deteriorate its activity. Stereocomplex formation is observed solely between the L-enantiomeric peptides and the D-enantiomers of PLA. Notably, when the L-enantiomeric peptide and protein moieties are combined with the L-enantiomeric PLA, no stereocomplex is formed, indicating the strict stereospecificity requirement for the stereocomplexation.[63]

Thermodynamic simulations based on the Flory−Huggins model reveal the structural compatibility between polylactic acid (PLA) and poly(amino acid) backbones. The predictions indicate favorable compatibility and minimal steric hindrance between PLA and amino acids, responsible for their good nucleation efficiency.[5] Wei et al. used Zinc salts of amino acids as a new class of biocompatible nucleating agents for PLLA. The nucleation mechanism was further explained by epitaxial nucleation based on lattice matching.[92] Carbone et al. report the use of polypeptides as nucleating agents for the crystallization of PLA. The addition of poly(amino acids) as heterogeneous nucleating agents makes sense, given that their chemical structure is similar to that of PLA, which may facilitate the crystal nuclei formation driven by stereocomplexation.[5]

One of the earliest reports of heterostereocomplexation between peptide and polyesters was reported by our group.[90] PLA and peptides have quite similar composition with the obvious difference of an ester bond instead of an amide bond. Specially, in aprotic nonaqueous solvents, peptides cannot form many hydrogen bridge bonds. Thus, the crystallization and packing in peptide and PLA moieties are comparable in organic solvents. When D-PLA is mixed with peptide moieties like Leuprolide and Vapreotide dissolved in an organic solvent such as acetonitrile, a physical complex precipitates out (with a high yield of even >90%), accompanied by a decrease in crystallinity.[59,62] The stereocomplex between Leuprolide and PLA shows a rapid increase in the small-angle X-ray scattering intensity. Acetonitrile has been shown to be a preferred solvent for stereocomplexation and is reported in many studies. Its property as a helix-inducing agent is attributed to facilitating the stereocomplexation process.[63,93,94]

Stereocomplexation is entirely physical and reversible without any chemical reaction. Stereocomplexation can be reversed by surfactants like span 80 that can detangle the complexation between the peptide and D-PLA, with almost 100% peptide recovered from the stereocomplex. In DSC measurements, isotactic PLA shows a sharp peak corresponding to its melting point around 175 °C. The formation of homostereocomplexion between D-PLA and L-PLA is accompanied by an increase in the melting point, characterized by a sharp peak at around 230 °C. However, on the other hand, heterostereocomplexation between D-PLA and L-Peptide shows two transition endotherms. Specifically, leuprolide with D-PLA shows one peak for the α form that melts at 178 °C and another for the β form around 169 °C. This decrease in melting point may be attributed to the β-helical form, implying that to form a heterostereocomplex with peptide moiety, the PLA helix undergoes a confirmational change from the α to the β form. The β form confirmation is contrary to usual conditions in which the α-helix is the thermodynamically preferred confirmation. This complexation is also accompanied by a decrease in the enthalpy directly proportional to the amount of peptide added to D-PLA. The plausible reason behind the decrease in enthalpy is a decrease in crystallinity or confirmational change from the α to the less stable β form.[57]

The heterostereocomplex formed by this interaction spontaneously forms colloidal assemblies that range in size from a few nanometers to a few microns and without the need for any specific surfactants or additives. The heterostereocomplex between L-Peptide and D-PLA generates particles of variable morphologies depending on the molecular weight of the polymer systems. For instance, leuprolide with D-PLA of larger molecular weight (100 kDa) initially forms elongated particles with 30 × 40 nm dimensions, which transform into spherical particles of 50 nm diameter after a few hours. On the other hand, 10 kDa D-PLA with leuprolide forms 50 nm spherical particles within 2 min after mixing. Vapreotide, a cyclic octapeptide somatostatin analogue, is also shown to form a stereocomplex with D-PLA by simply mixing together for 3 days at 60 °C in water and an acetonitrile solution. The resultant stereocomplex forms microspheres of 1−3 μm diameter.[62] These particles exhibit a fibrous structure with a large surface area, unlike conventional smooth surface microspheres of PLA. These stereocomplex particles show sustained first-order drug release for up to a period of 3 months under both *in vitro* and *in vivo* conditions without any sign of burst release. An *in vivo* set up stereocomplex of D-PLA with leuprolide, when administered subcutaneously to Wistar rats, controlled the testosterone level for over a period of 6 weeks, implying sustained release of leuprolide from the complex over that period of time.[59] The release of peptide therapeutics from the complex system primarily depends on the degradation of D-PLA and the subsequent detachment of the D-PLA/protein complex. Unlike conventional polymeric particles, drug release from the stereocomplex does not depend on the diffusion of the active peptide through the polymer matrix, circumventing the risk of an uncontrolled burst release associated with conventional polymeric carrier systems.[62,90]

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Insulin, a 51 amino acid unit peptide chain, is also shown to form a stereocomplex with D-PLA, resulting in fibrous microspheres in the size range of 1−3 μm. The stereocomplexation simply takes place by mixing insulin with D-PLA at 60 °C. Notably, insulin also demonstrates the ability to form stereocomplexes with the PEGylated copolymer of D-PLA (PEG−D-PLA). This suggests that stereocomplexation in PLA heavily depends on the ester backbone of PLA. Stereocomplexes can be established even with modified PLA moieties containing different functional groups, as long as the chiral ester backbone remains intact.[11,63] This has significant implications as a hydrophilic PEG moiety conjugated with D-PLA can be employed to form stereocomplexes in aqueous media without requiring any organic solvents. Water-soluble PEG-D-PLA can be used to produce nano- and microparticle therapeutics with peptide and proteins that are insoluble in nonaqueous solvents or susceptible to degradation in the presence of organic solvents.

Stereocomplexation has also been shown to be obtained by mixing a peptide with the triblock copolymers of D-PLA (D-PLA−PEG−D-PLA) even at room temperature. However, these di- and triblock copolymers tend to form a gel like stereocomplex, and they form a sticky substance upon drying. However, incorporating the L-PLA homopolymer with PEG-D-PLA copolymers produces microparticulate systems through stereocomplexation. The microparticulate systems resulting from the stereocomplexation between insulin and D-PLA or PEGylated D-PLA copolymers exhibit sustained insulin release under *in vitro* conditions with no burst release.[63]

2.2.2. Stereocomplexation between Peptides and Dendrimers. Peptide and protein moieties are also shown to form a stereocomplex with dendrimer systems. Matsui and co-workers also show that a second-generation polyamideamine (PAMAM) dendrimer bearing right-handed helices to its eight terminals can accommodate eight left-handed helices via stereocomplex formation, generating molecular assemblies with a diameter of 13−14 nm and a thickness of 6 nm.[95] They further utilized stereocomplexation between left- and right-handed helical peptides in host dendrimer and guest peptide moieties, respectively, to form nanoparticles with a hydrodynamic diameter in the range of 27 nm. The peptides were incorporated into the host dendrimer with tight helix packing and an antiparallel helix dipole arrangement, exhibiting a long-lifetime in the bloodstream and good tumor targeting.[96]

3. PREREQUISITES FOR STEREOCOMPLEXATION IN PEPTIDE AND PROTEIN MOIETIES

Stereocomplexation in peptide and protein moieties requires certain prerequisite conditions such as chirality, torsion angles, bond lengths of stereocomplex-forming moieties, and the length of polymer chains.

Stereocomplexation occurs when two polymers with opposite stereo configurations are mixed. A chiral backbone is one of the most important prerequisite requirements for stereocomplexation between two polymeric structures. While stereocomplexation is generally favored between the identical chemical structures (homostereocomplexation), heterostereocomplexation between polymer moieties with different chemical structures is also observed if their chiral backbones are compatible. For instance, peptide and protein moieties with an amide backbone are shown to form stereocomplexes with a chemically different polyester backbone in D-PLA due to similarities in their torsion angles and bond lengths.[8,12,91,57−59,61−64,90]

Given that stereocomplexation hinges on the chiral backbone, various stereocomplexes can form even in chiral polymer systems with nonchiral functional groups, as long as their interacting chiral backbones remain intact. L-Insulin, for example, forms stereocomplexes with D-PLA containing a nonchiral PEG side chain. However, a minimum length of the chiral PLA backbone (≈11−16 D-lactide monomer units) and a specific number of helical turns (two) are shown to be critical for stereocomplexation between PEG-D-PLA and insulin.[64]

If side chain substitution in the main chiral polymer backbone is too bulky, then it may hinder stereocomplexation. Studies have shown that the length of side chains in poly(γ-alkylglutamate) affects stereocomplex formation. A short alkyl side chain is conducive to stereocomplexation ($n \leq 3$ in the case of the poly(γ-alkyl-glutamate) stereocomplex system), emphasizing the importance of side-chain characteristics.[10]

While having two isotactic polymers with opposite chiral configurations is a clear prerequisite, other factors contribute to stereocomplexation. Mixing D- and L-chiral moieties does not always guarantee stereocomplex formation; sometimes homochiral assemblies may be preferred over heterochiral stereocomplex assemblies.[89] This preference for homocomplex formation over stereocomplex formation aligns with "Wallach's rule" as elucidated by Li, X. and co-workers in their recent report, in which they studied the homochiral and heterochiral assembly between β-sheet peptides. Their findings suggest a general preference for heterochiral packing over homochiral arrangements in crystalline structures, resulting in denser solid forms and a preference for racemic crystal formation, a phenomenon termed as "Wallach's rule". Their observations suggest that the formation of heterochiral mixtures of β-sheet peptides, and occasionally rippled β-sheets, is driven by solid-state packing forces. However, in solution phases, in which the influence of crystal packing is absent, the formation of rippled β-sheets is not favored, and homochiral assembly is favored.[78]

Further reaction conditions also influence the stereocomplexation. For instance, acetonitrile has been shown to be the preferred solvent for the stereocomplexation between D-PLA and peptide moieties. In aprotic nonaqueous solvents, peptides cannot form many hydrogen bridge bonds. Thus, the crystallization and packing in peptide and PLA moieties is comparable. Acetonitrile's property as a helix-inducing agent is attributed to facilitating the stereocomplexation process.[63,93,94]

It is important to note that the discussed prerequisites for stereocomplexation in peptide and protein moieties are not exhaustive and are still areas of active research. Identifying these premises is important for harnessing the advantages that stereocomplexation offers.

4. CHARACTERIZATION OF STEREOCOMPLEXATION IN PEPTIDE AND PROTEINS

Stereocomplexation is accompanied by changes in different physiochemical properties such as enthalpy, morphology, etc. Different techniques are employed for the characterization of these changes, including thermodynamic, optical, mechanical, and spectroscopic techniques. Most stereocomplex products exhibit insolubility in the solvents in which the enantiopure parent polymers are typically soluble.[8,62,63,81,86,90] The aggregation of stereocomplex structures in the reaction media increases the turbidity, often monitored through transmission reduction over time.[57,60,91,97] Stereocomplexation

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is also accompanied by characteristic changes in enthalpy, melting point, and crystallinity. These alterations are often attributed to the close packing of polymer chains and the ensuing intermolecular interactions during stereocomplexation. Differential scanning calorimetry (DSC) is the most commonly employed technique to study these thermodynamic changes.[85,98]

A combination of different spectroscopic techniques is used to study the structural changes, intermolecular interactions, and alterations in the molecular arrangement accompanied by stereocomplexation. Lyophilized powder samples of stereocomplex are characterized using powder diffraction to determine the crystal structure and to provide insights into the arrangement of the molecules in the stereocomplex system, including their packing and symmetry.[14] A combination of scattering (small- and wide-angle) techniques is used to extract the information about the crystal structure, orientation, and molecular packing in the stereocomplex. WAXS data analysis yields crucial information regarding the crystallinity and structural details of stereocomplexes.[99−102,14,103] On the other hand, small-angle X-ray scattering (SAXS) is specifically used to gain further insights into global morphology and its nanostructure by calculating the size and shape of the stereocomplex domains, the interparticle spacing, and their organization.[8]

Circular dichroism (CD) is a spectroscopic technique used to study the secondary structure of chiral molecules. Specific optical rotation measurements are used to study and monitor the stereocomplexation reaction.[89] Fluorescence and energy transfer spectrometry techniques are used to study the stereocomplexation. In these techniques, a reactive fluorescence label like nitroxide radical, carbazole, and anthracene is attached to the stereocomplex forming polymer moieties. Chemiluminescence data are measured to monitor the stoichiometry and reduced mobility associated with the stereocomplexation process.[104,105] NMR spectroscopy is another spectroscopic technique used to study the stereocomplexation process. By monitoring changes in the chemical shift over time or under different experimental conditions, NMR can offer insights into the kinetics and thermodynamics of conformational changes. By monitoring changes in the chemical shift upon ligand binding, NMR can provide information about the binding affinity and specificity of the ligand, as well as the location and nature of the binding site.[106,107,78,108,109]

The anticipated hydrogen bonding or ionic interactions between the enantiomeric polymer moieties on stereocomplexation can be investigated by using a combination of Raman and infrared spectroscopy. Infrared (IR) spectra are specifically employed to study the secondary structure of the protein or peptide in the stereocomplex system and for probing the kinetics of polymer−peptide conformation changes by analyzing the shift of amide bands in the infrared spectra. Near-infrared spectroscopy is used to provide information about the hydrogen bonding interactions between the polymer and protein molecules. Raman spectroscopy is specifically used to provide structural information on the specific functional groups involved in the stereocomplex formation and the intermolecular bonding by detecting changes in vibrational modes of the molecules.[61,110,111,85]

Dynamic light scattering is also used to characterize the stereocomplexation and to monitor the size of the stereocomplex particles in combination with microscopic techniques.[8] Electron microscopy techniques are standard methods used to study the morphology of stereocomplex systems. Heterochiral assembly between D- and L-peptides shows distinct morphology compared to the parent or homochiral assembly between enantiopure peptide moieties.[108,112] Atomic force microscopy (AFM) is a widely used technique to characterize stereocomplexation products. AFM is widely used to monitor the change in morphology of the stereocomplex particles and can also be used to measure the atomic level physical force interactions between the stereocomplex forming moieties. In this technique, one of the enantiomer moieties is grafted onto the substrate, and the other complementary enantiomer moiety is grafted onto the tip of the AFM cantilever. Using a piezo transducer, the cantilever tip is microscopically advanced toward and retracted away from the substrate surface. Deflection of the cantilever in the approach and retraction are detected using a laser focused detector on the cantilever. The complexation is monitored by a change in morphology, which is imaged by the AFM-tapping mode. In addition to visualization of morphological changes, AFM also enables the monitoring of phase differences, providing qualitative information about the material microstructure on the nanometre scale. In the resulting images, regions with disparate mechanical properties, such as friction, elastic modulus, and viscoelasticity, are depicted as areas of distinct contrast. The changes in the van der Waals forces as a function of distance between the two counter moieties and the topography of the stereocomplex can also be recorded. The degree of affinity between the counter moieties is quantified by measuring the force required for protein puling to detach the stereocomplex, overcoming the attractive van der Waals forces. The acquired images can be used to assess the helices area and their size and thus the morphology of the stereocomplex system. The information acquired can be used to extract the kinetic and thermodynamic parameters of the interaction.[57,113,114]

Stereocomplexation between two enantiomeric polymers is also accompanied by a change in the mechanical properties of the resulting stereocomplex product compared with the enantiopure parent polymers. Therefore, measurement of different mechanical properties is also used to characterize the stereocomplexation process. In general, stereocomplex materials usually display a change in the tensile strength and rheological properties compared to those of their parent enantiopure materials. Thus, the determination of these parameters is helpful in the characterization of the stereocomplexes.[8,11,115,112,116]

5. APPLICATIONS OF STEREOCOMPLEXATION IN PEPTIDE AND PROTEINS

The phenomenon of stereocomplexation has great implications in biomaterials science because the stereocomplexes have distinct mechanical and thermal properties compared to their homochiral polymers. The heterochiral assembly of peptides enantiomers creates a stereocomplex product with distinct biochemical, biophysical, and biological properties compared to enantiopure parent peptides.[11,12,77]

5.1. Biomaterials. *5.1.1. Hydrogels.* Stereocomplexation in peptides and proteins has diverse applications. Notably, it can be used to drive the formation of fibrils and hydrogels. Several amphiphilic peptides have been shown to form fibrils and hydrogel networks upon self-assembly. Stereocomplexation has been shown to be an important design tool to control the

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mechanical properties of these self-assembled peptide hydrogel systems. Hydrogels prepared from the heterochiral assembly of enantiomeric peptides show enhanced rigidity and thermodynamic stability compared to hydrogels prepared from the self-assembly of enantiopure peptides.[77,117] For example, β-hairpin peptides such as MAX1 and DMAX1 are shown to self-assemble to produce fibril hydrogels. The racemic mixture of these peptides coassembles to form a heterochiral pleat-like β-sheet in a manner similar to the rippled sheet structure proposed by Pauley and Corey. This heterochiral assembly creates a uniquely nested hydrophobic interaction between mirror image peptides due to hydrogen bonding and the arrangement of hydrophobic valine side chains, absent in enantiopure peptides. This arrangement enhances inter-residue contacts within the fibrils, resulting in hydrogels with four times higher mechanical rigidity compared to those derived from enantiopure peptides. Moreover, the fibrils prepared from racemic mixtures are also shown to form faster than the corresponding enantiopure systems.[77,78,117,118]

The hydrogels formed by the heterochiral assembly are not only stronger but in certain cases also found to be more resistant to proteolytic degradation than the hydrogel formed by the homochiral peptides assembly.[77,78,85,115] For example, Swanekamp and co-workers studied hydrogel systems formed by the heterochiral assembly of D- and L-enantiomers of Ac-(FKFE)2-NH2 peptide (a peptide with an acetyl group, two repetitions of the phenylalanine (F), lysine (K), phenylalanine (F), and glutamic acid (E) sequence, and an amino group). The hydrogel systems comprised of rippled β-sheet fibrils of coassembled D- and L-Ac-(FKFE)2-NH2 peptides show enhanced proteolytic stability and improved rheological strength compared to hydrogel systems comprised of self-assembled L-Ac-(FKFE)2-NH2 pleated β-sheet fibrils.[115]

Nahhas et al. report the formation of a nanosheet stereocomplex system, when right- and left-handed tripeptide supramolecular hydrogels are physically mixed in aqueous solution without requiring any external stimulus. Stereocomplexation between two peptide moieties reduces their molecular mobility responsible for an increase in mechanical rigidity and improved thermal stability.[119] Modifying the ratios of L- and D-peptides in the coassembled rippled β-sheet fibrils can also be used to control the mechanical properties and degradation profiles of these hydrogel systems to produce new biomaterial useful for a variety of applications, ranging from wound healing to drug delivery to tissue engineering.[119]

Stereocomplexation can serve as a molecular design tool for fine-tuning peptide-based biomaterials with the desired properties. In a recent and interesting report, Israt Jahan Duti and co-workers explain that blending L- and D-enantiomers of KYFIL (a pentapeptide) results in a network of plates, exhibiting less stiffness compared to the fibrous hydrogel formed from enantiopure peptides. The most plausible reason behind this anomaly is that the enantiopure form of KYFIL forms fibers that entangle to create a stiff hydrogel, but blending of D- and L-enantiomer produces stereocomplex plates that cannot entangle. Consequently, stereocomplexation offers a method to modify mechanical properties and regulate the biodegradation profile of biomaterials, enabling the creation of novel biomaterials tailored for controlled therapeutic release and tissue-mimicking regeneration materials.[7]

5.2. Biological Studies/Biomedical Applications. The application of stereocomplexation in peptides is not only limited to design of biomaterials, but it has potential therapeutic solution to tackle several disease problems. The heterochiral assembly in peptides has been used to characterize and study fibril formation of Aβ_{40} and Aβ_{42} and can provide a possible submolecular mean to tackle Alzheimer's disease.[78,79] Amyloid fibers, insoluble in nature, are a pathological hallmark in numerous human diseases. In addressing these conditions, one potential therapeutic approach involves the inhibition of amyloid formation.[120] Racemates typically exhibit less solubility compared with enantiopure compounds, and the racemic mixing of enantiomers can increase the aggregation tendency of peptides. Amyloid β (Aβ) 42 is a peptide that tends to aggregate, and it plays an important role in Alzheimer's disease. Soluble Aβ42 aggregation intermediates (oligomers) have emerged as being particularly neurotoxic. Dutta and Group report on a strategy to promote an Aβ oligomer-to-fibril transition using a mirror-image peptide as the molecular chaperone. This effect, termed Aβ Chiral Inactivation, leads to a protection of neuronal model systems from Aβ neurotoxicity. Mixing an equimolar amount of D- and L-enantiomer Aβ42 leads to accelerated nontoxic fibril formation and reduces the formation of soluble toxic oligomers produced by natural enantiopure L-Aβ42. As a consequence, cells are shielded from the L-Aβ42 associated toxicity.[121]

The stereocomplexation interaction between mirror image peptides and protein moieties has also shown the potential to modulate amyloid aggregation. The study of these interactions provides mechanistic tools to investigate the cellular interactions that are useful to identify the key positions in protein sequences having a role in the amyloid fibril aggregation.[122] It has been shown that the mirror-image amyloid beta (Aβ) can act as a molecular chaperone to promote oligomer-to-fibril conversion of the natural Aβ enantiomer, which reduces Aβ neurotoxicity against different neuronal cell models.[78,122,123]

The stereocomplexation phenomenon can prove to be an important tool in designing chiral inhibitors of amyloid formations.[77,120] Gray et al. report that heterochiral complexation between D- and L-enantiomeric dipeptide repeats (DPRs) is more favorable than homochiral complexation. That strategy can be utilized to design biopolymer systems conjugated with D-DPRs that can sequester and clear the toxic L-DPRs associated with amyotrophic lateral sclerosis (ALS). This can be utilized to design new therapies to treat ALS.[98] Kar et al. conclude that mirror-image D-polyglutamine can recruit L-polyglutamine and induce the formation of potentially toxic inclusion bodies in cell-based assays.[124] These studies indicate the importance of stereocomplexation with different biological consequences.

5.3. Nanostructures. Stereocomplexation serves as an important tool in the design of peptide-based nanostructures with specific morphologies. Ueda et al. studied the stereocomplexation between mirror image D- and L-enantiomers of helical peptides to design peptide nanostructures of different morphology and dimensions.[125−128] Temperature-triggered stereocomplexation leads to fusion of left-handed and right-handed amphiphilic helical peptides, which lead to the formation of vesicles with varying diameters and lengths.[126,129,130] Ueda et al. studied the stereocomplexation between mirror image peptides to examine the role of different molecular geometries on the morphology of these molecular assemblies. Peptidic flat-rods with precise morphology and dimensions are produced by stereocomplexation between

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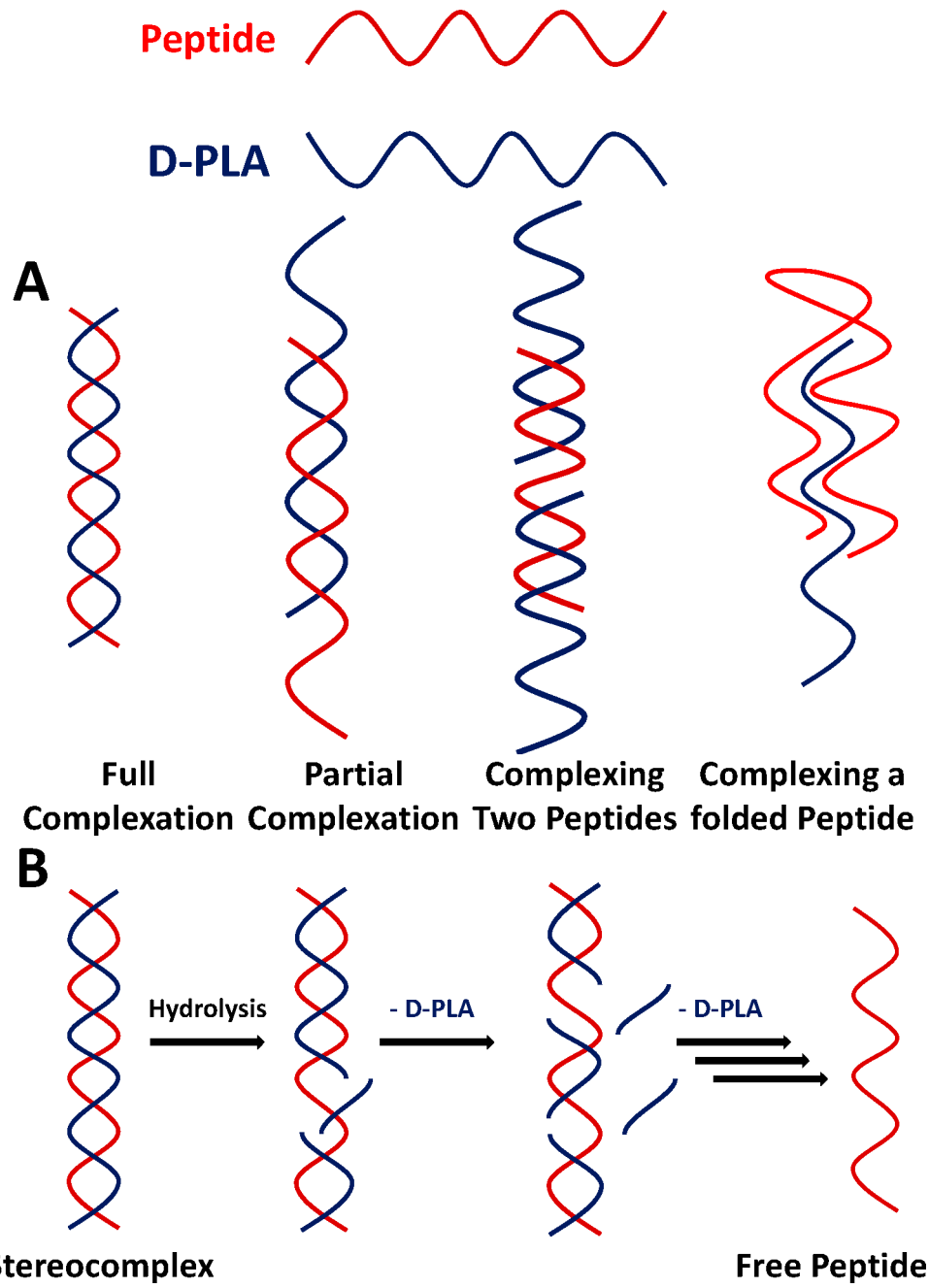

Figure 4. (A) Illustration of stereocomplexation between D-PLA and L-peptide and (B) peptide drug release from the stereocomplex by DPLA chain hydrolysis and detachment of the peptide moiety. Adapted from ref 64 (Shapira-Furman T. et al.).

mirror image T-shaped amphiphilic block copolypeptides. This provides a great degree of control over macroscopic higher-order assembly.[114] Further, stereocomplexation between D- and L-enantiomers of amphiphilic block polypeptides can be used to produce nanovesicles, with tunable viscoelasticity. The membrane's viscoelasticity can be controlled by adjusting the combination of the helical blocks and the interdigitation of side chains among helices within the stereocomplex system.[127] Mirror imaged amphiphilic peptides with an interdigitated helix structure can be used to produce unsymmetrical vesicular membranes with a great degree of control over the dimensions and morphology of the vesicles.[131]

The dendrimer systems bearing peptide moieties with left- and right-handed helices can be used to produce molecular assemblies having potential application in drug delivery.[95] Matsui and co-workers utilized stereocomplexation between left- and right-handed helical peptides in host dendrimer and guest peptide moieties, respectively, to fabricate nanoparticles with a hydrodynamic diameter in the range of 27 nm. The peptides were incorporated in the host dendrimer with tight helix packing and an antiparallel helix dipole arrangement, exhibiting long-lifetime in the bloodstream and good tumor targeting.[96]

Peptide nanotubes with left- and right-handed helical segments, when mixed together, transform into a vesicle structure by membrane fusion driven by stereocomplexation between the mirror imaged helical peptide segments. The study of these processes is useful in elucidating the

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Table 2. Stereocomplexation between Peptide Therapeutics and D-PLA for Controlled Drug Release Applications

Stereocomplex pair	Drug delivery carrier
D-PLA and Leuprolide[57−59,61,62]	Produces porous microparticles with a mean particle size of about 1.7 μm
	The stereocomplex system provides sustained first order drug release over a variable period of time (up to 3 months), depending upon the molecular weight of the polymer system and drug loaded, with negligible burst release.
	The stereocomplex system provides control over the plasma testosterone levels for over a period of 25 days in rats after a single subcutaneous administration.
D-PLA and Vapreotide[62]	Produces highly porous uniform spheres of 2−5 μm in diameter
	The stereocomplex system provides the first order sustained drug release for over a period of one month.
D-PLA and Octreotide[91]	Produces spherical porous microparticles of 1.5 to 4 μm diameter
	The stereocomplex system releases the complexed peptide for over a period of 40 days.
D-PLA and Insulin[63]	Produces porous 1−3 μm particles
	The stereocomplex system provides the sustained drug release with less than 30% of the complexed insulin released over a period of 2 weeks.
PEG-D-PLA and Insulin[64]	Produces ≈400 nm size spherical particles
	The stereocomplex system exhibits controlled release of insulin for over a period of 3 months.
	The stereocomplex system provides control over the blood glucose levels for over a period of 17 weeks in an Akita mouse model of diabetes after a single subcutaneous injection.

bioprocesses and design of biomaterials. Stereocomplexation introduces a new approach for preparing complex morphologies through a simple molecular design and preparation process.[129,130,132] So, the stereocomplex can be used to produce materials with tunable properties to interact with living cell and tissue systems and to design new biomaterials.

5.4. Peptide and Protein Heterostereocomplexes for Controlled Drug Delivery Therapeutics. The phenomenon of stereocomplexation in peptides and proteins has a major application in developing new peptide and protein therapeutics. L-Enantiomeric peptide and protein therapeutics can form a stereocomplex with mirror imaged D-enantiomeric polymers. Our group found that D-PLA shows complementarity with the L-configured backbone of peptide moieties, such as leuprolide, insulin, vapreotide, and octreotide. This provides an alternative approach for the controlled delivery of peptides and proteins. The stereocomplex between PLA and peptide therapeutics produces spherical particles in the size range of a few nanometers to several micrometers, which in turn depends upon the molecular weight of the polymer system and the ratio of peptide to D-PLA moieties in the stereocomplex system. The resulting stereocomplex particles provide controlled drug release for the complexed peptide therapeutics. For example, leuprolide forms stereocomplex particles with a D-PLA of about 1.7 μm diameter. These microsize stereocomplex systems show sustained first order drug release for over a period of up to 3 months in *in vitro* experiments. Subcutaneous administration of D-PLA and the leuprolide stereocomplex system in rats provides control over the plasma testosterone levels for over a period of 25 days.[57−59,61,62]

The implications of this approach are considerable but are largely unexplored. First, this kind of interaction is physical, without involving any chemical bonds. Thus, interaction does not affect the chemical structure of the peptide moiety and thereby does not affect its therapeutic activity. Second, the heterostereocomplex by this interaction spontaneously forms nano- to microsized assemblies that range in size from a few nanometers to a few micrometers and without the need for any specific surfactants or additives. Furthermore, the formed stereocomplex system shows a controlled release of peptide drugs that depends on the degradation of D-PLA and the subsequent disruption of the D-PLA/protein complex. Drug release does not depend on the diffusion of the active peptide through the polymer matrix, circumventing the risk of an uncontrolled burst release associated with conventional polymeric carrier systems (Figure 4).

Further, there is ample scope for modification in the side-chain of the D-PLA moiety, without affecting the stereocompatibility of the polymer. This can be used to functionalize the polymer chain with a hydrophilic group like PEG and can be used to achieve stereocomplexation in the aqueous environment without using organic solvents. For example, PEG-D-PLA can form a stereocomplex with insulin by simply mixing in an aqueous environment without requiring any organic solvent or surfactant system. The transformed insulin and PEG-D-PLA stereocomplex system produces nanoparticles of around 400 nm diameter, providing a controlled release of insulin for over a period of 3 months. The stereocomplex system provides control over the blood glucose levels for over a period of 17 weeks in an Akita mouse model of diabetes after a single subcutaneous injection.[64] It also implies that the PLA moiety can be functionalized with a targeting moiety to achieve targeted drug delivery. Most importantly, the major advantage is its simplicity and universality, and it is anticipated that it can be used with a wide variety of peptide and protein drug candidates. Table 2 summarizes the examples of stereocomplexation between peptide therapeutics and D-PLA for controlled drug release applications.

6. CONCLUSIONS AND FUTURE PROSPECTS

Homochiral assembly in peptide structures is a well-studied phenomenon since it is ubiquitous in nature. All ribosomal peptide and protein moieties are composed of L enantiomeric amino acids. However, heterochiral assembly in peptides and proteins has been only recently validated and garnered attention, owing to recent revelation of their crucial role in biomedical science. Moreover, homostereocomplexation between peptide structures among themselves is still studied, but heterostereocomplexation between peptide and nonpeptide structures has been only very scantly explored. The number of polymer combinations having the ability to form heterostereocomplexes is very limited compared to the number of polymers forming homostereocomplexes. The reliance of stereocomplexation in peptides on the chiral backbone presents a versatile landscape, allowing for the formation of diverse stereocomplexes even with substituted polymer moieties bearing different functional groups as long as the chiral peptide backbone remains intact. This flexibility

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facilitates the modification of the polymer side-chain without compromising stereocompatibility, thereby enabling the functionalization of peptide and protein therapeutics with desired properties. However, any substitution in the polymer system that disrupts the chiral backbone can impede stereocomplexation. Unfortunately, the current field lacks adequate models to predict these stereocomplexation interactions. Moreover, conventional characterization techniques often fall short in comprehensively studying stereocomplexation, particularly in the case of heterostereocomplexation. There is a pressing need to develop methodologies that can better elucidate the nature of interactions between heterochiral pairs, addressing the existing limitations in predicting stereocomplexation between two polymer systems. An additional constraint in stereocomplexation lies in its impact on bulky protein therapeutics, where the three-dimensional structure is crucial for bioactivity. While short peptide and protein therapeutics have demonstrated the formation of stable stereocomplexes without compromising bioactivity, the stability and bioactivity of larger protein therapeutics may be affected. Further exploration is warranted to form stereocomplexes without compromising the stability and bioactivity of these therapeutics. Further research in this direction holds great promise in the field of biomaterial science. Specifically, heterostereocomplexation has great implications that can lead to development of newer peptide and protein therapeutics with greater stability, overcoming delivery challenges associated with peptide and protein therapeutics.

AUTHOR INFORMATION

Corresponding Author

Abraham J. Domb − *School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem 91120, Israel;* orcid.org/0000-0002-2241-7726; Email: avid@ekmd.huji.ac.il

Author

Ravi Saklani − *School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem 91120, Israel*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.4c00178

Notes

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