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Perspective

Biology-Inspired Supramolecular Peptide Systems

Ayala Lampel[1,*]

Supramolecular living matter is remarkably complex in composition and structure and often exists far from equilibrium. The biological manufacturing process involves compartmentalization and confinement, supramolecular templating, and cascades of enzymatic reactions to produce structures at a variety of size scales that are formed in highly heterogeneous environments. In contrast, laboratory-based materials are typically formed as single- or bi-component systems in homogeneous, chemically "sterile" environments, therefore destined to be significantly less complex. Fortunately, over the last two decades, major developments in supramolecular chemistry and systems chemistry have made the construction of life-like peptide-based materials increasingly attainable. Peptide structures can now be designed with controlled hierarchy and symmetry, as ordered or disordered or dynamic assemblies, for the generation of complex responsive materials that adapt their properties in response to chemical or physical stimuli. In this Perspective, we highlight these recent efforts by focusing on the underlying molecular design and discuss where the field is heading.

The Bigger Picture

Challenges and opportunities:

- Designing peptides that self-assemble into materials with life-like properties from first principles.
- Utilizing supramolecular order and disorder or multicomponent building blocks to expand the repertoire of material properties.
- Increasing cross-disciplinary efforts to implement dynamic, far-from-equilibrium, and adaptive materials in living systems.

INTRODUCTION

Biological systems exploit tremendous molecular complexity and precise spatial and temporal control to construct exquisite structures and functional materials. The tunability, reversibility, adaptability, and dynamic features of biological materials rely on the chemical and structural properties of the building blocks and on the emerging properties of the resulting structures. This tremendous complexity and control is demonstrated by the hierarchical structures of bone and nacre,[1] the cooperative assembly of the extracellular matrix (ECM) polysaccharides and proteins,[2] and the dynamic cytoskeleton components,[3] to name a few. While just a decade ago the design of man-made materials was mostly focused on bulk structures formed by a single component, we now understand that designing synthetic materials with life-like properties necessitates higher levels of molecular complexity and requires the incorporation of elements of the biological manufacturing process into laboratory-based protocols.

Supramolecular self-assembly offers tremendous opportunities for the construction of bioinspired functional materials.[4] The process relies on non-covalent interactions between molecular building blocks, including hydrogen bonding, and hydrophobic, π-π, and electrostatic interactions. Although these interactions are weaker than covalent bonds, their summation and diversity give rise to the properties of the resulting supramolecular structures and materials. These functions may enable the materials to exhibit reversibility and tunability, functions, which may be very applicable for use in health care.[5] Peptides are particularly attractive building blocks for supramolecular materials because they are much simpler structurally than proteins,

are easy to synthesize, and from a technological point of view,[6,7] lend themselves readily to scaled up production. Peptides possess a diverse set of simple chemical functions provided by the aromatic, basic or acidic, polar, and aliphatic side-chain groups that, in combination, enable a rich and versatile chemical repertoire.[8]

Recent developments in the field include the preparation of life-inspired supramolecular peptide materials with properties that resemble those of biological materials and in some instances, outperform them. In this Perspective, we highlight these developments by focusing on the molecular design of the materials rather than on their applications. We review the design trends in the field and discuss where we are heading and how far we are from designing living peptide materials. The order of the systems discussed here reflects the level of design complexity, starting with hierarchical and reversible self-assembly, which results in solid-like structures, progressing to systems that utilize ordered or disordered building blocks and dynamic complexes formed by liquid-liquid phase separation, and concluding with active and adaptive supramolecular systems.

DESIGNED HIERARCHY AND SYMMETRY

Inspired by β-sheet fibers formed by silk and amyloid proteins, the most common supramolecular organization of designed peptides in 1D structures is β-sheet packing. Yet, most of the hierarchical biological structures rather rely on the organization of protein subunits that adopt a α-helix conformation including bacterial compartments, virus capsids, and collagen fibers. These exquisite protein structures have inspired the design of helical peptide assemblies,[9] and the most recent examples of hierarchical structure formation driven by helical organization are reviewed here.

Controlled Symmetry of Peptide Assemblies

Woolfson and coworkers designed a set of α-helical coiled coil peptide structures with a distinct symmetry[10] inspired by that of collagen. The structures were composed of peptides containing heptad (seven amino acids) repeat sequences consisting of hydrophobic (h) and polar (p) amino acids arranged as hpphppp, which self-assemble into coiled coil α-helical barrels.[11] The authors studied the atomic-level architecture of the structures formed by peptides containing systematic variations of aliphatic amino acids in positions 1 and 4. X-ray crystal structure of the peptides revealed that although they all self-assembled into coiled coil hexamers, subtle changes to the heptad sequence had a dramatic effect on their architecture. Peptides with two isoleucines (I) at positions 1 and 4 or leucine (L) and I at positions 1 and 4, respectively, assemble into symmetric α-helical barrels with defined channels (Figure 1A, 1 and 2), whereas reversing the L and I positions results in slipped barrel architecture (Figure 1A, 3 and 4). Replacing glutamic acid (E) (Figure 1A, 3) with serine (S) in position 7 (Figure 1A, 4) gives rise to an incomplete barrel of 5 helices, and substituting I with L in the latter variant (Figure 1A, 5) significantly reduces the symmetry. This work demonstrated that coiled-coil structures with defined symmetry can be designed and provides the guiding principles to do so.

By utilizing much shorter sequences, Gazit and coworkers succeeded in developing hierarchical helical fibrils[12] composed of the highly aggregative tripeptide[14] proline-phenylalanine-phenylalanine (PFF). The observation that PFF forms a helical architecture is quite surprising given that the dipeptide FF, which differs only in a single amino acid (P), self-assembles into typical amyloid-like β-sheet fibrils.[15] Circular dichroism (CD) and Fourier transform infrared spectroscopy (FTIR) revealed that the helical organization of PFF has peaks that are slightly shifted from those in typical

[1]George S. Wise Faculty of Life Sciences, School of Molecular Cell Biology and Biotechnology, Tel Aviv University, Tel Aviv 6997801, Israel

*Correspondence: ayalalampel@tauex.tau.ac.il

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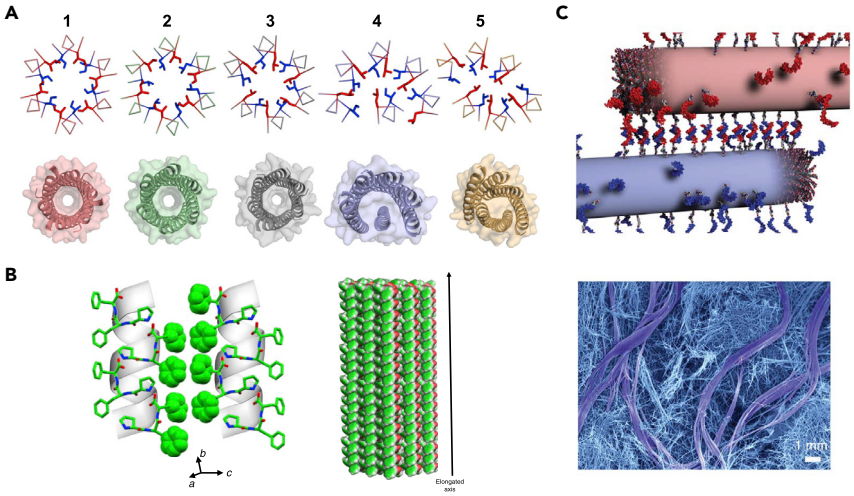

Figure 1. Hierarchical, Symmetrical, and Reversible Peptide Structures

(A) Atomic structure of helical peptide assemblies shows barrels with defined channels (1 and 2), slipped barrel (3), slipped and collapsed structure (4), and collapsed structure (5). Orthogonal views of the structures are shown on the bottom. Reprinted from Rhys et al.,[10] licensed under CC BY 4.0.

(B) Single-crystal structure of the tripeptide PFF shows interactions between adjacent helical-like assemblies along the c direction through aromatic stacking. Reprinted with permission from Bera et al.[12] Copyright 2019 Springer Nature.

(C) Schematics of cross-linked PA-DNA fibers through DNA hybridization and SEM image (bottom) of the resulting hydrogel show twisted bundles and individual fibers. Reprinted with permission from Freeman et al.[13] Copyright 2018 AAAS.

helical structures. The single-crystal X-ray structure of PFF indicated the presence of a helical-like architecture stabilized by head-to-tail intermolecular hydrogen bonds, where a terminal intermolecular hydrogen bond replaces the fourth amino acid that is required to complete a single helical turn. Although the center of this helix is hydrophilic, its surface is dry and composed of the two F side chains pointing in the same direction and stabilizing the interaction between adjacent helix-like structures (Figure 1B) through T-shape π-π stacking. A similar dry aromatic zipper that excludes water molecules was reported by Marchesan and coworkers[16] for heterochiral tripeptides F-X-F, where X is an aliphatic D-amino acid. Modifying Pro to hydroxyproline in PFF significantly increased the already highly rigid PFF helical-like structures to a level similar to the stiffness of collagen matrix, although no structural alterations were detected at the atomic level.

Both studies[10,12] demonstrated high levels of symmetry[10] and hierarchy[12] control in the formation of supramolecular assemblies that are inspired by natural collagen. Leveraging this level of structural control to design reversible, dynamic, and adaptive systems holds great promise for the construction of sophisticated life-like materials.

Reversible Hierarchical Peptide Materials

Reversibility is a trait of biological matter that is critical for most physiological processes. This includes the buildup and breakdown of cytoskeletal structures, which are important for maintaining cell shape and mechanical integrity,[3] and the remodeling of the ECM, which is critical for the release of growth factors as well as for regulation of cell morphology, motility, and growth.[2] The remarkable reversibility of natural assemblies has inspired efforts to design hierarchical protein structures that reversibly assemble and disassemble.[17] In an attempt to construct a reversibly

cross-linked hydrogel, Freeman et al.[13] developed hierarchical nanofibers formed by co-assembly of a peptide amphiphile (PA) with a C_{16} alkyl tail and oligonucleotide-conjugated PA (PA-DNA). These hierarchical fibers form intertwined bundles when mixed with fibers formed by PA co-assembled with PA-DNA with a complementary sequence (Figure 1C). Stochastic optical reconstruction microscopy (STORM) and coarse-grained (CG) molecular dynamics (MD) simulations revealed that the bundling is driven by dynamic redistribution of monomers through DNA hybridization. Thus, redistribution and clustering of the PA-DNA building blocks within fibers, their cross-linking, and the resulting bundling depend on the molar concentration of DNA segments. The process can be disrupted by the inclusion of the complementary DNA sequence (intruder DNA) that competitively hybridizes with one of the PA-DNA building blocks. Bundling growth rate is dictated by the relative intra- versus inter-fiber energy, which can be controlled by molecular design. The intra-fiber energy relies on the molecular attraction within the fibers provided by β-sheet formation and hydrophobic collapse of the aliphatic segment of the PA, whereas the inter-fiber energy relies on DNA hybridization between fibers. Given that the bundling and formation of superstructures are driven by DNA hybridization, they are completely reversible, and the process can be triggered either by thermal annealing or by introduction of an intruder DNA with a flanking sequence to break the superstructures followed by an anti-intruder DNA that complements the intruder's flanking sequence. The reversible bundling approach was also applied to a non-DNA system of co-assembled PAs with oppositely charged peptide segments, giving rise to bundles of intertwined fibers.[13]

Although these studies and others reviewed elsewhere[18] demonstrate how careful building-block design provides a remarkable control of the hierarchy, symmetry, and even reversibility of peptide assemblies at the sub-nanometric level, applying this level of control across different length scales remains an extremely challenging task. In contrast to the studies reviewed thus far, whose aim was to design structures with distinctive order and hierarchy, the systems described in the next section represent a new trend of leveraging structural disorder in order to control the properties of peptide materials.

ORDERED AND DISORDERED PEPTIDE ASSEMBLIES

Intrinsically disordered proteins (IDPs) play key roles in a variety of cellular processes,[19] including signaling and, as described in the next section, the formation of liquid intracellular organelles.[20,21] Unlike the hydrophobic buried domains of globular proteins, the unstructured flexible regions of IDPs can readily self-interact or bind guest biomolecules through multivalent interactions, and thus IDPs can be employed as building blocks of bioinspired materials.[22,23] In addition, there have been recent efforts to design simpler and shorter intrinsically disordered building blocks. One example of such materials is given by peptide brushes formed by a 32-mer peptide containing lysine-serine-proline (KSP) repeats derived from the IDP human neurofilament heavy subunit (NF-H).[24] To gain insights into the electrostatic contribution associated with phosphorylation of IDPs to the peptide brushes structure, S was replaced with aspartic acid (D). The introduction of the acidic D results in condensation and collapse of the brush with increasing salts, resulting in stimuli-responsive material that reduces nonspecific adsorption of materials at the solid-liquid interface.

The IDP amelogenin, which is involved in biomineralization,[25] inspired Mata and coworkers to guide mineralization by using elastin-like polyprotein (ELP).[26]

This ELP contains the hydrophobic repeating sequence VPGIG and a modified repeating sequence with I-to-K substitution. The resulting polypeptide conformation transitions from a random coil to a β-sheet upon dehydration in dimethylformamide (DMF). This, in turn, triggers self-assembly into β-sheet fibrils through hydrophobic interactions and the formation of a membrane structure. In contrast, cross-linking of the disordered polypeptide through the K side chains results in the formation of 3D spherulites after dehydration. The membrane and 3D spherulites act as a template for the formation of ordered mineralized structures after the addition of supersaturated fluorapatite. Scanning electron microscopy (SEM) imaging revealed that the fluorapatite nanocrystals grow radially and nucleate from the spherulites or are vertically aligned on the membrane surface. These mineralized nanocrystals have an X-ray diffraction (XRD) pattern similar to that of natural fluorapatite. Tuning the level of intramolecular order can control the geometry of the mineralized structures and their mechanical properties. Thus, increasing the disorder/order ratio in favor of a random coil conformation over a β-sheet by increasing the cross-linking level promotes the formation of spherulite structures and results in a switch from concentric rings to radial prisms. Surprisingly, there was a reverse correlation between the stiffness of ELP assemblies and that of the mineralized structures that they nucleate.[26] Similarly, amelogenin, which is mostly unstructured, undergoes a disorder-to-order transition upon binding to other enamel matrix proteins or enamel crystals.[25] These results illustrate how intramolecular and supramolecular order and disorder can be used as a design principle to increase the repertoire of properties of hierarchical materials, including the geometry, architecture, and stiffness of mineralized structures.

Supramolecular order and disorder of much simpler building blocks (tripeptides) were employed for templating and controlling the formation of melanin-like materials.[27] Melanin pigments are omnipotent in nature and provide a range of protective functions but have limited technological potential because of their disordered (chemically and structurally) nature and poorly controlled laboratory-based synthesis.[28] Melanin biosynthesis is a highly controlled process, both spatially and temporally, and involves enzymatic oxidative polymerization of tyrosine. Given that a balance between order and disorder is key for the controlled formation of melanin mimics, tyrosine-containing tripeptides with varying level of supramolecular order and disorder were designed as substrate building blocks. These peptides preorganize tyrosine side chains within the supramolecular assemblies according to the differential level of order or disorder and in this way act as a template for the enzymatic oxidation and polymerization of tyrosine into pigment materials with a range of colors, particle size, radical content, and energy storage capacities.[27] Atomistic MD simulations of the tripeptide sequence isomers containing the amino acids F, Y, and D in solution accompanied by powder and single-crystal XRD, FTIR, and electron microscopy of the assemblies before and after oxidative polymerization demonstrated that the proximity of the aromatic amino acid position is critical for supramolecular order and disorder and the pigment properties. Non-adjacent aromatics restrict the formation of stacking interactions, resulting in complete disorder. When the aromatics are adjacent and situated in the N-terminal and second positions, their side chains point in the same direction in a "syn" conformation, which promotes the formation of intramolecular salt bridges and stacking, resulting in suspensions and opaque hydrogels containing disordered aggregates and nanofibers. Having adjacent aromatics in the C-terminal and second positions results in an "anti" conformation in which the side chains point in different directions. This conformation promotes the formation of intermolecular hydrogen-bonding, salt-bridge, and stacking interactions, resulting in highly ordered 1D structures. As expected, a

loss of supramolecular order was observed after oxidation and polymerization, although pigment materials formed by oxidation of the more ordered assemblies have the highest levels of morphology control, storage capacity, and conversion yields and retain the specific d-spacing typical of β-sheets. The findings demonstrate the role of the peptide sequence in encoding supramolecular order and disorder even in ultrashort peptides and show how the latter can be leveraged to direct enzymatic catalysis into pigment formation. Yan and coworkers[29] and Natarajan and coworkers[30] reported on additional melanin-like materials obtained by oxidation of non-supramolecular tyrosine-containing peptide.

These examples[24,26,27] clearly show that intra- and supramolecular order and disorder can be designed for obtaining a specific function and can be leveraged for expanding the repertoire of material properties. The level of order or disorder in structures formed by protein and polypeptide building blocks relies on the (lack of) adopted conformation as the main design principle, whereas that of materials formed by shorter peptide building blocks that do not adopt a secondary structure relies on the complexity of the intermolecular interaction networks. The latter system offers substantial opportunities for sequence-structure relationships to be established and to understand the contribution of individual amino acids to supramolecular order and disorder because the shorter the peptide, the more critical each chemical group is for the consequent supramolecular structure. Despite significant advances, the available tools for studying supramolecular order and disorder are still limited and basically amount to analyzing loss of order or lack of crystallinity and periodicity by CD, FTIR, nuclear magnetic resonance (NMR), and XRD. It will be necessary to develop them further in order to provide a means of measuring supramolecular order and disorder.

DYNAMIC PEPTIDE ASSEMBLIES OR ENSEMBLES

With the objective of mimicking the dynamic properties of biological materials, increasing effort is being invested in the design of dynamic peptide structures, which are replacing the traditional design scheme of ordered, thermodynamically stable assemblies. This rising trend originates, in part, from the emerging field of liquid-liquid phase separation of membraneless organelles[20,21] and from origin-of-life research, which fascinates cell biologists, biological and chemical engineers, polymer chemists, and physicists. Membraneless organelles (or liquid organelles) are supramolecular disordered compartments formed by complex coacervation and liquid-liquid phase separation mediated by electrostatic interactions between IDPs and other biomolecules, predominantly nucleic acids. Examples of these organelles include stress granules, nucleoli, and Cajal bodies, among others.[21] The biological role of liquid organelles in condensing and concentrating various proteins and other biomolecules has inspired efforts to use polypeptide or peptide building blocks to design liquid droplets suitable for protein and enzyme encapsulation. Here, we highlight the molecular designs underlying a few of these systems by starting from the most simplified design scheme of polypeptides as charged polymer chains and progressing to more complex schemes that introduce various chemical functionalities to create complex intermolecular networks.

Peptide Materials Based on Liquid-Liquid Phase Separation

Peptide coacervation is considered to be entropy driven[31] and mainly mediated by electrostatic interactions. Perry and coworkers showed that the strength of electrostatic interactions between coacervate-forming polypeptides could be controlled by modification of the charge patterning.[32] Polyglutamic acid (polyE) was used as

the polyanion and the polycations consisted of 25 K and 25 G moieties, where the K distribution within the polypeptides could be adjusted. The results of computational and experimental methods using Monte-Carlo-informed phase-diagram calculations, turbidity measurements, and optical microscopy indicated that the charge distribution, or more likely the charge pattern of the polycations, is critical for coacervation. Thus, modifying the pattern from alternating positive and neutral charges (alternating K and G) to blocks of charge consisting of 12 K and 12 G repeats approximately doubles the critical salt concentration for coacervation. Isothermal titration calorimetry (ITC) analysis indicated that the entropic contribution to coacervation increases when the charge pattern changes from alternating positive and neutral moieties to charged blocks and when the block length is increased. Simulations to characterize counterion condensation in the dilute phase revealed that the counterions are increasingly confined near the charged blocks and consequently gain more entropy upon release, leading to the observed increased entropy of coacervates formed by charged blocks compared with charge alternates (Figures 2A and 2B).

Perry and Tirrell[35] showed previously that manipulating the chirality of polylysine (polyK) and polyglutamic acid (polyE) is an effective tool for inducing polypeptide conformation, formation of either electrostatic interactions or hydrogen bonding, and consequently, coacervation or formation of solid-like assemblies. Racemic polypeptides that co-assembled with the oppositely charged homochiral polypeptides remained mostly unstructured, as determined by FTIR, and exclusively formed coacervates. In contrast, co-assembly of homochiral polyK and polyE—D + L, L + L, or D + D (where D and L are enantiomers)—predominantly produced β-strand solid precipitates. Interestingly, whereas the formation of the solid precipitates is mediated by intermolecular hydrogen bonding (they completely disassemble in urea), coacervate assembly and disassembly are mediated by electrostatic interactions and are completely salt dependent. Peptide chirality was previously shown by Marchesan and coworkers[16] to be critical for the assembly pathway of short peptides by dictating peptide conformation and, in turn, their packing.

Moving from electrostatically driven complex coacervation to phase separation mediated by hydrophobic interactions, Chilkoti and coworkers[36] designed stimuli-responsive, injectable materials by using ELP building blocks. The polypeptides contain both disordered regions containing the tropoelastin repeating sequence VPGXG, which are thermally responsive and promote phase separation, and ordered α-helix domains composed of polyalanine. Functionally, the polypeptides exhibit thermally dependent phase separation, which starts from coacervation and evolves into hierarchical self-assembly of viscoelastic networks. The coacervation and assembly network is thermally reversible and is controlled by the composition and mass of the disordered and ordered domains within the polypeptide.

The spontaneous liquid-liquid phase separation of certain proteins of marine organisms, including the mussel foot protein and the squid beak protein,[37] gives rise to underwater metastable adhesives that are mediated by cation-π interactions. Waite and coworkers[38] were the first to demonstrate that the mussel foot protein Mfp-3S forms coacervates through a single-component liquid-liquid phase separation (unlike complex coacervation). The protein consists of two cationic amino acids and is rich in G, the polar N, and the aromatic amino acids Y, W, and 3,4-dihydroxyphenylalanine (DOPA). This phase separation is pH and salt dependent, unlike that of tropoelastin, which is mainly mediated by hydrophobic attractions. The single-component coacervation of the mussel foot protein inspired Hwang and coworkers[39] to design liquid droplets comprising two like-charged building blocks,

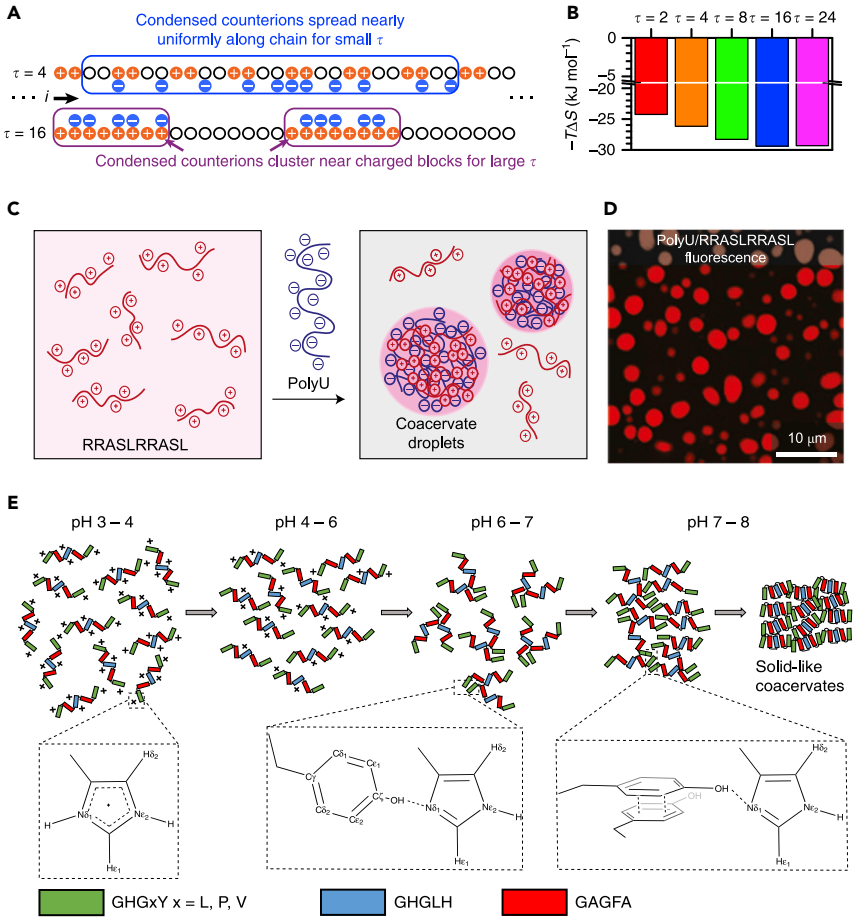

Figure 2. Liquid-Liquid Phase Separation of Peptide Building Blocks

(A and B) Schematics of the effect of charge distribution on peptide coacervation show confined counterions arranged along blocks of charge rather than homogeneous charge distribution (A), and an increase in block length (τ) increases the entropic contribution to the free energy (−TΔS) calculated computationally and corresponds to ITC data (B). Reprinted with permission from Chang et al.[32]

(C and D) Schematics of complex coacervation of RNA and a cationic decapeptide (C) and fluorescence image of the resulting droplets using a TAMRA-labeled peptide (D). Reprinted with permission from Aumiller et al.[33] Copyright 2015 Spriner Nature.

(E) Schematics of His-rich peptide coacervation involves hydrogen-bonding and stacking interactions. At low pH, the peptide is in an oligomeric form as a result of electrostatic repulsion between peptides. Upon His deprotonation, nuclei of liquid-liquid phase separation are formed by transient interactions between histidine (His) and tyrosine (Tyr). Tyr-Tyr intermolecular stacking and hydrophobic intra-molecular interactions trigger droplet formation. Reprinted from Gabryelczyk et al.,[34] licensed under CC BY 4.0.

namely the recombinant mussel foot protein and a cationic polyelectrolyte. Coacervation of this system is clearly mediated by cation-π interactions that exceed the electrostatic repulsion between the two cationic building blocks.

With the objective of understanding the molecular mechanisms underlying liquid-liquid phase separation, Miserez and coworkers[34] designed a series of peptides derived from the His-rich squid beak proteins and studied their dynamic self-assembly by using NMR spectroscopy, small-angle X-ray scattering (SAXS), dynamic light scattering (DLS), and optical microscopy. Their results indicated a role for the GHGLY repeating motif and peptide length in coacervation. They discovered that a minimum

of four repeats is critical for coacervation and that combinations of two repeats with the hydrophobic GAGFA repeats and increasing peptide length promote coacervation under a wider range of conditions, i.e., peptide concentration, salt, and pH. In addition, the results identified specific roles for each amino acid in phase separation. Introducing the hydrophobic A and F gave rise to condensed liquid droplets forming a hydrogel-like material. Solution-state NMR analysis of this structure shows a transient hydrogen bonding between Y and H side chains, whereas solid-state NMR analysis indicates that Y is locked in a rigid structure due to tyrosine-tyrosine stacking interactions. The authors proposed a multistep model for the liquid-liquid phase separation of this type of histidine-rich peptides (Figure 2C), which includes an initial deprotonation of H side chains upon pH increase, a transient histidine-tyrosine hydrogen bond, tyrosine-tyrosine intermolecular stacking, and intramolecular hydrophobic interactions, giving rise to liquid droplets.

These studies represent a clear trend whereby the molecular design of liquid-liquid phase separation of peptide and polypeptide building blocks starts from self-assembly that is purely driven by electrostatic interactions,[31,35] and progresses to materials dependent on hydrophobic interactions[36] and cation-π interactions[37,39] and, more recently, to designs that introduce hydrogen bonding and π-π stacking interactions.[34] The results of such studies and especially those with the more complex materials, suggest that the classic classification of liquid-liquid phase separation building blocks as disordered is too simplistic and even misleading considering the multiplicity of interactions involved among partially ordered domains. Moreover, these designs open opportunities to manipulate material properties at the nano-, micro-, and mesoscales by controlling peptide charge distribution, chirality, and obviously, chemical composition.

Dynamic Multicomponent Peptide Ensembles

The rich set of properties of biological structures relies on the chemical complexity of their building blocks. This complexity is observed, among other examples, in the ECM, where there is a cooperative assembly of glycosaminoglycans and protein assemblies, or intracellularly, in the protein-RNA complexes of membraneless organelles. A large number of supramolecular materials have been designed to mimic this complexity, which typically involves interactions between peptides and additional biomolecules, i.e., nucleic acids, oligo-, or polysaccharides. Although multicomponent hierarchical assemblies of such type are discussed elsewhere,[40,41] we focus on dynamic structures formed by peptide-nucleic acid ensembles, which are especially intriguing in the context of the origin of life.[42]

One of the earliest examples of dynamic peptide-nucleic acid complexes was reported by Mann and coworkers,[43] who designed the first membrane-free protocell model formed by complexation of varying lengths of cationic polyK and nucleotide triphosphates (ATP, UTP, GTP, CTP, and TTP), deoxytriphosphates (dATP and dCTP), diphosphates (ADP), or monophosphates (AMP). Interaction with nucleic acids induced the otherwise disordered peptides to adopt a helical conformation. As expected, this interaction and the partitioning of organic dyes, inorganic nanoparticles, proteins, and enzymes are pH dependent.

Liquid droplets formed by complex coacervation of phosphorylated peptide and nucleic acid were designed by Keating and coworkers.[33] Droplet formation is electrostatically driven by co-assembly of a cationic decapeptide (RRASLRRASL) and poly uracil or transfer RNA (tRNA) (Figures 2C and 2D). Similar to the findings discussed in the previous section, increasing peptide length by an additional RRASL pentamer decreases the critical coacervate concentration and promotes droplet

stability in high salt. Phosphorylation of the Ser was used to control peptide charge and, as a result, to inhibit the formation of droplets. Thus, an autonomous, reversible system of liquid droplet assembly and disassembly can be obtained by the addition and removal of phosphates with a kinase and a phosphatase, respectively. Ulijn and coworkers[44] have used phage display to screen for peptides that bind and complex with ATP. NMR spectroscopy revealed that the pentapeptide ADARYKS, which does not resemble any known nucleotide binding protein motif, interacts with the charged phosphate and the aromatic ring of ATP. The results of both NMR and atomistic MD simulations indicated highly dynamic hydrogen bonding interactions between the peptide and ATP and that the peptide adopts a variety of conformations upon ATP binding. This research, as well as the report by Miserez[34] discussed above, suggests that hydrogen bonding mediates the formation of dynamic intermediate structures during simple[34] and complex[44] coacervation.

The complexation of a 50-mer polyK and DNA was recently studied by Tirrel and coworkers,[45] revealing a remarkable difference between the liquid droplets formed by single-stranded DNA (ssDNA) compared with the solid disordered precipitates formed by double-stranded DNA (dsDNA). Fluorescence recovery after photobleaching (FRAP) confirmed the formation of dynamic structures by polyK-ssDNA complexation and the solid structures of polyK-dsDNA. The results from FTIR and melting studies using fluorescence microscopy revealed the expected electrostatic interactions between the DNA phosphates and polyK and an interesting role for nucleobase hybridization and coacervation. Annealing dsDNA into ssDNA by increasing the temperature triggered a phase transition from solid precipitates to liquid droplets through electrostatic interactions between the ssDNA and the peptide. The reverse liquid droplet-to-solid precipitates transition could be triggered by the addition of complementary DNA strands to pre-formed droplets to induce hybridization. These results highlight the role of DNA hybridization in complex coacervation and suggest that only ssDNA molecules are involved in intracellular liquid-liquid phase separation during the formation of membraneless organelles.

The increasing number of studies on peptide liquid-liquid phase separation, including those already discussed, emphasize the important role of peptide building blocks as model systems for biological phase separation underlying membraneless organelles formation or construction of structures formed by marine organisms' proteins. Although still in its infancy, this field of peptide self-assembly paves the way toward understanding how proteins interact with nucleic acid or other biomolecules to form dynamic functional intracellular assemblies, insights that are nearly impossible to obtain at the molecular level using biological systems. However, there are only a limited number of characterization techniques that can be used to obtain a molecular level understanding of the formation of dynamic structures, especially those formed by peptide ensembles. Currently, NMR and MD simulations are the most promising tools available. Thus, although we aspire to design dynamic multicomponent peptide structures that are one step closer to biological structures than their single-component counterparts, we will also have to focus on advancing the current spectroscopy and microscopy tools to accommodate these needs.

RESPONSIVE AND ADAPTIVE PEPTIDE SYSTEMS

Similar to living materials, active and adaptive synthetic materials are designed to respond to external triggers such as reaction conditions, mechanical force, radiation, magnetic field, or the addition of guest molecules by a change in their physical properties or chemical composition. This ability is incredibly important for the interface of synthetic materials with living systems, which often exist far from equilibrium

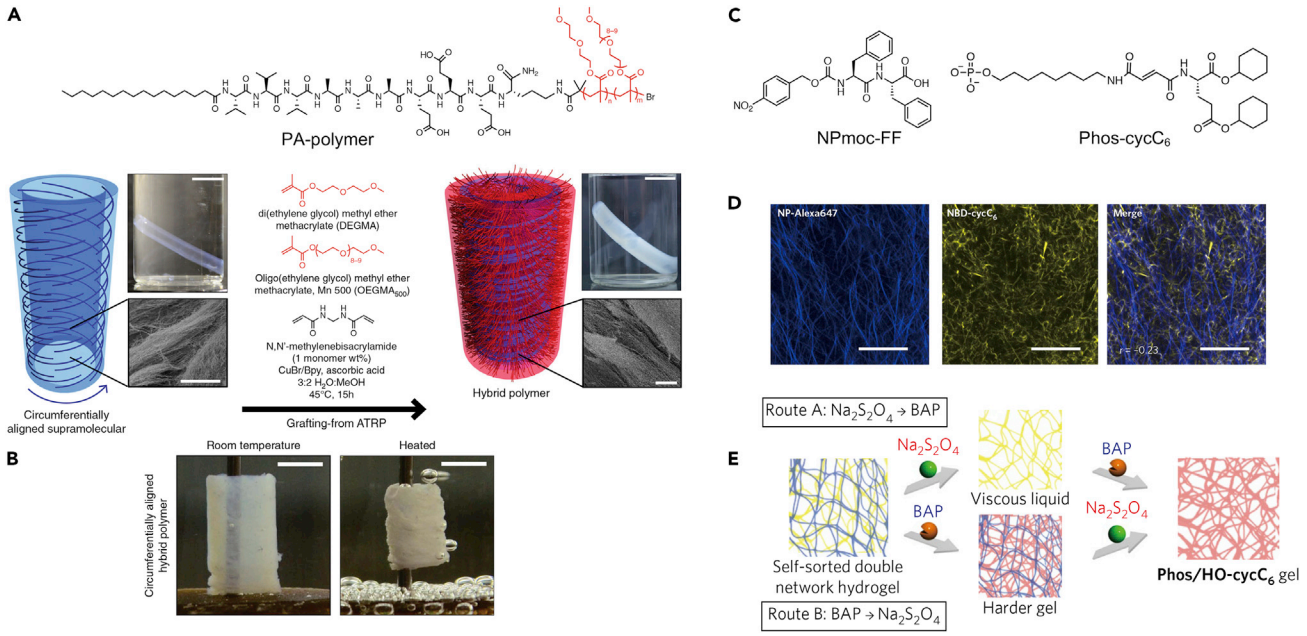

Figure 3. Thermally and Chemically Responsive Supramolecular Peptide Systems

(A) PA nanofibers circumferentially aligned by rotational shear force, resulting in gel formation in the presence of $CaCl_2$ solution. Covalent chains are grafted radially from the nanofiber surface by the PA building block after ATRP. Macroscopic images show increased gel opacity (scale bars: 1 cm), and SEM micrographs show retention of fibrous morphology (scale bars: 10 μm).

(B) Macroscopic images show anisotropic actuation of the covalent-supramolecular gel system by thermal trigger (scale bars: 3 mm).

(A and B) Reprinted from Chin et al.,[47] licensed under CC BY 4.0.

(C) Chemical structures of peptide-based and lipid-based building blocks of the self-sorting system.

(D) High-resolution confocal microscopy images of the resulting fibers (scale bars: 5 μm).

(E) Schematic representation of the chemically responsive self-sorting hydrogel system. "Route A" shows the gel-sol-gel transition following $Na_2S_2O_4$ and BAP addition, and "route B" shows the gel-gel-gel transition with the reverse order of stimuli. Color code: NPmoc-FF fibers, blue; Phos-cycC_6 fibers, yellow; Phos/HO-cycC_6 fibers, light red.

(C–E) Reprinted with permission from Shigemitsu et al.[48] Copyright 2018 Springer Nature.

and undergo continuous changes. This section will consider two main types of active and adaptive systems: (1) those that are found in thermodynamic equilibrium and are designed to change their properties only in response to external trigger and (2) materials that are inherently metastable and exist out of equilibrium.

Responsive Peptide Materials

The stimuli responsiveness of polymeric systems, including peptide-based materials designed for applications related to the medical field, was recently reviewed by van Hest and coworkers.[46] One of the major design challenges of these materials is to maintain a balance between responsiveness and stability. Stupp and coworkers[47] addressed this challenge by employing polymerization of supramolecular peptide assemblies. These structures are formed by co-assembly of PAs, which are composed of the core sequence VVVAAAEEE, with one conjugated to the bromoisobutyryl moiety, which initiates an atom-transfer radical polymerization (ATRP). When subjected to a weak shear force, the nanofibers first form a millimeter-size tubular hydrogel and are then polymerized radially from the nanofiber surface by ATRP (Figures 3A and 3C). Unlike purely covalent polymers or tubes that are only supramolecular, these hybrid tubes display muscle-like thermoresponsive anisotropic actuation and display reversible contraction and re-swelling in response to a thermal trigger such that samples are able to lift up to 380 times their dry weight (Figure 3B).

A large number of studies have described materials that respond to physical stimuli, including some that can form the dynamic assemblies discussed above, but there are only a few examples of peptide materials that respond to chemical signals. Kumar et al.[49] designed an organic semiconductor naphthalene diimide building block, conjugated to D- and L-tyrosine methyl esters, which form supramolecular sheet-like assemblies. These structures respond to individual amino acids added as chemical cues by their either supramolecular or covalent incorporation. The latter occurs through amide bond formation using the enzyme α-chymotrypsin. The enantioselectivity of the enzyme in favor of L over D gives rise to different kinetic pathways of self-assembly. The system exhibits specific chemical responsiveness to the amino acids by chemical, structural, and functional changes to the supramolecular assemblies. Thus, peptide bond formation and incorporation through the L end followed by hydrolysis of the D end is observed for aliphatic amino acids, and incorporation without hydrolysis is observed for aromatic amino acids. Addition of polar amino acids results in their supramolecular incorporation, a morphological change from sheet-like to 1D nanofibers, and hydrolysis of both L and D ends of the building block. This, in turn, affects the supramolecular chirality as analyzed by CD, where E gives rise to a transient left helix, L leads to a delayed right helix, and aromatics result in no chirality. The helicity direction is controlled by the chirality of the amino acid that is found in the fiber core so that replacing the chirality at the core by using a D-enantiomer of L (^{D}L) produces a left-hand helix. The supramolecular incorporation of E also results in time-dependent conductivity of the nanofibers, demonstrating how structures that respond to chemical signals *in situ* can be leveraged for transient functionality, a property that is critical for implementation in biological systems.

Hamachi and coworkers[48] described a self-sorting multicomponent hydrogel system in which each component responds to a different stimulus, resulting in changes to material properties that are sensitive to the stimuli sequence. The system consists of a peptide-based building block (NPmoc-FF) and a lipid-based building block (Phos-cycC_6) that assemble into two distinct supramolecular nanofibers (Figure 3D). NPmoc-FF fibers disassemble following addition of a reducing reagent ($Na_2S_2O_4$), resulting in a gel-to-sol transition, whereas addition of bacterial alkaline phosphatase (BAP), which hydrolyzes organophosphates, triggers structural modification of Phos-cycC_6 fibers, resulting in a sol-to-gel transition. This unique mechanical-responsiveness was leveraged to obtain a controlled release of proteins, where the protein release is triggered by $Na_2S_2O_4$ addition and can be shut off by the addition of BAP. Interestingly, reversing the order of the stimuli produced a gel-to-gel transition, where the gel becomes stiffer after BAP addition and then softer after $Na_2S_2O_4$ (Figure 3E).

Out-of-Equilibrium Peptide Systems

The emerging field of systems chemistry[50] and its relevance to the origin of life has fueled recent developments in the design of peptide materials with transient architecture and functionality. These developments include self-replicating peptides,[51,52] dynamic chemical networks,[53] out-of-equilibrium peptide-based materials,[54,55] and dissipative self-assembly in dynamic droplets.[56] All these materials were recently extensively reviewed by Otto,[57] Ashkenasy and Lynn,[50] and Boekhoven and coworkers[58] and are therefore not discussed in this Perspective.

SUMMARY AND OUTLOOK

After more than two decades of peptide self-assembly research, we now understand how to build peptide structures with tailored architectures that self-assemble at will. It is

therefore about time to start adopting a "systems materials" approach and design active, life-like materials from peptide building blocks by considering elements from the living world such as out-of-equilibrium systems, feedback loops, and communication between components in multicomponent systems. In addition, we should also adopt elements from the manufacturing processes of biological materials, including compartmentalization and confinement of reactions, as has been well noted by Grzybowski and Huck.[59] The use of peptide assemblies to compartmentalize and confine chemical reactions is far from exhausted, and the exploitation of peptide building blocks, especially relatively short ones, to construct dynamic droplets is in its infancy and is expected to be developed in the near future. However, with all the progress that has been made, it is still not trivial to predict liquid-liquid phase-separating peptide sequences from first principles. Whether machine learning can aid in a prediction and automation of peptide building blocks that includes considerations of order and disorder, dynamic self-assembly and out-of-equilibrium systems is yet to be explored. Another major challenge in the design of dynamic and out-of-equilibrium peptide systems is how to apply the molecular design at the nanoscale to the mesoscale and macroscale. In order to design and analyze materials on different scales, it will obviously become necessary to combine materials science approaches with those of supramolecular chemistry.

There is no doubt that supramolecular peptide materials have tremendous potential in healthcare applications, as indicated from cell-culture[60] and animal-model studies of thermodynamically stable, hierarchical peptide assemblies designed for applications in drug delivery or tissue regeneration.[4,6] Yet, the interface of dynamic assemblies, multicomponent ensembles, and far-from-equilibrium adaptive materials with living systems has not yet been rigorously explored; therefore, the stability, integrity, efficiency, and responsiveness of these materials in complex biological environment are not clear yet. Collaborative efforts between supramolecular chemists and biologists or bioengineers will be critical for the implementation of these dynamic peptide materials in living systems for practical and successful medical applications.

Finally, the more complex the design approach is and the more life-like elements are included, the more challenging it is to characterize the resulting materials—heterogeneous mixtures and dynamic multicomponent ensembles cannot be analyzed with the same tools as homogeneous crystalline materials. This brings us to the biggest challenge we currently face on the way to building living peptide matter: the need for advanced tools, both experimental and computational, that will allow us to characterize complex and dynamic materials at sufficient temporal and spatial resolution to provide a molecular-level understanding of how these materials are built.

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