CHEMICAL REVIEWS

pubs.acs.org/CR

Review

Peptide-Based Supramolecular Systems Chemistry

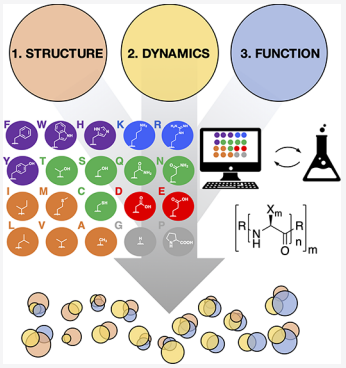
Fahmeed Sheehan,[#] Deborah Sementa,[#] Ankit Jain,[#] Mohit Kumar,[#] Mona Tayarani-Najjaran, Daniela Kroiss, and Rein V. Ulijn*

Cite This: *Chem. Rev.* 2021, 121, 13869−13914

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: Peptide-based supramolecular systems chemistry seeks to mimic the ability of life forms to use conserved sets of building blocks and chemical reactions to achieve a bewildering array of functions. Building on the design principles for short peptide-based nanomaterials with properties, such as self-assembly, recognition, catalysis, and actuation, are increasingly available. Peptide-based supramolecular systems chemistry is starting to address the far greater challenge of systems-level design to access complex functions that emerge when multiple reactions and interactions are coordinated and integrated. We discuss key features relevant to systems-level design, including regulating supramolecular order and disorder, development of active and adaptive systems by considering kinetic and thermodynamic design aspects and combinatorial dynamic covalent and noncovalent interactions. Finally, we discuss how structural and dynamic design concepts, including preorganization and induced fit, are critical to the ability to develop adaptive materials with adaptive and tunable photonic, electronic, and catalytic properties. Finally, we highlight examples where multiple features are combined, resulting in chemical systems and materials that display adaptive properties that cannot be achieved without this level of integration.



CONTENTS

1. Introduction 13870
2. Peptide Designs for Order/Disorder 13872
  2.1. Sequence Dictates Molecular Conformation and Supramolecular Assembly 13872
  2.2. Static and Stable Structures with High Stiffness 13872
  2.3. Hydrogels with Varying Mechanical Properties 13873
  2.4. Flexible Peptide Structures and Biomolecular Condensates 13873
  2.5. Interplay of Flexible and Rigid Peptide Nanostructures 13875
3. Compositional Complexity: Coassembly and Interfaces 13876
  3.1. Cooperative and Disruptive Assemblies 13876
  3.2. Interface Stabilization 13879
  3.3. State of the Field: Order and Disorder in Supramolecular Peptide Systems 13880
4. Structures in and out of Equilibrium 13880
  4.1. Adaptive Equilibrium Structures 13880
  4.2. Nonequilibrium Structures 13883
    4.2.1. Fueled and Transient Systems 13883
    4.2.2. Chemically Fueled Systems 13884
    4.2.3. Enzymatically Regulated Systems 13885
    4.2.4. In Situ Regulation in Dynamic Systems 13886
    4.2.5. Spatially Controlled Assembly 13887
  4.3. Chemomechanical and Mechanochemical Supramolecular Responsiveness 13888
  4.4. State of the Field: Spatial and Temporal Control in Supramolecular Peptide Systems 13890
5. Functional, Complex Peptide Nanostructures 13890
  5.1. Photonic, Electronic, Protonic, and Ionic Conduction and Communication 13891
    5.1.1. Supramolecular Self-Assembly of Aromatic Short Peptides for Biophotonic and Electronic Wires 13892
    5.1.2. Peptides with Chromophoric Conjugation 13895
    5.1.3. Light-Harvesting Properties of Peptide Nanostructures Functionalized with Aromatic Groups 13897
  5.2. Catalysis and Reactivity 13898
  5.3. Multicomponent Functional Supramolecular Systems toward Metabolic Systems Chemistry 13901
  5.4. State of the Field: Functional Supramolecular Peptide Systems 13902

Special Issue: Molecular Self-Assembly

Received: January 30, 2021
Published: September 14, 2021

6. Conclusions and Future Directions 13903
Author Information 13904
Corresponding Author 13904
Authors 13904
Author Contributions 13904
Notes 13904
Biographies 13904
Acknowledgments 13905
References 13905

1. INTRODUCTION

All currently known life forms share the same conserved sets of building blocks and interactions. Of the classes of biomolecules, peptides and proteins produced from 20 amino acids are arguably the most versatile, giving rise to structures and functions that orchestrate the chemistry of life. When organized into oligomers or polymers with specific sequences, these chemically simple amino acids give rise to structures with folded and flexible domains, giving rise to wide-ranging functionalities including molecular recognition, self-assembly, catalysis, fluorescence, shapeshifting, and more. Furthermore, at the next level of complexity, the dynamic interactions between collections of these functional biopolymers lead to complex systems, resulting in Nature's remarkable feats of molecular engineering, including motility, morphogenesis, sensing, growth, and healing.

Contemporary understanding of the organizational principles in space and time of complex biomolecular ensembles is limited. Beyond much progress in the elucidation of sequence-to-structure-to-function relationships in individual biomolecules, leading to powerful design rules, a next frontier is to elucidate how complex adaptive functions emerge through a systems-level consideration of large numbers of interacting biomolecules. Analogous to biological systems, it is increasingly apparent that integration of multiple different interacting biomolecules provides routes to system-level functions that are not accessible by using the more traditional minimalist approach. For such systems, functionality emerges not only from primary sequence and its supramolecular self-assembly but as a consequence of adaptive and multifaceted interactions between numerous building blocks and changes in these interactions over time. Systems chemistry[1−7] has been defined as the science of studying networks of interacting molecules, to create new functions from a set (or library) of molecules with different hierarchical levels and emergent properties. Examples that make use of holistic, systems-level design approaches, include self-selection of complex and self-folding structures,[8] self-replication of supramolecular structures,[9] emergence of positive-feedback catalytic functions with through recruitment of cofactors,[10,11] reaction-diffusion catalytic systems,[12] chemical oscillators, and designed clock-reactions.[13] It has been recognized that peptides are particularly useful as components of designed complex chemical systems.[14] Typically, the approach involves reducing (compared to their biological counterparts) the complexity of individual building blocks (designed peptides instead of proteins) and study how their multicomponent interactions give rise to new structures (Figure 1A (1), how their dynamics (2) change over time or in response to applied stimuli, and ultimately give rise to designed properties and functions (3).

The field of supramolecular peptide systems integrates systems chemistry[6,14−17] with peptide nanotechnology.[15,18]

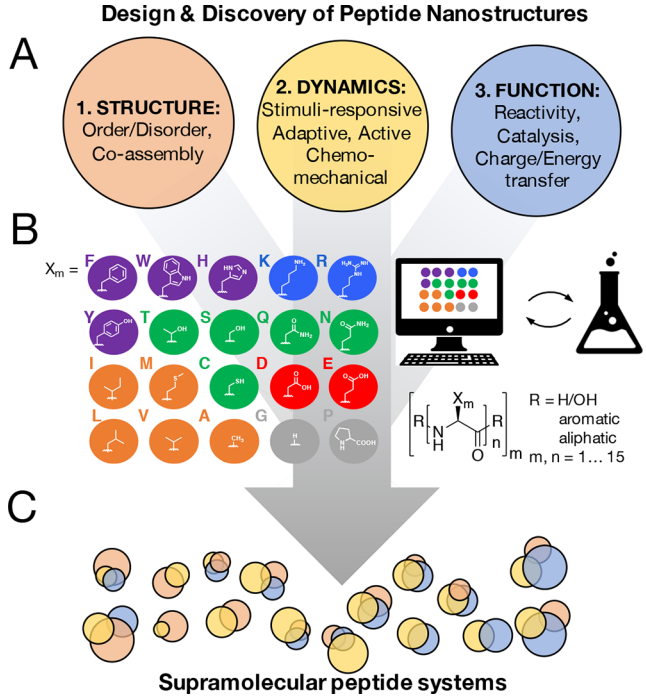


Figure 1. Peptide-based supramolecular systems chemistry. (A) Outline of the topics covered in this review. (B) Color-coded amino acid building blocks which build up to functional peptides, discovered through computation and experimentation. (C) Schematic representation of peptide systems that combine structure, dynamics, and function.

Peptide and protein nanotechnology exploits insights of biomolecular folding and assembly to create materials and structures with designed functions that are typically much simpler than their biological counterparts. In this field, the ultimate aim is typically to simplify or repurpose biomolecular structures as components of nanoscale devices and materials for biomedical, environmental, and technological applications.[17−20] This review addresses efforts that start to bridge minimal peptide nanotechnology design, typically precisely understood, but with limited functionality, and systems-level complex functions observed in biological systems. We review literature examples that show integration of multiple components to produce rational and tractable systems with designable properties that rely increasingly on the organization, in space and time, of multiple components.

While breaking up complex systems into components is inherently not true to the systems thinking, in order to produce a review that is logically organized, we have decided to divide the review into the following parts according to the emphasis of the examples that we discuss. They are, first, how multicomponent peptide systems give rise to multicomponent structures. A key focus in this section is the balancing of strong folding, stable structures, and adaptivity and flexibility (section 2). When interactions are too strong, irreversible phase separation and aggregation can occur, and when they are too weak, favorable folds or interactions are insufficiently stabilized to promote the emergence of supramolecular functions. Thus, what is crucial is the balancing and rebalancing of components that favor supramolecular order versus disorder. Indeed, the role of dynamic, induced, or disordered motifs in the functioning of proteins that navigate a shallow energy landscape has become increasingly clear[15,21,22] and has started

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

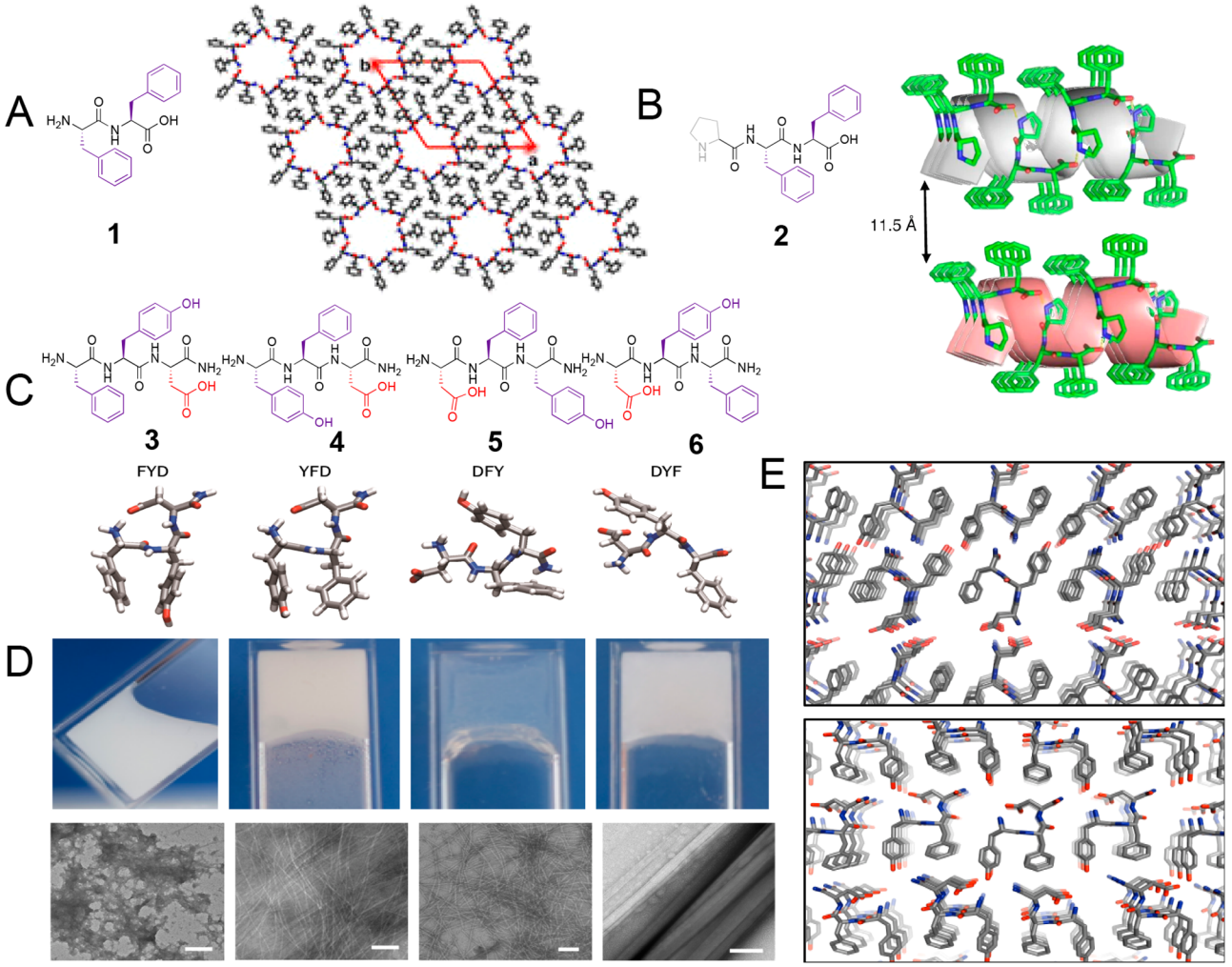


Figure 2. Supramolecular crystals produced from short peptides. (A) FF and its crystal structure. The unit cell is highlighted in red, encapsulating most of the aromatic zipper. (B) PFF and its helical structure. (C) Amidated tripeptides FYD, YFD, DFY, and DYF along with their preferred molecular conformation as obtained by molecular dynamics simulations. (D) Macroscopic appearance of the above structures assembled in water, with corresponding TEM images, showing the different structures formed by the isomers. Scale bars 100 nm. (E) Crystal structures of DYF (top) and YFD. (A) Reproduced with permission from ref 58. Copyright 2016 Springer Nature. (B) Reproduced with permission from ref 59. Copyright 2019 Springer Nature. (C–E) Reproduced with permission from ref 40. Copyright 2019 AAAS.

to make its way into peptide nanotechnology. New disordered structures are being designed, such as biomolecular condensates, which complement rigid and ordered structures.[23,24] Section 3 covers integration of multiple different types of peptide building blocks or components where properties are observed that are not achievable with single building blocks, including coassembly and assembly at interfaces. Besides structural and spatial aspects, supramolecular peptide systems are characterized by rich, dynamic, and temporal features. Remarkable active and adaptive properties that are not accessible without this level of integration have been demonstrated (section 4). We then focus on systems that display new properties and functions that arise from their multicomponent and dynamic properties, discussing emergence of properties such as reactivity, catalysis, charge, and energy transport. Finally, we will discuss systems that show integration of structure, dynamics, and functions in their designs, where we are starting to see the power of *complex chemical systems* of multiple reactive biomolecules to achieve new functions (section 5).

In this review, we focus on a subset of the literature where building blocks are short peptides and short peptide derivatives (typically less than 15 amino acids in length) to create supramolecular functions with increasing levels of integration and complexity. These peptide designs themselves are often not of biological origin but specifically designed to achieve desired material properties. The peptide designs may be linear or cyclic, and they may be functionalized with aliphatic or aromatic groups in order to influence their self-assembly properties and/or to introduce chemical functionality. Examples are also included that incorporate reversible covalent conjugation or oligomerization steps, so that both collective covalent and noncovalent exchange of bonds are possible. The assembly rules for these types of short peptide building blocks have been developed using both experimental and computational approaches,[25] and we refer to a number of recent reviews for details on the basic sequence-to-structure relationships for aliphatic peptides,[26] short peptides, and aromatic peptide amphiphiles,[27−31] as well as cyclic structures.[32,33] We use the single letter code (Figure 1B) throughout the review. Unless otherwise noted, peptide termini should be assumed to be

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

unprotected ($-NH_2$ and $-COOH$). Biomedical applications have been reviewed extensively elsewhere and are beyond the scope of the current review.[34−36] We recognize that it is not possible to do this fast-moving field full justice in the scope of one review, but we hope to have covered some of the main developing themes in this field and are able to inspire new thinking in this area. While we aim to cover the key recent developments in this field as much as possible, we do apologize for the omission of many works that we could not include in the current review.

2. PEPTIDE DESIGNS FOR ORDER/DISORDER

Many functional proteins in living organisms spontaneously fold into a stable 3-D structure as dictated by their amino acid composition. However, intrinsically disordered proteins (IDPs) are increasingly recognized as being prevalent in eukaryotes, characterized by regions that do not have a specific secondary or tertiary structure but rather fluctuate between various conformations. In the context of short peptides, the same understanding applies: ordered peptides typically self-assemble predictably into a rigid structure, whereas disordered peptides either assemble weakly or not at all. IDPs typically have highly repetitive sequences of low complexity, typically rich in "disorder-promoting" amino acids. The prevalence of amino acids in (dis-)ordered regions in the Protein Data Bank has been mapped[37] and can inform design selection of short order or disorder promoting regions. Literature examples illustrate that even simple peptides are incredibly versatile in regulating order/disorder and consequent mechanical properties, with the entire range of structures from crystals, gels, liquid condensates, and solutions accessible. We will discuss the latest developments in sequence, from stiff crystals to free-flowing liquid condensates. We note that these peptide nanostructures cover the full range of mechanical properties observed both in nature and in synthetic materials, up to that of bone and Kevlar. We also refer to reviews focusing on assessment of mechanical[38] and structural properties[39] of peptide-based soft matter.

2.1. Sequence Dictates Molecular Conformation and Supramolecular Assembly

Lampel et al. studied the sequence-dependent mechanical properties of six sequence isomers of (C-terminal amidated) tripeptides composed of D, F, and Y.[40] From this analysis, it was found that the peptide sequences could be grouped in pairs depending on the positioning of the aspartic acid, with YDF-NH_2 and FDY-NH_2 separating the aromatic dyad motif and consequently not showing self-assembly, FYD-NH_2 (**3**) and YFD-NH_2 (**4**) forming amorphous assemblies and nanofibrous gels, while DFY-NH_2 (**5**) gave rise to nanofibrous gels and DYF-NH_2 (**6**) to crystals (Figure 2C,D). Single crystal and molecular dynamics analyses reveal that each of these peptides have specific preferred conformations which influence the availability of different intermolecular interactions (Figure 2E). In DYF-NH_2, the aromatic groups have a dihedral angle of roughly 140° to each other, extending each molecule to produce a beta-sheet-like stacking within the crystal sustained by amide−amide H bonds laterally and tyrosine−tyrosine H-bonds vertically. YFD-NH_2 instead has an aromatic dihedral angle around 30°, with the aromatic groups oriented on the same side and aromatic interactions supporting the structure internally and laterally instead. The work represents a culmination of the progress in peptide engineering, with sequence isomers of a tripeptide assembling into crystals, gels, and amorphous assemblies. Remarkably, single peptide molecular dynamics simulations to calculate spectroscopic and crystallographic data aligned well with these preferred orientations, suggesting predictive potential based on the peptide sequence (Figure 2C). There are more and more examples in the literature of computationally guided sequence design,[41−43] leaving us hopeful that the deliberate design of ordered/disordered peptides is not far out of reach.

We note that single- or few amino acid mutations in self-assembling peptide sequences can lead to significant difference in the order/disorder of peptide sequences and consequent self-assembly behavior. Son et al. demonstrated a range of related peptide sequences (roughly in the form of aromatic−aliphatic-charged) that were designed as enzyme-responsive nanomaterials. It was found that an A → P mutation in the aliphatic region resulted in the formation of fibers versus spherical aggregates with no apparent structure in a series of decapeptides. Introducing a mutated P into self-assembling peptides is known to introduce kinks into the chain.[44] In this study, the P-mutated peptides were cleaved both faster and more completely by a matrix metalloprotease (MMP-9). This is thought to be due to the disordered domain introduced by the mutation, which has enhanced accessibility compared to the rigid fiber structure of the unmutated peptide.[45]

2.2. Static and Stable Structures with High Stiffness

Although peptidic supramolecular structures are largely held together by the collective contributions of many relatively weak intermolecular forces, their combined effect can give rise to some astonishing macroscale mechanical properties that approach those of the stiffest known natural and synthetic materials. Key features that control the stiffness are supramolecular interactions between building blocks, relative growth in different dimensions to favor 1D, 2D, or 3D structure growth, and the tendency for hierarchical assembly.

Single aromatic amino acids (F) have been observed to self-assemble into rigid fibers, giving rise to Young's moduli near 6 GPa[46] due to the favorable packing involving both electrostatic stabilization and aromatic stacking. FF (**1**) nanotubes have been established to be even more rigid, with AFM experiments determining their Young's modulus to be ∼19 GPa[47] and a beam-bending model calculating stiffnesses as high as 27 ± 4 GPa.[48] This rigidity has been attributed mainly to dispersive forces, primarily arising from the dense aromatic zipper motif found in the nanotubes (Figure 2A).[49] A recent paper showed that FF-derived molecules that were optimized to form denser aromatic packing were even more rigid.[50] We refer to several instructive reviews on FF and FF-inspired molecules for further details.[51−53]

Bera et al. described a peptide that combines features of FF with those of collagen. The tripeptide PFF (**2**) self-assembles into novel crystalline α-helical stacked sheets comprised of a hydrophilic center and hydrophobic phenylalanine-rich outside (Figure 2B).[54] Multiple fibers bundle together to form a dry aromatic zipper. Of note is the fact that substitution of the proline for hydroxyproline, normally known to substantially alter the higher-order structures of self-assembling peptides,[55,56] retains the unique α-helical stacking of the parent peptide while adding an additional hydrogen bonding site between fibers. This extra hydrogen bond further strengthens bundling, leading to a Young's modulus of 102 GPa comparable to a collagen matrix and distinctly higher than

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

PFF at 44 GPa. The peptide was derived from the computational mapping of all 8000 possible tripeptides,[57] which gave rise to guiding principles for tripeptides to favor self-assembly, in particular dyads of aromatic amino acids flanked by charged residues. It is worth noting that PFF was identified as the tripeptide with the highest self-assembly propensity.

2.3. Hydrogels with Varying Mechanical Properties

While many short peptides form micrometer scale fibers, tubes, or crystals, others preferentially form thinner fibers, which, provided that they display surface functionality that favors interactions with the solvent, entangle to form gel-phase materials. It is known that these supramolecular gels often represent metastable structures due to the restricted molecular dynamics in the gel state. In other cases, the gel state is thermodynamically favored. Self-assembling peptides are typically amphiphilic, thus giving rise to a combination of solvophobic and solvophilic moieties where a level of solvent exposure at the nanostructure surface is favorable. Sasselli et al. introduced a simple packing model, based on prisms with faces of different nature (solvophobic and solvophilic) and variable interaction parameters, to represent amphiphile self-assembly. They demonstrate that, by tuning "self" or "solvent" interaction parameters, either the 1D fiber or 3D crystal may form.[60] Therefore, the amphiphilic nature and consequent balance between self-assembly interactions and solvent interactions dictates gelation versus crystallization. Upon comparing a number of related FF derivatives functionalized at the N terminus with a variety of capping groups,[61] fluorenylmethoxycarbonyl (Fmoc)-FF was shown to form much thinner nanofibers that entangle to form hydrogels.[62,63] These gels show very high, yet tunable storage moduli, ranging from 1 kPa up to 20 kPa based on preparation conditions and pH.[64]

On the basis of the insight into the required balance between self-assembling and polar, solvent-facing groups in supramolecular hydrogelation, Frederix et al. performed an extensive combinatorial coarse-grained molecular dynamics analysis of the entire tripeptide sequence space.[57] These simulations provided insights into self-assembly propensities via quantifiable aggregation propensity scores, and when these were analyzed in terms of the two seemingly contradicting qualities of low hydrophobicity (through log P) and high self-assembly propensity, a number of new tripeptides could be identified and experimentally verified to form hydrogels, including KYF, KYY, KFF, and KYW. KYF in particular has been shown to have a high storage modulus around 1200 kPa upon biocatalytic inducement of self-assembly.[65] A library of pentapeptides that also contained the KYF motif (variants of KYFIL) also formed hydrogels that demonstrated storage moduli between 10 and 17 000 Pa with subtle variations in the amino acid sequence.[66] K, F, and L had to be conserved to maintain gelation. Replacing Y for A induces an apparent pK_a shift and increased electrostatic repulsion between peptides. Changing residues at the fourth position altered gel strength as a function of hydrophobicity or, in the context of self-assembling peptides, β-sheet forming propensity, with more β-sheet forming tendencies leading to more stable gels.

Illustrating that peptides of identical composition (so identical overall hydrophobicity) but different sequence can give rise to differential self-assembly and gelation properties, Clarke et al. tested three different pentapeptide sequence isomers of D_2I_3–NH_2, isoleucine chosen for its propensity to form β-sheets and aspartic acid to enhance solubility of the peptide.[67] The three pentapeptides tested were DIIID (terminal charged groups), DDIII (N-terminal charged), and IDIDI (alternating charges), each with an amide C-terminal. Each of the pentapeptides formed hydrogels at a 1% and 2% concentration by weight, with the higher concentration greatly enhancing storage moduli. While IDIDI formed high-aspect ratio fibers, there was limited entanglement in the fibers compared to the other two peptides. Greater entanglement led to stiffer gels: 1% IDIDI had a storage modulus of 2 kPa, while 2% DDIII was much stiffer at 200 kPa. These values are similar to those found in soft tissues. It is thought that the charged groups on the termini aid in interfiber H-bonding, while the consecutive isoleucine residues promote greater β-sheet entanglement.

In a peptide amphiphile (PA) structure[68] with a β-sheet forming middle region, the specific sequence and choice of residues can affect the mechanical stiffness of the resultant hydrogel and it could be correlated to the nature and positioning of the β-sheet forming region. Pashuck et al. determined three design trends that allowed for variation of mechanical stiffness: increasing length of the β-sheet forming region, introducing more residues with β-sheet forming propensity (in this case, valine vs. alanine), and placing these β-sheet forming residues closer to the hydrophobic tail of the amphiphile rather than near the head for better alignment of H-bonding residues in the β-sheet.[69] To determine these trends, the authors designed a series of peptides with 16-carbon tails, three glutamic acid residue heads, and variable β-sheet regions containing valine or alanine. V_4A_2 had the highest storage modulus at nearly 100 kPa. A_3V_3 had the lowest at under 5 kPa. Thus, the rules proposed can be exploited to control stiffness over scales of several orders of magnitude.

Besides the de novo designs and β-sheet-based structures described so far, collagen, the primary structural protein in the extracellular matrix and by far the most prevalent protein in animals by weight, has inspired a vast body of research trying to create collagen-like mimics with similar function. Much of this research is inspired by the predictable molecular structure of collagen, which contains an abundance of tripeptide repeats of the form Gly-Xaa-Yaa, where X and Y are frequently proline and hydroxyproline. GPO (O: hydroxyproline) is therefore the target of many studies, and successful collagen-like assemblies have been created using (GPO)repeats.[70,71] In an effort to create supramolecular variants composed of much shorter peptides, Ghosh et al. studied the self-assembly propensity of minimalistic Fmoc-GPO. While the tripeptide GPO dissolves in water, Fmoc-GPO was found to readily crystallize into a left-handed helix, resembling a polyproline II architecture as seen in single collagen helices.[72] X-ray analysis of the crystal structure seems to suggest that the interactions governing interhelix interactions are still present in Fmoc-GPO. Uniquely, Fmoc-FF coassembled with Fmoc-GPO forms a hydrogel where the dominant structure appears to be a polyproline II-type helix rather than the β-stacked structure commonly found in FF-based assemblies. A 1:2 coassembly of Fmoc-GPO and Fmoc-FF have a storage modulus of 24 kPa, a 3-fold increase over the storage modulus of Fmoc-FF at 8 kPa.

2.4. Flexible Peptide Structures and Biomolecular Condensates

One key structural differentiator between folded and disordered peptide structures, beyond amino acid composition,

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

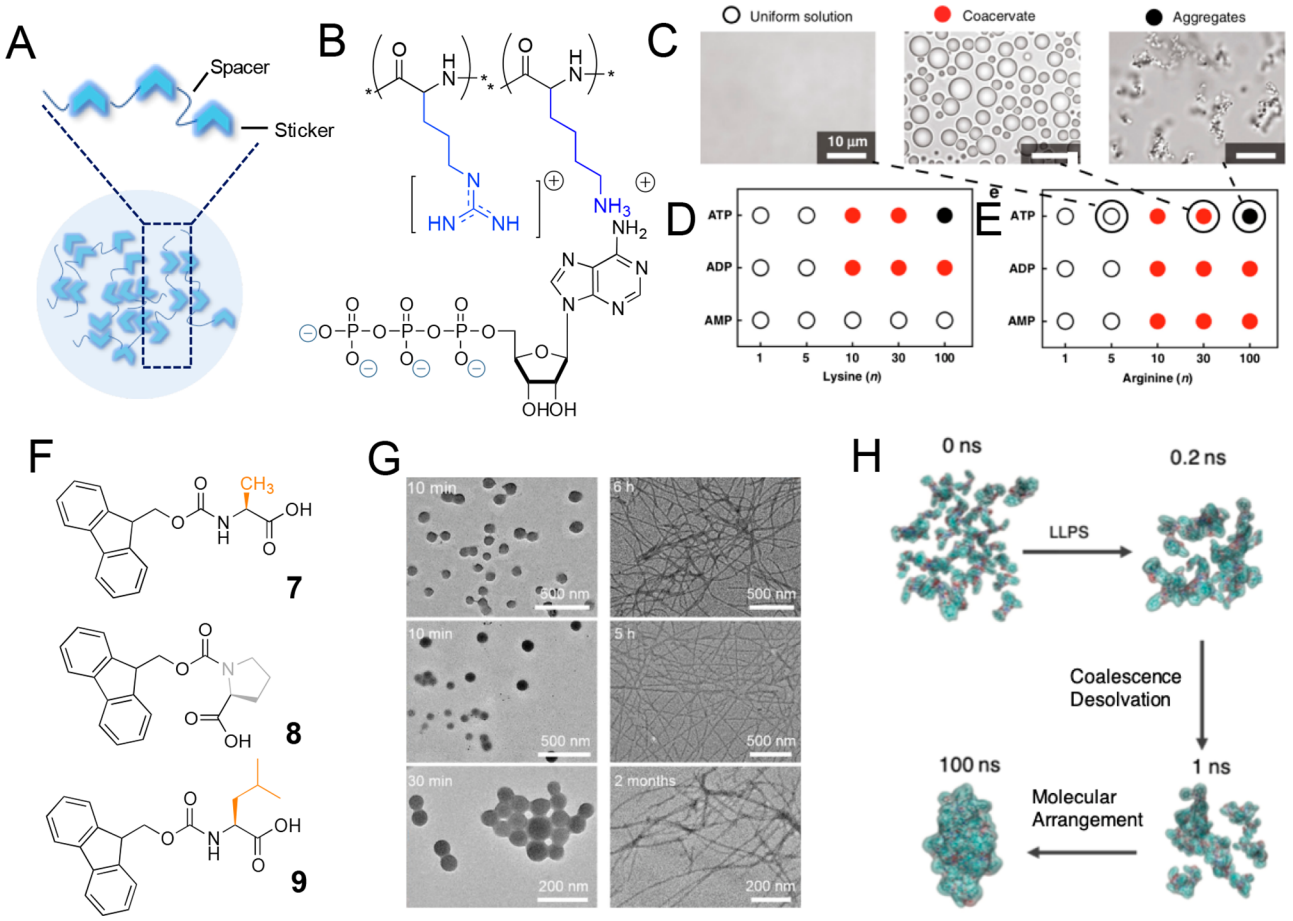


Figure 3. Peptide-based condensates. (A) Schematic illustration of a sticker-and-spacer containing construct associated with liquid−liquid phase separation (simple coacervation). (B−E): (B) Structures of negatively and positively charged moieties used as polyanions and polycations involved in coacervates formation. (C) Optical microscope images of uniform solutions (white circles), coacervates (red circles), and aggregates (black circles). Libraries of combinations of (Lys)1−100 (D) and (Arg)1−100. (F−H): Structures of Fmoc-Ala, Fmoc-Pro, Fmoc-Leu (F), and TEM images of their nucleation process (G). (H) All-atom molecular dynamics simulations of the nucleation and growth of Z-FF nanofibrils. (B−E) Reproduced with permission from ref 81. Copyright 2020 Creative Commons. (F−H) Reproduced with permission from ref 82. Copyright 2019 John Wiley and Sons.

is that the former are typically dominated by conserved hydrogen bonding motifs leading to specific secondary and tertiary structures, while the latter transiently interact through patterns of amino acid side chain functionality that are separated by unstructured domains (so-called *stickers* and *spacers*, Figure 3A).[73] As a consequence, complex combinatorial interactions involving electrostatics, $\pi-\pi$ and cation$-\pi$ interactions and side chain hydrogen bonds may be involved.[74] Methods for the design, discovery, and analysis of biomolecular functionality with structural agility are still in their infancy, and concrete design principles are largely elusive.[21,22]

Condensates and disordered peptide structures navigate shallow energy landscapes. This refers to the existence of multiple energy states with similar energies and low barriers among them; thus, they interconvert rapidly through thermal fluctuations. This is characteristically opposite to the thermally equilibrated deep energy wells of aggregative fiber-forming (hydrogelating) species and crystals discussed in the previous sections. Such shallow energy landscapes may arise from designs that have features in common with intrinsically disordered domains found in protein structures. Disordered domains are classified as such due to lack of a defined secondary or tertiary structure. These shallow energy landscapes of disordered domains bestow upon them higher adaptability to environmental change, which is of utmost importance while designing peptide-based materials with adaptive properties. Apparently, they also came with an evolutionary advantage, as evident from the fact that most eukaryotes have higher than 32% intrinsically disordered proteins (IDPs) while most prokaryotes have 29% or lower.[75,76] Perhaps in the case of IDPs, complexity begets flexibility. The resultant disordered regions have various functionalities, including scaffolding and recruitment of binding partners, regulation of post-translational modifications, and conformational adaptability. Following from the recent explosion in research activities in the area of biomolecular condensates,[77,78] there is interest in the development of minimalistic mimics of flexible structures, with a number of recent examples highlighted here.

Biological ligand recognition typically involves interconversion of disordered and ordered structures triggered by introduction of ligands, via an "induced-fit" mechanism, where peptides rearrange upon complexation to accommodate binding ligands. Designing minimalistic compounds with such adaptability is challenging, as they involve subtle conformational changes that are energetically similar. Kroiss et al.

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

adapted the phage display technique to identify peptides as short as heptamers that could bind to small molecules, as exemplified by ATP and UTP. In particular, they identified the unstructured peptide ADARYKS that was shown to have modest, sequence specific binding affinity for ATP,[79] and later, using an integrated experimental screening and molecular modeling approach, the peptide KAIHPMR-NH_2 was demonstrated to form a stable, induced loop upon recognition of UTP.[43] Peptide sequences selected using phage display were refined computationally and correlated with experimental K_D values down to sub-mM.

Unstructured functional peptides may form condensates that engage in the formation of spatially confined compartments in a phenomenon known as coacervation, as has been known in polymer chemistry since the 1920s,[80] or liquid−liquid phase separation (LLPS). This phase separation occurs generally when polymers or proteins interact with themselves (simple coacervation) or with oppositely charged oligomers (complex coacervation), where they start demixing with the surroundings via entropic release of water. This demixing results in a separate phase that is usually still "liquid" with a higher concentration of its components than in the bulk solution.

There is an emerging interest in developing designed peptide-based analogues of such systems, with the objective to introduce dynamic features that are not accessible using conventional peptide design approaches that typically focus on stable folds. While the use of short peptides in this area is still rare, a number of synthetic systems have been designed to study the rules that underpin the formation of these phase-separated compartments, in addition to their ability to recruit, concentrate, and activate biomolecules within these new phases.[83] It is perhaps prudent to mention that unique strategies to understand dynamics of water in aggregated and condensed phases[84−86] and the governing interactions is necessary to elucidate the internal workings of a condensate.[87] Molecular association is a driving force in coacervation and is dependent on a number of interactions. These interactions include a diverse set of electrostatic, $\pi-\pi$, cation$-\pi$, and H-bonding interactions, all encoded in an equally diverse palette of amino acids sequences.[88]

A plethora of information can be derived from in vivo counterparts for designing biomaterials in vitro. A recent example of this is Abbas et al.'s minimal approach on conventional stickers and spacers[89] model for protein coacervation.[90] These authors have designed a unique small peptide-based motif that spontaneously separates into a liquid phase in water. The design of the molecule is essentially composed of two dipeptides (stickers) on either end with N-termini connected via a variety of possible spacers, including cystamine, 2,2′-(ethylenedioxy)bis(ethylamine) and 1,4-bis(3-aminopropoxy)butane as linkers. For stickers, dipeptides used included FF (**1**), LL, and WW and dipeptide combinations of similar amino acids. Three key parameters have been outlined to achieve coacervation: a threshold of sticker hydrophobicity, zero to negative solvation free energy of the spacer, and the dipole moment of the spacer. Along with an appropriate amount of sticker hydrophobicity to initiate molecular association, a spacer should work toward not only solubilizing the sticker but also having the necessary dipole moment for solvent interactions.

One insofar unmet challenge is the elucidation of basic design principles of liquid−liquid phase separation, with most systems described to date focusing on variations on biologically derived sequences. However, a number of recent studies pave the way toward a rational design of condensates. pH-sensitive spherical compartments have been prepared by employing the stoichiometric charge neutralization of cationic peptides and anionic mononucleotides, retracing a prebiotic organization.[91] With the aim of investigating the lower-multivalency polyelectrolytes that are able to drive coacervate formation,[92] cationic peptides, $(Lys)_n$ or $(Arg)_n$, and anionic peptides, $(Asp)_n$ or $(Glu)_n$, in a range of lengths (n = 1, 5, 10, 30, or 100) were combined (Figure 3B). Coacervate droplets have been observed when at least one component is a decapeptide and the other is a pentapeptide. The four combinations at these peptide lengths led to uniformly mixed solution in the case of the shortest oligomers, while solid aggregates were formed with the longest (Figure 3C−E). Interestingly, the mixing of $(Glu)_n$ with the oppositely charged polycation resulted in solid formation over different numbers of residues, whereas $(Asp)_n$ formed aggregates only in the presence of $(Arg)_{100}$. Additionally, a coacervate library provided by pairing $(Lys)_n$ or $(Arg)_n$ with anionic mono-, di-, and triphosphates of adenosine (AMP, ADP, and ATP) showed that oligoarginine-based coacervates had a higher salt stability compared to the ones formed via oligolysines. Such behavior can be brought on by the presence of cation$-\pi$ interactions among the arginine residues and adenosine nucleobases, as well as differences in hydrophobicity and hydrogen bonding.

In this context, it is noteworthy that dramatic divergencies in viscosity have been reported between Arg and Lys containing droplets.[93] In fact, a complete condensate inversion has been observed upon addition of polyR to polyK-UTP complexes, with polyArg competing for UTP and causing polyLys release. Moreover, partitioning the solutes, coacervates have the capability to increase the local concentrations of reactants speeding up the reactions. Cationic decapeptides in the presence of RNA aptamers, for example, can function as microreactors, enhancing RNA-cleavage reactions catalyzed by multiple ribozymes and a DNAzyme.[94]

2.5. Interplay of Flexible and Rigid Peptide Nanostructures

Recent investigations show that condensates form a key intermediate in protein aggregations that result in amyloidogenic diseases.[95] This behavior has also been shown in smaller peptides, suggesting not only that condensate mediated aggregation could be a general pathway, but that it can in principle be leveraged in future biomaterial design or as model systems to better understand diseased states. A highly dynamic process, LLPS also plays a role in the nucleation of supramolecular nanofibrils.[96] Experimental results, supported by all-atom molecular dynamics simulations, demonstrated that the carboxybenzyl (Z)-protected diphenylalanine dipeptide (Z-FF) is initially demixed into liquid droplets, which then, reorganizing and merging, give rise to thermodynamically favorable nanofibrils, in line with Ostwald's step rule, with Fmoc-A (**7**), Fmoc-P (**8**), and Fmoc-V(**9**) displaying similar behavior (Figure 3G,H). It should be noted that this behavior in short peptides might be more general than previously thought. Spherical structures observed upon self-assembly of several short peptides and peptide derivatives that were previously not identified as condensates but referred to as (micellar) aggregates need a thorough revisiting, in particular previously reported YF, Fmoc-YQ, Nap-Y-OH, and FS/FD mixtures.[97−100]

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

While some condensates are thermodynamically stable, others are observed as intermediates prior to formation of more stable self-assembled structures, typically fibers. For example, Rengifo et al. studied HHQALVFFA-NH_2 as a peptide of interest and observed a cooperative growth of amyloid fibers from an unstructured condensate intermediate.[101] Through detailed isotope labeled IR and NMR experiments, they conclude that the condensate is rich in an ensemble of β-sheet conformations stabilized by hydrogen bonds. The transition of disordered phase to an ordered fibrous structure has also been shown by Kumar et al., albeit through a chemically fueled reaction.[102] These authors demonstrate a novel complex coacervate between positively charged poly(diallyldimethylammonium chloride) (PDDA) and negatively charged Fmoc-^{D}Ala-^{D}Ala in its carboxylate form (Fmoc-AA). PDDA and Fmoc-AA complex together to form coacervates. Because one of the components in this coacervate is rich in hydrophobic residues and also pH sensitive (Fmoc-AA), a proton source can thus not only disrupt the droplet architecture by interfering with the charge balance but can also ultimately trigger assembly driven by the hydrophobically rich core. Glucono-δ-lactone (GDL) hydrolysis can be used advantageously in such a situation as it can consume hydroxyl ions to lower the pH of the solution.[103] Addition of GDL to this coacervate triggers a transformation from disordered spherical aggregates to highly ordered fibers.

3. COMPOSITIONAL COMPLEXITY: COASSEMBLY AND INTERFACES

While the regime of self-assembling peptides already lends itself to a high degree of structural tunability, further complexity approaching that seen in biological proteins can be achieved through cooperative assembly. Such heterogeneous systems have seen rising interest, and peptide-based coassembling structures have been reviewed extensively.[38,104−106] Thus, the state of the field at large will not be reviewed here; rather, a series of instructional examples will be presented in the context of modulating the structures of peptide materials. A brief section on interface-stabilizing peptides has been included as an example of a hierarchical emergence of complexity involving at least three components.

3.1. Cooperative and Disruptive Assemblies

Co-assembly has emerged as a strategy to meld together the functions and properties of two (or more) peptides or combinations of peptides with other synthetic materials.[107−109] In the context of tunable mechanical properties, self-sorted coassemblies are often most desirable, where the two components self-assemble separately into distinct structures in close proximity, usually enhancing stiffness through the formation of denser networks.[110] When the two components of coassembly are very similar or can interact with one another as strongly as with molecules of its own type, cooperative assemblies may be observed. This generally enhances macroscale properties of the material. On the other hand, disruptive assemblies serve to weaken bulk mechanical properties, resulting from the interference on the assembly of one (or both) assembling species due to the presence of the second assembler.[110] Another type of coassembly is self-sorted or orthogonal assembly, where each assembling species assembles independently. In some cases, the individual components display distinct self-assembled behavior, but they collectively interact, such as in a core−shell assembly.[104] Different interactions are utilized to develop coassembled peptide structures, including through electrostatic,[111−113] aromatic,[110] and chirally matched[114,115] interactions. It should be noted that what follows are examples of coassembled structures toward the development of tunable mechanical peptide platforms. Co-assembly has broadly been employed to incorporate different functionalities to peptide systems, examples of which will be highlighted in later sections.

In an effort to rationalize the criteria that determine the impact of component peptides on mechanical properties of the coassembled structure, Adams' group examined the coassembly of a series of naphthalene dipeptides.[120] Six different peptides were tested: Br-Nap-VA, Br-Nap-AA, Nap-AA, Nap-VG, Nap-IF, and Nap-ML, based mainly on their differences in pK_a and gel-forming propensity. Assembly is triggered using a gradual reduction in pH.[103] Briefly, glucono-δ-lactone spontaneously hydrolyzes slowly in water into gluconic acid, reducing the pH of the solution steadily. Compared to acidification using HCl, this approach leads to the formation of more homogeneous gel structures with more consistent rheological properties. The results of the experiments are summarized in Table 1. Br-Nap-VA and Br-Nap-AA coassemble synergistically to produce a much stiffer gel. The peptides were shown to self-sort, and the crystals of Br-Nap-AA were shown not to interfere with the assembly over time. Nap-AA and Nap-VG also assemble independently, but the final gel is weaker than that of Nap-VG assembling alone. Nap-IF and Nap-ML demonstrate disruptive assembly. As the pH decreases, Nap-IF begins to form a gel, but the crystalline precipitates created by Nap-ML frustrate the gel and lead to a turbid solution. Finally, the assembly of Br-Nap-VA and Nap-AA produces a gel without self-sorting that has enhanced mechanical properties. While specific rules for designing peptides that coassemble following a specific path remain elusive, the work highlights the range of possible coassemblies.

Table 1. Properties for Gels Formed by Single Dipeptides and Coassemblies of Dipeptides (Adapted from ref 120)

gelator	properties of gel at pH ∼ 4	storage modulus (Pa)	pK_a
Br-Nap-VA	transparent gel	13600	5.9
Br-Nap-AA	transparent gel, crystallizes over time	6000	5.0
Nap-AA	transparent solution	6	5.3
Nap-VG	transparent gel	14900	4.5
Nap-IF	slightly turbid gel	2200	6.4
Nap-ML	opaque, compact aggregates	2	5.9
Br-Nap-VA + Br-Nap-AA	transparent gel	157000	N/A
Nap-AA + Nap-VG	transparent gel, delayed formation	8030	N/A
Nap-IF + Nap-ML	Turbid solution	63	N/A
Br-Nap-VA + Nap-AA	Transparent gel	61700	N/A

The rigid gels formed by Fmoc-FF (**10**) can be tuned through coassembly with a variety of peptidic and nonpeptidic molecules. Aa an example of disruptive assembly, a series of coassembled systems of Fmoc-FF and D-dinaphthylalanine formed crystalline structures that showed intermediate Young's moduli to single component self-assembled systems of either. Reducing relative amounts of FF led to lower stiffness, ranging from between 3.5 GPa for 80% FF to around 1.2 GPa for 20%

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

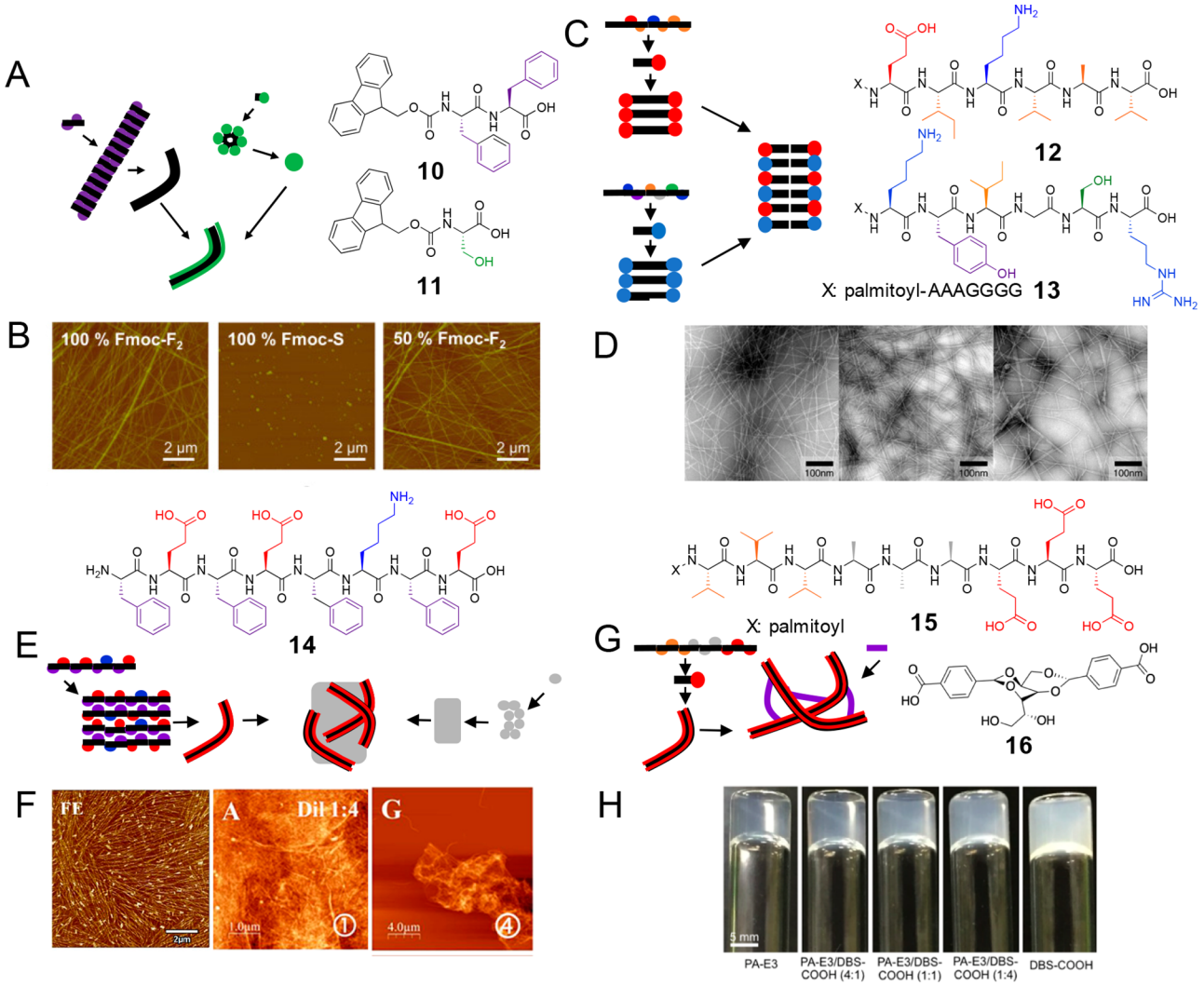


Figure 4. Examples of cooperative assemblies involving short peptides. (A) Coassembly of aromatic amphiphiles involving self-assembling Fmoc-FF and Fmoc-S forming core−shell decorated fibers, with (B) AFM image showing Fmoc-FF fibers, Fmoc-S aggregates, and their coassembled morphology. (C) PAs of opposite charges, along with (D) TEM images of individual fibers along with coassembled fibers. (E) Peptide + inorganic flakes, such as graphene oxide. (F) AFM image of FEFEFKFE nanofibers alone, followed by noninteracting peptide and GO, followed by fibers assembled on the flakes. (G) Self-assembling peptide and low molecular weight gelator molecule, exemplified by DBS. (H) Hydrogels formed by decreasing ratios of PA with DBS. (B) Reproduced with permission from ref 116. Copyright 2015 Elsevier. (D) Reproduced with permission from ref 117. Copyright 2003 ACS. (F) Feproduced with permission from ref 118. Copyright 2018 ACS. (H) Reproduced with permission from ref 119. Copyright 2019 ACS.

FF. On the other hand, a coassembly of Fmoc−pentafluorophenylalanine and Fmoc-FF produced hydrogels with dramatically higher rigidity than either system alone, with the 1:1 combination having the highest storage modulus at 190 kPa.[121] Fmoc-FF is thus amenable to both cooperative and disruptive assemblies.

Alakpa et al. showed that Fmoc-FF (**10**) coassembled with Fmoc-S (**11**) produces nanofibers that have the same β-sheet rich molecular structure of Fmoc-FF but with polar S residues displayed on the fiber surface in a core−shell morphology (Figure 4A,B).[116] Gelation is triggered by exposure to calcium ions, which cross-links surface exposed Fmoc-S carboxylic acids. It was found that the stiffness of these gels can be modulated by changing the concentration of Fmoc-FF/Fmoc-S, with oscillatory rheology of the gels showing elastic moduli ranging from 0.1 to 32 kPa, thus covering a range of gel stiffnesses that consequently could be exploited to modulate differential of stem cells grown on these gels.

Small peptide-based hydrogels are commonly employed as extracellular matrix (ECM) mimics. The peptide sequence RGDS, identified in 1984 as a recognition motif in the ECM protein fibronectin,[122] has frequently been employed in peptide amphiphile systems to enhance cell recognition.[123,124] It was shown that a disruptive coassembly using Fmoc-FF and Fmoc-RGD at ratios of under 30% Fmoc-RGD produced hydrogels that could sustain anchorage-dependent human dermal fibroblasts.[125] Different ratios of Fmoc-FF or Fmoc-RGD allowed for control over gel stiffness and, consequently, cell viability and proliferation. Another approach based on cysteine-mediated disulfide bridges to conjugate bioactive molecules to a peptide successfully demonstrated the conjugation and activity of CRGDS. CRGDS was shown to conjugate to Ac-LIVAGKC, a unique gel-forming peptide that helical fibers rather than β-stacks. Both species were mixed under H_2O_2, which facilitated disulfide bond formation. This

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

versatile approach lends itself to the use of bioactive motifs other than RGD.[126]

Biocatalytic peptide self-assembly, where an inactive precursor of a gelling peptide is activated by an enzyme, has been a widely used tool to exert kinetic control over the self-assembly process (more on this in section 4.2.3).[127,128] It was shown that the cooperative coassembly of Fmoc-FY with Fmoc-S, Fmoc T, or Fmoc-RGD could be triggered by enzymatic dephosphorylation. Upon enzymatic hydrolysis of the tyrosine phosphate ester, Fmoc-FY changes morphology from micelle-like structures to nanofibers[129] that serve as a hydrophobic (sticky) scaffold, leading to a core−shell type fiber for Fmoc-S and a more complex structure for Fmoc-T and Fmoc-RGD driven by Fmoc groups anchoring onto the hydrophobic Fmoc-FY fiber surface. Enzyme kinetics affect gel strength, with higher concentrations of enzyme leading to shorter assembly times and stiffer gels. Reaction kinetics in an enzyme-triggered assembly process seems to have an opposite structural role compared to the kinetics in a pH-triggered assembly, where a slower acidification process produces stiffer gels.

Recent research has focused on ways to imitate the dynamism of the ECM through peptide hydrogels. Dynamic control entails both spatial and temporal control over dimensions of assembly. While spatial control over presentation of biochemical functionality (commonly referred to as epitopes) on supramolecular scaffolds has been studied extensively,[130,131] temporal control is more difficult to achieve. One approach presented by Redondo-Goméz et al. is to avoid covalently binding the recognition sequence to the peptide and instead rely on more fleeting complexes to present the epitope, such as the noncovalent host−guest complex formed by cyclodextrin and adamantane.[132] $V_3A_3K_3$-based amphiphiles linked to either β-cyclodextrin or adamantane were shown to coassemble cooperatively. A PA bearing adamantane coassembled with RGDS-β-cyclodextrin resulted in nanofibers where the RGDS motif was displayed on the surface of the nanofiber.[133] The noncovalent nature of the RGDS conjugation to a peptide fiber allows for both preassembly and postassembly functionalization in a tunable manner, as well as functionalization using multiple epitopes.

Fmoc-dipeptides coassembled with low concentrations of β-lactoglobulin or bovine serum albumin show tunable mechanical characteristics as a consequence of cooperative self-assembly.[134] In buffer, the proteins alone form disordered clusters, while each dipeptide studied formed hydrogels. The 0.03% of protein coassembled with each peptide formed enhanced the storage moduli of Fmoc-YL (from 1.4 kPa to ∼6 kPa) and Fmoc-VL (1.5 mPa to 3 mPa). The low concentration of protein clusters is thought to introduce geometric and motility constraints to the hydrogel, increasing their strength. However, at 2% protein, the mechanical properties diminish, thought to be a result of high interaction between protein and peptide, leading to the formation of thicker, coated fibers that limit interfiber interactions.

Brito et al. found that Fmoc-FF coassembled with either Fmoc-glucosamine sulfate or the equivalent phosphate analogue in aqueous conditions forms nanofibrous gels at a peptide−sugar ratio of 2:1.[135] Spectroscopy results indicate that a core−shell fiber system is formed, with hydrophobic Fmoc groups shielded from solvent by the outward-facing carbohydrates. Compared to Fmoc-FF alone, the coassembled fibers appear to be thicker, stiffer, and susceptible to bundling and branching. The coassembly reduces the Young's moduli of the fibers to between 7 and 10 GPa. Interestingly, the coassembled gels have greater storage moduli than Fmoc-FF gels, especially in the presence of multivalent cations like calcium, owing to the anionic display on the surface of the fibers. Phosphates were the most potent ion, having a storage modulus around 11 kPa in the presence of calcium ions. Fmoc-FF with Fmoc-glucosamine sulfate reached 7 kPa in comparison, with Fmoc-FF at 5 kPa. Okesola et al. recently demonstrated coassembly of a peptide amphiphile with a different sugar-based low-molecular-weight gelator.[119] A standard PA of the form $C_{16}V_3A_3E_3$ (**15**) assembled with 1,3(*R*):2,4(*S*)-dibenzylidene-D-sorbitol (DBS, **16**) produced a self-sorted nanofibrous gel, where the degree of network formation and consequently stiffness could be tuned by DBS concentration in a linear fashion, ranging between 27 kPa for the single component DBS to 9.5 kPa for the pure PA (Figure 4G,H). These are both instances of orthogonal assembly, where intermediate stiffnesses are obtained that are tunable based on the ratio of assembling components.

Bridging the peptide supramolecular chemistry and graphene nanomaterials in order to modulate mechanical properties of composite structures, Saiani's group studied peptide−graphene coassemblies to establish the relationship between molecular interactions in these systems and their bulk mechanical properties (Figure 4E,F).[118] Three β-sheet forming octapeptides, VEVKVEVK, FEFKFEFK, and FEFEFKFE (**14**), were chosen to differ between the influences of hydrophobicity, charge, and $\pi-\pi$ interactions. After observing the structural features of assemblies between each peptide and graphene oxide (GO) derivatives with different charge and surface chemistry, it was determined that favorable electrostatic and strong hydrophobic interactions enabled favorable interactions between peptide fibers and graphene flakes. This resulted in enhanced gel mechanical strength by up to 2-fold for the VEVKVEK peptide and up to 4-fold for FEFEFKFE. FEFKFEFK was a unique case where the lone peptide produced a very stiff gel which was broadly weakened by interactions with graphene flakes, thought to arise from GO binding, limiting the ability of the fibers to cross-link.

Boothroyd et al. coassembled a FEFEFKFK with a double-length version, FEFEFKFK-GG-FKFKFEFE.[136] FEFEFKFK is known to assemble in water into an antiparallel β-sheet-rich gel, driven by hydrophobic interactions of the F and the electrostatic complementarity of E and K. Each arm of the double-length peptide provides an ionic complementary binding site for FEFEFKFK. The flexible GG region allows both arms of the peptide to connect between two independently growing fibers. Thus, by "doping" FEFEFKFK with its double-length peptide, the rheological properties of the hydrogel can be tuned, with the authors reporting a 30-fold increase in storage moduli. However, in some cases, it may still be thermodynamically favorable for self-sorting to occur. Sahoo et al. demonstrate that when the tetrapeptides DWDW and KWKW are allowed to self-assemble together at pH 6 (where both peptides possess opposite charges), DWDW preferentially assembles into fibers while KWKW forms spheres.[112] These spheres stick to fibers of DWDW. Curiously, the fiber-sphere hybrids show enhanced mechanical gel properties to pure DWDW fibers, likely through charge screening of the negatively charged fibers.

To obtain control over hierarchical assembly from the molecular to the microscale, the network topology of the

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

fibrous hydrogel can also be a point of adjustment to form tunable materials.[136,137] Fiber bundling is understood to increase gel stiffness and decrease gel elasticity. Gao et al. modified a β-sheet forming octapeptides by introducing arginine residues in place of lysine (changing FEFEFKFK to FEFEFRFK and FEFEFRFR).[137] The arginine replacement promoted greater fiber−fiber interaction due to the guanidinium moiety on arginine being capable of a wide variety of interactions: hydrogen bonding, electrostatic, and salt interactions. Pure FEFEFRFR formed large-scale aggregates that precipitate, while FEFEFRFK formed a hydrogel. Doping FEFEFKFK with 10% or 20% FEFEFRFR maintained the former's β-sheet forming ability but enhanced fiber bundling greatly, while pure FEFEFRFK seemed to support less aggressive fiber bundling. Thus, the single R replacement, while still increasing lateral fiber associations, does so in a slower, more controlled manner. The double R replacement causes much more pronounced fiber bundling, leading to precipitation in pure samples. FEFEFRFK has the highest storage modulus among all peptide systems tested, suggesting the slower fiber association is necessary for the best control over mechanical property. Other means of altering fiber bundling include changing the charge moduli of the peptide, with a higher charge moduli preventing fiber bundling due to electrostatic repulsion.[138]

Peptide amphiphile self-assembly can be triggered by neutralizing the charge on the amphiphile by introducing some multivalent metal ion, commonly Ca^{2+}.[139,140] Niece et al. showed an example of the coassembly of two oppositely charged PAs (**12**,**13**).[117] TEM images (Figure 4D) showed similar fiber morphologies for the coassembled and single-component fibers, suggesting that the coassembled fibers were thoroughly mixed rather than having local regions of highly unfavorable charge density. The valency and concentration of the metal ions affect the storage moduli of the gel but do not do so in a predictable manner, convoluted by differences in hydration spheres and electronic structures of metal ions.[141] A very precise way of modulating PA gel stiffness is by introducing oppositely charged oligopeptides rather than metal ions. Metal salts are normally used to neutralize the charges on the peptide amphiphile to trigger self-assembly and gelation. Godbe et al. instead applied oligopeptides of various lengths (K_6, K_{10}, K_{15}) to probe the effect on mechanical stiffness of the peptide counterions.[141] The PA used was $C_{16}V_3A_3E_3$. There appears to be a direct, systematic relationship between the length of lysine used as a gelator and Young's modulus, with a ∼10.5 Pa increase in stiffness per lysine residue. The longer oligopeptides seem to be more efficient at electrostatic screening between nanofibers, with a lower concentration of oligopeptide required as peptide length is increased. Thus, larger changes in stiffness can be achieved through molecular modification of the peptide amphiphile and finer adjustments made by deliberate choice of gelator.

Chirality is a less traditionally explored means by which to tune the assembly of hydrogels.[46,114,142] It has long been known that altering the chirality of even a single amino acid in a short peptide can influence self-assembly propensity.[114,143] The introduction of racemic mixtures of a small peptide can drastically alter mechanical properties. Racemic mixtures of aromatic single amino acids, which can produce hydrogels by themselves but offer little in the way of customizability, can have up to 10 times the stiffness of D or L amino acids by themselves, with F standing out for producing hydrogels with a Young's modulus of 53.1 GPa compared to 5.8 GPa for the D-enantiomer and 1.8 GPa for the L-enantiomer alone.[46] In both circumstances, there is thought to be energetically favorable interactions between the enantiomers. For racemic F, electron spray ionization mass spectrometry reveals the formation of an enantiomeric dimer. Although the specific structure of the self-assembled material is unknown, the high stiffness suggests close molecular packing. The longer peptide $(VK)_8$VPPT-$(VK)_8$, known to assemble into fibrillar structures, shows different storage moduli when assembled alongside its enantiomeric counterpart $(VK)_8{}^{D}$VPPT$(VK)_8$, with a storage modulus of around 800 Pa compared to the 200 Pa for the single-component gels.[142] Later, Nagy et al. determined the molecular basis for the enhanced mechanical rigidity in this mixed enantiomer assembly: the racemic mixture allows for hydrophobic interactions not possible in pure enantiomer assembly, maximizing inter-residue interactions.[144]

3.2. Interface Stabilization

Surfactant-like peptides (SLPs) are a class of self-assembling peptides that assemble as 2D films, micelles, tubes, or vesicles, rather than gels. A conceptually intuitive, yet influential design that combines a hydrophilic head with a hydrophobic tail (A_6D, V_6D, and V_6D_2, for example) led to the formation of nanotubes and vesicles where the tails preferentially faced away from water to give rise to surfactant-like structures observed plentifully in biology but using designs not known in biological systems.[145] The hydrophobicity and β-sheet-forming propensity of SLPs are recognized to have important roles in determining the nanostructures formed by these peptides. SLPs can assemble at interfaces, forming effective diffusion barriers[146] and, in some cases, stabilizing emulsions and dispersions.[147] These stabilizing peptides can compartmentalize synthetic molecules such as drugs and carbon nanotubes, representing a bridging medium between biology and synthetic chemistry. A wider range of peptide structures are now recognized to show surfactant-like properties,[146,148,149] including simple dipeptides.[150]

Capito et al. presented an early example of the powerful utility of PAs as interface stabilizing molecules.[146] A PA solution mixed with a solution of hyaluronic acid (HA), a high-molecular-weight polysaccharide, led to formation of macroscale membranes at their interface. This was achieved through several regimes, including injection of either solution into the other to obtain enclosed cavities (sacs) of PA/HA. These sacs were shown to be self-healing and hydrolytically stable for several months. The membrane is composed of a nanofibrous PA network, whose assembly is triggered by ionic screening at the solution−solution interface. Remarkably, after the initial membrane formation, nanofiber formation perpendicular to the interface could be observed, growing toward the PA solution.

A subset of surfactant-like peptides show antimicrobial activity, reviewed comprehensively elsewhere.[151] The bolaamphiphilic SLP RAAAR has been shown to selectively restructure lipid membranes based on electrostatic interactions offered by the lipids.[152] In two model anionic/zwitterionic lipid membranes designed after bacterial membranes, RAAAR increased lipid−lipid correlation. The same peptide reduced bilayer correlation in a separate pair of lipid membranes designed after mammalian cell membranes. The peptide was shown to have appreciable antibacterial activity as well as reasonable cell viability.

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

A variety of surfactant-like peptides have been shown to stabilize membrane proteins.[153] A recent development in short-peptide-based materials has been the demonstration that these peptides can stabilize emulsions and other chemical compartments through self-assembly at the interface solvent−compartment interface. Many different short peptides have shown the ability to stabilize organic droplets in biphasic solvents by forming a nanofibrous monolayer at the interface, instead of the more traditional monolayer typical for surfactants, increasing the resistance of the emulsion to separation in a sequence-dependent manner.[150,154−156] Controllable release of incorporated substances, such as drugs, can be achieved through enzymatic cleavage of the peptide.[45,157−159] These enzyme-sensitive peptides are designed to contain an enzyme-cleavable site specific to the desired application, a hydrophobic motif to support self-assembly, and a final tunable motif containing charged residues, generally to tune peptide functionality or stimuli recognition/responsiveness. These examples show that kinetics, not just thermodynamics, can be exploited to design adaptive systems comprised of dynamic structures whose formation and breakdown can be controlled.

3.3. State of the Field: Order and Disorder in Supramolecular Peptide Systems

Collectively, short peptide designs lend themselves remarkably well to the creation of materials with customizable mechanical properties that cover a significant range through molecular self-assembly at different hierarchical length scales. It is not always possible or advisible to generalize among the examples that have been studied so far, but some trends are evident. Abundance maps derived from analysis of vast collections of proteins show that rigid sections and folds are typically rich in aliphatic and aromatic amino acids, while flexible folds are typically rich in polar and charged groups. The same rules appear to hold for the design of much shorter peptides. Moreover, controlling cooperative assembly processes can follow biology's lead, in the use of designs that involve deliberate sticky (sticker) sites and flexible (spacer) regions. The balance between affinity for other copies of itself versus solvent dictates the propensity for formation of 1D fibers or crystals,[60] the nature of the peptide amphiphile can impact the balance between growth in orthogonal directions to create 1D vs 2D nanostructures,[160] and fiber bundling can be engineered by selection of appropriate surface functionality.[161] Further modes of influencing mechanical properties include enhancing interfiber interactions through concentration increase,[162] charge screening,[136] enhanced H-bonding, and fiber entanglement.[117] The previous examples show the various ways in which to rationally tune each of these properties using peptide design. However, even in these minimal systems, there are complex interactions at play which prevent simple, linear relationships to be drawn.[163] Massively parallel computation simulations[42] and, increasingly, machine learning[164] can be useful to provide general correlations and design rules for short peptides, including predicting gelation[42] and order−disorder relationships[40] and even cooperative self-assembly.[165]

4. STRUCTURES IN AND OUT OF EQUILIBRIUM

Navigating complex potential energy surfaces in the presence of many competing opportunities for chemical reactions and interactions is critical in living systems and should inform the design of advanced supramolecular structures. The ability to select preferred structures or interactions leads to features such as self-selection and structure-based amplification that can be powerful in both the identification and optimization of peptide structures. In addition, such systems that take advantage of supramolecular complexity through competition can provide important insights into understanding how a supramolecular design may operate in a complex medium. Peptide-based supramolecular materials are often designed based on thermodynamic considerations, and the ability to navigate these landscapes through dynamic covalent chemistry has been explored both in the thermodynamic optimization and selection of optimized supramolecular structures and their consequent functions. The approaches discussed here have a distinct "systems" approach, in that structures emerge through thermodynamic selection in complex mixtures of interacting molecules.

4.1. Adaptive Equilibrium Structures

Discovery of (combinations of) peptide sequences typically relies on combinatorial experimental or modeling approaches.[166] In such experiments, it is attractive when compositions and sequences can be systematically compared in direct competition. One approach to exploring the peptide sequence space for structure and function is to create a dynamic peptide library, a system where a multitude of peptide sequences can form reversibly by dynamic exchange of peptide (or orthogonal) bonds that are able to recombine peptide modules. In such systems, it has been shown that the equilibrium peptide distribution that is eventually reached reflects the propensity of each formed peptide for self-assembly. Dynamically exchanging peptide mixtures covering the chemical space dictated by a variety of input dipeptides has proven to be a useful method to elucidate peptide design rules for supramolecular materials.[100]

Another way to access the functionality of the amino acid palette involves pseudopeptidic molecules. Jiang et al.'s work on finding a reversible, noncompetitive, and selective inhibitor for β-tryptase is one such example.[167] Their research combined oligopeptides, end functionalized by hydrazide moieties, and multivalent aldehydes, giving rise to not just sequence selectivity but also spatial selection of the ensuing binders the dynamic library was exposed to on the enzyme. Equilibrated libraries were added to heparin-stabilized human rhSkin β-tryptase. On a high throughput assay, enzyme activity was analyzed for this mixture using a fluorescent substrate Toc-Gly-Pro-Arg-AMC. As the substrate is cleaved by the enzyme, free AMC (7-amino-4-methylcoumarin) is released resulting in a switching on of AMC emission, which can be used as a marker for enzyme activity. The resulting system was selected for trivalent rigid linkers with positively charged peptide residues on its adduct termini.

Otto's group, over the past decade, have shown remarkable phenomena that spontaneously emerge from peptide libraries governed by redox behavior of thiols.[170] Dithiol functionalized peptides, which are amenable for cyclic disulfide adducts of various sizes, can be leveraged toward selective ring architecture based on self-assembly or system environment requirements. They have been successful in showing complex systems chemistry concepts such as self-replication, speciation, and even parasitic behavior in these networks.[171] While these systems undergo thermodynamic selection, they can also be kinetically biased, as was demonstrated using differential mechanical agitation (shaking versus stirring; see also section

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

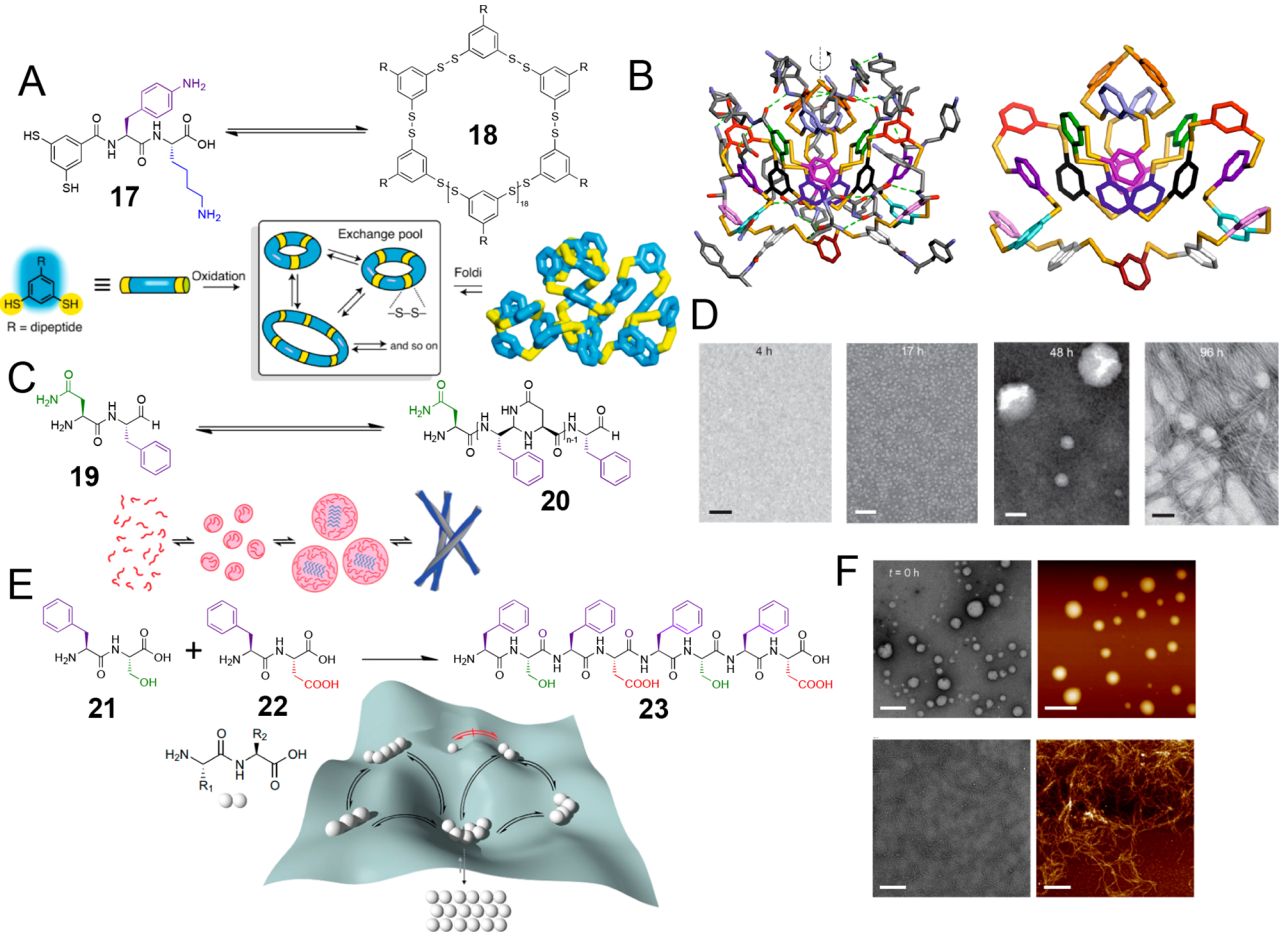


Figure 5. Covalent adaptive peptide systems. (A) Molecular structures representing monomers and one of its macrocyclic products in dynamic library with dithiol building blocks. Schematic represents the molecular network driven by macrocyclic stabilization. (B) Crystal structure of **18** with H-bonds and just the backbone. Green dashed lines show the peptide side chains involved in hydrogen bonds. Benzene rings are differentially colored depending on their environment. (C) Molecular structures of reaction equilibrium ensued by formation of *N*,*N*-acetal bonds. Schematic represents the multiphasic outcome of the reaction equilibrium. (D) TEM micrographs of the reaction progress at respective times. Scale bars, 100 nm. (E) Reaction depicting a dynamic peptide library with the corresponding schematic showing energy landscape of the process. (F) TEM micrographs and AFM images, respectively, of the reaction process with **21** and **22** to form **23** at $t = 0$ and 400 h, respectively. Scale bars, 500 nm; *z*-scales for the AFM images, −18.9 to 23.6 nm and −50 to 50 nm, respectively. (A,B) Reproduced with permission from ref 168. Copyright 2020 Springer Nature. (C,D) Reproduced with permission from ref 169. Copyright 2017 Springer Nature. (E,F) Reproduced with permission from ref 100. Copyright 2016 Springer Nature.

4.2) that directs these structures toward different compositional and structural fates.[172] In a follow-up, they show diversification of a system containing mutually compatible supramolecular replicators. These replicating species have overlapping pathways due to competing building blocks giving rise to diversified progeny that replicate as well.[173] Recent studies also show that precise low symmetry macrocyclic architectures can be obtained, which is reminiscent of the folding complexity of protein structures, particularly in that sterics and dynamic topologies dictate that there is only one final preferred folding solution when many similar weak forces are at play simultaneously (Figure 5B).[8,174] Pappas et al. show that monomers with stabilizing supramolecular interactions can form large macrocycles with a frustrated conformation. In a dynamic combinatorial mixture, monomers such as **17** were designed to include both a dynamic-bond forming potential (disulfide bonds) as well as a dipeptide which encodes limited directional hydrogen-bonding functionality to give rise to large macrocycles with chain lengths up to 23 monomers, such as **18**. This macrocycle contains features of hydrophobic collapse akin to a globular protein and is internally stabilized by a plethora of supramolecular interactions (Figure 5B). This example clearly elucidates the potential that this approach has in studying behavior of nonbiological reaction networks. The lessons and patterns of species that develop can be a clear guiding tool for advanced biological simplifications. The chemical complexity is still limited here, with only one dipeptide sequence giving this level of complexity when allowed to dynamically exchange toward equilibrium. One can only imagine the level of complexity and potential functionality available when multipeptide systems are investigated, although emergence of any specific sequences will present substantial analytical challenges.

Pseudopeptide library approaches have also been crucial in putting forth a driven search of materials that focuses on the behaviors of libraries rather than the outcomes. For example, Chen et al. have shown in an aldehyde substituted pseudopeptidic library, with tetrahydro-4-pyrimidinone as dynamic frameworks, how dynamic reactions can evolve as a multiphasic chemical network (Figure 5C).[169] Di- and

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

tripeptide aldehydes such as NF-CHO (**19**) and NFF-CHO were designed, such that the imine condensation via cyclization of the N-terminal asparagine side chain forms an *N*,*N*-acetal polymer (**20**). These networks elucidate the evolution of species in supramolecular contexts, with the final fate of these products observed to be the formation of amyloid-like aggregates, mimicking transitions observed in biological systems (Figure 5C,D). As a dynamic bond is involved in polymer formation, its propagation and adaptability to changes in the system, such as the presence of a preformed template, has been further studied. In a recent follow-up of the work the chemistry has been expanded to *N*,*O*-acetal linkage using threonine motifs.[175] A control on the chemistry and the ensuing kinetics has allowed the authors to decipher the multiphasic mixture in their libraries. We note that complexity of exchange libraries quickly renders fully tractable analysis of structure formation over time extremely challenging, and these defined mixtures that can combine in finite ways are advantageous for analysis and understanding of multiphasic chemical networks.

A fundamental challenge in creating reversible peptide sequence exchange libraries under physiological conditions is that the energy barriers for peptide bond formation and hydrolysis are significant; indeed, half-lives of peptide bonds are estimated to be 267 years.[176] If these barriers can be overcome, the systems lend themselves well to dynamic libraries as the free energy change for peptide bond formation is modest, −4 kJ/mol, so that peptides and their hydrolysis or condensation products always coexist in appreciable quantities.[177] In biological systems, amide bonds are hydrolyzed with ease by using a repertoire of proteases that significantly lower this free energy barrier, enabling rapid hydrolysis (or condensation) of peptide bonds. A relatively nonselective endoprotease, thermolysin, has been effectively used to enable formation of dynamic peptide libraries where the bias for hydrolysis is shifted toward condensation (peptide bond formation) by relative thermodynamic stabilization of peptides oligomers through supramolecular self-assembly.[178] The dynamic covalent approach operates under thermodynamic control and is therefore useful in thermodynamic optimization of charge transfer nanostructures.[179] This approach works well in creating diverse and complex covalent peptide mixtures that are more open ended, which has provided insights on global tendencies of amino acid reactivity under heterogeneous reaction conditions. These libraries create high information wealth ensembles under kinetically unbiased conditions. While the construction of dynamic libraries typically involves relatively weak dynamic bonds, for stronger linkages, such as amide bonds that connect amino acids in peptides, enzymatic approaches can be used to lower energy barriers. The efficacy of this concept was first demonstrated by Swann et al.,[180] with limited yields, at the time, owing to unfavorable thermodynamics. Ulijn et al. have since explored the approach to explore self-assembly landscapes. It was shown that gelator peptides (Fmoc-peptides) continuously exchange amino sequences via an enzymatically catalyzed making and breaking of amide bonds. This approach demonstrated that sequence selection as well as peptide length selection is possible, with assembly and gelation as the driving force.[177,181−183] Since then, this approach has been used for the dynamic selection of modified peptides to identify charge transfer hydrogels[184] and energy transfer hydrogels.[185] Peptide-based energy and charge transfer gels will be discussed in section 5.1.

Fully reversible peptide condensation and hydrolysis has also been used to alleviate the self-assembly of systems that exists in their kinetic states with suboptimal supramolecular organization, limiting their performance. In a work by Nalluri et al., charge transfer structures with naphthalene diimide (NDI) conjugated amino acids (Y in this case) can facilitate formation of an ordered assembly of charge transfer complexes by condensation reaction with F-NH_2 in the presence of thermolysin.[179] Wijerathne et al. demonstrated a peptide−porphyrin gel that assembles in aqueous buffer at pH 8.[186] Tetrakis(4-carboxyphenyl)porphyrin (TCPP) was coassembled with Fmoc-TL-NH_2, which was dynamically and reversibly formed in situ through enzymatic condensation of Fmoc T and L-NH_2. Fluorescence spectroscopy confirmed the efficient energy transfer from the Fmoc moiety to the porphyrin.

Pappas et al. explored a design of assembly driven dynamic peptide library based on unmodified peptides (Figure 5E) by taking advantage of the propensity of oligopeptides to aggregate into nanostructures.[100,181] In these libraries, dipeptides form as initial feeds and the nonselective protease thermolysin can scramble the sequences, ultimately enriching the sequence that overpowers the equilibrium by self-assembling and thereby purging the entire equilibrium in its direction. Specifically, for longer oligopeptides with binary patterns, it was demonstrated that a single sequence octapeptide (FDFSFDFS) (**23**) was formed starting from a binary mixture Phe-Ser (FS) (**21**) and Phe-Asp (FD) (**22**) as input dyads (Figure 5E). Microscopically starting reaction started as spheres resembling unstructured condensates and then matured as supramolecular fiber as reaction progressed (Figure 5F). The FDFSFDFS octapeptide was resynthesized and was shown to form β-sheet rich structures. As shown by TEM and rheology analyses, the peptide assembled into a hydrogel that consists of an entangled fibrillar network. DPL has also been used by Guilbaud et al. to show that it can be utilized to drive the synthesis and gelation of ionic octapeptides from tetrapeptide (FEFK) inputs, giving rise to predominantly octapeptide-based hydrogels.[187]

In addition to aggregation, equilibrium networks can be incentivized to adapt their composition through coassembly. Peptide libraries' amplification of species that electrostatically bind to supramolecular oligosaccharides has been demonstrated by Abul-Haija et al.[188] Fmoc-protected basic (R, K, H) and acidic (E,D, cystic acid (CA)) amino acids were mixed with oppositely charged polysaccharides (chitosan and heparin) in the presence of F-NH_2 and thermolysin. CA and K stood out as they produced the highest condensation product, Fmoc-KF-NH_2 and Fmoc-CAF-NH_2, in the presence of heparin and chitosan, respectively, clearly elucidating a charge-based, binding-driven adaptive equilibrium system. Williams et al. show that Fmoc-L reacting in the presence of LL and immobilized thermolysin has the product percentage of Fmoc-L_3 rise from 54% to 80% with minimal side products like Fmoc-L_5 in the presence of laminin.[189] These authors also show that Fmoc-L_3 aggregates into β-sheets further stabilized by $\pi-\pi$ interactions from Fmoc, and these interactions are not hampered by inclusion of laminin in the supramolecular structure. Supramolecular coassembly can also be directed in an adaptive manner through reversible noncovalent interactions.[190] Freeman et al. show that supramolecular bundling of peptide amphiphiles can be controlled through the dynamic inclusion of molecules with programmable cross-linkers and

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

spacers. These cross-linkers can vary from complementary nucleotide sequences to charge complementary peptide sequences. Such adaptive dynamicity of the supramolecular fibers is reminiscent of constant remodeling in the extracellular matrix (see section 5.3).

Adaptive equilibrium can be achieved through combinations of dynamic covalent and noncovalent interactions, increasing the complexity of the energy landscape. Sadownik et al. coupled a dynamic peptide forming equilibrium system with oxidation-sensitive disulfide formation by starting their reaction with F-OMe and Fmoc-C in the presence of thermolysin, increasing the ensemble to six components (two reactants and four possible products).[191] The energy landscape between these products can now be leveraged to external conditions that affect either of the coupled equilibria (air oxidation and heat). The authors demonstrated pathway complexity in which product distributions change drastically depending on whether amide exchange happens in the presence or absence of an oxidizing environment. This also gives rise to a unique supramolecular inhibition of disulfide formation as amide exchange is first carried out in a nonoxidizing environment before being exposed to air. Evidently the supramolecular assembly of the amide condensation product Fmoc-CF-OMe is responsible for such an inhibition (or locking) to disulfide formation. This landscape is subsequently rearranged to disulfide linked Fmoc-CF-OMe as heat is applied, resulting in disruption of the supramolecular structure of the reduced thiolated form of the peptide). Another unique coupling of amide condensation equilibrium is shown by Sahoo et al., who employed Y with an Azo-protected N-termini (Azo-Y) as one of the reactants for thermolysin-catalyzed amide condensation.[192] The authors observed that while the *trans* form of Azo-Y condensed in individual reactions with amidated F (84%), L (59%), and V (63%) to form hydrogels, the *cis* form showed reduced condensation (7.6% for F-NH_2) and no gel formation. This reduced yield implies the impact of unfavorable aggregation of the *cis* isomer on the light-independent amide condensation. Coupled enzymatic reactions were shown by Sahoo et al. by using alkaline phosphatase in combination with thermolysin.[193] In this approach, a substrate for the amide condensation started with phosphorylated Fmoc-tyrosine coupling in the presence of F-NH_2. Depending on the order of enzymes used on the substrates, the amphiphilicity of the products changed drastically, giving rise to an energy landscape with a number of distinct nanostructures that could be accessed transiently.

We note that polymerization of amino acids may also be achieved through (nonenzymatic) dehydration-induced condensation reactions that have been both theoretically and experimentally studied.[194,195] Methodologies that study diverse product spaces under a variety of environmental selection pressure are being developed.[196,197] We do not discuss these approaches in detail here because they do not operate under thermodynamic control, but we note that they show potential in the identification of new structures without enzymatic bias.

As has been discussed, peptide mixtures and ensuing product libraries can not only be a useful tool in understanding complex supramolecular phenomena relevant to the molecular basis of life but also specifically in the identification of thermodynamically optimized functional materials. Research in our group is currently focused on the use of dynamic peptide libraries to include and search for disorder-oriented sequences, which could aid in further discovery of ligand binding adaptive sequences. This research is relevant to the design of life-like matter, considering the omnipresence of chemical networks that connect reactions across bilayers and through various assembly states in biological systems.

4.2. Nonequilibrium Structures

Living systems are always in flux. The dynamic aspects of nature are controlled by a tightly regulated network of reactions, which are performed in precise spaces and over defined time regimes. This manifests in the programmable formation and dismantling of self-assembled nanostructures, each process independently regulated but closely related. Control over nucleation and growth processes provides a major challenge in supramolecular self-assembly. The study of systems that display subtle, dynamic responsiveness to applied stimuli, as well as systems that are heterogeneous in nature in terms of reactivity using nanoscale organization, presents strides toward controlling complexity in a manner approaching nature itself.

The chemistry of life is sustained by energy consumption. As in the case of biological networks, there is significant interest in the development of supramolecular structures, where the components populate high-energy states that interact in a fashion that is dictated by kinetics and (nonequilibrium) thermodynamics. According to the Second Law of thermodynamics, this is possible only by providing a constant flow of energy (fuel) in dissipating conditions.[2,4,198−201] These types of systems have been extensively studied in the past decade, and the terminology and definitions have been debated.[202,203] In the current section, we use a broad definition to inform discussions of systems whose properties are of interest in thermodynamic states that do not represent the global minimum. These are either dynamic systems that require continuous energy input to support their existence, or they are supramolecular systems that exist at a local energy minimum (and consequently may be en route to thermodynamic equilibrium).

4.2.1. Fueled and Transient Systems. Natural metabolism serves as an example of the ability to regulate chemical processes through energetically favorable and unfavorable (fueled) reactions. As in a natural metabolic pathway, at least two complementary sets of reactions, anabolic and catabolic, are required to enable out-of-equilibrium supramolecular self-assembly. A well-known example from biology is the dynamic supramolecular polymerization and depolymerization of actin, where in the presence of ATP, the nonassembling G-actin (globular) monomers readily self-assemble to form F-actin (filamentous) following a nucleation−elongation mechanism; the subsequent hydrolysis of ATP to ADP drives the decay of the microfilaments, resulting in the reformation of the original monomers.[204]

Inspired by these biological systems, a set of key requirements can be identified in order to produce synthetic systems with similar out-of-equilibrium self-assembly behaviors. First, an *anabolic* reaction promotes the activation of precursors at the expense of the fuel provided, resulting in the buildup of self-assembling molecules under kinetic control (i). Then, there is the need for a competing *catabolic* reaction which reverts the system to the initial condition, breaking down the kinetic ensemble and producing waste (ii). It is critical that these forward and backward reactions follow distinct routes, preferably with orthogonal kinetic rate constants so that they can be regulated independently, to create materials that build

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

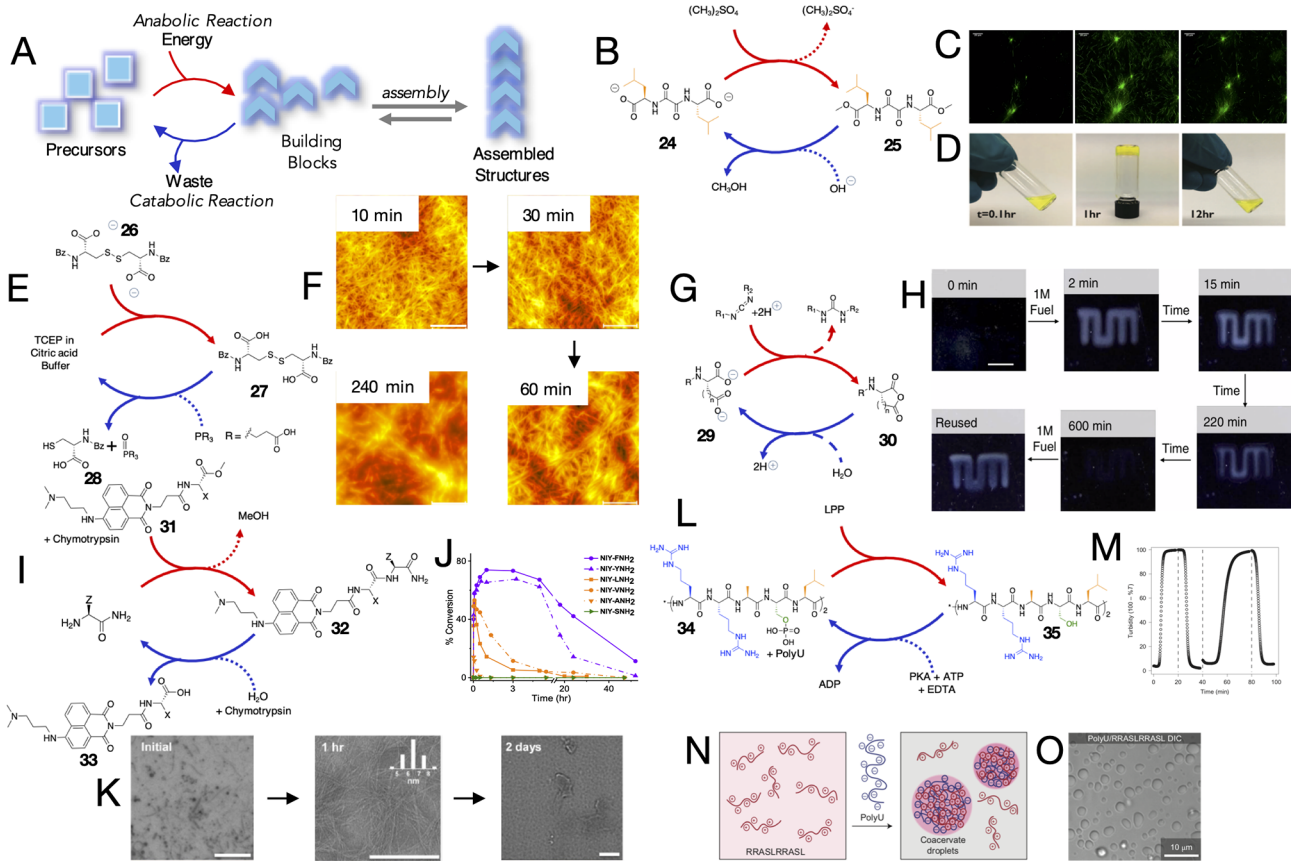


Figure 6. Fueled and transient systems. (A) Schematic representation of a synthetic systems with out-of-equilibrium self-assembly behavior. (B–D) Reaction cycle of the gelator (B), and its micro- (C) and macroscopic images (D). (E,F): (E) catabolic and anabolic reactions involved in the transient hydrogel formation; (F) time-resolved atomic force microscopy of the gelator (scale bars represent 1 μm). (G,H) Scheme of the chemical reaction network (G) and photographs of gels over time (H). (I–K): (I) General reaction scheme of the fluorophore containing structures, (J) HPLC time course showing transient behavior of self-assembling dipeptides, (K) TEM images of the transient nanofibers NI–Y–OMe with F-NH_2. Scale bars 400 nm. (L–O) Schematic illustration of the reaction cycle (L) and corresponding turbidity plot (M), representation (N), and a transmitted light differential interference contrast (DIC) image of polyU/RRASLRASL coacervate phase droplets (O). (B–D) Reproduced with permission from ref 208. Copyright 2015 AAAS. (E,F) Reproduced with permission from ref 209. Copyright 2018 ACS. (G,H) Reproduced with permission from ref 210. Copyright 2017 Creative Commons. (I–K) Reproduced with permission from ref 211. Copyright 2020 John Wiley and Sons. (L–O) Reproduced with permission from ref 212. Copyright 2015 Springer Nature.

up and degrade over time in a programmable manner (iii). In addition, the conditions need to be such that the self-assembly building blocks accumulate to a level where they (temporarily) exceed the critical aggregation concentration (iv). Feedback regulation can be introduced in these systems if the self-assembled structure is either more or less reactive compared to the unassembled precursors (v). Clearly, the material can then only exist under conditions when the anabolic reaction is, at least initially, much faster compared to the catabolic reaction. Moreover, a steady-state dynamic material can be achieved when these rate constants of build-up and breakdown are equal and give rise to a concentration level where self-assembly is observed. The material will cease to exist when fuel runs out or when the catabolic reaction takes over. The overall features of such a system are summarized in Figure 6A.

With these design principles in mind, scientists have developed a wide array of chemical fuel-driven out-of-equilibrium systems.[2,201,202] A broad palette of chemical reactions have been incorporated in artificial metabolic patterns, providing an extensive catalogue of chemistries for self-assembly and disassembly processes.[205] We focus on examples based on short peptide and short peptide derivatives only, but it should be noted that several designed systems not involving peptides have been reported, as recently reviewed.[206]

4.2.2. Chemically Fueled Systems. As reported in a pioneering system by Boekhoven et al., the precursor of low-molecular- weight gelator dibenzoyl-(l)-cystine (DBC, **26**) can be involved in a versatile chemical reaction cycle.[207] In this example, a methylating agent (methyl iodide, MeI) was used as fuel for the temporary removal of the intramolecular Coulombic repulsion between the carboxylate groups of the unassembled entities to gain self-assembled diesters (DBC-OMe_2). The resulting aggregates were transiently stabilized by cooperative hydrogen bonds and hydrophobic interactions. In the aqueous environment, a competing (general acid catalyzed) deactivating hydrolysis reaction takes place, reforming the anionic COO^- moieties and producing methanol as waste. The group subsequently investigated the use of a more reactive methyl donor and leucine derivative pregelators (**24**/**25**), which had the advantage of speeding up the reactions and consequently reducing the lifetime of the assemblies to hours instead of 5–15 days (Figure 6B–D).[208] It was shown that the time-scale of the fibrillar assemblies can be fine-tuned by regulating pH, with higher pH levels (above 10) boosting the hydrolytic breakdown of the structures and the

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869–13914

dissolution of the gels, while lower pH value of 9 reduced the hydrolysis rate. The time scales were compatible with real-time visualization using confocal microscopy, which provided fundamental insights of fiber growth and breakdown. Interestingly, when the fuel is exhausted, hydrolysis takes over, starting from the fiber ends, and no fractures are observed along the length of the nanostructures. Then, above the critical hydrolysis level, the unstable fibers undergo a fast, stochastic collapse. Thus, at the microscopic level, the transiently formed fibers showed behaviors reminiscent of microtubule dynamics.

Subsequently, an alternative approach was developed using the same DBC (**26**) building block, taking advantage of the cystine disulfide bridge core to tune the lifetime of transient supramolecular hydrogels[209] by using tris(2-carboxyethyl) phosphine (TCEP), a thiol-free reducing agent frequently used to break disulfide bonds within and between peptide-based molecules (Figure 6E,F). Compared to other reductants, this water-soluble phosphine triggers the hydrolysis of the bond rapidly and quantitatively in water at pH 3. At this pH value, DBC's two carboxylic acid groups are protonated (**27**), resulting in the self-assembly into a fibrous hydrogel scaffold, which can be dissolved upon reductive breaking of the S−S core to give **28** upon consumption of TCEP. In this case, the activation/deactivation reactions are autonomously controlled by independently affected rate constants of supramolecular hydrogel formation and breakdown. Moreover, by programming the starting concentration of TCEP, it is possible to refuel the system by adding DBC.

As an alternative to protonation or methylation, the ability of carbodiimide fuels to activate carboxylic acid can be harnessed for the conversion of two terminal carboxylates (one provided by the backbone of the peptide, the other by the side chain) into intramolecular anhydrides as reaction products that are prone to self-assembly (Figure 6G,H).[210] In this kinetically controlled process, the subsequent hydrolysis of the metastable anhydride leads back to the formation of the dicarboxylate precursors (**29**,**30**). It was found that by using Fmoc-protected amino acid and peptide derivatives with different self-assembly propensities, it was possible to regulate the morphologies of the active materials. Specifically, precursors are N-Fmoc-protected short peptides with aspartic or glutamic acid termini, which are soluble and negatively charged in buffered water at pH 6. Fmoc-peptides are well-documented as versatile self-assembling molecules that give rise to a variety of sequence-dependent nanoscale structures.[27] According to rule (iv), the molecular design dictates the self-assembly propensity, which in turn dictates the lifetime of these carboxylic-based materials and their morphology diversity. If the fuel is available, alanine-bearing sequences (Fmoc-AAD, Fmoc-AAE) turn into anisotropic fibrillar hydrogels because of the β-sheet forming propensity of the dialanine motif. Fmoc-D was, upon anhydride formation, shown to assemble into spherulites that were subsequently used as self-erasing ink with a lifetime that can be varied over a substantial range by modifying the fuel concentration. The introduction of the glycine moiety within the above-mentioned sequence (Fmoc-GD), as well as the substitution of aspartic acid with glutamic acid (Fmoc-E), leads to the formation of colloidal particles.[213] A key feature of these systems is that upon self-assembly the anhydrides are excluded from water, exerting a feedback inhibition on their deactivation reaction, thereby prolonging the lifetime of these materials.

The same chemical activation approach has been employed for chemical fuel-driven complex coacervation with Ac-FRGRGRGD and RNA (Poly U) as counterparts.[214] 1-Ethyl-3-(3-(dimethylamino)propyl)carbodiimide (EDC) condenses the C-termini and the Asp side chain into an anhydride, changing the net charge of the peptide from +1 to +3. The resulting product is a much better counterpart to RNA for coacervation. In turn, water molecules slowly hydrolyze the anhydride to the starting peptide, reducing the net charge to +1 and dissolving the metastable coacervates. Interestingly, the combination of the activated cationic peptide and the anionic homo-(poly(styrenesulfonate) (PSS) homo polymer) and block copolymer (poly(ethylene glycol) (PEG) + PSS copolymer) gives rise to different coacervate based structures (droplets, worm-like micelles, or large-compound micelles).[215]

Another study that takes advantage of carbodiimide-based activation demonstrated the formation of metastable lipid tailed amphiphilic nanostructures (C18H), synthesized by simple stearoylation (C18) of histidine.[216] Notably, the in-built base form of histidine's imidazole assists the formation of the kinetically stable ester bond, thereby inducing self-assembled fibrillar networks within 10 min. The deactivation reaction is then driven by the same moiety via intramolecular nucleophilic catalysis, achieving different lifetimes by varying the concentration of the reactants and through the inductive effect of different substituents of the phenol group. Refueling by application of another batch of the coupling agent resulted in regelation of the sample up to three times. Furthermore, it has been demonstrated that free terminal histidine residues were able to hydrolyze neighboring ester bonds, disassembling helical nanostructures, and thus providing negative feedback on their stability.[217] Also, the histidine driven hydrophobic collapse has been adopted to gain spatiotemporal control over electrical conductivity of short peptide-based amyloid networks.[218]

4.2.3. Enzymatically Regulated Systems. A programmable supramolecular pathway can also be regulated by making use of biocatalytic reactions, adhering to the *anabolic/catabolic* pair of reactions rule. To overcome the activation energy barriers associated with the build-up and breakdown of the macromolecules, living systems make extensive use of enzymes for almost all metabolic processes. The possibility of using the catalytic activity of enzymes in synthetic responsive and adaptive materials has been explored following from pioneering work by Yang et al., demonstrating the use of a phosphatase to control Fmoc-Y self-assembly by in situ dephosphorylation.[219] A first example of enzyme-controlled transient self-assembly and gelation behavior orchestrated in situ made use of α-chymotrypsin, which regulates both the competing assembly (anabolic) and disassembly (catabolic) reactions but via a different mechanism.[220] α-Chymotrypsin is a serine protease and preferentially hydrolyzes peptide bonds wherein the carboxyl side is a hydrophobic amino acid (such as tyrosine, tryptophan, phenylalanine, and, at slower rates, leucine and methionine). The enzyme is also known as an amide bond-forming catalyst where it requires an activated acyl donor which enables peptide bond formation under kinetic control, giving appreciable yields even in aqueous media, provided that the formation reaction kinetics exceed those of peptide breakdown via hydrolysis.[221,222] Taking advantage of this method, a naphthalene-functionalized amino acid acyl donor (specifically, Nap-Y-OMe) reacts with an amino acid amide (precursor X-NH_2: L-tyrosine amide Y-NH_2, L-phenylalanine amide F-NH_2, or L-leucine amide L-NH_2), in the presence of α-chymotrypsin, to form the self-assembling

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

dipeptide amide Nap-YX-NH_2 through a trans-acetylation reaction. Meanwhile, the competing reaction leads to the hydrolysis of Nap-YX-NH_2 to Nap-Y and X-NH_2, but this reaction takes place at a lower rate. It has been demonstrated that the transient assembly is remarkably dependent on the sequence of the transient dipeptide that is formed due to variations of the self-assembly propensity (as per rule (iv). For example, the equilibrium concentration of Nap-YF-NH_2 that is formed exceeds the critical gelation concentration (CGC) and therefore remains a nanofibrous gel. By contrast, by replacing the F moiety, the products Nap-YY-NH_2 and Nap-YL-NH_2 result in a situation where the equilibrium concentration is lower than the CGC, resulting in nonequilibrium gelation. Consequently, the systems show kinetically controlled formation of fibers and consequent gelation, but over time the degradation reaction takes over and eventually the peptide concentration drops below the CGC, reverting the system back to a clear solution. These examples show that peptide aggregation propensity can be used as a design parameter to enable the selection of transient versus permanent gels. Moreover, refueling the system by adding additional Nap-Y-OMe at the complete hydrolysis stage is possible and it could be repeated up to three cycles, although yields reduced in each cycle due to product inhibition. It is noteworthy that this hybrid biosynthetic system has been validated using deliberately a "poor assembler", Nap-YY, in order to produce nanostructure whose formation should be unfavorable at equilibrium.[223]

The reaction scheme was subsequently applied to a peptide-based system which did not contain any non-natural components but was produced from a dipeptide methyl ester (the low-calorie sweetener aspartame, DF-OMe) and amino acid amides. As a result of π-stacking interactions between the aromatic moieties of the amino acids and hydrogen bonding linking the peptide backbones, α-chymotrypsin gives rise to the formation of transient tripeptide DFX-NH_2 starting from the substrate DF-OMe (aspartame) and a range of structurally diverse amino acid amides X-NH_2 (X: F, Y, W, L, V, S, T). Depending on the choice of the amino acid in third position, the lifetime of the hydrogel can be tuned significantly. For example, DFF-NH_2 provides a stable gel for 24 h; while a less stable (4 h) gel can be obtained including Y within the sequence, while with other amino acids hydrolysis of aspartame was observed after 30 min. In addition, direct competition among F-NH_2 and Y-NH_2 reveals the kinetic selection of nanostructures rather than thermodynamic, leading to the formation of DFY-NH_2, the kinetically favored product.

The ability of gaining temporal control over the formation of nanostructures has been extended by mapping the sequence space of multiple peptide amphiphiles (Figure 6I–K) was demonstrated by Kumar et al.[211] By employing the same enzymatic approach and using a 1,8-naphthalimide (NI) amino acid methyl-esters (F, Y, L) as substrates (**32**), it is possible to predictably vary the half-life of the transient nanofibers relying on physical parameters, such as log *P* and the resultant self-assembly propensity. The inclusion of a fluorophore into the molecular design allowed for in situ imaging of the transient nanostructures with stimulated emission depletion (STED) based super-resolution fluorescent microscopy, increasing signal-to-noise ratio and resolution compared to traditional confocal imaging techniques, without the need for external staining.

Imparting a transient nature to synthetic materials is possible also by combining enzymatic oxidation and metal coordination, taking advantage of two distinct functional groups of the peptide-based monomers.[224] Specifically, the substrates are FF peptides (or FFX, with X = I, L, F) protected at the N-terminus with the BPmoc (borono-phenylmethoxycarbonyl). Thus, the carboxylic terminus can work as a coordination site for the Zn^{2+} ions triggering the hydrogelation; thereafter, the generated H_2O_2 upon treatment with glucose oxidase GOx/glucose system leads to the oxidation of BPmoc group and the collapse of the nanofibers. Interestingly, the reaction network has been adopted to control force generation by a propagating wave of BPmoc-F_3 peptide-based nanofibers.[225]

In metabolic pathways, reversible reactions are typically catalyzed by pairs of orthogonal enzymes. For example, the transient formation of condensates can be achieved between the positively charged peptide $(RRASL)_{1-3}$ (**34**) and poly-U RNA (Figure 6L–O).[212] The serine residues are the enzymatic substrates and RRASL is the peptide-based consensus sequence for protein kinase A (PKA): once the peptide is dephosphorylated (**35**), it can interact with its anionic counterpart forming condensates, which are then disrupted by the addition of the kinase in the presence of ATP; subsequently, serine dephosphorylation repristinates the coacervation. Consequently, the system is fueled by ATP and produces ADP and inorganic phosphate (Pi) as waste. This drawback can be overcome by the introduction of a membrane reactor, enabling the continuous trade of fuel and waste.[226] In this example, the sequence LRRASL has been used to symmetrically functionalize the 3,4,9,10-perylene diimide (PDI) moiety. The dialysis cassette selectively seizes the PDI-based molecules and the enzymes inside while letting ATP, ADP and Pi cross the membranes, avoiding accumulation of waste that may otherwise inhibit the system. The inase/phosphatase couple has also been adopted for the transient phosphorylation of hydrogels, leading to their dissolution and reassembly, as in the case of the naphthyl-protected pentapeptide Nap-FFGEY.[128] The sol–gel phase transition has been reproduced in vivo, proving to be a strategy for minimally invasive delivery of biomaterials. A similar approach has been adopted for controlling the release of the cancer drug doxorubicin from protein kinase A (PKA)-responsive supramolecular nanofibers (KRRASVAGK[C_{12}]-NH_2).[227]

4.2.4. In Situ Regulation in Dynamic Systems. Dynamic regulation of properties can be observed in systems that are en route to the thermodynamic minimum. In this context, the functional properties of such supramolecular chemical complexes are gained from the building block components, but they are also dependent on the preparative pathway in a hysteresic way.[228−230] Kinetics and life times of the assembled networks in this case are dependent on energy input, including chemical stimuli[231,232] and, as discussed below, mechanical triggers.[9,150]

As an alternative to electrostatic control of the dynamic self-assembly through catalytic (de-) methylation of carboxylic acid groups, so-called dynamic buffers have been used to dynamically regulate protonation, deprotonation, and consequent self-assembly.[233] Such a system is regulated by temporarily changing the ionization state by controlled alteration of the environment, switching between different contexts, and consequently different equilibrium situations. For example, Heuser et al. designed their precursor such that the peptide contains basic pH responsive moieties, specifically the

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

ornithine-containing sequence Ac-QQ-Orn-F-Orn-W-Orn-F-QQQ-NH_2. Thus, in an alkaline regime, a so-called activator (base or alkaline buffer) induces the formation of the nanostructures and at the same time gradually hydrolyses the deactivator (ester-containing molecule) to acid; the latter reaction leads to the disassembly process lowering the pH.[233] By varying the deactivator, temporal control of the assemblies is achieved over a substantial range: from a few minutes (gluconic acid δ-lactone) to hours (methyl formate) or days (ε-caprolactone). The activator/deactivator pair can be also applied to an acidic pH profile, switching the chemical nature of the components.[234]

Another approach to building up reversible peptide-based supramolecular assemblies involves controlling the overall bulk environmental conditions in a dynamic manner. Angulo-Pachon et al. took advantage of the well-characterized baker's yeast (*Saccaromyces cerevisiae*), which hydrolyzes sucrose into ethanol and CO_2.[235] In this work, the amphiphilic amino acid-based (V, I, F) precursors are endowed with aliphatic amines at the C-terminus, while the N-terminal is functionalized with a succinyl moiety. The decrease of the pH due to the CO_2 produced in solution can trigger the transient protonation of carboxylate-containing sequences. The consequent self-assembly persists until the pH turns to basic condition upon CO_2 elimination. The hydrogels can be reformed with batch-wise addition of sucrose. Interestingly, the different hydrophobic character of the amino acids included into the precursors does not affect the pH range at which the sol−gel transition occurs. By contrast, a short alkyl tail (provided by hexylamine instead of dodecylamine) cannot drive the gel formation in water. In addition, glucose-fueled assemblies can be sustained by glucose oxidase, allowing the development of dendritic peptide-based thermogels.[236]

4.2.5. Spatially Controlled Assembly. Spatial control of supramolecular assembly in biology is usually achieved by release of catalyst at a specific location, which either generates self-assembled structures in its vicinity or acts as nucleation site for self-assembly, e.g., in plants, spatial control over cell growth and shape are governed by localized secretion of catalysts and building blocks to form cell walls.[237] Nature also uses localized nucleation-crystallization as a method for growth of teeth enamel with spatial control, where enamel proteins act as a nucleation site for enamel formation with desirable orientation.[238] Also, the reaction diffusion and chemical gradients have been extensively utilized in nature to achieve spatial control over structures and functions.

Supramolecular materials are typically bulk materials, where nucleation and growth, let alone the locale of structure formation, is challenging to control. Attempts to achieve spatial control in peptide assembly have mainly followed a bioinspired approach of catalytic site-controlled assembly. A catalytic site is chosen where self-assembling molecules are locally generated, and if the self-assembly rate is faster than molecular diffusion, it would promote local accumulation such that the critical assembly concentrations are rapidly reached in close proximity to the site. This results in spatially controlled assembly at the site of catalytic reaction. The catalytic site can be on an enzyme surface, where biocatalytic self-assembly happens around the enzyme active site or through the immobilization or generation of catalyst on a surface, either synthetic or biological.

An early example of enzymatic reaction controlled self-assembly of an amino acid derivative was demonstrated by Yang et al.[219] Here, enzymatic dephosphorylation of Fmoc-Y-phosphate by alkaline phosphatase produced the more hydrophobic Fmoc-Y, leading to hydrogelation nucleating at the catalytic site of the enzyme. Although the authors did not mention it, in hindsight it is probably the earliest example of spatially controlled nucleation of a self-assembly process. Subsequent detailed mechanistic investigation by Thornton, Abul-Haija et al. demonstrated that Fmoc-Y indeed nucleates around the enzyme surface as spherical aggregates before transforming into 1-D nanofibers.[239] Therefore, it was demonstrated that enzymatic surface can be used for nucleation and growth of self-assembled nanostructures.

Spatial control of self-assembly can be obtained on synthetic surfaces by immobilization or generation of catalyst on the surface. In this regard, Williams et al. demonstrated that spatially defined enzyme immobilization on glass surface promoted localization of self-assembling peptide nanostructures on the patterned surface.[177] Thermolysin was covalently immobilized on a patterned glass surface. Upon immersion of enzyme functionalized surface to a solution containing Fmoc-L and LL resulted in self-assembling peptide (Fmoc-L_n) through biocatalytic condensation. Because self-assembling Fmoc-L_n was formed by thermolysin, it resulted in localized nucleation and growth of nanostructures only in areas where the enzyme was immobilized on glass surface, thus demonstrating spatially confined supramolecular self-assembly of peptide nanofibers. More recently, a variety of surfaces like TEM grids, glass, and magnetic nanoparticles could be used to immobilize various proteases and spatiotemporal control over formation of peptide nanofibers could be demonstrated.[240−242]

The ability to control nucleation in the assembly process was achieved by incorporating nucleation sites on a solid polymer surface was studied by Jierry et al. This approach used a grafted seed layer of hydrogelator monomers on top of an enzyme layer on a polymeric film.[243,244] The seed layer was composed of a polyelectrolyte matrix which was covalently functionalized with hydrogelator molecule (Fmoc-FFY) acting as a seed and was placed over an alkaline phosphatase layer. Addition of phosphorylated Fmoc-FFY(PO_4^{2-}) in solution triggers an enzymatic reaction to form Fmoc-FFY hydrogelator, which nucleates on top of the seed layer to form a nanofibers network.

In eukaryotic cells, the pH gradient is known to control the spatially defined tip growth for cellular extensions.[245] This has inspired several proton catalyzed (pH controlled) peptide self-assemblies which involve the modification of either the charged side chain residues of amino acids or the $-NH_2$ and $-COOH$ termini to regulate amphiphilicity and thus initiate assembly. Therefore, processes that can generate protons at specific locations would provide an effective means to control supramolecular self-assembly spatially. One such approach utilized the surface grafting of a proton producing enzyme, which resulted in localized formation of a peptide hydrogel.[246] To demonstrate this concept, glucose oxidase (GOx) was anchored on polymeric film, which oxidized glucose to yield H^+, creating a proton gradient on the surface. The localized acidic medium protonated the Fmoc-dialanine (Fmoc-AA-OH) to create a hydrogel. Interestingly, immobilization of two enzymes (GOx and horseradish peroxidase-HRP) in a multilayer architecture promoted a cascade of reactions where hydrogen peroxide produced by GOx was utilized by HRP for in situ generation of O_2, which is essential for GOx to produce H^+ for peptide self-assembly. In another example,

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

passive diffusion of protons from a surface resulted in the spatially controlled peptide assembly in a carboxylate terminated benzene-1,3,5-tricarboxamide (BTA) peptide conjugate. A poly(dimethylsiloxane) (PDMS) layer soaked in HCl was dipped in BTA−peptide conjugate solution, and controlled release of H^+ from PDMS surface created a proton gradient propagating front of supramolecular polymerization from the PDMS surface. It was observed that the kinetics of proton diffusion had to be slower than the nucleation time of nanostructures for proper spatial control over assembly.

Local change in pH can alternatively be induced by electrochemical reaction on an electrode surface. This has been utilized to demonstrate spatial control over self-assembly of an aromatic peptide amphiphile on an electrode surface. In this case, the electrochemical oxidation of hydroquinone on a gold electrode results in formation of benzoquinone with release of two protons near the electrode surface. The pH drop observed near the electrode surface was used for the protonation of Fmoc-LG, which consequently resulted in their self-assembly to form a thin film of hydrogel on the electrode surface.[247] Properties of these gels could be controlled by precisely regulating the current supplied at the electrode. Payne et al. utilized a similar strategy of pH gradient near an anodic surface to spatially control Fmoc-F gel growth both in the normal and lateral directions.[248] The gels could be simultaneously formed and erased on two electrodes of different polarity and their thickness controlled by the polarity, magnitude, and duration of the current supplied.

Adams and co-workers demonstrated a local pH-controlled peptide assembly to form spatially resolved multicomponent gels. The two aromatic peptide amphiphile gelators with distinct apparent pK_a values (which is dictated by the hydrophobicity of these peptides) were subjected to a globally decreasing solution pH through autonomous hydrolysis of GLD.[249] This led to sequential assembly of peptides with higher pK_a peptides assembling first, resulting in a self-sorted network of two types of fibers made up of individual peptides. The controlled pH change could also be obtained at the electrode surface by regulating the current supplied, resulting in spatial, temporal, and compositional control of the hydrogel on the electrode surface. The authors further develop their system to incorporate a photoswitchable stilbene unit into one of the peptide hydrogelators.[250] Consequently, photoirradiation of the two component gel leads to selective sol to gel transition of one of the components, resulting in the formation of a patterned fiber network with precise spatial control over hydrogel composition and rheological properties.

Having shown that a catalyst-generating surface can be used for spatial control over assembly, specific cells which release enzymes could be used for biocatalytic peptide self-assembly in proximity to cells. Xu et al. reported a design of enzyme-instructed self-assembly of peptide derivatives which form nanofibers in cellular environments. In cells, enzyme distribution is naturally variable, and Xu et al. took advantage of specific enzymes that are either localized inside (cytoplasmic enzymes) or on the surface (ectoenzyme) of cells. By matching the biocatalytic self-assembly system with these enzymes, the formation of peptide nanostructures can be spatially confined.[251] For example, when phosphatase overexpressed *E. coli* cells were incubated with a phosphorylated tyrosine derivative, in situ dephosphorylation resulted in formation of peptide nanofibers that were spatially confined inside the cytoplasm.[252] This eventually led to bacterial cell death due to a change in viscosity inside the cells. Similarly, using an ectoenzyme alkaline phosphatase which are overexpressed on cancer cell surfaces, a peptide nanofiber network could be formed on the pericellular region, resulting in the apoptosis of cancer cells.[253] Additionally, they developed a peptide substrate which can simultaneously react with phosphatase and esterase to result in peptide assembly and disassembly, respectively.[254] This system exploits esterase downregulation of self-assembly of peptides for selective inhibition of cancer cells without affecting other cells. Furthermore, conjugation of a dye (4-nitro-2,1,3-benzoxadiazole) to the peptide could be used as an imaging probe to monitor in situ phosphatase activity on cell surfaces.[255] Thus, enzyme secretion from cells provide a spatiotemporal control over formation of peptide nanofibers for their application in controlling cell fate and monitoring cellular chemical reactions.

Kalafatovic et al. have utilized the spatially controlled assembly of peptides for the site-specific release of drug molecules for effective therapy.[256] This was achieved by design of a micellar peptide nanostructure that assembles as hydrophobic fibers only upon enzymatic activation and therefore spatially confined to areas where the target enzymes are overexpressed. The peptide sequence, Phac-FFAGLDD, upon MMP-9 catalyzed hydrolysis and undergoes a supramolecular reconfiguration from micelles to fibers due to a change in amphiphilicity. Because MMP-9 is an overexpressed enzyme on tumor cells, antitumor drug doxorubicin loaded peptide micelles could reconfigure into nanofibers near diseased cells. This resulted in spatially controlled slow release of drug and inhibition of tumor in animal model.

4.3. Chemomechanical and Mechanochemical Supramolecular Responsiveness

Living systems display remarkably efficient shape-changing phenomena by reacting autonomously to external stimuli, which may include dynamically alternating their structure from nano- to macroscale. In addition to the chemical, physical, and biochemical stimuli discussed so far, mechanical stimuli offer an effective means to exchange energy with supramolecular systems, both through mechanical activation of chemical events and chemical activation of mechanical changes.

Monreal Santiago et al. demonstrated that the peptide GLKFK, activated with an N-terminus 1,3-benzenedithiol group (**36**), cyclizes upon oxidation in buffer to produce a library of different sized peptide macrocycles. Remarkably, the outcome of the experiment is strongly influenced by mode of agitation, i.e., whether the sample is shaken or stirred.[257] The autocatalytic peptide system, upon self-assembly and photoactivation, can catalyze the synthesis of its own precursors in a process that is strongly dependent on mechanical energy supplied to the system. **36** forms mainly with 3 or 4 monomer cycles, but from spontaneous disulfide exchange can form hexamers. Hexamers are capable of fiber formation, depleting the pool of hexamers and triggering replenishment. Furthermore, mechanical agitation causes fiber breakage, increasing the number of growing ends and resulting in exponential growth of the fiber system, so long as an oxidant is present in the system to activate uncyclized monomers. These results establish that mechanical forces can act as a selection pressure in the competition between replicators and can determine the outcome of a covalent synthesis.

The design of supramolecular materials that are adaptive and flexible and can interconvert rapidly in response to chemical

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

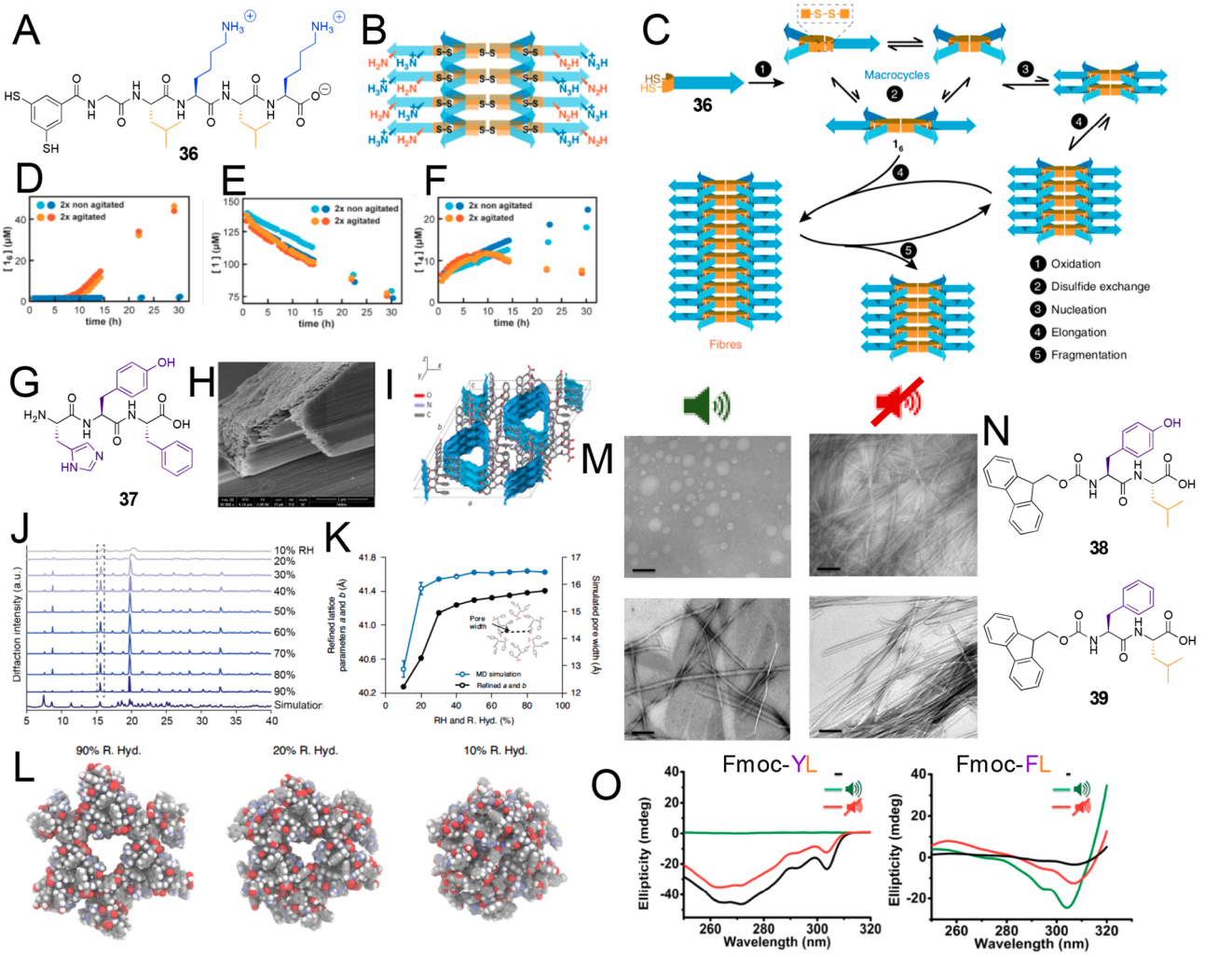


Figure 7. Chemomechanical and mechanochemical energy transitions in peptide-based systems. (A–F) Peptide-based chemical structure of the building block monomer (A) and schematic illustration of its integration in the macrocycles (B). (C) Mechanism of self-replication. Concentration of 1_6 (D), 1 (E), and 1_4 (F) over time. (G–L) Chemical structure of HYF (G) and its scanning electron microscope image (scale bar, 2 μm) (H). (I) Rhombohedral crystal structure of HYF. (J) Powder XRD of HYF at different relative humidities. (K) Evolution of lattice parameter with relative humidity, as determined by molecular dynamics. (L) Molecular dynamics simulated crystal structure of an aqueous pore structure of HYF. (M–O) TEM images of Fmoc-YL and Fmoc-FL upon ultrasound exposure and after it is off (scale bar 200 nm) (M) and their chemical structures (N). (O) Circular dichroism spectra of Fmoc-YL and Fmoc-FL before (black line), after 5 min ultrasound exposure (green line) and when the sound is switched off (red line). (A–F) Reproduced with permission from ref 257. Copyright 2020 Springer Nature. (G–L) reproduced with permission from ref 258. Copyright 2020 Springer Nature. (M–O) Reproduced with permission from ref 263. Copyright 2015 RSC.

triggers is currently in its infancy. We have investigated nanoporous peptide-based crystals as actuating materials which have distinct (stiff) H-bonding and (soft) aromatic domains and are prone to sequence-dependent reconfigurations. While crystals are typically perceived as stiff and brittle entities, it has been recognized that crystals that are stabilized by supramolecular interactions can be remarkably pliable and they can be designed to respond to applied stimuli, showing rapid reconfiguration and shape change. Specifically, we demonstrated tripeptide crystals of reversible and sequence-dependent lattice deformation in response to changes in relative humidity (Figure 7J–L).[258] Piotrowska et al. used a self-assembled hydrophobic HYF (**37**), which enable favorable interactions with water. HYF crystals exhibit a dual network of strong, reconfigurable H-bonding between water and peptides and soft π–π stacked aromatic regions (Figure 7I). Changes in surrounding relative humidity led to reversible, structural rearrangement, and volume change. Decreasing humidity induce strengthening of water–peptide H-bonding that drives pore contraction, leading to mechanical stress dissipation through interconnected, deformable aromatic areas in the crystal lattice. This mechanism and the enhancement of water bonding inside the pore amplifies the capillary force that induces expansion and contraction of the crystal. Interestingly, analogue tripeptide DYF-NH_2 (**6**) (Figure 2E) shares common features such as hierarchical structure, and aqueous pores do not show H-bonding strengthening. This indicates that the role of water in the pores and dual network of the integrated stiff, deformable, and soft domain is crucial for water responsiveness.

The flexibility afforded by a peptide backbone creates readily deformable pores in peptide–metal frameworks.[259−262] Katsoulidis et al. designed a flexible metal–organic framework (MOF) based on Zn using the peptide linker GGH, hypothesizing that the tripeptide would allow the MOF to access multiple structures.[261] The structure itself is comprised

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

of rigid Zn-His layers, the imidazole on the histidine coordinating to Zn^{2+}. The MOF crystal structure was found to vary among nine different conformations based on the presence of different solvents (DMF, H_2O, methanol, DMSO) and guest molecules (dioxane, cyclopentanol, furfural, furfuryl alcohol). The pores of the MOF are inaccessible to any guest in DMSO, but introduction of DMF or water to them displaces the DMSO to permit adsorption of guests. XRD analysis revealed that in each conformation, the peptide linker adopts different torsion angles, which in turn affects the intermolecular forces between consecutive linkers or between linkers and the solvent. Different linkers of the form G/A-pyrazole were later tested to expand the conformational reach of the frameworks.[262] These examples show that a level of mechanical configuration can be achieved based on chemical potential differences of free and bound molecules.

Inostroza-Brito et al. utilized the coassembly of elastin-like polypeptides (ELPs, negatively charged) with peptide amphiphiles (positively charged) to create a hybrid protein−peptide membrane.[264] Interestingly, by externally controlling the way in which the two components were brought together, the system could form a 3-D open tubes, which could be spatiotemporally manipulated to obtain the desired shape of self-assembled membrane. The example demonstrates the utilization of supramolecular interactions at the nanoscale combined with external application of directional motility to control the formation of macroscopic materials, which were subsequently utilized to support and guide the growth of stem cells for potential tissue engineering applications.

Mechanical waves also hold some promise to trigger multiscale supramolecular responses but are a relatively under-researched area. The idea is based on the concept that mechanical force can rearrange supramolecular interactions to relieve the applied force. Mechanically induced chemical reactions have been established for affecting matter at various energy levels, including breaking of covalent bonds and reorganization of noncovalent structures.[265] Mechanical transductions have been used to trigger aggregation and macroscopic superstructure alignments, in turn impacting on mechanical properties of the system.[266] Biology provides some outstanding examples on how soft matter can be impacted by acoustics, leading to mechanical transduction through soft structures, coupled with electrical or chemical activity, as seen in hearing and touch, for example.[267,268] For peptide-based supramolecular materials there have been examples showing acoustic responses dictated by molecular composition as well as induced alignment across hundreds of micrometers.[269] It has been shown that two closely related dipeptide amphiphiles, Fmoc-YL (**38**) and Fmoc-FL (**39**), upon ultrasound energy application, are subject to supramolecular reconfiguration depending on their electron densities.[270] Specifically, in the case of Fmoc-YL, the fibers formed in an aqueous buffer were converted to spherical aggregates upon 5 min of ultrasound exposure, reverting back to the initial network when the external stimulus was turned off. Moreover, after sonication, extreme syneresis has been observed in FF dipeptides, still conserving their gel-like behavior.[271] Cbz-FF-OH, (Cbz = benzyloxycarbonyl) showed an order of magnitude variation in the mechanical properties achieved through gelation via a heat−cool method versus of sonication.[272] Interestingly, hydrogelation ability can be modulated also for a single amino acid (Ala-hyd, bringing an aromatic N-protecting group and a hydrazide moiety at its C-terminus) by varying the initial concentration.[273]

An example worth noting in this context are peptide-assisted molecular machines for disrupting cell membranes.[274] Light-activated molecular machines have recently been utilized to controllably adjust membrane channels.[275] García-Lopéz et al. combined such a molecular machine with a peptide receptor sequence (DGEA and SNTRVAP) to target prostate cancer cells. Of the two sequences, the shorter showed no enhancement in cellular disruption, but the longer sequence displayed up to 50% higher mechanically induced necrosis in cells compared to untargeted machines.

4.4. State of the Field: Spatial and Temporal Control in Supramolecular Peptide Systems

The discussed examples begin to show steps toward the bottom-up synthesis of laboratory-based systems that show features such as competition, cooperation, and compartmentalization that are possible because of a combination of kinetic and thermodynamic features. In self-assembly experiments, a key development is the formation of self-assembling structures in situ by covalent modification of precursors, often regulated by catalytic reactions. These can typically be rationally designed, often starting from a known self-assembling system and then incorporating covalent handles or blocking groups to enable dynamic covalent control. With access to these building blocks, researchers can explore kinetics, in addition to thermodynamic considerations, to demonstrate new behaviors. While many of the systems studied to date are transient and on their way to thermodynamic equilibrium, the use of open systems is allowing systems to truly be studied out-of-equilibrium, where new properties emerge.[276] Recent trends focus on the inclusion of disordered regions and coacervates to create dynamic compartments,[277] thus blending and integrating design features discussed in sections 3 and 4. Within these compartments the chemical environment is different from the bulk, and this aspect can be exploited to create feedback control, for example, through enhancing catalysis upon structure formation that in turn triggers disassembly,[217] thus starting to mimic features of nature's fueled biopolymers.

Clearly, mechanical energy can be used to influence nanostructure assembly and disassembly mechanisms across length scales, which, in turn, changes the mechanical properties of these same materials. Vice versa, changes in supramolecular interactions can trigger mechanical responses. Collectively, the nonequilibrium systems presented here endowed man-made assembly of structures with functionality. The intriguing challenge is to regulate energy flow in space and time, through signaling and hierarchically organized systems, which should ultimately enable the creation of synthetic matter with adaptive functions currently only known in the living world.

5. FUNCTIONAL, COMPLEX PEPTIDE NANOSTRUCTURES

We have taken a close look at peptide design for structure, both in homogeneous settings and in heterogeneous mixtures, as well as principles behind the design of dynamic systems that exist in and out of equilibrium and display adaptive changes in space and time under the influence of applied chemical or mechanical energy. What follows in this section are an assortment of papers that integrate the design rules established so far to produce functional peptidic systems with complex properties, as represented in Figure 1C. These examples have

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

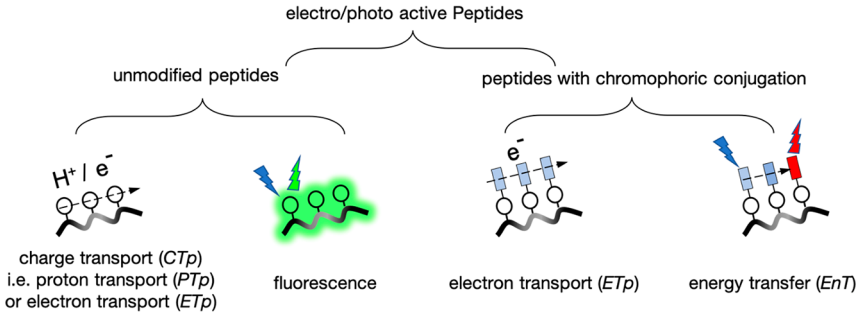


Figure 8. Peptides as building blocks for electro-/photoactive materials. Schematic illustration of the classification of electroactive and photoactive peptide conjugates used in this review along with various types of charge and photon migration processes discussed.

wide-ranging implications across broad fields of chemistry, biotechnology, and materials science, ranging from controllable mechanical response in water-responsive peptide crystals,[258] molecular motors that disrupt cellular membranes under light,[274] conducting nanowires that transiently disassemble,[218] and pH-switchable short peptide catalysts.[278] The section culminates in several works that illustrate complex dynamic properties and functions that can only be achieved by using a systems approach, with consideration for spatial, temporal, kinetic, and thermodynamic features of multicomponent systems.

5.1. Photonic, Electronic, Protonic, and Ionic Conduction and Communication

An essential aspect of living systems and synthetic devices alike is their ability to efficiently send and receive signals across length scales to direct the flow of energy and information. In biological systems, such communication efforts are often mediated by photons and charged particles like electrons, protons, or other ionic species and regulated by various chemical switches like ligand−receptor interactions and three-dimensional conformational changes. Communication through light and electrons are central to many functions, including vision, neuronal signaling, photosynthesis, and redox processes.[279] Simultaneously, charge mobility is a critical component of electronic devices, integral to devices and sensors. While both biological and synthetic devices utilize the same carriers for information and energy, the designs used are typically completely different. Whereas electronics in the context of life take the form of dynamic, hydrated and reconfigurable structures, synthetic electronic and photonic devices typically rely on static, dry, and fixed environments. There is substantial interest in developing bioinspired materials as devices for artificial photosynthesis, bioelectronics, wearable, and biodegradable electronics that combine concepts from the biological world with those used in devices. Peptide-based design holds much promise as materials that integrate synthetic and biological systems, or as building blocks for new types of electronics that combine concepts from both the biotic and abiotic worlds. This area of research has been reviewed numerous times in recent years, and we refer the reader to several excellent comprehensive reviews.[280−283] In this section, we focus on those areas where designed short peptide nanostructures have been used to facilitate electronic and photonic communication, where peptides provide either structure or function. We focus on areas where structural, spatial, and temporal aspects are considered, and where dynamic features are incorporated toward active and adaptive electronic and photonic functions.

Charge conduction in biology is mainly mediated by proteins where conductive efficiency is dependent on their secondary or quaternary structure.[284] Light-controlled processes in biology typically involve nonprotein chromophores organized within the protein assembly. As a minimalistic biomimetic approach, self-assembling short peptides and their chromophoric conjugates are increasingly explored as building blocks for materials that display charge and photon mediated properties.[281] The electronic and photo activity can be achieved either by taking advantage of the communication between amino acid side chains or through functionalization of short peptides with organic chromophores. In the latter case, the peptides provide the structural components that are responsible for the geometric organization of the chromophores.

Terminology is not always consistently applied, and we start by defining different modes of transfer and transport of charge and energy (Figure 8) that underpin these systems. (1) Electron transfer (ET) usually refers to the exchange of electrons in a redox process between a peptide/protein and an electrolyte. Because it is mainly a molecular process, it will not be discussed in this review. (2) Charge transport (CTp) is broadly defined as the phenomenon of movement of charges through a (supra-)molecular structure, such as a protein or peptide assembly. CTp can be of two types: (2a) Electron transport (ETp) refers solely to the phenomenon of flow of electrons through a conducting material, whereas (2b) proton transport (PTp) corresponds to movement of protons through a material.[283] (3) Energy transfer (EnT) is typically inspired by the photosynthetic process, where light energy is absorbed by the donor chromophore and the energy is transferred to the acceptor molecules. (4) Charge transfer (CT), on the other hand, involves either the donation of electrons from an electron-rich donor molecule to the electron-deficient acceptor molecules or merely the interaction between the donor−acceptor molecules.[285] As a general rule, transfer processes occur at local molecular scales, whereas transport usually suggests longer length ranges.

A significant amount of research has been dedicated to elucidating the mechanism and refining the photonic and charge-related properties in peptides and peptide-based molecules. Our focus here is not necessarily on the performance of these materials and systems but rather on the approach to supramolecular peptide design to control function. The electro-/photoactive peptide structures that are described in this section will be discussed based on similarities in chemical design as shown in Figure 8. Section 5.1.1 will discuss self-assembled materials made up of unmodified peptides, which will be categorized into charge transporting

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

(CTp) materials and fluorescent self-assembled peptides. Section 5.1.2 will discuss peptides which are conjugated with chromophores into either electron transporting (ETp) systems or as energy transfer (EnT) systems.

5.1.1. Supramolecular Self-Assembly of Aromatic Short Peptides for Biophotonic and Electronic Wires. Minimalistic peptide design consisting of natural amino acids has proven powerful in mimicking structural and functional properties of proteins. They can be studied, modeled, and engineered more effectively than whole proteins. One approach in the design of electro-/photoactive structures takes advantage of the inherent properties of aromatic amino acids, sometimes combined with charged residues, to form assemblies that can transport charges or photon energy through their nanostructures.

Because supramolecular organization is an important factor in charge transport (CTp), a critical role is assigned to versatile and tunable self-assembly. In a simple design, minimalistic self-assembling dipeptides such as FF and FW were investigated for their ETp properties. The origin of the semiconducting properties of such peptides has been reviewed recently by Tao et al.[282,288] Briefly, the assembly of peptides containing aromatic residues can behave like semiconductors when the interactions involved in the self-assembly process itself, including aromatic stacking and hydrogen bonding, collectively reduce the band gap of the interacting molecules within the nanostructure. Conductivity then arises from the presence of quantum confined structures in supramolecular peptide nanomaterials, as shown by the reported two-dimensional quantum well confinement in these structures.[289] Specifically, the self-assembly of FF micro- and nanotubes is known to be driven by the formation of an aromatic zipper, with the hydrophobic phenyl rings oriented toward each other and stacking one on top of the other (Figure 2A).[282] This "dry" region forms a dense π network of delocalized electrons, producing the observed conductivity. Amdursky et al. compared the electrical conductivity of dipeptides FF and FW to establish the role of these aromatic amino acids.[290] The conductivity of the self-assembled peptides was measured on a drop-casted dry film using conductive probe AFM. They observed that while the FF and FW films had similar topography, the conductivity of the FW was 5 times higher than that of FF, with FF effectively behaving as an insulator. The authors suggest that the lower optical band gap of tryptophan was responsible for the difference in conductivity and further suggested that inclusion of tryptophan in short peptides can broadly enhance conduction.

To further understand the role of amino acid sequence in general and the presence of tryptophan in particular, Cahen et al. investigated the ETp property of homomeric (tetra- to hepta and 20 mer) peptides composed of A, E, K, and W. They measured the conduction through a monolayer molecular junction (rather than supramolecular assembly) of these peptides placed between gold electrodes. The peptide's C terminus was free (−COOH), whereas the N terminus was thiolated, which allowed for the formation of a molecular junction between gold electrodes. By varying the nature of constituent amino acid (A, E, K, or W), the length of peptide chain and the secondary structure of the peptides (helical vs disordered), they established the crucial interdependence between ETp and peptide sequence. The ETp efficiency increased for peptides with more protonated side-chain amino acids like K and W and exponentially decreased upon increasing the chain length from 4 to 7. Furthermore, helical homoalanine formed by 20-mer oligo-alanine showed a 400 times higher ETp compared to disordered hepta-alanine, indicating the strong impact of secondary structure.[291] Using electronic structure calculations, the authors suggest an off-resonance tunneling mechanism of conduction in these peptides. Additionally, they investigated ETp in hepta-alanine with a single tryptophan substitution acting as a dopant.[292] As expected, the ETp across self-assembled monolayer showed a 10-fold increase in conductance when the tryptophan was placed close to the electrode (i.e., near the thiolated terminal), highlighting the role of improved peptide−electrode coupling in such a setup.

Although aromatic amino acids have been major source of ETp in peptides, self-assembled FF films by themselves have been shown to be electrical insulators.[290] Interestingly, upon cyclization of FF into cyclo-FF, which was previously shown to occur spontaneously during their vapor phase self-assembly,[293] the peptides show increased semiconducting properties.[294] The reason for the enhanced conductance in the cyclic state is likely due to the enhanced rigidity of this system, which leads to a lower band gap compared to the linear FF. Additionally, Amit et al. studied the ETp in the longer FF-containing peptide sequence AAKLVFF, derived from amyloid β peptide.[295] In this system, the replacement of both the phenylalanines with non-natural amino acids such as 2-thienylalanine leads to an appreciable enhancement of the relatively low conductivity of pure peptide nanotubes. The authors attribute the difference in conductivity to the morphology of assemblies, noting that the presence of aromatic side residues was essential. Bal et al. utilized a nonequilibrium self-assembly of a related cross β amyloid peptide HLVFFAE in a related study.[218] The peptide was functionalized in situ at the side chain of E with 4-nitrophenol using ester coupling agent EDC, which resulted in self-assembly. However, presence of H at the N terminal catalyzed the hydrolysis of the nitrophenol ester, leading to disassembly. As described in section 4.2, the competing formation and degradation reaction resulted in temporal control over self-assembly. Thus, the authors achieved a temporally controllable electronic conducting network. This integration of non-equilibrium self-assembly with control over the ETp provides a significant step toward bioinspired electronic devices that may be seamlessly integrated with living systems through provision of on-demand electronic connectivity with feedback control.

In aqueous systems, there are typically contributions from both ETp and proton transport in conductive protein and peptide-based systems. It is now recognized that, in general, humidity influences both processes differently, with high relative humidity supporting greater proton conduction. Humid proton conduction requires the presence of water, yet there is also interest in the development of dry proton conductivity for certain devices that may require operation that is not compatible with the presence of liquid water, e.g., in the design of fuel cells. Ashkenasy et al. surveyed a series of nanotube-forming cyclic octapeptides, based on the original design by Ghadiri's group of alternating D,L stereochemistry,[296] hypothesizing that the presence of polar amino acids together with aromatic residues would enhance (dry) proton transport.[297] The cyclic peptide sequence investigated were cyclo-$(KW)_4$, cyclo-$(KY)_4$, cyclo-$(EW)_4$, and cyclo-$(KA)_4$. The authors observed that, in dry conditions, long-range order

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

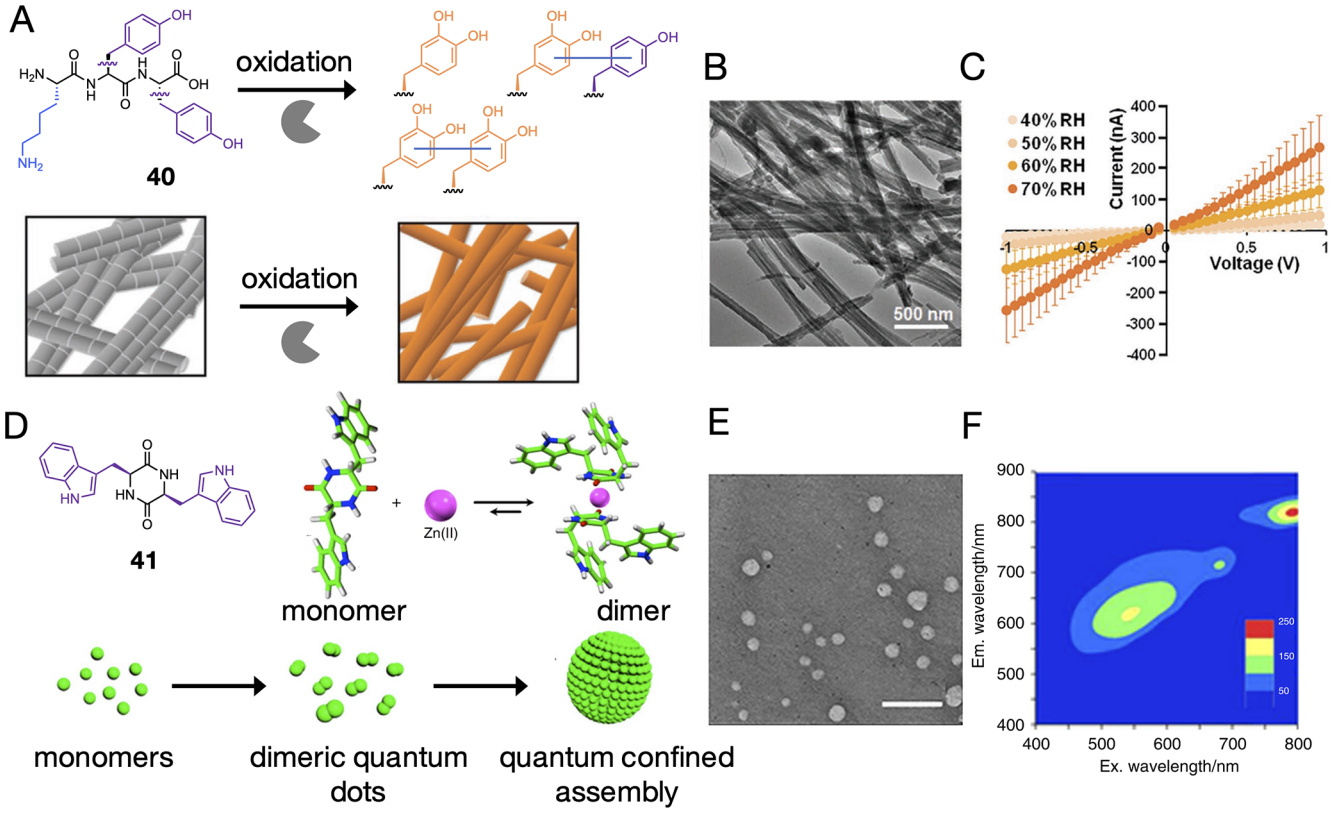


Figure 9. Peptides as building blocks for electro-/photoactive materials. (A) Chemical structure of self-assembling tripeptide KYY (**40**) and its chemical, supramolecular transformation upon enzymatic oxidation. The resultant oxidized product self-assembled into one-dimensional nanowires as seen by (B) TEM micrographs. (C) The conductance was measured at various relative humidity (RH) and the plot of current vs voltage confirmed that the conductivity is mainly mediated by PTp. (D) Chemical structure of cyclic dipeptide cyclo-WW (**41**) and the schematic representation of possible Zn (II) coordination resulting in formation of dimeric quantum dots and resultant quantum confined superstructures. The self-assembly resulted in formation of nanoparticles as seen with (E) TEM. Scale bar 300 nm. (F) The fluorescent nanostructure exhibited excitation wavelength dependent broad emission from visible to near-infrared spectral regions. (A−C) Reproduced with permission from ref 286. Copyright 2020 John Wiley and Sons. (D−F) Reproduced with permission from ref 287. Copyright 2018 Creative Commons.

had a greater impact on proton conductivity, favoring the incorporation of aromatic residues, whereas for conduction in hydrated conditions, presence of carboxylate moieties had a greater role. The same authors have shown that, similar to living system, both electron and protons can simultaneously be responsible for the total charge transport.[298] As expected, the contribution of proton transport is lower under low humidity and proton transport significantly increases upon increase in humidity, and their insights provide design rules to optimize these features.

The proton conduction in a linear peptide can also be enhanced by the introduction of a charged amino acid to the peptide sequence, given other self-assembly parameters are kept intact. For example, swapping the lysine to a glutamic acid residue in AAKLVFF enhanced the proton conductivity of the peptide by 28 times.[299] This is explained by the fact that acidic groups in glutamic acid contribute more charge carriers to the material than basic groups, as well as the ability of acidic charge carriers to be more mobile. Recently, Reddy et al. have investigated proton transport in KYY (**40**) tripeptide assemblies and subsequently in the enzymatically oxidized versions where covalent connections between Y residues along the length of the fibers dramatically enhanced the observed conductance through the formation of polyphenolic 1-dimensional nanofibers reminiscent of melanin (Figure 9A,B).[286] Interestingly, the enzymatically oxidized KYY fibers were an order of magnitude more conductive than their native KYY fibers due to the oxidative tyrosine linkage, providing conductive tracks for proton transport. By performing electrical measurements at different relative humidities (Figure 9C), the authors confirmed that the PTp is the main mechanism of conduction. The role of tyrosine oxidation in forming covalent conducting tracks for proton transport in response to a biological amplification mechanism (enzymatic oxidation) can potentially be a generalized design for enhancing conductance in peptide-based fibers in situ.

Fluorescence in biology has been investigated in proteins like green fluorescent proteins (GFPs), which has led to a variety of applications in biophotonics, sensing, fundamental cell biology and biochemistry, and diagnostics.[300] In GFPs, the inherent fluorescence originates from the conjugated chromophores that are formed by sterically induced SYG tripeptide cyclization in the β-barrel core of these proteins. Remarkably, in a minimalistic biomimetic approach, the self-assembly of aromatic peptides has shown promise as inherently fluorescent materials in the visible range. Similar to the observed self-assembly induced formation of semiconducting structures, the fluorescence property of the self-assembled peptide is mainly a product of the formation of quantum confined structures, which gives rise to lower electronic band gaps.[282,289] The quantum confinement in peptides containing aromatic amino acid originates from the tightly packed self-assembly involving

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

rigidification of aromatic interactions. The inherent fluorescence property of these self-assembled peptide particles holds promise for fluorescence imaging, as an alternative to external fluorescent dye labeling strategies.[301]

Supramolecular crystals formed from individual aromatic amino acids were shown to give rise to red-shifted fluorescence upon self-assembly and therefore show promise as biophotonic wires. The aromatic amino acids W, Y, and F were shown to assemble into amyloid-like structures, which showed intrinsic fluorescence in the blue region (450 nm).[302] Additionally, the intrinsic fluorescence could be used as a means to self-report the formation and distribution of amyloid-like metabolite self-assembly inside living cells.

Amdursky et al. demonstrated that crystalline FF peptide nanotubes, as a consequence of the aforementioned semiconducting band gap, display intrinsic fluorescence properties due to the supramolecular interactions in the self-assembly and crystallization process.[289] Detailed spectroscopic investigations revealed the emergence of a new absorption band near 300−370 nm along with a corresponding fluorescence peak in the visible region (450 nm). The source of the fluorescence was the unique zipper-like aromatic interlocks observed in FF assembly, resulting in an extremely stiff structure and efficient π-electron delocalization.[282] This works showed the importance of structural morphology and the molecular mechanism responsible for physiochemical properties.

Besides π-electrons delocalization, cation−π interactions can play a role in shifting spectral properties. This was demonstrated in several aromatic/cationic dipeptides by Juszczak et al., who reported intrinsic fluorescence in the visible range in other dipeptides. They observed that placing aromatic amino acids, such as tyrosine and tryptophan adjacent to cationic amino acids like arginine and histidine, led to visible light emission.[303] Detailed analysis confirmed the origin of fluorescence from the cation−π interactions in the self-assembled structure and formation of aromatic radicals. The dipeptides RW, YW, and HW displayed an intense pink color in the solid state, confirming the role of supramolecular organization in the emergence of these optical properties.

An interesting class of peptides which has received significant attention in regulation of optical properties are cyclic peptides. The optoelectronic properties of peptides in the cyclic form are significantly enhanced compared to the linear form due to their structural rigidity, stronger intermolecular interactions due to the presence of two peptide bonds, unique hydrogen bonding pattern, and improved proteolytic stability.[304] Gazit's group presented tryptophan based aromatic cyclo-dipeptides like cyclo-FW and cyclo-WW (**41**) where photoluminescence originated from the formation of a quantum confined assembly (Figure 9D).[287] Interestingly, the emission could be tuned from the visible to near-infrared spectral regions (420−820 nm) as shown in Figure 9F. Such a tunability was obtained through the change of various parameters, including amino acid substitution, metal ion coordination, oxidation, and solvent replacement, all of which directly affected the self-assembled structures of the dipeptides. The coordination of Zn^{2+} to cyclo-WW resulted in the formation of nanoparticles (Figure 9E), which were utilized for in vivo imaging and light-emitting diodes. Furthermore, cyclo-HH coordinated to Zn^{2+} exhibited bright fluorescence emission with 70% quantum yield, which is among the highest reported so far for peptide-derived materials.[305] The enhanced fluorescence was hypothesized to be due to the self-assembly locking strategy, whereas the Zn^{2+} co-ordination rigidifies the chromophore, thereby reducing fluorophore mobility. The material was utilized for the development of a prototype light-emitting device and as a nanocarrier for intracellular drug delivery and bioimaging. The same group also reported the crystallization of cyclo-GW, which showed aggregation-induced blue emission and wave guiding properties.[306] Additionally, the crystals demonstrated significant piezoelectric responses by generating high and stable currents under force. These bioinspired materials could, in the future, act as an alternative to inorganic counterparts for optoelectronic applications.

The oxidative dimerization of tyrosines provides a route toward shifting emission to the visible region.[307] Min et al. investigated the self-assembly of a Y containing peptide, which was subsequently dimerized using the well-known UV-induced cross-linking of tyrosine to form dityrosines.[308,309] Ruthenium catalyst-mediated photo-oxidation of Y in the peptide YYAYY resulted in the formation of fluorescent peptide nanoparticles with blue emission at 410 nm, owing to the tyrosine−tyrosine covalent bond. Interestingly, these particulate nanogels could be utilized as a bioreactor for the formation of gold and platinum nanoparticles without an external reducing agent, resulting in a versatile metal−peptide hybrid material. Lampel et al. performed the enzymatic oxidation of Y on a self-assembling KYF tripeptide to obtain melanin-like chromophoric microparticles with unique optical properties.[310] The work shows that the preorganization of Y on self-assembled KYF was crucial during enzymatic oxidation to form green emitting nanostructures (λ_{em} = 533 nm). Detailed chemical analysis revealed the formation of new catechol and quinone units, which further polymerize to form stable assemblies. Interestingly, incorporation of additional amino acids (F, I, C, and others) led to remarkable shifts in emission due to the formation of new chromophores by chemical incorporation. For example, by adding F to the particles formed by enzymatic oxidation of KYF led to a 40 nm red-shifted yellow emission with greater intensity. Addition of other amino acids like C resulted in a blue-shifted, yellow solution, whereas isoleucine gave rise to a distant red emission at 621 nm. Taken together, oxidation of aromatic amino acids in self-assembled peptides represents a new method to diversify the field of fluorescent peptides for biophotonic applications.

Apart from materials produced from unmodified short peptide, simple chemical modification has been proven to enhance photonic properties. Kong et al. constructed a ferrocene-functionalized tyrosine-based oligopeptide that displays green fluorescence combined with cell permeability for cell imaging.[311] The peptide consisted of a YY dipeptide motif, a GPGR motif to enable cell penetration and a ferrocene group to enhance self-assembly. The sequence of the final structure is Fc-YYGCGPGRC, with the two cysteines dimerized to create a cyclic construct. The peptide displayed a bright-green emission at 520 nm. Interestingly, like GFP, the peptide showed fluorescence only upon deprotonation, confirming that the phenolate form of Y is responsible for emission. The peptides were cell penetrable and thus could be used for fluorescent imaging of HeLa cells.

In summary, short aromatic peptides hold much promise as novel photonic materials where the inherent fluorescence emerges from a range of interactions like aromatic stacking and cation−π. Furthermore, emission is enhanced by structural rigidification, providing aromatic amino acids with a central

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

Table 2. Different Classes of Fluorescent Peptides Investigated and Their Emission Maxima

single amino acid[302]	sequence	F	Y	W			
	λ_{em} (nm)	435	460	480			
dipeptide[289,303]	sequence	FF	WF	YW	HW	RW	WS
	λ_{em} (nm)	465	390	522	501	519	506
cyclic dipeptide[287,306]	sequence	cyclo FF	cyclo YY	cyclo HH	cyclo FW	cyclo WW	cyclo GW
	λ_{em} (nm)	530	570	430, 460	460	425, 520	420
cyclic dipeptide with Zn(II)[287,305]	sequence	cyclo FW + Zn(II)	cyclo HH + Zn(II)	cyclo WW + Zn(II)			
	λ_{em} (nm)	465	500	520, 610, 712, 817			

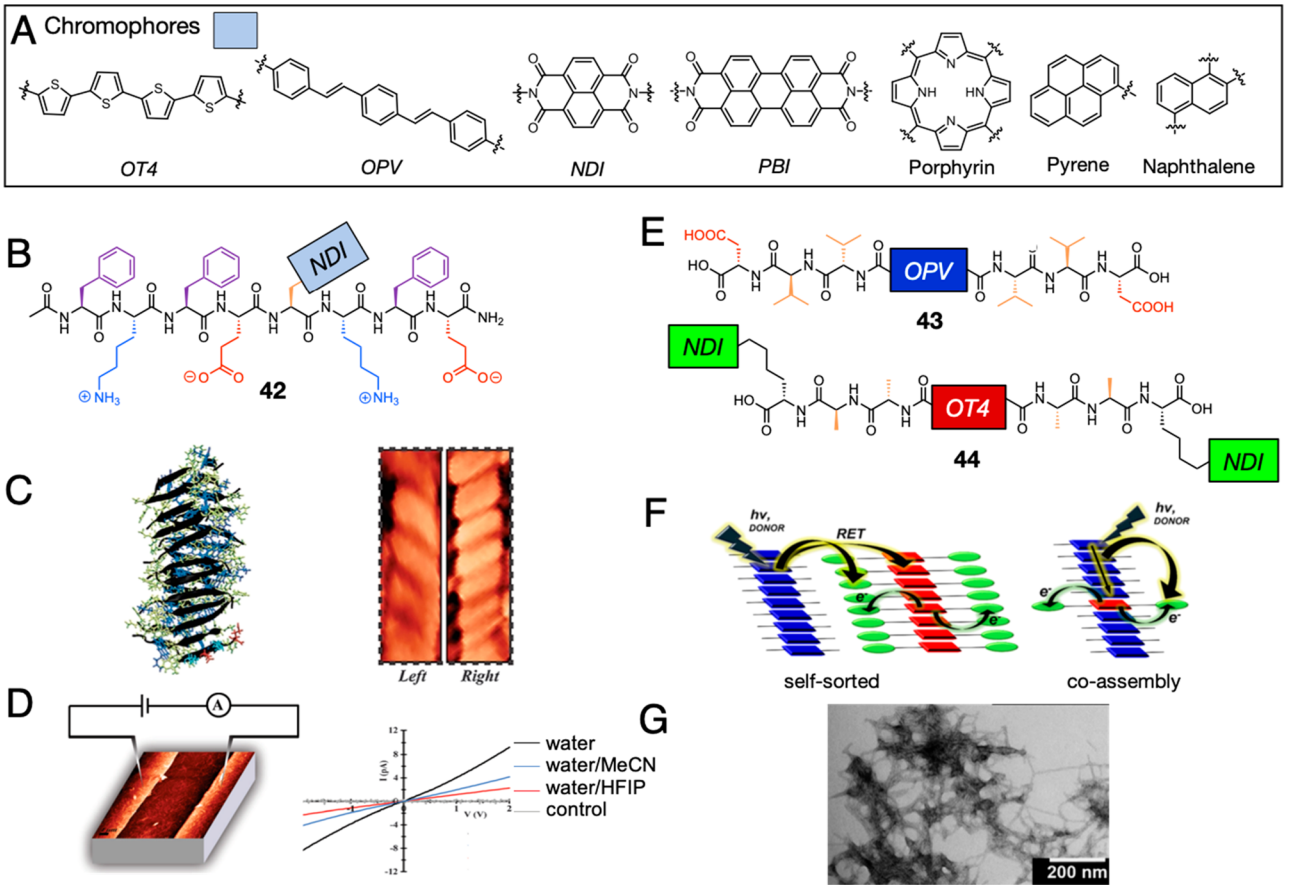


Figure 10. Chromophore modified peptides as electro-/photoactive material. (A) chemical structure of various chromophores used for conjugation with peptides. (B) Chemical structure of NDI−peptide conjugate (**42**), which shows polymorphic self-assembly in different solvent mixture of water with MeCN (acetonitrile) or water with HFIP (hexafluoro-2-propanol) to result in either majorly left-handed or right-handed helical assembly which could be imaged by (C) AFM. The conductivity measurement was performed using a device shown in (D) and current vs voltage graph data were plotted for different polymorphic forms. (E) Chemical structure of peptide conjugate with OPV (**43**) and with NDI and thiophene (**44**). (F) Schematic illustration of multichromophoric self-assembly of **43** with **44**, which under kinetic control can form either self-sorted assembly or coassembly resulting in a complex EnT and CT process. (G) TEM micrograph showing self-assembled structure of 1:1 mixture of **43** and **44**. (B−D) Reproduced with permission from ref 312. Copyright 2016 John Wiley and Sons. (E−G) Reproduced with permission from ref 313. Copyright 2017 ACS.

role in determining fluorescence. As seen in Table 2, it is not trivial to provide simple design rules for developing fluorescent self-assembling peptides. However, some conclusions can be drawn. As a general observation, W containing peptides shows more red-shifted emission compared to Y and F. The self-assembly itself is necessary for fluorescence, whereas additional rigidification methods like cyclization and metal co-ordination of peptides give rise to higher wavelength (visible to red) emission. Furthermore, these systems hold much promise for incorporation in complex functional systems. Because the emission can be dynamically regulated by covalent modifications, such as through chemical or enzymatic oxidation, can implement extended conjugation and improve emission properties, which is expected to find uses in dynamic reporting of metabolite levels in complex media as well as the formation of biophotonic wires in response to localized catalytic activity.

5.1.2. Peptides with Chromophoric Conjugation. In living organisms, most of the electron and photon transfer

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

processes are mediated by proteins that incorporate macrocyclic chromophores, resulting in properties such as charge migration and photosynthesis. Biomimetic peptide structures have also taken advantage of chromophore conjugation, either covalently or noncovalently, to enhance their inherent properties or introduce new functions. Both biomimetic structures derived from porphyrins and completely synthetic structures derived from organic electronics have been studied in this context for charge transport and artificial light harvesting.

To improve the low absolute conductivity of many purely peptide-based systems, a wide range of organic chromophores have been incorporated into peptides, either through covalent attachment or noncovalent coassembly. In such cases, the peptides act as the structural motif to provide suitable organization, and the chromophores act as the functional conducting motif. Through supramolecular organization, the chromophores' inherent electronic properties are further enhanced by peptide-mediated structures. However, despite the well-understood relationship between peptide design, structure, and function, the influence of sequence, morphology, and order are not additive properties. Thus, the challenge posed by peptide-mediated electronic materials cannot be solved purely by a reductive approach but rather through holistic design principles catered uniquely to each system. Moreover, combining electronic wires with active and adaptive designs will give rise to aqueous-compatible, biomimetic electronic circuits that cannot be developed using conventional organic electronics.

Aromatic π-conjugated units like naphthalene diimide (NDI) and thiophene (Figure 10A) are well-suited for supramolecular electronics[314,315] because of their strong intermolecular aromatic−aromatic interactions which contribute to self-assembly, while their inherent n-type or p-type organic semiconducting characteristics confer desirable ETp properties. In this regard, Diegelmann et al. introduced a bola-amphiphilic peptide-based design for 1-D optoelectronic nanostructures incorporating a π-conjugated moiety within an asymmetric, self-assembling oligopeptide.[316] In this design, the chromophore of choice was flanked by two peptides, creating a conducting track which is partially shielded from the solvent environment. This class of aromatic peptide amphiphiles has the distinction of assembling in aqueous conditions into helical nanofibers. The first published examples had the composition NNDFA-bithiophene-DFANA, where the bithiophene π-conjugated functionality is embedded within the peptide rather than presented terminally for enhanced ETp properties. More recently, the aromatic-bola-amphiphile was developed further into an efficient electrically conducting material and to combine photoexcitation and energy transfer.[280] The authors applied the heat−cool injection method to align a peptide gel with an embedded chromophore to introduce macroscale alignment. Peptide functionalized α-quaterthiophene, i.e., OT4 in Figure 10A (EEA-OT4-AEE) and 1,4-distyrylbenzene (VEVAG-benzene-GAVEV) were investigated.[317] As envisaged, alignment introduced anisotropic electronic and photophysical properties and led to an order of magnitude increase in hole mobility.

Lee et al. reported an organic−inorganic hybrid self-assembly of peptide conjugated with a thiophene-based π-conjugated unit.[318] The peptide sequence GAVEV assembles upon acidification. Interestingly, slow addition of HCl resulted in formation of KCl in situ, which mineralized on the peptide assembly using E as a template. The self-assembly resulted in highly conductive dendritic microstructures, whose conductivity was comparable to the metals. The enhanced ETp property was proposed to be due to the proton doping of π conjugated unit and closely spaced chromophores in the biomineralized dendritic structures. This example clearly demonstrates the potential of amino acid templated biomineralization and inorganic-peptide−chromophores hybrid structures in developing electrically efficient biomaterials.

In peptide−π systems, the sequence to optoelectronic function correlation is poorly understood. In this regard, molecular dynamics (MD) simulation is an efficient exploratory method.[57] However, considering the massive chemical space, with 8000 different possibilities for tripeptides alone, it becomes computationally challenging to traverse the sequence space of larger peptides. Shmilovich et al. reported an active learning directed coarse grained MD simulation of an DXXX-oligo(*p*-phenylenevinylene)-DXXX based tetrapeptide−π−tetrapeptide system to identify the best assembling XXX tripeptide sequences.[319] Active learning and MD simulations independently have relatively good screening speeds, but combining them together accelerated the search by efficiently screening just 2.3% of the total chemical combinations. The study confirmed the already known designs, but importantly they also revealed some nonintuitive sequences. It predicted that good assemblers would be enriched in intermediate-hydrophobic residues like G, A, L, V, and I and would lack aromatic residues like F, Y, and W. Methionine broadly had no effect on assembly, except when adjacent to the core of the molecule. This approach combining active learning and MD simulation provides an efficient means to improve performance of existing systems and develop new peptide−π conjugate for optoelectronic applications.

To study the influence of NDI substitution, Ivnitski et al. investigated the self-assembly of an octapeptide FKFEF*KFE, where the fifth F residue was substituted by NDI (**42**).[312] Interestingly, they observed greater than a 2 order increase in conductivity upon NDI substitution, confirming the crucial role of NDI in ETp. Additionally, the system exhibited polymorphic self-assembly under different environmental conditions. For example, in a water/acetonitrile mixture, it formed long, left-handed fibers, while much shorter right-handed fibers were found in water/HFIP. Assembly in water at pH 5.5 produced fibers with a racemic mix of both orientations (Figure 10C). It was observed that the organization of NDI in polymorphic forms can affect the ETp properties. Measurement of electronic conductivity of the same peptide−NDI conjugate in different polymorphic state demonstrated a 5-fold difference in conductivity (Figure 10D). Despite the very low absolute conductivities observed in these peptides, the large increases in relative conductivity for different polymorphs reveal that the selection of solvent environments can dictate morphology and consequent function and thus provides an important processing design parameter to tune when devising new conductive peptides.

Xu et al. utilized biocatalytic self-assembly of Fmoc functionalized short peptides (Fmoc-L_3), which formed nanotubular gels upon enzymatic hydrolysis from the corresponding methyl ester in aqueous medium.[320] They showed that the formation of helical β-sheet structures aided in the positioning of fluorenyl groups to permit π−π contact. The conductive measurement of the xerogel was performed using impedance spectroscopy, which confirmed the conducting

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

nature of the nanostructures. Detailed mechanistic investigation of the system revealed the contribution of electronic transport in vacuum as well as charge transport through adsorbed moisture in the air.

Doping is a well-established procedure in inorganic semiconductor industry to improve the electrical properties of devices. Similarly, the ETp property of purely n-type or p-type semiconducting supramolecular structures can be enhanced by doping with an electron acceptor or donor. Alternatively, formation of charge transfer complexes with alternating stacks of acceptors and donors molecules can also enhance conductivity.[285] Following that principle, Khalily et al. designed a system containing of 1-D peptide nanowires with highly ordered donor−acceptor stacks.[321] Electron donor pyrene was conjugated to the N terminal of VVAGKK to form the p-type peptide, whereas electron acceptor NDI was conjugated to the N terminal of VVAGEE to form the n-type peptide. The charge complementarity of the two peptides and their β-sheet promoting sequences could be exploited to assist in formation of a coassembled charge transfer complex. The two peptides coassembled through H-bonding, charge transfer interactions, and electrostatic interactions. These interactions resulted in uniform conducting nanowires. The donor−acceptor charge transfer nanowires were vastly more conductive than individual nanostructures, with the conductivity 10 times greater than the p-type peptide and 2400 times greater than the n-type peptide, confirming the potential of charge transfer complexes.

Several other peptide charge transfer complexes have been investigated for enhanced electronic properties. For example, the organic donor molecule, tetrathiafulvalene, was conjugated with FF-NH_2 to form p-type semiconducting nanofibers with limited conductivity.[322] However, upon doping with an electron acceptor, tetracyano-*p*-quinodimethane or iodine, a charge transfer complex was formed with up to 4 times enhanced conductivity compared to the p-type nanofibers. One limitation of the design was its incompatibility with aqueous medium, as all samples were drop-casted from chloroform solution. To design an aqueous compatible system, the well-known acceptor−donor pair NDI and naphthalene were used.[285,323] Peptide-functionalized NDI was also shown to form efficient charge transfer complex with naphthalene using biocatalytic self-assembly.[179]

ETp can also be mediated through the absorption of photons by chromophores. In this case, the absorption of light by a chromophore generates exciton, which under an applied voltage can generate a photocurrent. This approach has resulted in the demonstration of several photoconductive peptide−chromophore conjugates. Roy et al. investigated the photocurrent response of peptide functionalized perylene bisimide (PBI) chromophores.[324] Conjugation of PBI with Y on both sides resulted in the formation of hydrogel. Interestingly, the photoresponse of the xerogel under visible and white light showed high current conduction compared to dark current. The current could be switched on and off multiple times with current on/off ratio of 18 and 51 for visible and white light. Draper et al. investigated multiple PBI conjugated with either A, H, F, or V, which formed dark-red-colored gel.[325] Photocurrent measurement of both the dried solution and xerogel showed ohmic response with significant increase of current under illumination. Interestingly, the photocurrent persisted for up to 8 h after illumination, which was explained through the formation of very stable radical anions. The same group reported the first example of peptide functionalized diketopyrrolopyrrole hydrogelator.[326] The xerogel formed a photoconductive film whose conductivity could be enhanced by radial shear-induced alignment of self-assembled fibers. These examples demonstrate that chromophores functionalized peptide can be useful as a photoconductive material for potential applications in organic solar cells that may ultimately be biodegradable and recyclable.

5.1.3. Light-Harvesting Properties of Peptide Nanostructures Functionalized with Aromatic Groups. Photosynthesis is the process of light harvesting performed in the living organism. Fundamentally, it is a process where chromophoric chlorophyll molecules absorb sunlight and transfer the energy to various reaction centers for conversion into chemical potential. Two important requirements of such a process are the appropriate organization of various chromophoric molecules and suitable matching of the optical band gap. In this regard, self-assembling peptides have been used as a well-organized matrix for the coassembly of chromophores to convert light into more useful forms.

Porphyrins are one class of biological chromophores found in the chloroplasts of plant cells that crucial for harvesting solar energy. The specific electronic arrangement of porphyrins facilitates efficient photoabsorption. Synthetic porphyrins tend to aggregate in solution, although these aggregates by themselves do not show sufficient light-harvesting activity owing to their lack of well-defined organization. In recent years, coassembly of porphyrins with short peptides has been shown to enhance the functionality of porphyrins, a promising first step toward bioinspired light-harvesting materials.

In 2012, Park's group demonstrated the coassembly of tetra(*p*-hydroxyphenyl) porphyrin (THPP, porphyrin derivative in Figure 10A) with FF dipeptide nanotubes.[327] Inspired by natural photosynthesis, Pt nanoparticles were decorated on the nanotube surface to act as an electron separator to increase the efficiency of photoinduced electron transfer. It was shown that Pt decorated nanotubes performed visible light photocatalytic regeneration of NADH efficiently. Kai et al. coassembled anionic porphyrin with cationic dipeptide KK, through electrostatic interactions, to form fiber bundles capable of catalyzing the oxidation of iodide to triiodide.[328] The porphyrin coassembled peptide fibers also catalyzed the synthesis of Pt nanoparticles with light through peptide nanostructure templated catalysis.

In section 2.1, we discussed that peptides with aromatic amino acids can directly absorb and emit light energy in the form of fluorescence. Additionally, in a multichromophoric system, they can perform energy transfer (EnT, Figure 9). The process is mainly useful in converting nonusable ultraviolet-blue light into more useful visible light. As one of the simplest example of such peptide conjugates, Nap-FF donor molecules perform efficient energy transfer when an acceptor chromophore (anthracene, dansyl derivatives) is coassembled in a fully aqueous media.[329] Excitation of the Nap-FF at 280 nm resulted in the emission at 485 nm from the dansyl derivative, confirming energy transfer.

Ardoña et al. developed a multichromophoric self-assembling system that could absorb energy from light and apply it toward electron transfer (Figure 10E−G).[313] The peptide functionalization of three different chromophores were investigated. Specifically, two sides of oligo(*p*-phenylenevinylene) i.e., OPV were conjugated with the N terminal of VVD tripeptides (**43**), whereas the two sides of quaterthiophene *i.e.*,

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

OT4, was functionalized with the N terminal of AAK. The side chain amine of K in AAK was further conjugated with NDI to form NDI−peptide−OT4 conjugate (**44**) as shown in Figure 10E. Additionally, the three chromophores were chosen to have different optical band gaps and were spatially positioned within 1-dimensional peptidic nanofibers to facilitate energy transfer and electron transfer processes. Light absorption by OPV in **43** funnels energy to OT4 in **44**. Because **44** is terminated on both ends with electron acceptor NDI groups, it resulted in excited-state electrons transfer from the OT4 to NDI as shown schematically in Figure 10F. Interestingly, kinetic control over the self-assembly through slow acidification resulted in the formation of self-sorted coassembly due to the difference in pK_a of the two peptides. However, rapid protonation of peptides resulted in a randomly mixed coassembly. Although both the self-sorting and mixed assemblies resulted in nanofibers and gel formation (Figure 10G), the energy transfer and exciton migration were most efficient in the self-sorted coassembly. This is a unique example of a system with multiple degrees of optical and electronic control for future bioelectronic applications.

A number of self-assembling peptides exist in kinetic states with suboptimal supramolecular organization, limiting their performance. Addressing this challenge, Wijerathne et al. designed a peptide−porphyrin gel that assembles in aqueous buffer at pH 8. Tetrakis(4-carboxyphenyl)porphyrin (TCPP, porphyrin in Figure 10A) was coassembled with Fmoc-TL-NH_2, which was dynamically and reversibly formed in situ through enzymatic condensation.[186] Fluorescence spectroscopy confirmed the efficient EnT from the Fmoc moiety to the porphyrin. As discussed in section 4.2, due to the fully reversible nature of biocatalytic peptide formation, the self-assembly was under thermodynamic control, which could be responsible for efficient EnT. Interestingly, they report a TCPP concentration-dependent two-step self-assembly process into either a homogeneous coassembly or heterogeneous self-aggregated assembly of TCPP. Such a two-step assembly provided additional control over the EnT process.

5.2. Catalysis and Reactivity

Enzymes are still the envy of chemists when it comes to the ability to utilize chemomechanical actuation and appropriately positioned functional groups to catalyze reactions with high selectivity.[330,331] Enzymes are complex supramolecular machines, and their catalytic activity arises from their three-dimensional structure. The focus is often on the preorganized active site which forms a binding pocket, but it is appreciated that catalysis is further facilitated by the utilization of flexible structural motifs comprising a network of complementary "networked" amino acid residues to optimally bind and position the substrate in favorable configurations to react with the catalytic amino acid residue(s). The importance of this 3D flexible network around the active site is perhaps best illustrated by the dramatic impact that some mutations may have that are remote from the active site. In addition to catalytic amino acid residues, metal ions, especially divalent cations, play a crucial role in protein-mediated catalysis in nature. They function as redox centers, activating reactive species and stabilizing intermediates and transition states. Despite these insights, experimental efforts to synthetically mimic enzyme performance has proven anything but simple.

Curiously, biology has selected only a relatively small number of ordered protein folds from the myriad of theoretically possible structures, suggesting that appropriate scaffold designs can be repurposed for a variety of functionalities. Furthermore, while enzymes typically consist of hundred or more amino acids, it has been proposed that they evolved from much simpler functional precursors, suggesting that simple sequences can perform catalytic functions. These observations serve as inspiration for the development of peptide-based catalytic materials.

Several examples of short peptides capable of catalyzing chemical reactions, albeit with modest efficiency, have been reported in the literature. In the early 1980s, Behmoaras et al.[332] discovered that the tripeptides KWK and KWW are able to detect apurinic sites in double-stranded DNA by $\pi-\pi$ stacking interactions and cleave the DNA backbone. The details of the mechanism by which this remarkable catalyzed reaction occurs, however, remained unclear. Even shorter peptides, such as Ser-His (SH) and related analogues, designed to provide a minimalistic mimic of the catalytic center of serine proteases, have been used to catalyze the hydrolysis of amides and phosphodiesters in biopolymers,[333] in addition to the formation of peptide bonds between activated substrates through transacylation,[334] although the effectiveness of SH as a catalyst has been disputed.[335] Separately, the use of amino acids and peptide-based catalysis has long been explored in conventional (nonaqueous) organic chemistry.[336]

It has been suggested that self-assembling peptides, which spontaneously form supramolecular structures, could be powerful candidates for the design of novel, minimalistic catalysts. The approach could enable incorporation of features that are not present in natural catalysts. Important factors to be considered are the primary sequence to control the composition of chemical functionality, presentation of arrays of active residues on scaffolds, multivalency, and the presence of metal cofactors,[337] giving rise to "catalytic materials". Consequently, the scientific community has started to explore various self-assembly strategies as well as metal-binding sequences to produce catalytic systems.[338]

It is beyond the scope of the current review to provide a complete overview of all the excellent work that is emerging in this field, and we refer to recent reviews for the interested reader.[338] Our focus here is to consider in particular those examples that illustrate design approaches unique in minimalistic peptide design and in particular those involving consideration of coassembly, order−disorder, and induced fit in design.

In one of the earlier examples, Guler et al. reported on the design of a cylindrical peptide nanostructure that was designed to present catalytic histidines to the fiber surface. The design was based on aliphatic amphiphiles and contains a His-residue on the surface and catalyzes hydrolysis of 2,4-dinitrophenylacetate (DNPA), a substrate commonly used to investigate ester hydrolysis.[339] Importantly, the results demonstrated that morphology impacts catalysis, as the observed reaction rates were greater for highly ordered nanofibers than for less ordered spherical aggregates. A related peptide amphiphile was shown to bind to heme groups and behave as a peroxidase, catalyzing the oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB). For equivalent peptides, micellar morphologies enhanced catalytic activity compared to their fibrous counterparts.[340] Another approach has been to create catalytic triads such as Ser-His-Asp by coassembly of peptide amphiphiles containing Ser, His, and Asp individually. These authors show that the coassembled triad nanostructure had highest esterase activity followed by

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

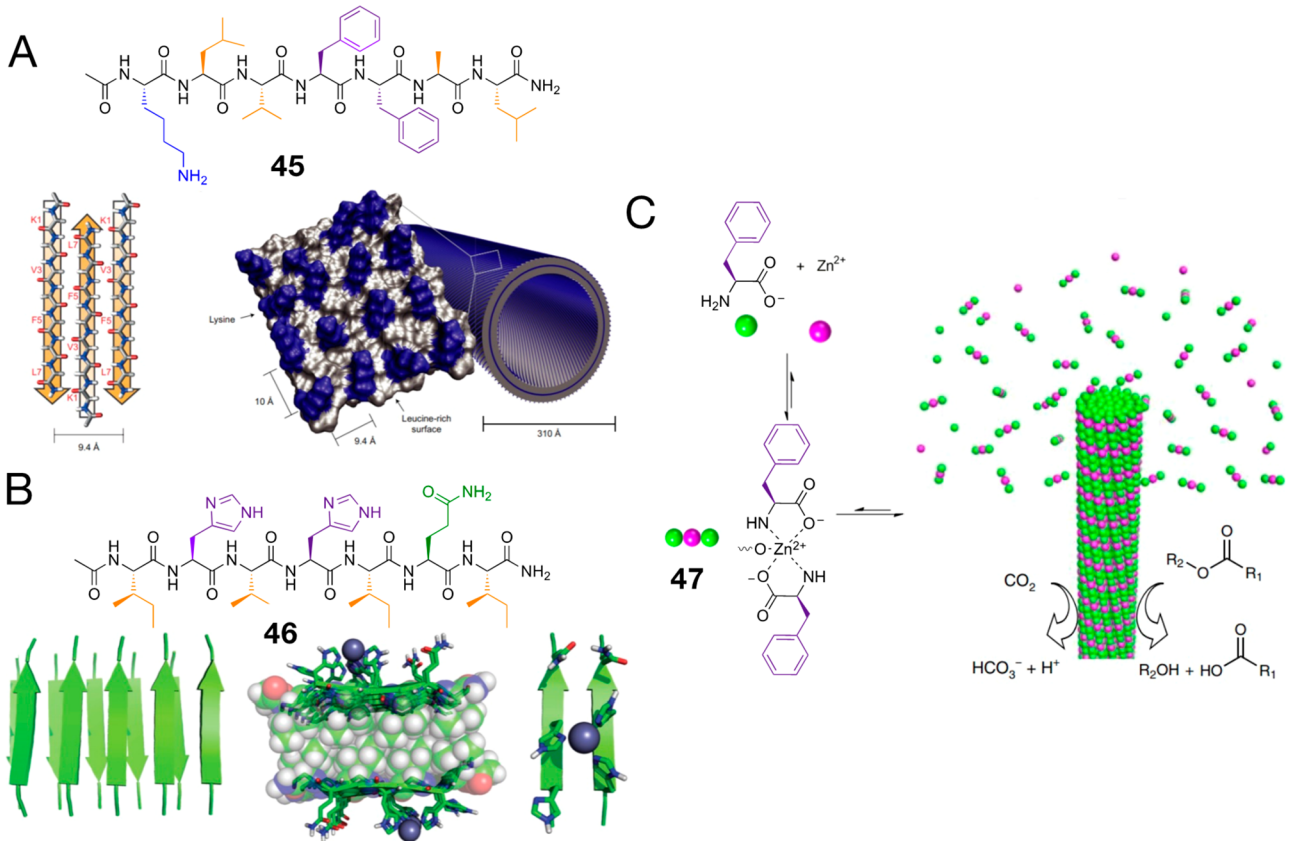


Figure 11. (A) Molecular structure and schematic representing the β sheet packing of Ac-KLVFFAL-NH_2. (B) Molecular structure and schematic representing the β sheet packing of Ac-IHIHIQI-NH_2 along with binding of Zinc ions. (C) Reversible reaction of zinc ion induced complexation and ensuing assembly. Catalytic reactions performed by the supramolecular fiber are also shown. A, B, and C reproduced with permission from refs 342, 343, and 347, respectively. Copyright 2017, 2014, and 2019, respectively, Springer Nature.

combinations of His-Ser, His-Asp and Ser-Asp nanostructures.[341]

Omosun et al. constructed short peptides that self-assemble into amyloid fibers, thus providing a paracrystalline surface that catalyzes retro-aldol condensation.[342] The observed peptide-mediated catalysis is enantiospecific, and the exchange of single amino acids could be applied to predictably control the self-assembled structure and consequently the catalytic potential. The sequence Ac-KLVFFAL-NH_2 (**45**) (Figure 11B), which was modified after the Aβ peptide of Alzheimer's disease, was found to increase the rate of aldol condensation $>10^4$ times more compared to Lys alone. The sequence Ac-RLVFFAL-NH_2, which only differs in the C-terminal Arg-residue, however, did not display catalytic activity as compared to Lys. Moreover, shortening the amine tether by replacing Lys with ornithine and β-alanine resulted in structures with significantly higher initial rates of conversion. Thus, the authors conclude that the position of the primary amine group is crucial for efficient catalysis of the reaction.

Rufo et al. combined the integration of metal-ions with the use of supramolecular structures and applied amyloid-forming heptapeptides to achieve metal-mediated ester hydrolysis.[343] The original peptide sequence was modified to obtain rational mutants with a stable β-sheet structure and the capability to bind metal ions. The heptapeptide sequence Ac-IHIHIQI-NH_2 (**46**) (Figure 11B) was found to be the most catalytically active of the ones that were tested, with a k_{cat}/K_M value of 360 M^{-1} s^{-1}. This is approximately 7-fold lower than the activity observed for the enzyme carbonic anhydrase. However, on a molecular weight basis, the peptide hydrolyses *p*NPA more efficiently. As discussed above, the formation of an ordered structure is crucial to the catalytic activity of these peptides. It was later determined that amyloid-forming heptapeptides interact synergistically with one another in binary mixtures to enhance catalytic activity, although a structural and mechanistic reasoning for this behavior is yet to be determined.[115]

Copper-binding peptide amyloids have been shown to efficiently catalyze hydrolysis of phosphoesters.[344] The heptapeptide Ac-IHIHIYI-NH_2, which is the Q6Y mutant of a previously reported ester-hydrolyzing peptide, was found to accelerate hydrolysis of paraoxon, a widely used pesticide, by more than 3 orders of magnitude. The formation of ordered amyloid structures is induced by binding of Cu^{2+} to the peptide and the maximum catalytic activity was detected at a 1:1 molar ratio of peptide to metal. However, no particular mechanism of catalysis was proposed.

Supramolecular self-assembly can also provide with structural order that may be exploited to induce enhanced catalytic prowess to external chromophores. Recently, the spontaneous cyclization and self-assembly of dipeptide methyl esters was reported, where the intramolecular aminolysis leads to the formation of cyclic self-assembled structures in aqueous media.[168] The obtained morphologies were found to be sequence-specific, e.g., the dipeptide LF-OMe yields *c*(LF) fibers that result in gelation, whereas the sequence LL-OMe assembles into tapes and the dipeptide DF-OMe forms spherical aggregates. It was found that cooperative assembly

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

of *c*(LF) and the metalloporphyrin Fe^{III}-TMPyP produced nanofibers that catalyze the oxidation of organic phenol in water. Additionally, chromophores like diketopyrrolopyrrole (DPP) can be imparted significant photocatalytic activity when coupled with peptide-based supramolecular gelation.[345] In this example, the aqueous self-assembly process of the poorly soluble bola-amphiphile was facilitated by in situ enzymatic hydrolysis from a methyl ester precursor.

Singh et al. found that two amphiphilic peptide monomers self-sort and form discrete supramolecular fibers without coassembling with each other in aqueous conditions, isolating an acidic catalytic group on one fiber from a basic catalytic group on the other.[346] One was a valine-containing bola-amphiphile with carboxylic acid termini, while the other had a PV dyad at the N terminus, followed by a 12-C alkyl tail. Hydrogels of these molecules by themselves have been shown to catalyze aldol condensation and deacetalization reactions. The charge isolation permits a one-pot deacetalization-aldol reaction, which is not achieved in a coassembled system where the two monomers did not self-sort.

Moreover, aromatic peptide amphiphiles have been designed with esterase activity by incorporation of histidine residues, as well as residues that further assist catalytic activity.[337] The sequences Fmoc-FFH and Fmoc-FFR were based on the well-known sequence Fmoc-FF, which is known to self-assemble into nanotubes. The tripeptide amphiphiles coassemble into stable β-sheet structures that hydrolyze the esterase-substrate *p*-nitrophenyl acetate. The common feature that these aromatic peptides share with the aliphatic ones is the highly ordered supramolecular structure. Histidine-based amphiphiles have been also shown to form Fe^{3+} binding metallo-gels that show higher pNPA hydrolytic activity when compared to their supramolecular counterparts with no metal coordination.[348]

Dolan et al. recently found that aliphatic peptide amphiphiles of the general sequence ^{D}PX-C_{16} (with X representing all 20 genetically encoded amino acids) associate with iridium to produce highly enantioselective catalysts for transfer hydrogenation of a vast array of ketones.[349] The optimal peptide had the form HN-^{D}PL-C_{16}, although many PX dipeptides showed some amount of activity. Although appreciable enantiomeric specificity is observed prior to any self-assembly, enantiomeric excesses of greater than 90% are regularly observed afterward for a variety of ketones, comparable to the specificity of established iridium-based catalysts complexed with monotosyl ethylenediamine.

Tena-Solsona et al. demonstrated the emergent catalytic behavior of PEF and PFE-based peptides, finding a relationship between accessibility of the active site and catalytic efficiency, in this case as an aldolase.[350] PFE-based peptides were generally more efficient than PEF-based designs, hypothesized to be due to phenylalanine's role in secondary interactions at the catalytic site. Introduction of a hydrophobic alkyl tail, which assists in self-assembly, dramatically increased catalytic efficiency and yield. An important structural parameter identified here was the substrate's access to the catalytic site: the gel $PFEC_{12}$ was able to incorporate one equivalent of cyclohexanone, while the aggregates of $PEFC_{12}$ could only incorporate 0.5 equivalent. The structure is thus closely related to the catalytic effectiveness.

Recent work by Makam et al.[347] showcased a nonproteinaceous hydrolase that displayed the highest catalytic efficiency among all reported artificial biomolecular hydrolases despite its low molecular mass (Figure 11C). Formation of the catalytic amyloid-like supramolecular structure is achieved by assembly of only the amino acid phenylalanine coordinated with zinc ions (**47**).

Collectively, these examples demonstrate that short peptides can catalyze a variety of chemical reactions by combining supramolecular self-assembly and incorporation of appropriate chemical functionality through (combinations of) amino acid side chains, cofactors, and/or metals. However, catalytic activities are typically still orders of magnitude lower compared to those observed in enzymes and despite elaborate designs, the catalytic efficiencies remain stubbornly low.[230] Analogous to the design of supramolecular materials based on peptides, the focus has typically been on identification of peptides that form stable "supramolecular conformations", i.e., there is an emphasis on presenting and positioning functional amino acids appropriately for catalysis to occur, with less consideration of dynamic organization or *induced fit*. As proposed by Emil Fischer 125 years ago,[351] many protein−ligand interactions are initially weak, but increase in strength upon structural rearrangement of the receptor that favors complexation.[352]

Regulation of enzyme-like catalysis requires introduction of flexible domains to enable preorganization and induced fit, which is expected to give rise to more subtle regulation of catalytic activity. These features are not commonly incorporated into designed peptide catalysts. Zhang et al. demonstrated a designed supramolecular peptide catalyst that could be switched on and off through a pH-induced conformation switch,[278] which relied on a subtle and reversible bringing together of a catalytic histidine with a hydrophobic binding site to (in)activate catalysis. This structure presented among the highest reported activity of a designed peptide nanostructure that does not use a metal cofactor. The peptide consists of two β-strands (VK repeats) that become connected by a type II′ β-turn upon H-bonding between the two strands. The catalytically active His-residue is located at the N-terminus, connected to the VK repeats via a dipeptide linker. At neutral pH, the protonation of the Lys side chains results in repulsion of the β-strands, inducing a random coil conformation. However, upon increasing the pH to 9, the peptide assembles into ordered β-sheets. The His-residues are thereby positioned in an ordered array, creating a catalytic microenvironment. With a k_{cat}/K_M of 19 M^{-1} s^{-1}, the catalytic efficiency of this peptide is moderate, yet it still constitutes one of the most efficient metal-independent peptide hydrolases reported thus far. Zhao et al. showed that a photoswitchable hydrolase could be achieved by incorporating an azobenzene group to peptide sequence GFGH.[353] Upon UV irradiation, the azobenzene moiety switches to a trans conformation, the β-sheet nanofibers break down to random coils and catalytic efficiency decreases by about 20%. Repeated cycles of activation and deactivation appeared to harm the supramolecular architecture, reducing catalytic ability.

Very recently, the use of disordered phases was shown to provide suitable environments for catalysis. Abbas et al. successfully shows enhanced rates of aldol condensation and hydrazone formation inside minimalistic synthon-based droplets. These molecules are derived on a sticker and spacer model with dipeptides like FF are used as rigid stickers and flexible cystamine spacers. They conclude that internal environment of the droplets is responsible for the behavior.[90]

Most of the currently available catalytic peptides were obtained via rational design or through simplification of sequences that are found in proteins. Because this approach is

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

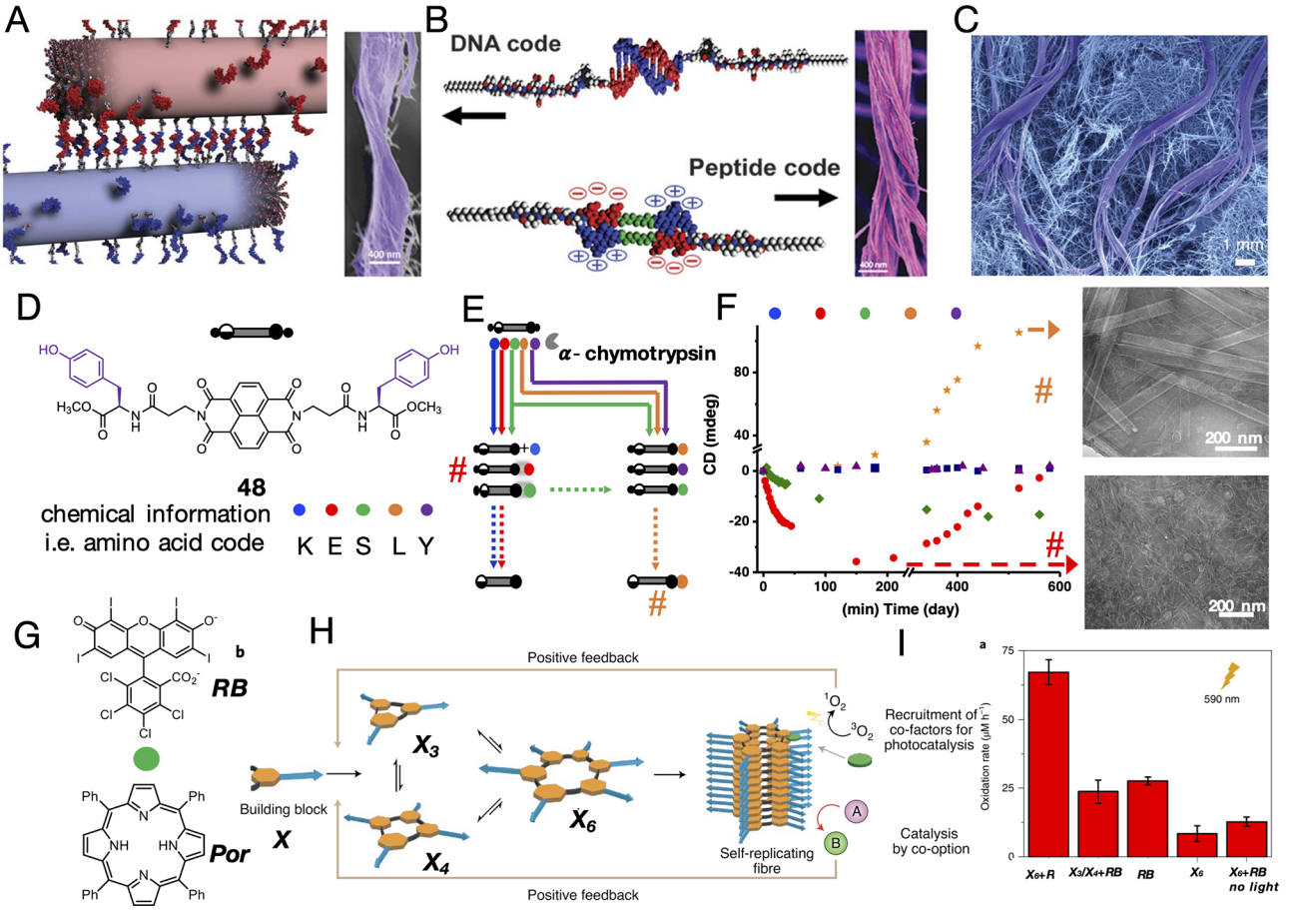


Figure 12. Toward metabolic systems chemistry. (A) Schematic of peptide amphiphile fibers connected by DNA hybridization. (B) Schematic representing molecular conjugation by complementary DNA (top) and charge complementary peptides (bottom) on either side are corresponding fiber bundling as observed by SEM. (C) SEM image of the DNA cross-linked hydrogel. Two distinct populations of hierarchy are differentially colored. (D) Chemical structure of NDI derivative 48. (E) schematic illustration of the amino acid encoded biocatalytic reaction pathways and their supramolecular trajectories and (F) is the corresponding time dependent variation in circular dichroism (CD) intensity of self-assembled 48 upon biocatalytic reaction with different amino acid. (G) Chemical structure of photosensitizer (photocatalyst), i.e., RB (rose Bengal) and Por (porphyrin). (H) Schematic illustration of self-replicating species X6 along with their precursor monomer, schematic of cofactor recruitment, and the catalysis process. (I) The rate of oxidation of *X* in presence or absence of RB, with and without light. (A−C) Reproduced with permission from ref 190. Copyright 2018 AAAS. (D−F) Reproduced with permission from ref 355. Copyright 2018 Springer Nature. (G−I) Reproduced with permission from ref 257. Copyright 2020 Springer Nature.

inherently biased toward sequences that are prevalent in nature, there is a great interest in the development of novel methods to identify peptides with catalytic activity, which may be especially powerful for the development of catalysts that are to operate in nonconventional media, so extremes of temperature, pH, and nonaqueous solvents as well as solvent-free conditions. A proof-of concept was provided by Maeda et al., wherein phage display was applied to unbiased selection of oligopeptides that catalyze the hydrolysis/condensation of amide and ester bonds in an unbiased fashion.[354] The screening was performed using precursor molecules that self-assemble to form nanostructures upon catalytic action. Thus, incubation of a phage library with these precursors allows for the identification of peptide sequences that show the desired catalytic activity, as they result in the formation of self-assembled aggregates on the surface of the phage. Using *p*-nitrophenyl acetate as a substrate, the best performing sequence (DLRSCTACAVNA) showed moderate level of amidase activity, as well as esterase activity that was approximately 2.5-fold above background. Interestingly, this sequence does not contain His, but two Cys-residues and one Arg-moiety that are expected to play a role in substrate binding.

Overall, it can be concluded that the integration of common features present in biological enzymes, such as divalent metals, multivalency, and the ability to form well-ordered structures, into minimalistic peptide-based systems is a powerful strategy for the development of novel biocompatible catalysts. Future efforts will focus on revealing the exact chemical mechanisms underlying these reactions as well as on the development of screening strategies to select potential candidates. Moreover, there will be an emphasis on developing strategies to screen for, or even rationally design, peptide sequences that display an element of adaptive substrate binding through preorganization and "induced fit".

5.3. Multicomponent Functional Supramolecular Systems toward Metabolic Systems Chemistry

The crucial role of metabolism, by which biological organisms convert resources in the environment into energy, is widely recognized as a highly efficient means to direct energy and information to produce complex chemicals in remarkably efficient ways. A logical next step for peptide-based supra-

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

molecular systems chemistry is development of *metabolic systems chemistry*, the holistic study of energy harvesting, chemical signaling, interactions, and transformations in compartments, and feedback regulation to drive chemical processes. While no fully synthetic examples of metabolic systems have been demonstrated to date, a number of papers show remarkable levels of integration of adaptive, mechanical, reactive features to create materials with properties achieved through holistic design and systems-level consideration of interactions to achieve desired functions.

Freeman et al. demonstrated the complexity in supramolecular structure and its manifestation into macroscopic properties through control of dynamic and hierarchical behavior in response to specific chemical signals.[190] The authors employed ssDNA functionalized peptide amphiphiles that were incorporated via cooperative assembly into fibers composed of unfunctionalized peptide amphiphiles in varying percentages. They observe that when two distinct population of such fibers with complementary strand DNA dopants are mixed, large scale fiber reorganization occurs along with extensive fiber bundling, demonstrating spontaneous hierarchical organization which was fully governed by balancing noncovalent interactions (Figure 12A−C). The authors show extensive simulations and detailed microscopy analysis that such reorganization and bundling results due to a narrow regime of intermolecular interactions (5−10 k_BT, k_B being the Boltzmann constant and T being temperature. Their product signifies thermal energy). They showed that if the β-sheet interactions among peptide amphiphiles is too strong, there would not be a monomer reconfiguration, and if it is too weak, they would not assemble at all, demonstrating a balance of order and disorder. The observed hierarchical structures are reversible depending on specific chemical (competing DNA strands) and physical stimuli (temperature).

The authors further observe that hydrogels with such extensive bundling have superior mechanical properties. Moreover, such cross-linking interactions are not limited to peptide amphiphiles that were functionalized with DNA sequences. They can also be brought about by peptide sequences which show charge complementarity. Clusters of E and K when functionalized on the amphiphiles can bring about similar clustering. The diameter of these bundles can further be modulated by introducing PEG spacers between E and K clusters. This work represents a unique dynamic behavior induced by specific chemical signals among supramolecular systems and is reminiscent of other dynamic behaviors in biology like the ECM.

Another example showed integrated catabolic, anabolic processes that could be regulated by chemical signals and ultimately give rise to tunable and transient electronic properties. In this example, the chemical information was actively encoded, edited on demand with spatiotemporal precision. Kumar et al. reported an amino acid encoded biocatalytic self-assembly for temporal control over supramolecular structure and function.[355] The design consist of a semiconductor NDI (Figure 12D) core flanked by "*L*" and "*D*" enantiomers of the Y methyl ester on both sides (**48**). Because of the enzyme's kinetic preference of reaction for the "*L*" enantiomer over the "*D*" enantiomer of amino acid, the system has an in-built kinetic competition. In the presence of enzyme α-chymotrypsin and various amino acids, **48** reacts to incorporate different amino acid uniquely into its structure. Interestingly, the chemical nature of amino acid encodes the time dependent chemical and supramolecular trajectory (Figure 12E), e.g., addition of amino acid E result in the formation of left-handed helical assembly of **48**, which transiently form and degrade over time. However, in the presence of other amino acid like L resulted in permanent right-handed tubular nanostructures (Figure 12F). Additionally, when both E and L were added in direct competition, the chemical information was actively incorporated into the assembly to result in a complex supramolecular transformation from racemic to left-handed nanofibers to right-handed tubes from hours to 2 weeks' time. Interestingly, the formation and degradation of helical structure over time resulted in formation of transient electroconducting nanowires, whose conductance could be temporally modulated, encoded by the amino acid. Thus, the system integrates chemical information instructed assembly with temporal control over nanostructures for emergent functions.

It is proposed that coordinated performance of replication, metabolism, and compartmentalization in a synthetic chemical system can greatly advance the understanding of the origin of life problem and develop smart materials. In this regard, Santiago et al. reported a self-replicating system which utilizes external energy to perform protometabolic process.[257] The chemical design consists of the previously discussed GLKFK peptide-based dithiol precursor (*X*), which upon reversible oxidation forms self-replicating hexameric disulfide macrocycle (Figure 12H, X6). Interestingly, the assembly of self-replicator can incorporate photosensitizers like rose Bengal (RB, Figure 12G) and porphyrin dyes (Por), reminiscent of biochemical cofactors. The coassembly of the dye with the self-replicator assembly utilizes light energy to convert $^3O_2$ to more reactive $^1O_2$ form. This enhances the formation of oxidized disulfide, which in turn increases the rate of formation of self-replicator, thus providing a positive feedback loop. The rate of the oxidation could be enhanced up to 2.3 times (Figure 12I), mainly due to enhanced photocatalytic activity of the dye upon coassembly and improved solubility within the hydrophobic pocket of self-replicator assembly. Thus, they demonstrate the working of a chemical metabolic system, which utilizes external light energy to synthesize the precursor of self-replication species from inert material.

5.4. State of the Field: Functional Supramolecular Peptide Systems

Supramolecular functionality relies on supramolecular order. Conventionally, designs were often focused on thermodynamic optimization of the most stable structures, which in some cases can be aided by using nonpeptidic components to lock structures, leading to remarkable optical and electronic properties.[305] It is clear, however, that the most stable structures are often not optimal to create agile and adaptive structures. Moreover, the level of order and anisotropy best suited for the function depends on the application, with short-range transfer processes occurring through dynamic interfaces, while long-range transport required order at much larger length ranges. Despite the well-understood relationship between peptide design, structure, and function, the influence of sequence, morphology, and order are not additive properties and combinatorial screening experiments involving side-by-side comparisons are often good start points to elucidate design rules[356] Active learning and MD simulations are also suitable to reduce the search space and identify designs.[319] Similarly, for the optimization of reactivity and catalysis,

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

preformed binding sites enhance catalytic activity by recruitment of reactants, but reactivity can be further enhanced by the incorporation of induced or triggered organization.[278]

6. CONCLUSIONS AND FUTURE DIRECTIONS

Life itself is not an additive collection of multiple functional units, but an integrated whole of complex interdependent processes. Therefore, a holistic design, where multiple factors like supramolecular structures, encoded chemical functions, spatiotemporal control, catalysis, and molecular recognition come together and are collectively considered, is required for the development of active and adaptive matter with life-like properties. Although we are still far from creating chemical structures that resemble the active and adaptive properties of living systems, recent examples discussed in this review show that short peptides possess many properties relevant to life and that, increasingly, the design of these properties can be integrated to create functional materials with properties that rely on the chemical nature of the components and, crucially, on interactions and reactions between these components. Functionality in biology often emerges from molecular ensembles that combine stable folds and flexible regions to enable preorganization and induced-fit, thought critical to achieve enzyme-like catalysis but also to enable opening and closing of pores, action of molecular machinery to create motion, and to activate communication pathways. It is not possible to design mimics of such systems without holistic design.

The move from a concept where peptide sequence and structure dictates properties and functions, to one where molecular interactions and reactions between multiple peptide modalities, and in some cases under the influence of externally applied energy, collectively dictate properties represents a new way in which chemistry can be understood, studied, and applied, thus being a true paradigm shift. The collection of papers reviewed here clearly demonstrates that short peptides are exceptionally well suited to systems chemistry investigations because they are chemically rich and modular, and they can be designed to perform a wide range of functions, including catalysis, self-assembly, compartmentalization, and supramolecular recognition. Systems-level design approaches with a focus on dynamic structural and temporal features is particularly important to achieve adaptive functions that are challenging to design by chemists, including induced fit, allostery, enzyme-like catalysis, pressure sensitivity, and ultimately designed chemical metabolisms and channeled reaction pathways.

Further progress in peptide-based supramolecular systems chemistry must, by necessity, invoke advances in synthesis, kinetics, self-assembly, analytical chemistry, and theory beyond chemical equilibrium. Embracing complexity in supramolecular peptide systems requires a focus on analytical tractability in order to follow independently the interactions and fate of individual interacting peptides as part of a complex whole. It has been recognized for some time[357] that simplicity (of building blocks) provides a good approach to study combinatorial complexity of interactions in space and time. It is therefore expected that more effort will be placed on developing minimalistic peptides that *collectively* provide active and adaptive properties but much beyond the current complexities which typically do not exceed 2−3 components. Studying cooperative behavior of dozens of molecules would sill enable the tractable searching of collective peptide functionality and will no doubt provide new insights relevant to understanding of life functions. Exploiting tractable systems and significant recent advances in analytical and computational methods, early examples of integrated chemical systems demonstrated new emergent functions by design. For future work, perhaps an effort into the deconvolution of systems-level properties into chemical lessons would be beneficial, as it not only furthers our understanding but also informs the design of better systems. It is expected that selective labeling techniques will be critical to trace the behavior of individual molecules in dynamic ensembles.

Advances are being made to further our understanding and predict possible functional outcomes of peptide design. This is not only being extended to construct better materials but also to predict self-assembling characteristics of a large subsection of peptides, warranting further collaboration to decipher the mathematical aspects of a complex chemical system.[164,358,359] Machine learning approaches are undoubtedly going to feature increasingly in this field, with some recent examples showing much promise,[164] but we note that the advantage of speed and large data sets can be offset by the black box character of these methods, which do not always allow correlations to be rationalized at the molecular level. Integrating MD and machine learning is a good way forward in that regard and of course has been tremendously successful in elucidation of related challenges, including through the recent giant steps in solving the protein folding problem using artificial intelligence by Google's Alphafold.[360]

There have been efforts to develop sequences and materials de novo and specifically for nonbiological applications, including examples with no known biological equivalent. These approaches in principle provide access to folds, structures, and ultimately functions that have not been exploited in biological systems, thus providing opportunities for the application of peptides and proteins outside biology. Systems thinking in chemical design is also relevant to the development of synthetic versions of nature's remarkable ability to produce 100% recyclable materials and devices that often display superior performance compared to the synthetic structures that we use today. Biology's materials provide ideas for bioplastics, energy sources, building materials, cosmetics products, electronics, and sensors, all with reduced environmental impact. Green bioinspired nanotechnology will require systems thinking and has the potential to revolutionize energy, materials, and technology sectors for the benefit of the environment.

In conclusion, artificial biomolecular materials and systems have a long-term potential to lead to entirely new sustainable and degradable materials, dynamically tunable sensors, new types of autonomous medical interventions, and new modes of energy production and conversion. With further developments in computational design, improved analytical techniques better able to deal with complex systems, heterogeneous, and dynamically adaptive mixtures, along with contributions from automation and artificial intelligence, peptide-based systems chemistry will ultimately give rise to new solutions and insights that have not been explored by biology, and we expect the designs will be robust and rational.

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

AUTHOR INFORMATION

Corresponding Author

Rein V. Ulijn − *Advanced Science Research Center (ASRC) at the Graduate Center, City University of New York, New York, New York 10031, United States; Department of Chemistry, Hunter College City University of New York, New York, New York 10065, United States; Ph.D. Program in Chemistry, The Graduate Center of the City University of New York, New York, New York 10016, United States; Ph.D. Program in Biochemistry, The Graduate Center of the City University of New York, New York, New York 10016, United States;* orcid.org/0000-0002-7138-1213; Email: rulijn@gc.cuny.edu

Authors

Fahmeed Sheehan − *Advanced Science Research Center (ASRC) at the Graduate Center, City University of New York, New York, New York 10031, United States; Department of Chemistry, Hunter College City University of New York, New York, New York 10065, United States; Ph.D. Program in Chemistry, The Graduate Center of the City University of New York, New York, New York 10016, United States*

Deborah Sementa − *Advanced Science Research Center (ASRC) at the Graduate Center, City University of New York, New York, New York 10031, United States*

Ankit Jain − *Advanced Science Research Center (ASRC) at the Graduate Center, City University of New York, New York, New York 10031, United States*

Mohit Kumar − *Advanced Science Research Center (ASRC) at the Graduate Center, City University of New York, New York, New York 10031, United States; Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), Barcelona 08028, Spain;* orcid.org/0000-0003-0083-7217

Mona Tayarani-Najjaran − *Advanced Science Research Center (ASRC) at the Graduate Center, City University of New York, New York, New York 10031, United States; Department of Chemistry, Hunter College City University of New York, New York, New York 10065, United States; Ph.D. Program in Chemistry, The Graduate Center of the City University of New York, New York, New York 10016, United States*

Daniela Kroiss − *Advanced Science Research Center (ASRC) at the Graduate Center, City University of New York, New York, New York 10031, United States; Department of Chemistry, Hunter College City University of New York, New York, New York 10065, United States; Ph.D. Program in Biochemistry, The Graduate Center of the City University of New York, New York, New York 10016, United States;* orcid.org/0000-0003-1483-9096

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.chemrev.1c00089

Author Contributions

#F.S., D.S., A.J., and M.K. contributed equally.

Notes

The authors declare no competing financial interest.

Biographies

Fahmeed Sheehan, after graduating from the Macaulay Honors College at Hunter College with a B.A. in Chemistry, remained with the City University of New York (CUNY) to earn a Ph.D. in Chemistry. As an undergraduate, he worked on designing a system using diphenylalanine gels to trap small molecules in solution. He is currently a Ph.D. student under the supervision of Dr. Rein Ulijn. His current research seeks to identify responsive short peptide materials that approach the substrate responsiveness and catalytic efficiency of natural enzymes.

Deborah Sementa received her Ph.D. from University of Naples—Federico II with a thesis on the development of peptide-based anticancer therapeutics. As part of her Ph.D. studies, she has been a visiting scholar at Massachusetts Institute of Technology, investigating the intracellular stability of mixed chirality proteins. After joining the Royal College of Surgeons in Ireland as a Marie Curie Postdoctoral Research Fellow for one year, she is currently working in Prof. Rein Ulijn's lab as a Research Associate. Her research interests focus on the design of minimalistic peptides for understanding the sequence determinants of liquid phase separations of proteins.

Ankit Jain obtained his B. Tech degree in Biotechnology from SASTRA University, India. In 2011, he joined the Jawaharlal Nehru Centre for Advanced Scientific Research, India, as a Ph.D. student under Prof. Subi J. George. His research work focused on dynamic charge transfer aggregates and temporal control of their self-assembly. He has also worked on clay−chromophore conjugates and their pertinent photochemistry. Currently, he is working with Prof. Rein Ulijn as a postdoctoral research associate at the Advanced Science Research Center (ASRC), CUNY. His work mainly focuses on development of dynamic peptide libraries towards answering questions pertaining to chemical origin of life.

Mohit Kumar did his Master's in Chemistry from Sri Sathya Sai Institute of Higher Learning, India. Thereafter, he completed his Ph.D. with Prof. Subi J. George at Jawaharlal Nehru Centre for Advanced Scientific Research, India, in 2014. This was followed by a postdoctoral stay with Prof. Rein Ulijn at the ASRC, CUNY in New York, USA, with research focus on nonequilibrium assemblies of peptide derivatives. Currently he is a La Caixa Junior leader fellow at the Institute for Bioengineering of Catalonia in Barcelona, Spain, where he is developing active self-assembly for targeted drug delivery.

Mona Tayarani-Najjaran is currently a postdoctoral researcher in Rein Ulijn's group at the ASRC, CUNY. She has completed her Ph.D. in Nanotechnology and Material Chemistry at CUNY in the USA. Her Ph.D. research was focused on discovery of visible fluorescence short tripeptides. Her current research focuses on the application of these fluorescence peptide nanoparticles in bioimaging and biomedical applications. She has also worked on anticancer activity of different organic and inorganic compounds during her masters in Iran.

Daniela Kroiss obtained her B.Sc. in Food Science and Biotechnology and her M.Sc. in Medical Biotechnology from the University of Natural Resources and Life Sciences Vienna (Austria) after completing a final year project on human antibody receptors. After receiving her Ph.D. in Chemistry under the supervision of Rein Ulijn on peptide-based supramolecular systems relevant to the origin of life, she has moved back to Austria as the Head of Research at Burg Design GmbH.

Rein Ulijn's fundamental research question that drives his work is how the molecular building blocks and design concepts of life can be simplified and repurposed to produce materials, systems, and molecular technologies with adaptive functionalities that cannot be

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

achieved using existing chemical design approaches. He is founding Director of the Nanoscience Initiative at the Advanced Science Research Center at CUNY, New York. Prof. Ulijn has held several personal fellowships and has won a number of awards, including the Vannevar Bush Faculty Fellowship, the RSC Norman Heatley Medal, Royal Society Merit Award, and was elected as a Fellow of the Royal Society of Edinburgh. He is the Einstein Professor of Chemistry of Hunter College at CUNY.

ACKNOWLEDGMENTS

We thank the Air Force Office of Scientific Research (AFoSR) for funding of R.V.U, F.S., and D.S. (grant FA9550-21-1-0091), U.S.−Israel Binational Science Foundation for funding of R.V.U. and M.K. (grant 2017510), and U.S. Army Research Laboratory and U.S. Army Research Office for funding of R.V.U. and M.K. (grant W911NF-16-1-0113).

REFERENCES

(1) Ashkenasy, G.; Hermans, T. M.; Otto, S.; Taylor, A. F. Systems Chemistry. *Chem. Soc. Rev.* **2017**, *46*, 2543−2554.
(2) Grzybowski, B. A.; Huck, W. T. The Nanotechnology of Life-Inspired Systems. *Nat. Nanotechnol.* **2016**, *11*, 585−592.
(3) Epstein, I. R.; Xu, B. Reaction-Diffusion Processes at the Nano- and Microscales. *Nat. Nanotechnol.* **2016**, *11*, 312−319.
(4) Mattia, E.; Otto, S. Supramolecular Systems Chemistry. *Nat. Nanotechnol.* **2015**, *10*, 111−119.
(5) Grover, M. A.; Hsieh, M. C.; Lynn, D. G. Systems Analysis for Peptide Systems Chemistry. *Life* **2019**, *9*, 55.
(6) Bai, Y.; Chotera, A.; Taran, O.; Liang, C.; Ashkenasy, G.; Lynn, D. G. Achieving Biopolymer Synergy in Systems Chemistry. *Chem. Soc. Rev.* **2018**, *47*, 5444−5456.
(7) Azevedo, H. S.; Perry, S. L.; Korevaar, P. A.; Das, D. Complexity Emerges from Chemistry. *Nat. Chem.* **2020**, *12*, 793−794.
(8) Pappas, C. G.; Mandal, P. K.; Liu, B.; Kauffmann, B.; Miao, X.; Komaromy, D.; Hoffmann, W.; Manz, C.; Chang, R.; Liu, K.; et al. Emergence of Low-Symmetry Foldamers from Single Monomers. *Nat. Chem.* **2020**, *12*, 1180−1186.
(9) Carnall, J. M.; Waudby, C. A.; Belenguer, A. M.; Stuart, M. C.; Peyralans, J. J.; Otto, S. Mechanosensitive Self-Replication Driven by Self-Organization. *Science* **2010**, *327*, 1502−1506.
(10) Monreal Santiago, G.; Liu, K.; Browne, W. R.; Otto, S. Emergence of Light-Driven Protometabolism on Recruitment of a Photocatalytic Cofactor by a Self-Replicator. *Nat. Chem.* **2020**, *12*, 603−607.
(11) Liu, B.; Pappas, C. G.; Ottele, J.; Schaeffer, G.; Jurissek, C.; Pieters, P. F.; Altay, M.; Maric, I.; Stuart, M. C. A.; Otto, S. Spontaneous Emergence of Self-Replicating Molecules Containing Nucleobases and Amino Acids. *J. Am. Chem. Soc.* **2020**, *142*, 4184−4192.
(12) Chen, R.; Neri, S.; Prins, L. J. Enhanced Catalytic Activity under Non-Equilibrium Conditions. *Nat. Nanotechnol.* **2020**, *15*, 868−874.
(13) Semenov, S. N.; Wong, A. S.; van der Made, R. M.; Postma, S. G.; Groen, J.; van Roekel, H. W.; de Greef, T. F.; Huck, W. T. Rational Design of Functional and Tunable Oscillating Enzymatic Networks. *Nat. Chem.* **2015**, *7*, 160−165.
(14) Sadownik, J. W.; Ulijn, R. V. Dynamic Covalent Chemistry in Aid of Peptide Self-Assembly. *Curr. Opin. Biotechnol.* **2010**, *21*, 401−411.
(15) Ulijn, R. V.; Jerala, R. Peptide and Protein Nanotechnology into the 2020s: Beyond Biology. *Chem. Soc. Rev.* **2018**, *47*, 3391−3394.
(16) Zhang, S. Fabrication of Novel Biomaterials through Molecular Self-Assembly. *Nat. Biotechnol.* **2003**, *21*, 1171−1178.
(17) Fletcher, J. M.; Harniman, R. L.; Barnes, F. R.; Boyle, A. L.; Collins, A.; Mantell, J.; Sharp, T. H.; Antognozzi, M.; Booth, P. J.; Linden, N.; et al. Self-Assembling Cages from Coiled-Coil Peptide Modules. *Science* **2013**, *340*, 595−599.
(18) Zhang, S. Fabrication of Novel Biomaterials through Molecular Self-Assembly. *Nat. Biotechnol.* **2003**, *21*, 1171−1178.
(19) Webber, M. J.; Appel, E. A.; Meijer, E. W.; Langer, R. Supramolecular Biomaterials. *Nat. Mater.* **2016**, *15*, 13−26.
(20) Aida, T.; Meijer, E. W.; Stupp, S. I. Functional Supramolecular Polymers. *Science* **2012**, *335*, 813−817.
(21) Oldfield, C. J.; Dunker, A. K. Intrinsically Disordered Proteins and Intrinsically Disordered Protein Regions. *Annu. Rev. Biochem.* **2014**, *83*, 553−584.
(22) van der Lee, R.; Buljan, M.; Lang, B.; Weatheritt, R. J.; Daughdrill, G. W.; Dunker, A. K.; Fuxreiter, M.; Gough, J.; Gsponer, J.; Jones, D. T.; et al. Classification of Intrinsically Disordered Regions and Proteins. *Chem. Rev.* **2014**, *114*, 6589−6631.
(23) Abbas, M.; Lipiński, W. P.; Wang, J.; Spruijt, E. Peptide-Based Coacervates as Biomimetic Protocells. *Chem. Soc. Rev.* **2021**, *50*, 3690−3705.
(24) Ulijn, R. V.; Lampel, A. Order/Disorder in Protein and Peptide-Based Biomaterials. *Isr. J. Chem.* **2020**, *60*, 1129−1140.
(25) Frederix, P. W. J. M.; Patmanidis, I.; Marrink, S. J. Molecular Simulations of Self-Assembling Bio-Inspired Supramolecular Systems and Their Connection to Experiments. *Chem. Soc. Rev.* **2018**, *47*, 3470−3489.
(26) Cui, H.; Webber, M. J.; Stupp, S. I. Self-Assembly of Peptide Amphiphiles: From Molecules to Nanostructures to Biomaterials. *Biopolymers* **2010**, *94*, 1−18.
(27) Fleming, S.; Ulijn, R. V. Design of Nanostructures Based on Aromatic Peptide Amphiphiles. *Chem. Soc. Rev.* **2014**, *43*, 8150−8177.
(28) Draper, E. R.; Adams, D. J. How Should Multicomponent Supramolecular Gels Be Characterised? *Chem. Soc. Rev.* **2018**, *47*, 3395−3405.
(29) Du, X.; Zhou, J.; Shi, J.; Xu, B. Supramolecular Hydrogelators and Hydrogels: From Soft Matter to Molecular Biomaterials. *Chem. Rev.* **2015**, *115*, 13165−13307.
(30) Fichman, G.; Gazit, E. Self-Assembly of Short Peptides to Form Hydrogels: Design of Building Blocks, Physical Properties and Technological Applications. *Acta Biomater.* **2014**, *10*, 1671−1682.
(31) Lampel, A.; Ulijn, R. V.; Tuttle, T. Guiding Principles for Peptide Nanotechnology through Directed Discovery. *Chem. Soc. Rev.* **2018**, *47*, 3737−3758.
(32) Dougherty, P. G.; Sahni, A.; Pei, D. Understanding Cell Penetration of Cyclic Peptides. *Chem. Rev.* **2019**, *119*, 10241−10287.
(33) Hill, T. A.; Shepherd, N. E.; Diness, F.; Fairlie, D. P. Constraining Cyclic Peptides to Mimic Protein Structure Motifs. *Angew. Chem., Int. Ed.* **2014**, *53*, 13020−13041.
(34) Spicer, C. D.; Jumeaux, C.; Gupta, B.; Stevens, M. M. Peptide and Protein Nanoparticle Conjugates: Versatile Platforms for Biomedical Applications. *Chem. Soc. Rev.* **2018**, *47*, 3574−3620.
(35) Zhang, P.; Cui, Y.; Anderson, C. F.; Zhang, C.; Li, Y.; Wang, R.; Cui, H. Peptide-Based Nanoprobes for Molecular Imaging and Disease Diagnostics. *Chem. Soc. Rev.* **2018**, *47*, 3490−3529.
(36) Gelain, F.; Luo, Z.; Zhang, S. Self-Assembling Peptide Eak16 and Rada16 Nanofiber Scaffold Hydrogel. *Chem. Rev.* **2020**, *120*, 13434−13460.
(37) Oldfield, C. J.; Dunker, A. K. Intrinsically Disordered Proteins and Intrinsically Disordered Protein Regions. *Annu. Rev. Biochem.* **2014**, *83*, 553−584.
(38) Draper, E. R.; Adams, D. J. How Should Multicomponent Supramolecular Gels Be Characterised? *Chem. Soc. Rev.* **2018**, *47*, 3395−3405.
(39) Guilbaud, J.-B.; Saiani, A. Using Small Angle Scattering (Sas) to Structurally Characterise Peptide and Protein Self-Assembled Materials. *Chem. Soc. Rev.* **2011**, *40*, 1200−1210.
(40) Lampel, A.; McPhee, S. A.; Park, H.-A.; Scott, G. G.; Humagain, S.; Hekstra, D. R.; Yoo, B.; Frederix, P. W. J. M.; Li, T.-D.; Abzalimov, R. R.; et al. Polymeric Peptide Pigments with Sequence-Encoded Properties. *Science* **2017**, *356*, 1064−1068.

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

(41) Fjell, C. D.; Hiss, J. A.; Hancock, R. E. W.; Schneider, G. Designing Antimicrobial Peptides: Form Follows Function. *Nat. Rev. Drug Discovery* **2012**, *11*, 37−51.
(42) Frederix, P. W. J. M.; Scott, G. G.; Abul-Haija, Y. M.; Kalafatovic, D.; Pappas, C. G.; Javid, N.; Hunt, N. T.; Ulijn, R. V.; Tuttle, T. Exploring the Sequence Space for (Tri-)Peptide Self-Assembly to Design and Discover New Hydrogels. *Nat. Chem.* **2015**, *7*, 30−37.
(43) Kroiss, D.; Aramini, J. M.; Ragoonath, S.; Ulijn, R. V.; Tuttle, T. Combinatorial Discovery and Validation of Heptapeptides with Utp Binding Induced Structure. *ChemSystemsChem* **2021**, *3*, No. e2000025.
(44) Yaron, A.; Naider, F.; Scharpe, S. Proline-Dependent Structural and Biological Properties of Peptides and Proteins. *Crit. Rev. Biochem. Mol. Biol.* **1993**, *28*, 31−81.
(45) Son, J.; Kalafatovic, D.; Kumar, M.; Yoo, B.; Cornejo, M. A.; Contel, M.; Ulijn, R. V. Customizing Morphology, Size, and Response Kinetics of Matrix Metalloproteinase-Responsive Nanostructures by Systematic Peptide Design. *ACS Nano* **2019**, *13*, 1555−1562.
(46) Bera, S.; Xue, B.; Rehak, P.; Jacoby, G.; Ji, W.; Shimon, L. J. W.; Beck, R.; Král, P.; Cao, Y.; Gazit, E. Self-Assembly of Aromatic Amino Acid Enantiomers into Supramolecular Materials of High Rigidity. *ACS Nano* **2020**, *14*, 1694−1706.
(47) Kol, N.; Adler-Abramovich, L.; Barlam, D.; Shneck, R. Z.; Gazit, E.; Rousso, I. Self-Assembled Peptide Nanotubes Are Uniquely Rigid Bioinspired Supramolecular Structures. *Nano Lett.* **2005**, *5*, 1343−1346.
(48) Niu, L.; Chen, X.; Allen, S.; Tendler, S. J. B. Using the Bending Beam Model to Estimate the Elasticity of Diphenylalanine Nanotubes. *Langmuir* **2007**, *23*, 7443−7446.
(49) Azuri, I.; Adler-Abramovich, L.; Gazit, E.; Hod, O.; Kronik, L. Why Are Diphenylalanine-Based Peptide Nanostructures So Rigid? Insights from First Principles Calculations. *J. Am. Chem. Soc.* **2014**, *136*, 963−969.
(50) Basavalingappa, V.; Bera, S.; Xue, B.; O'Donnell, J.; Guerin, S.; Cazade, P.-A.; Yuan, H.; Haq, E. U.; Silien, C.; Tao, K.; et al. Diphenylalanine-Derivative Peptide Assemblies with Increased Aromaticity Exhibit Metal-Like Rigidity and High Piezoelectricity. *ACS Nano* **2020**, *14*, 7025−7037.
(51) Yan, X.; Zhu, P.; Li, J. Self-Assembly and Application of Diphenylalanine-Based Nanostructures. *Chem. Soc. Rev.* **2010**, *39*, 1877−1890.
(52) Wei, G.; Su, Z.; Reynolds, N. P.; Arosio, P.; Hamley, I. W.; Gazit, E.; Mezzenga, R. Self-Assembling Peptide and Protein Amyloids: From Structure to Tailored Function in Nanotechnology. *Chem. Soc. Rev.* **2017**, *46*, 4661−4708.
(53) Tao, K.; Levin, A.; Adler-Abramovich, L.; Gazit, E. Fmoc-Modified Amino Acids and Short Peptides: Simple Bio-Inspired Building Blocks for the Fabrication of Functional Materials. *Chem. Soc. Rev.* **2016**, *45*, 3935−3953.
(54) Bera, S.; Mondal, S.; Xue, B.; Shimon, L. J. W.; Cao, Y.; Gazit, E. Rigid Helical-Like Assemblies from a Self-Aggregating Tripeptide. *Nat. Mater.* **2019**, *18*, 503−509.
(55) Bretscher, L. E.; Jenkins, C. L.; Taylor, K. M.; DeRider, M. L.; Raines, R. T. Conformational Stability of Collagen Relies on a Stereoelectronic Effect. *J. Am. Chem. Soc.* **2001**, *123*, 777−778.
(56) Horng, J.-C.; Raines, R. T. Stereoelectronic Effects on Polyproline Conformation. *Protein Sci.* **2006**, *15*, 74−83.
(57) Frederix, P. W. J. M.; Scott, G. G.; Abul-Haija, Y. M.; Kalafatovic, D.; Pappas, C. G.; Javid, N.; Hunt, N. T.; Ulijn, R. V.; Tuttle, T. Exploring the Sequence Space for (Tri-)Peptide Self-Assembly to Design and Discover New Hydrogels. *Nat. Chem.* **2015**, *7*, 30−37.
(58) Andrade-Filho, T.; Martins, T. C.; Ferreira, F. F.; Alves, W. A.; Rocha, A. R. Water-Driven Stabilization of Diphenylalanine Nanotube Structures. *Theor. Chem. Acc.* **2016**, *135*, 185.
(59) Bera, S.; Mondal, S.; Xue, B.; Shimon, L. J. W.; Cao, Y.; Gazit, E. Rigid Helical-Like Assemblies from a Self-Aggregating Tripeptide. *Nat. Mater.* **2019**, *18*, 503−509.
(60) Ramos Sasselli, I.; Halling, P. J.; Ulijn, R. V.; Tuttle, T. Supramolecular Fibers in Gels Can Be at Thermodynamic Equilibrium: A Simple Packing Model Reveals Preferential Fibril Formation Versus Crystallization. *ACS Nano* **2016**, *10*, 2661−2668.
(61) Reches, M.; Gazit, E. Self-Assembly of Peptide Nanotubes and Amyloid-Like Structures by Charged-Termini-Capped Diphenylalanine Peptide Analogues. *Isr. J. Chem.* **2005**, *45*, 363−371.
(62) Jayawarna, V.; Ali, M.; Jowitt, T. A.; Miller, A. F.; Saiani, A.; Gough, J. E.; Ulijn, R. V. Nanostructured Hydrogels for Three-Dimensional Cell Culture through Self-Assembly of Fluorenylmethoxycarbonyl-Dipeptides. *Adv. Mater.* **2006**, *18*, 611−614.
(63) Mahler, A.; Reches, M.; Rechter, M.; Cohen, S.; Gazit, E. Rigid, Self-Assembled Hydrogel Composed of a Modified Aromatic Dipeptide. *Adv. Mater.* **2006**, *18*, 1365−1370.
(64) Raeburn, J.; Pont, G.; Chen, L.; Cesbron, Y.; Lévy, R.; Adams, D. J. Fmoc-Diphenylalanine Hydrogels: Understanding the Variability in Reported Mechanical Properties. *Soft Matter* **2012**, *8*, 1168−1174.
(65) Moreira, I. P.; Piskorz, T. K.; van Esch, J. H.; Tuttle, T.; Ulijn, R. V. Biocatalytic Self-Assembly of Tripeptide Gels and Emulsions. *Langmuir* **2017**, *33*, 4986−4995.
(66) Tang, J. D.; Mura, C.; Lampe, K. J. Stimuli-Responsive, Pentapeptide, Nanofiber Hydrogel for Tissue Engineering. *J. Am. Chem. Soc.* **2019**, *141*, 4886−4899.
(67) Clarke, D. E.; Parmenter, C. D. J.; Scherman, O. A. Tunable Pentapeptide Self-Assembled B-Sheet Hydrogels. *Angew. Chem., Int. Ed.* **2018**, *57*, 7709−7713.
(68) Hartgerink, J. D. Self-Assembly and Mineralization of Peptide-Amphiphile Nanofibers. *Science* **2001**, *294*, 1684−1688.
(69) Pashuck, E. T.; Cui, H.; Stupp, S. I. Tuning Supramolecular Rigidity of Peptide Fibers through Molecular Structure. *J. Am. Chem. Soc.* **2010**, *132*, 6041−6046.
(70) Feng, Y.; Melacini, G.; Taulane, J. P.; Goodman, M. Acetyl-Terminated and Template-Assembled Collagen-Based Polypeptides Composed of Gly-Pro-Hyp Sequences. 2. Synthesis and Conformational Analysis by Circular Dichroism, Ultraviolet Absorbance, and Optical Rotation. *J. Am. Chem. Soc.* **1996**, *118*, 10351−10358.
(71) Melacini, G.; Feng, Y.; Goodman, M. Acetyl-Terminated and Template-Assembled Collagen-Based Polypeptides Composed of Gly-Pro-Hyp Sequences. 3. Conformational Analysis by ^{1}H-Nmr and Molecular Modeling Studies. *J. Am. Chem. Soc.* **1996**, *118*, 10359−10364.
(72) Ghosh, M.; Bera, S.; Schiffmann, S.; Shimon, L. J. W.; Adler-Abramovich, L. Collagen-Inspired Helical Peptide Coassembly Forms a Rigid Hydrogel with Twisted Polyproline Ii Architecture. *ACS Nano* **2020**, *14*, 9990−10000.
(73) Martin, E. W.; Holehouse, A. S.; Peran, I.; Farag, M.; Incicco, J. J.; Bremer, A.; Grace, C. R.; Soranno, A.; Pappu, R. V.; Mittag, T. Valence and Patterning of Aromatic Residues Determine the Phase Behavior of Prion-Like Domains. *Science* **2020**, *367*, 694−699.
(74) Broom, A.; Rakotoharisoa, R. V.; Thompson, M. C.; Zarifi, N.; Nguyen, E.; Mukhametzhanov, N.; Liu, L.; Fraser, J. S.; Chica, R. A. Ensemble-Based Enzyme Design Can Recapitulate the Effects of Laboratory Directed Evolution in Silico. *Nat. Commun.* **2020**, *11*, 4808.
(75) Uversky, V. N. In *Protein Folding and Structure*; 1st ed.; Springer International, 2014; DOI: 10.1007/978-3-319-08921-8.
(76) Ward, J. J.; Sodhi, J. S.; McGuffin, L. J.; Buxton, B. F.; Jones, D. T. Prediction and Functional Analysis of Native Disorder in Proteins from the Three Kingdoms of Life. *J. Mol. Biol.* **2004**, *337*, 635−645.
(77) Uversky, V. N. Recent Developments in the Field of Intrinsically Disordered Proteins: Intrinsic Disorder-Based Emergence in Cellular Biology in Light of the Physiological and Pathological Liquid-Liquid Phase Transitions. *Annu. Rev. Biophys.* **2021**, *50*, 135−156.
(78) Fare, C. M.; Villani, A.; Drake, L. E.; Shorter, J. Higher-Order Organization of Biomolecular Condensates. *Open Biol.* **2021**, *11*, 210137.

(79) Kroiss, D.; Aramini, J. M.; McPhee, S. A.; Tuttle, T.; Ulijn, R. V. Unbiased Discovery of Dynamic Peptide-Atp Complexes. *ChemSystemsChem.* **2019**, *1*, 7−11.
(80) de Jong, H. G. B.; Kruyt, H. R. Koazervation: Entmischung in kolloiden Systemen. *Colloid Polym. Sci.* **1930**, *50*, 39−48.
(81) Fisher, R. S.; Elbaum-Garfinkle, S. Tunable Multiphase Dynamics of Arginine and Lysine Liquid Condensates. *Nat. Commun.* **2020**, *11*, 4628.
(82) Yuan, C.; Levin, A.; Chen, W.; Xing, R.; Zou, Q.; Herling, T. W.; Challa, P. K.; Knowles, T. P. J.; Yan, X. Nucleation and Growth of Amino Acid and Peptide Supramolecular Polymers through Liquid-Liquid Phase Separation. *Angew. Chem., Int. Ed.* **2019**, *58*, 18116−18123.
(83) Yewdall, N. A.; André, A. A. M.; Lu, T.; Spruijt, E. Coacervates as Models of Membraneless Organelles. *Curr. Opin. Colloid Interface Sci.* **2021**, *52*, 101416.
(84) Hwang, D. S.; Zeng, H.; Srivastava, A.; Krogstad, D. V.; Tirrell, M.; Israelachvili, J. N.; Waite, J. H. Viscosity and Interfacial Properties in a Mussel-Inspired Adhesive Coacervate. *Soft Matter* **2010**, *6*, 3232−3236.
(85) Ortony, J. H.; Qiao, B.; Newcomb, C. J.; Keller, T. J.; Palmer, L. C.; Deiss-Yehiely, E.; Olvera de la Cruz, M.; Han, S.; Stupp, S. I. Water Dynamics from the Surface to the Interior of a Supramolecular Nanostructure. *J. Am. Chem. Soc.* **2017**, *139*, 8915−8921.
(86) Ortony, J. H.; Newcomb, C. J.; Matson, J. B.; Palmer, L. C.; Doan, P. E.; Hoffman, B. M.; Stupp, S. I. Internal Dynamics of a Supramolecular Nanofibre. *Nat. Mater.* **2014**, *13*, 812−816.
(87) Nakashima, K. K.; Vibhute, M. A.; Spruijt, E. Biomolecular Chemistry in Liquid Phase Separated Compartments. *Front. Mol. Biosci.* **2019**, *6*, 21.
(88) Gomes, E.; Shorter, J. The Molecular Language of Membraneless Organelles. *J. Biol. Chem.* **2019**, *294*, 7115−7127.
(89) Choi, J. M.; Holehouse, A. S.; Pappu, R. V. Physical Principles Underlying the Complex Biology of Intracellular Phase Transitions. *Annu. Rev. Biophys.* **2020**, *49*, 107−133.
(90) Abbas, M.; Lipinski, W. P.; Nakashima, K. K.; Huck, W. T. S.; Spruijt, E. A Short Peptide Synthon for Liquid-Liquid Phase Separation. *ChemRxiv* **2020**, DOI: 10.26434/chemrxiv.12881288.v1
(91) Koga, S.; Williams, D. S.; Perriman, A. W.; Mann, S. Peptide-Nucleotide Microdroplets as a Step Towards a Membrane-Free Protocell Model. *Nat. Chem.* **2011**, *3*, 720−724.
(92) Cakmak, F. P.; Choi, S.; Meyer, M. O.; Bevilacqua, P. C.; Keating, C. D. Prebiotically-Relevant Low Polyion Multivalency Can Improve Functionality of Membraneless Compartments. *Nat. Commun.* **2020**, *11*, 5949.
(93) Fisher, R. S.; Elbaum-Garfinkle, S. Tunable Multiphase Dynamics of Arginine and Lysine Liquid Condensates. *Nat. Commun.* **2020**, *11*, 4628.
(94) Poudyal, R. R.; Guth-Metzler, R. M.; Veenis, A. J.; Frankel, E. A.; Keating, C. D.; Bevilacqua, P. C. Template-Directed Rna Polymerization and Enhanced Ribozyme Catalysis inside Membraneless Compartments Formed by Coacervates. *Nat. Commun.* **2019**, *10*, 490.
(95) Ray, S.; Singh, N.; Kumar, R.; Patel, K.; Pandey, S.; Datta, D.; Mahato, J.; Panigrahi, R.; Navalkar, A.; Mehra, S.; et al. A-Synuclein Aggregation Nucleates through Liquid-Liquid Phase Separation. *Nat. Chem.* **2020**, *12*, 705−716.
(96) Yuan, C.; Levin, A.; Chen, W.; Xing, R.; Zou, Q.; Herling, T. W.; Challa, P. K.; Knowles, T. P. J.; Yan, X. Nucleation and Growth of Amino Acid and Peptide Supramolecular Polymers through Liquid-Liquid Phase Separation. *Angew. Chem., Int. Ed.* **2019**, *58*, 18116−18123.
(97) Fan, Z.; Sun, L.; Huang, Y.; Wang, Y.; Zhang, M. Bioinspired Fluorescent Dipeptide Nanoparticles for Targeted Cancer Cell Imaging and Real-Time Monitoring of Drug Release. *Nat. Nanotechnol.* **2016**, *11*, 388−394.
(98) Hughes, M.; Debnath, S.; Knapp, C. W.; Ulijn, R. V. Antimicrobial Properties of Enzymatically Triggered Self-Assembling Aromatic Peptide Amphiphiles. *Biomater. Sci.* **2013**, *1*, 1138−1142.
(99) Debnath, S.; Roy, S.; Ulijn, R. V. Peptide Nanofibers with Dynamic Instability through Nonequilibrium Biocatalytic Assembly. *J. Am. Chem. Soc.* **2013**, *135*, 16789−16792.
(100) Pappas, C. G.; Shafi, R.; Sasselli, I. R.; Siccardi, H.; Wang, T.; Narang, V.; Abzalimov, R.; Wijerathne, N.; Ulijn, R. V. Dynamic Peptide Libraries for the Discovery of Supramolecular Nanomaterials. *Nat. Nanotechnol.* **2016**, *11*, 960−967.
(101) Rengifo, R. F.; Sementilli, A.; Kim, Y.; Liang, C.; Li, N. X. A.; Mehta, A. K.; Lynn, D. G. Liquid-Like Phases Preorder Peptides for Supramolecular Assembly. *ChemSystemsChem* **2020**, *2*, No. e2000007.
(102) Krishna Kumar, R.; Harniman, R. L.; Patil, A. J.; Mann, S. Self-Transformation and Structural Reconfiguration in Coacervate-Based Protocells. *Chem. Sci.* **2016**, *7*, 5879−5887.
(103) Adams, D. J.; Butler, M. F.; Frith, W. J.; Kirkland, M.; Mullen, L.; Sanderson, P. A New Method for Maintaining Homogeneity During Liquid-Hydrogel Transitions Using Low Molecular Weight Hydrogelators. *Soft Matter* **2009**, *5*, 1856−1862.
(104) Makam, P.; Gazit, E. Minimalistic Peptide Supramolecular Co-Assembly: Expanding the Conformational Space for Nanotechnology. *Chem. Soc. Rev.* **2018**, *47*, 3406−3420.
(105) Okesola, B. O.; Mata, A. Multicomponent Self-Assembly as a Tool to Harness New Properties from Peptides and Proteins in Material Design. *Chem. Soc. Rev.* **2018**, *47*, 3721−3736.
(106) Raymond, D. M.; Nilsson, B. L. Multicomponent Peptide Assemblies. *Chem. Soc. Rev.* **2018**, *47*, 3659−3720.
(107) Chen, L.; Revel, S.; Morris, K.; Spiller, D. G.; Serpell, L. C.; Adams, D. J. Low Molecular Weight Gelator-Dextran Composites. *Chem. Commun.* **2010**, *46*, 6738−6740.
(108) Morris, K. L.; Chen, L.; Raeburn, J.; Sellick, O. R.; Cotanda, P.; Paul, A.; Griffiths, P. C.; King, S. M.; O'Reilly, R. K.; Serpell, L. C.; Adams, D. J. Chemically Programmed Self-Sorting of Gelator Networks. *Nat. Commun.* **2013**, *4*, 1480.
(109) Wang, J.; Wang, Z.; Gao, J.; Wang, L.; Yang, Z.; Kong, D.; Yang, Z. Incorporation of Supramolecular Hydrogels into Agarose Hydrogels—a Potential Drug Delivery Carrier. *J. Mater. Chem.* **2009**, *19*, 7892−7896.
(110) Fleming, S.; Debnath, S.; Frederix, P. W. J. M.; Hunt, N. T.; Ulijn, R. V. Insights into the Coassembly of Hydrogelators and Surfactants Based on Aromatic Peptide Amphiphiles. *Biomacromolecules* **2014**, *15*, 1171−1184.
(111) Niece, K. L.; Hartgerink, J. D.; Donners, J. J. J. M.; Stupp, S. I. Self-Assembly Combining Two Bioactive Peptide-Amphiphile Molecules into Nanofibers by Electrostatic Attraction. *J. Am. Chem. Soc.* **2003**, *125*, 7146−7147.
(112) Sahoo, J. K.; VandenBerg, M. A.; Ruiz Bello, E. E.; Nazareth, C. D.; Webber, M. J. Electrostatic-Driven Self-Sorting and Nanostructure Speciation in Self-Assembling Tetrapeptides. *Nanoscale* **2019**, *11*, 16534−16543.
(113) Xu, X.-D.; Chen, C.-S.; Lu, B.; Cheng, S.-X.; Zhang, X.-Z.; Zhuo, R.-X. Coassembly of Oppositely Charged Short Peptides into Well-Defined Supramolecular Hydrogels. *J. Phys. Chem. B* **2010**, *114*, 2365−2372.
(114) Marchesan, S.; Easton, C. D.; Kushkaki, F.; Waddington, L.; Hartley, P. G. Tripeptide Self-Assembled Hydrogels: Unexpected Twists of Chirality. *Chem. Commun.* **2012**, *48*, 2195−2197.
(115) Marshall, L. R.; Jayachandran, M.; Lengyel-Zhand, Z.; Rufo, C. M.; Kriews, A.; Kim, M.-C.; Korendovych, I. V. Synergistic Interactions Are Prevalent in Catalytic Amyloids. *ChemBioChem* **2020**, *21*, 2611−2614.
(116) Alakpa, E. V.; Jayawarna, V.; Lampel, A.; Burgess, K. V.; West, C. C.; Bakker, S. C. J.; Roy, S.; Javid, N.; Fleming, S.; Lamprou, D. A.; et al. Tunable Supramolecular Hydrogels for Selection of Lineage-Guiding Metabolites in Stem Cell Cultures. *Chem.* **2016**, *1*, 298−319.
(117) Niece, K. L.; Hartgerink, J. D.; Donners, J. J. J. M.; Stupp, S. I. Self-Assembly Combining Two Bioactive Peptide-Amphiphile Molecules into Nanofibers by Electrostatic Attraction. *J. Am. Chem. Soc.* **2003**, *125*, 7146−7147.
(118) Wychowaniec, J. K.; Iliut, M.; Zhou, M.; Moffat, J.; Elsawy, M. A.; Pinheiro, W. A.; Hoyland, J. A.; Miller, A. F.; Vijayaraghavan, A.;

Saiani, A. Designing Peptide/Graphene Hybrid Hydrogels through Fine-Tuning of Molecular Interactions. *Biomacromolecules* **2018**, *19*, 2731−2741.
(119) Okesola, B. O.; Wu, Y.; Derkus, B.; Gani, S.; Wu, D.; Knani, D.; Smith, D. K.; Adams, D. J.; Mata, A. Supramolecular Self-Assembly to Control Structural and Biological Properties of Multicomponent Hydrogels. *Chem. Mater.* **2019**, *31*, 7883−7897.
(120) Colquhoun, C.; Draper, E. R.; Eden, E. G. B.; Cattoz, B. N.; Morris, K. L.; Chen, L.; McDonald, T. O.; Terry, A. E.; Griffiths, P. C.; Serpell, L. C.; et al. The Effect of Self-Sorting and Co-Assembly on the Mechanical Properties of Low Molecular Weight Hydrogels. *Nanoscale* **2014**, *6*, 13719−13725.
(121) Halperin-Sternfeld, M.; Ghosh, M.; Sevostianov, R.; Grigoriants, I.; Adler-Abramovich, L. Molecular Co-Assembly as a Strategy for Synergistic Improvement of the Mechanical Properties of Hydrogels. *Chem. Commun.* **2017**, *53*, 9586−9589.
(122) Pierschbacher, M. D.; Ruoslahti, E. Cell Attachment Activity of Fibronectin Can Be Duplicated by Small Synthetic Fragments of the Molecule. *Nature* **1984**, *309*, 30−33.
(123) Guler, M. O.; Hsu, L.; Soukasene, S.; Harrington, D. A.; Hulvat, J. F.; Stupp, S. I. Presentation of Rgds Epitopes on Self-Assembled Nanofibers of Branched Peptide Amphiphiles. *Biomacromolecules* **2006**, *7*, 1855−1863.
(124) Guler, M. O.; Soukasene, S.; Hulvat, J. F.; Stupp, S. I. Presentation and Recognition of Biotin on Nanofibers Formed by Branched Peptide Amphiphiles. *Nano Lett.* **2005**, *5*, 249−252.
(125) Zhou, M.; Smith, A. M.; Das, A. K.; Hodson, N. W.; Collins, R. F.; Ulijn, R. V.; Gough, J. E. Self-Assembled Peptide-Based Hydrogels as Scaffolds for Anchorage-Dependent Cells. *Biomaterials* **2009**, *30*, 2523−2530.
(126) Seow, W. Y.; Hauser, C. A. E. Tunable Mechanical Properties of Ultrasmall Peptide Hydrogels by Crosslinking and Functionalization to Achieve the 3d Distribution of Cells. *Adv. Healthcare Mater.* **2013**, *2*, 1219−1223.
(127) Winkler, S.; Wilson, D.; Kaplan, D. L. Controlling B-Sheet Assembly in Genetically Engineered Silk by Enzymatic Phosphorylation/Dephosphorylation. *Biochemistry* **2000**, *39*, 12739−12746.
(128) Yang, Z.; Liang, G.; Wang, L.; Xu, B. Using a Kinase/Phosphatase Switch to Regulate a Supramolecular Hydrogel and Forming the Supramolecular Hydrogel in Vivo. *J. Am. Chem. Soc.* **2006**, *128*, 3038−3043.
(129) Sadownik, J. W.; Leckie, J.; Ulijn, R. V. Micelle to Fibre Biocatalytic Supramolecular Transformation of an Aromatic Peptide Amphiphile. *Chem. Commun.* **2011**, *47*, 728−730.
(130) Sur, S.; Tantakitti, F.; Matson, J. B.; Stupp, S. I. Epitope Topography Controls Bioactivity in Supramolecular Nanofibers. *Biomater. Sci.* **2015**, *3*, 520−532.
(131) Webber, M. J.; Tongers, J.; Renault, M.-A.; Roncalli, J. G.; Losordo, D. W.; Stupp, S. I. Development of Bioactive Peptide Amphiphiles for Therapeutic Cell Delivery. *Acta Biomater.* **2010**, *6*, 3−11.
(132) Redondo-Gómez, C.; Abdouni, Y.; Becer, C. R.; Mata, A. Self-Assembling Hydrogels Based on a Complementary Host-Guest Peptide Amphiphile Pair. *Biomacromolecules* **2019**, *20*, 2276−2285.
(133) Redondo-Gómez, C.; Padilla-Lopategui, S.; Azevedo, H. S.; Mata, A. Host-Guest-Mediated Epitope Presentation on Self-Assembled Peptide Amphiphile Hydrogels. *ACS Biomater. Sci. Eng.* **2020**, *6*, 4870−4880.
(134) Javid, N.; Roy, S.; Zelzer, M.; Yang, Z.; Sefcik, J.; Ulijn, R. V. Cooperative Self-Assembly of Peptide Gelators and Proteins. *Biomacromolecules* **2013**, *14*, 4368−4376.
(135) Brito, A.; Abul-Haija, Y. M.; da Costa, D. S.; Novoa-Carballal, R.; Reis, R. L.; Ulijn, R. V.; Pires, R. A.; Pashkuleva, I. Minimalistic Supramolecular Proteoglycan Mimics by Co-Assembly of Aromatic Peptide and Carbohydrate Amphiphiles. *Chem. Sci.* **2019**, *10*, 2385−2390.
(136) Boothroyd, S.; Saiani, A.; Miller, A. F. Controlling Network Topology and Mechanical Properties of Co-Assembling Peptide Hydrogels: Co-Assembling Peptide Hydrogels. *Biopolymers* **2014**, *101*, 669−680.
(137) Gao, J.; Tang, C.; Elsawy, M. A.; Smith, A. M.; Miller, A. F.; Saiani, A. Controlling Self-Assembling Peptide Hydrogel Properties through Network Topology. *Biomacromolecules* **2017**, *18*, 826−834.
(138) Roberts, D.; Rochas, C.; Saiani, A.; Miller, A. F. Effect of Peptide and Guest Charge on the Structural, Mechanical and Release Properties of B-Sheet Forming Peptides. *Langmuir* **2012**, *28*, 16196−16206.
(139) Greenfield, M. A.; Hoffman, J. R.; Olvera de la Cruz, M.; Stupp, S. I. Tunable Mechanics of Peptide Nanofiber Gels. *Langmuir* **2010**, *26*, 3641−3647.
(140) Stendahl, J. C.; Rao, M. S.; Guler, M. O.; Stupp, S. I. Intermolecular Forces in the Self-Assembly of Peptide Amphiphile Nanofibers. *Adv. Funct. Mater.* **2006**, *16*, 499−508.
(141) Godbe, J. M.; Freeman, R.; Burbulla, L. F.; Lewis, J.; Krainc, D.; Stupp, S. I. Gelator Length Precisely Tunes Supramolecular Hydrogel Stiffness and Neuronal Phenotype in 3d Culture. *ACS Biomater. Sci. Eng.* **2020**, *6*, 1196−1207.
(142) Nagy, K. J.; Giano, M. C.; Jin, A.; Pochan, D. J.; Schneider, J. P. Enhanced Mechanical Rigidity of Hydrogels Formed from Enantiomeric Peptide Assemblies. *J. Am. Chem. Soc.* **2011**, *133*, 14975−14977.
(143) Marchesan, S.; Easton, C. D.; Styan, K. E.; Waddington, L. J.; Kushkaki, F.; Goodall, L.; McLean, K. M.; Forsythe, J. S.; Hartley, P. G. Chirality Effects at Each Amino Acid Position on Tripeptide Self-Assembly into Hydrogel Biomaterials. *Nanoscale* **2014**, *6*, 5172−5180.
(144) Nagy-Smith, K.; Beltramo, P. J.; Moore, E.; Tycko, R.; Furst, E. M.; Schneider, J. P. Molecular, Local, and Network-Level Basis for the Enhanced Stiffness of Hydrogel Networks Formed from Coassembled Racemic Peptides: Predictions from Pauling and Corey. *ACS Cent. Sci.* **2017**, *3*, 586−597.
(145) Vauthey, S.; Santoso, S.; Gong, H.; Watson, N.; Zhang, S. Molecular Self-Assembly of Surfactant-Like Peptides to Form Nanotubes and Nanovesicles. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 5355−5360.
(146) Capito, R. M.; Azevedo, H. S.; Velichko, Y. S.; Mata, A.; Stupp, S. I. Self-Assembly of Large and Small Molecules into Hierarchically Ordered Sacs and Membranes. *Science* **2008**, *319*, 1812−1816.
(147) Cousins, B. G.; Das, A. K.; Sharma, R.; Li, Y.; McNamara, J. P.; Hillier, I. H.; Kinloch, I. A.; Ulijn, R. V. Enzyme-Activated Surfactants for Dispersion of Carbon Nanotubes. *Small* **2009**, *5*, 587−590.
(148) Castelletto, V.; Seitsonen, J.; Ruokolainen, J.; Piras, C.; Cramer, R.; Edwards-Gayle, C. J. C.; Hamley, I. W. Peptide Nanotubes Self-Assembled from Leucine-Rich Alpha Helical Surfactant-Like Peptides. *Chem. Commun.* **2020**, *56*, 11977−11980.
(149) Hamley, I. W.; Castelletto, V.; Dehsorkhi, A.; Torras, J.; Aleman, C.; Portnaya, I.; Danino, D. The Conformation and Aggregation of Proline-Rich Surfactant-Like Peptides. *J. Phys. Chem. B* **2018**, *122*, 1826−1835.
(150) Bai, S.; Pappas, C.; Debnath, S.; Frederix, P. W. J. M.; Leckie, J.; Fleming, S.; Ulijn, R. V. Stable Emulsions Formed by Self-Assembly of Interfacial Networks of Dipeptide Derivatives. *ACS Nano* **2014**, *8*, 7005−7013.
(151) Fjell, C. D.; Hiss, J. A.; Hancock, R. E. W.; Schneider, G. Designing Antimicrobial Peptides: Form Follows Function. *Nat. Rev. Drug Discovery* **2012**, *11*, 37−51.
(152) Castelletto, V.; Barnes, R. H.; Karatzas, K.-A.; Edwards-Gayle, C. J. C.; Greco, F.; Hamley, I. W.; Seitsonen, J.; Ruokolainen, J. Restructuring of Lipid Membranes by an Arginine-Capped Peptide Bolaamphiphile. *Langmuir* **2019**, *35*, 1302−1311.
(153) Koutsopoulos, S.; Kaiser, L.; Eriksson, H. M.; Zhang, S. Designer Peptide Surfactants Stabilize Diverse Functional Membrane Proteins. *Chem. Soc. Rev.* **2012**, *41*, 1721−1728.
(154) Aviño, F.; Matheson, A. B.; Adams, D. J.; Clegg, P. S. Stabilizing Bubble and Droplet Interfaces Using Dipeptide Hydrogels. *Org. Biomol. Chem.* **2017**, *15*, 6342−6348.

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

(155) Mondal, S.; Varenik, M.; Bloch, D. N.; Atsmon-Raz, Y.; Jacoby, G.; Adler-Abramovich, L.; Shimon, L. J. W.; Beck, R.; Miller, Y.; Regev, O.; Gazit, E. A Minimal Length Rigid Helical Peptide Motif Allows Rational Design of Modular Surfactants. *Nat. Commun.* **2017**, *8*, 14018.
(156) Scott, G. G.; McKnight, P. J.; Tuttle, T.; Ulijn, R. V. Tripeptide Emulsifiers. *Adv. Mater.* **2016**, *28*, 1381−1386.
(157) Dragulska, S. A.; Chen, Y.; Wlodarczyk, M. T.; Poursharifi, M.; Dottino, P.; Ulijn, R. V.; Martignetti, J. A.; Mieszawska, A. J. Tripeptide-Stabilized Oil-in-Water Nanoemulsion of an Oleic Acids-Platinum(Ii) Conjugate as an Anticancer Nanomedicine. *Bioconjugate Chem.* **2018**, *29*, 2514−2519.
(158) Castelletto, V.; Edwards-Gayle, C. J. C.; Hamley, I. W.; Barrett, G.; Seitsonen, J.; Ruokolainen, J. Peptide-Stabilized Emulsions and Gels from an Arginine-Rich Surfactant-Like Peptide with Antimicrobial Activity. *ACS Appl. Mater. Interfaces* **2019**, *11*, 9893−9903.
(159) Cao, M.; Lu, S.; Wang, N.; Xu, H.; Cox, H.; Li, R.; Waigh, T.; Han, Y.; Wang, Y.; Lu, J. R. Enzyme-Triggered Morphological Transition of Peptide Nanostructures for Tumor-Targeted Drug Delivery and Enhanced Cancer Therapy. *ACS Appl. Mater. Interfaces* **2019**, *11*, 16357−16366.
(160) Hughes, M.; Xu, H.; Frederix, P. W. J. M.; Smith, A. M.; Hunt, N. T.; Tuttle, T.; Kinloch, I. A.; Ulijn, R. V. Biocatalytic Self-Assembly of 2d Peptide-Based Nanostructures. *Soft Matter* **2011**, *7*, 10032−10038.
(161) Duncan, K. L.; Ulijn, R. V. Short Peptides in Minimalistic Biocatalyst Design. *Biocatalysis* **2015**, *1*, 67−81.
(162) Clarke, D. E.; Parmenter, C. D. J.; Scherman, O. A. Tunable Pentapeptide Self-Assembled B-Sheet Hydrogels. *Angew. Chem., Int. Ed.* **2018**, *57*, 7709−7713.
(163) Draper, E. R.; Wallace, M.; Schweins, R.; Poole, R. J.; Adams, D. J. Nonlinear Effects in Multicomponent Supramolecular Hydrogels. *Langmuir* **2017**, *33*, 2387−2395.
(164) van Teijlingen, A.; Tuttle, T. Beyond Tripeptides Two-Step Active Machine Learning for Very Large Data Sets. *J. Chem. Theory Comput.* **2021**, *17*, 3221−3232.
(165) Moreira, I. P.; Scott, G. G.; Ulijn, R. V.; Tuttle, T. Computational Prediction of Tripeptide-Dipeptide Co-Assembly. *Mol. Phys.* **2019**, *117*, 1151−1163.
(166) Lampel, A.; Ulijn, R. V.; Tuttle, T. Guiding Principles for Peptide Nanotechnology through Directed Discovery. *Chem. Soc. Rev.* **2018**, *47*, 3737−3758.
(167) Jiang, Q.-Q.; Sicking, W.; Ehlers, M.; Schmuck, C. Discovery of Potent Inhibitors of Human B-Tryptase from Pre-Equilibrated Dynamic Combinatorial Libraries. *Chem. Sci.* **2015**, *6*, 1792−1800.
(168) Pappas, C. G.; Wijerathne, N.; Sahoo, J. K.; Jain, A.; Kroiss, D.; Sasselli, I. R.; Pina, A. S.; Lampel, A.; Ulijn, R. V. Spontaneous Aminolytic Cyclization and Self-Assembly of Dipeptide Methyl Esters in Water. *ChemSystemsChem* **2020**, *2*, No. e2000013.
(169) Chen, C.; Tan, J.; Hsieh, M.-C.; Pan, T.; Goodwin, J. T.; Mehta, A. K.; Grover, M. A.; Lynn, D. G. Design of Multi-Phase Dynamic Chemical Networks. *Nat. Chem.* **2017**, *9*, 799−804.
(170) Liu, B.; Pappas, C. G.; Zangrando, E.; Demitri, N.; Chmielewski, P. J.; Otto, S. Complex Molecules That Fold Like Proteins Can Emerge Spontaneously. *J. Am. Chem. Soc.* **2019**, *141*, 1685−1689.
(171) Ashkenasy, G.; Hermans, T. M.; Otto, S.; Taylor, A. F. Systems Chemistry. *Chem. Soc. Rev.* **2017**, *46*, 2543−2554.
(172) Carnall, J. M. A.; Waudby, C. A.; Belenguer, A. M.; Stuart, M. C. A.; Peyralans, J. J. P.; Otto, S. Mechanosensitive Self-Replication Driven by Self-Organization. *Science* **2010**, *327*, 1502.
(173) Sadownik, J. W.; Mattia, E.; Nowak, P.; Otto, S. Diversification of Self-Replicating Molecules. *Nat. Chem.* **2016**, *8*, 264−269.
(174) Liu, B.; Pappas, C. G.; Zangrando, E.; Demitri, N.; Chmielewski, P. J.; Otto, S. Complex Molecules That Fold Like Proteins Can Emerge Spontaneously. *J. Am. Chem. Soc.* **2019**, *141*, 1685−1689.
(175) Tan, J.; Zhang, L.; Hsieh, M.-C.; Goodwin, J. T.; Grover, M. A.; Lynn, D. G. Chemical Control of Peptide Material Phase Transitions. *Chemical Science* **2021**, *12*, 3025−3031.
(176) Radzicka, A.; Wolfenden, R. Rates of Uncatalyzed Peptide Bond Hydrolysis in Neutral Solution and the Transition State Affinities of Proteases. *J. Am. Chem. Soc.* **1996**, *118*, 6105−6109.
(177) Williams, R. J.; Smith, A. M.; Collins, R.; Hodson, N.; Das, A. K.; Ulijn, R. V. Enzyme-Assisted Self-Assembly under Thermodynamic Control. *Nat. Nanotechnol.* **2009**, *4*, 19−24.
(178) Toledano, S.; Williams, R. J.; Jayawarna, V.; Ulijn, R. V. Enzyme-Triggered Self-Assembly of Peptide Hydrogels Via Reversed Hydrolysis. *J. Am. Chem. Soc.* **2006**, *128*, 1070−1071.
(179) Nalluri, S. K. M.; Berdugo, C.; Javid, N.; Frederix, P. W. J. M.; Ulijn, R. V. Biocatalytic Self-Assembly of Supramolecular Charge-Transfer Nanostructures Based on N-Type Semiconductor-Appended Peptides. *Angew. Chem., Int. Ed.* **2014**, *53*, 5882−5887.
(180) Swann, P. G.; Casanova, R. A.; Desai, A.; Frauenhoff, M. M.; Urbancic, M.; Slomczynska, U.; Hopfinger, A. J.; Le Breton, G. C.; Venton, D. L. Nonspecific Protease-Catalyzed Hydrolysis/Synthesis of a Mixture of Peptides: Product Diversity and Ligand Amplification by a Molecular Trap. *Biopolymers* **1996**, *40*, 617−625.
(181) Das, A. K.; Hirst, A. R.; Ulijn, R. V. Evolving Nanomaterials Using Enzyme-Driven Dynamic Peptide Libraries (Edpl). *Faraday Discuss.* **2009**, *143*, 293−303.
(182) Hughes, M.; Frederix, P. W. J. M.; Raeburn, J.; Birchall, L. S.; Sadownik, J.; Coomer, F. C.; Lin, I. H.; Cussen, E. J.; Hunt, N. T.; Tuttle, T.; et al. Sequence/Structure Relationships in Aromatic Dipeptide Hydrogels Formed under Thermodynamic Control by Enzyme-Assisted Self-Assembly. *Soft Matter* **2012**, *8*, 5595−5602.
(183) Pappas, C. G.; Abul-Haija, Y. M.; Flack, A.; Frederix, P. W. J. M.; Ulijn, R. V. Tuneable Fmoc-Phe-(4-X)-Phe-Nh2 Nanostructures by Variable Electronic Substitution. *Chem. Commun.* **2014**, *50*, 10630−10633.
(184) Berdugo, C.; Nalluri, S. K. M.; Javid, N.; Escuder, B.; Miravet, J. F.; Ulijn, R. V. Dynamic Peptide Library for the Discovery of Charge Transfer Hydrogels. *ACS Appl. Mater. Interfaces* **2015**, *7*, 25946−25954.
(185) Nalluri, S. K. M.; Ulijn, R. V. Discovery of Energy Transfer Nanostructures Using Gelation-Driven Dynamic Combinatorial Libraries. *Chem. Sci.* **2013**, *4*, 3699−3705.
(186) Wijerathne, N. K.; Kumar, M.; Ulijn, R. V. Fmoc-Dipeptide/Porphyrin Molar Ratio Dictates Energy Transfer Efficiency in Nanostructures Produced by Biocatalytic Co-Assembly. *Chem. - Eur. J.* **2019**, *25*, 11847−11851.
(187) Guilbaud, J.-B.; Vey, E.; Boothroyd, S.; Smith, A. M.; Ulijn, R. V.; Saiani, A.; Miller, A. F. Enzymatic Catalyzed Synthesis and Triggered Gelation of Ionic Peptides. *Langmuir* **2010**, *26*, 11297−11303.
(188) Abul-Haija, Y. M.; Ulijn, R. V. Sequence Adaptive Peptide-Polysaccharide Nanostructures by Biocatalytic Self-Assembly. *Biomacromolecules* **2015**, *16*, 3473−3479.
(189) Williams, R. J.; Hall, T. E.; Glattauer, V.; White, J.; Pasic, P. J.; Sorensen, A. B.; Waddington, L.; McLean, K. M.; Currie, P. D.; Hartley, P. G. The in Vivo Performance of an Enzyme-Assisted Self-Assembled Peptide/Protein Hydrogel. *Biomaterials* **2011**, *32*, 5304−5310.
(190) Freeman, R.; Han, M.; Álvarez, Z.; Lewis, J. A.; Wester, J. R.; Stephanopoulos, N.; McClendon, M. T.; Lynsky, C.; Godbe, J. M.; Sangji, H.; et al. Reversible Self-Assembly of Superstructured Networks. *Science* **2018**, *362*, 808.
(191) Sadownik, J. W.; Ulijn, R. V. Locking an Oxidation-Sensitive Dynamic Peptide System in the Gel State. *Chem. Commun.* **2010**, *46*, 3481−3483.
(192) Sahoo, J. K.; Nalluri, S. K. M.; Javid, N.; Webb, H.; Ulijn, R. V. Biocatalytic Amide Condensation and Gelation Controlled by Light. *Chem. Commun.* **2014**, *50*, 5462−5464.
(193) Sahoo, J. K.; Pappas, C. G.; Sasselli, I. R.; Abul-Haija, Y. M.; Ulijn, R. V. Biocatalytic Self-Assembly Cascades. *Angew. Chem., Int. Ed.* **2017**, *56*, 6828−6832.

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

(194) Kitadai, N. Thermodynamic Prediction of Glycine Polymerization as a Function of Temperature and Ph Consistent with Experimentally Obtained Results. *J. Mol. Evol.* **2014**, *78*, 171−187.
(195) Rodriguez-Garcia, M.; Surman, A. J.; Cooper, G. J. T.; Suárez-Marina, I.; Hosni, Z.; Lee, M. P.; Cronin, L. Formation of Oligopeptides in High Yield under Simple Programmable Conditions. *Nat. Commun.* **2015**, *6*, 8385.
(196) Forsythe, J. G.; Petrov, A. S.; Millar, W. C.; Yu, S.-S.; Krishnamurthy, R.; Grover, M. A.; Hud, N. V.; Fernández, F. M. Surveying the Sequence Diversity of Model Prebiotic Peptides by Mass Spectrometry. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114*, E7652.
(197) Surman, A. J.; Rodriguez-Garcia, M.; Abul-Haija, Y. M.; Cooper, G. J. T.; Gromski, P. S.; Turk-MacLeod, R.; Mullin, M.; Mathis, C.; Walker, S. I.; Cronin, L. Environmental Control Programs the Emergence of Distinct Functional Ensembles from Unconstrained Chemical Reactions. *Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116*, 5387.
(198) van Rossum, S. A. P.; Tena-Solsona, M.; van Esch, J. H.; Eelkema, R.; Boekhoven, J. Dissipative out-of-Equilibrium Assembly of Man-Made Supramolecular Materials. *Chem. Soc. Rev.* **2017**, *46*, 5519−5535.
(199) Moscatelli, A. Chemistry in the Gap. *Nat. Nanotechnol.* **2017**, *2017*, 240.
(200) Arango-Restrepo, A.; Barragan, D.; Rubi, J. M. Self-Assembling Outside Equilibrium: Emergence of Structures Mediated by Dissipation. *Phys. Chem. Chem. Phys.* **2019**, *21*, 17475−17493.
(201) Tena-Solsona, M.; Boekhoven, J. Dissipative Self-Assembly of Peptides. *Isr. J. Chem.* **2019**, *59*, 898−905.
(202) Ragazzon, G.; Prins, L. J. Energy Consumption in Chemical Fuel-Driven Self-Assembly. *Nat. Nanotechnol.* **2018**, *13*, 882−889.
(203) Sorrenti, A.; Leira-Iglesias, J.; Markvoort, A. J.; de Greef, T. F. A.; Hermans, T. M. Non-Equilibrium Supramolecular Polymerization. *Chem. Soc. Rev.* **2017**, *46*, 5476−5490.
(204) Karsenti, E. Self-Organization in Cell Biology: A Brief History. *Nat. Rev. Mol. Cell Biol.* **2008**, *9*, 255−262.
(205) Singh, N.; Formon, G. J. M.; De Piccoli, S.; Hermans, T. M. Devising Synthetic Reaction Cycles for Dissipative Nonequilibrium Self-Assembly. *Adv. Mater.* **2020**, *32*, 1906834.
(206) Hossain, M. M.; Atkinson, J. L.; Hartley, C. S. Dissipative Assembly of Macrocycles Comprising Multiple Transient Bonds. *Angew. Chem., Int. Ed.* **2020**, *59*, 13807−13813.
(207) Boekhoven, J.; Brizard, A. M.; Kowlgi, K. N.; Koper, G. J.; Eelkema, R.; van Esch, J. H. Dissipative Self-Assembly of a Molecular Gelator by Using a Chemical Fuel. *Angew. Chem., Int. Ed.* **2010**, *49*, 4825−4828.
(208) Boekhoven, J.; Hendriksen, W. E.; Koper, G. J. M.; Eelkema, R.; van Esch, J. H. Transient Assembly of Active Materials Fueled by a Chemical Reaction. *Science* **2015**, *349*, 1075−1079.
(209) Wojciechowski, J. P.; Martin, A. D.; Thordarson, P. Kinetically Controlled Lifetimes in Redox-Responsive Transient Supramolecular Hydrogels. *J. Am. Chem. Soc.* **2018**, *140*, 2869−2874.
(210) Tena-Solsona, M.; Riess, B.; Grotsch, R. K.; Lohrer, F. C.; Wanzke, C.; Kasdorf, B.; Bausch, A. R.; Muller-Buschbaum, P.; Lieleg, O.; Boekhoven, J. Non-Equilibrium Dissipative Supramolecular Materials with a Tunable Lifetime. *Nat. Commun.* **2017**, *8*, 15895.
(211) Kumar, M.; Sementa, D.; Narang, V.; Riedo, E.; Ulijn, R. V. Self-Assembly Propensity Dictates Lifetimes in Transient Naphthalimide-Dipeptide Nanofibers. *Chem. - Eur. J.* **2020**, *26*, 8372−8376.
(212) Aumiller, W. M.; Keating, C. D. Phosphorylation-Mediated Rna/Peptide Complex Coacervation as a Model for Intracellular Liquid Organelles. *Nat. Chem.* **2016**, *8*, 129−137.
(213) Riess, B.; Wanzke, C.; Tena-Solsona, M.; Grotsch, R. K.; Maity, C.; Boekhoven, J. Dissipative Assemblies That Inhibit Their Deactivation. *Soft Matter* **2018**, *14*, 4852−4859.
(214) Donau, C.; Späth, F.; Sosson, M.; Kriebisch, B. A. K.; Schnitter, F.; Tena-Solsona, M.; Kang, H.-S.; Salibi, E.; Sattler, M.; Mutschler, H.; Boekhoven, J. Active Coacervate Droplets as a Model for Membraneless Organelles and Protocells. *Nat. Commun.* **2020**, *11*, 5167.
(215) Späth, F.; Donau, C.; Bergmann, A. M.; Kränzlein, M.; Synatschke, C. V.; Rieger, B.; Boekhoven, J. Molecular Design of Chemically Fueled Peptide-Polyelectrolyte Coacervate-Based Assemblies. *J. Am. Chem. Soc.* **2021**, *143*, 4782−4789.
(216) Bal, S.; Das, K.; Ahmed, S.; Das, D. Chemically Fueled Dissipative Self-Assembly That Exploits Cooperative Catalysis. *Angew. Chem., Int. Ed.* **2019**, *58*, 244−247.
(217) Afrose, S. P.; Bal, S.; Chatterjee, A.; Das, K.; Das, D. Designed Negative Feedback from Transiently Formed Catalytic Nanostructures. *Angew. Chem., Int. Ed.* **2019**, *58*, 15783−15787.
(218) Bal, S.; Ghosh, C.; Ghosh, T.; Vijayaraghavan, R. K.; Das, D. Non-Equilibrium Polymerization of Cross-B Amyloid Peptides for Temporal Control of Electronic Properties. *Angew. Chem., Int. Ed.* **2020**, *59*, 13506−13510.
(219) Yang, Z.; Gu, H.; Fu, D.; Gao, P.; Lam, J. K.; Xu, B. Enzymatic Formation of Supramolecular Hydrogels. *Adv. Mater.* **2004**, *16*, 1440−1444.
(220) Debnath, S.; Roy, S.; Ulijn, R. V. Peptide Nanofibers with Dynamic Instability through Nonequilibrium Biocatalytic Assembly. *J. Am. Chem. Soc.* **2013**, *135*, 16789−16792.
(221) Fastrez, J.; Fersht, A. L. Demonstration of the Acyl-Enzyme Mechanism for the Hydrolysis of Peptides and Anilides by Chymotrypsin. *Biochemistry* **1973**, *12*, 2025−2034.
(222) Salam, S. M. A.; Kagawa, K.; Kawashiro, K. A-Chymotrypsin-Catalyzed Peptide Synthesis Using N-Protected D-Amino Acid Carbamoylmethyl Esters as Acyl Donors. *Biotechnol. Lett.* **2005**, *27*, 1199−1203.
(223) Pappas, C. G.; Sasselli, I. R.; Ulijn, R. V. Biocatalytic Pathway Selection in Transient Tripeptide Nanostructures. *Angew. Chem., Int. Ed.* **2015**, *54*, 8119−8123.
(224) Ikeda, M.; Tanida, T.; Yoshii, T.; Kurotani, K.; Onogi, S.; Urayama, K.; Hamachi, I. Installing Logic-Gate Responses to a Variety of Biological Substances in Supramolecular Hydrogel-Enzyme Hybrids. *Nat. Chem.* **2014**, *6*, 511−518.
(225) Kubota, R.; Makuta, M.; Suzuki, R.; Ichikawa, M.; Tanaka, M.; Hamachi, I. Force Generation by a Propagating Wave of Supramolecular Nanofibers. *Nat. Commun.* **2020**, *11*, 3541.
(226) Sorrenti, A.; Leira-Iglesias, J.; Sato, A.; Hermans, T. M. Non-Equilibrium Steady States in Supramolecular Polymerization. *Nat. Commun.* **2017**, *8*, 15899.
(227) Webber, M. J.; Newcomb, C. J.; Bitton, R.; Stupp, S. I. Switching of Self-Assembly in a Peptide Nanostructure with a Specific Enzyme. *Soft Matter* **2011**, *7*, 9665−9672.
(228) Tantakitti, F.; Boekhoven, J.; Wang, X.; Kazantsev, R. V.; Yu, T.; Li, J.; Zhuang, E.; Zandi, R.; Ortony, J. H.; Newcomb, C. J.; et al. Energy Landscapes and Functions of Supramolecular Systems. *Nat. Mater.* **2016**, *15*, 469−476.
(229) Hirst, A. R.; Roy, S.; Arora, M.; Das, A. K.; Hodson, N.; Murray, P.; Marshall, S.; Javid, N.; Sefcik, J.; Boekhoven, J.; et al. Biocatalytic Induction of Supramolecular Order. *Nat. Chem.* **2010**, *2*, 1089−1094.
(230) Sahoo, J. K.; Roy, S.; Javid, N.; Duncan, K.; Aitken, L.; Ulijn, R. V. Pathway-Dependent Gold Nanoparticle Formation by Biocatalytic Self-Assembly. *Nanoscale* **2017**, *9*, 12330−12334.
(231) Jonkheijm, P.; van der Schoot, P.; Schenning, A. P.; Meijer, E. W. Probing the Solvent-Assisted Nucleation Pathway in Chemical Self-Assembly. *Science* **2006**, *313*, 80−83.
(232) Lloyd, G. O.; Steed, J. W. Anion-Tuning of Supramolecular Gel Properties. *Nat. Chem.* **2009**, *1*, 437−442.
(233) Heuser, T.; Steppert, A.-K.; Molano Lopez, C.; Zhu, B.; Walther, A. Generic Concept to Program the Time Domain of Self-Assemblies with a Self-Regulation Mechanism. *Nano Lett.* **2015**, *15*, 2213−2219.
(234) Heuser, T.; Weyandt, E.; Walther, A. Biocatalytic Feedback-Driven Temporal Programming of Self-Regulating Peptide Hydrogels. *Angew. Chem., Int. Ed.* **2015**, *54*, 13258−13262.
(235) Angulo-Pachon, C. A.; Miravet, J. F. Sucrose-Fueled, Energy Dissipative, Transient Formation of Molecular Hydrogels Mediated by Yeast Activity. *Chem. Commun.* **2016**, *52*, 5398−5401.

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

(236) Spitzer, D.; Rodrigues, L. L.; Strassburger, D.; Mezger, M.; Besenius, P. Tuneable Transient Thermogels Mediated by a Ph- and Redox-Regulated Supramolecular Polymerization. *Angew. Chem., Int. Ed.* **2017**, *56*, 15461−15465.
(237) Smith, L. G.; Oppenheimer, D. G. Spatial Control of Cell Expansion by the Plant Cytoskeleton. *Annu. Rev. Cell Dev. Biol.* **2005**, *21*, 271−295.
(238) Simmer, J. P.; Fincham, A. G. Molecular Mechanisms of Dental Enamel Formation. *Crit. Rev. Oral Biol. Med.* **1995**, *6*, 84−108.
(239) Thornton, K.; Abul-Haija, Y. M.; Hodson, N.; Ulijn, R. V. Mechanistic Insights into Phosphatase Triggered Self-Assembly Including Enhancement of Biocatalytic Conversion Rate. *Soft Matter* **2013**, *9*, 9430−9439.
(240) Conte, M. P.; Lau, K. H. A.; Ulijn, R. V. Biocatalytic Self-Assembly Using Reversible and Irreversible Enzyme Immobilization. *ACS Appl. Mater. Interfaces* **2017**, *9*, 3266−3271.
(241) Conte, M. P.; Sahoo, J. K.; Abul-Haija, Y. M.; Lau, K. H. A.; Ulijn, R. V. Biocatalytic Self-Assembly on Magnetic Nanoparticles. *ACS Appl. Mater. Interfaces* **2018**, *10*, 3069−3075.
(242) Vigier-Carrière, C.; Wagner, D.; Chaumont, A.; Durr, B.; Lupattelli, P.; Lambour, C.; Schmutz, M.; Hemmerlé, J.; Senger, B.; Schaaf, P.; et al. Control of Surface-Localized, Enzyme-Assisted Self-Assembly of Peptides through Catalyzed Oligomerization. *Langmuir* **2017**, *33*, 8267−8276.
(243) Vigier-Carrière, C.; Boulmedais, F.; Schaaf, P.; Jierry, L. Surface-Assisted Self-Assembly Strategies Leading to Supramolecular Hydrogels. *Angew. Chem., Int. Ed.* **2018**, *57*, 1448−1456.
(244) Vigier-Carrière, C.; Garnier, T.; Wagner, D.; Lavalle, P.; Rabineau, M.; Hemmerlé, J.; Senger, B.; Schaaf, P.; Boulmedais, F.; Jierry, L. Bioactive Seed Layer for Surface-Confined Self-Assembly of Peptides. *Angew. Chem., Int. Ed.* **2015**, *54*, 10198−10201.
(245) Gibbon, B. C.; Kropf, D. L. Cytosolic Ph Gradients Associated with Tip Growth. *Science* **1994**, *263*, 1419.
(246) Rodon Fores, J.; Martinez Mendez, M. L.; Mao, X.; Wagner, D.; Schmutz, M.; Rabineau, M.; Lavalle, P.; Schaaf, P.; Boulmedais, F.; Jierry, L. Localized Supramolecular Peptide Self-Assembly Directed by Enzyme-Induced Proton Gradients. *Angew. Chem., Int. Ed.* **2017**, *56*, 15984−15988.
(247) Johnson, E. K.; Adams, D. J.; Cameron, P. J. Directed Self-Assembly of Dipeptides to Form Ultrathin Hydrogel Membranes. *J. Am. Chem. Soc.* **2010**, *132*, 5130−5136.
(248) Liu, Y.; Kim, E.; Ulijn, R. V.; Bentley, W. E.; Payne, G. F. Reversible Electroaddressing of Self-Assembling Amino-Acid Conjugates. *Adv. Funct. Mater.* **2011**, *21*, 1575−1580.
(249) Raeburn, J.; Alston, B.; Kroeger, J.; McDonald, T. O.; Howse, J. R.; Cameron, P. J.; Adams, D. J. Electrochemically-Triggered Spatially and Temporally Resolved Multi-Component Gels. *Mater. Horiz.* **2014**, *1*, 241−246.
(250) Draper, E. R.; Eden, E. G. B.; McDonald, T. O.; Adams, D. J. Spatially Resolved Multicomponent Gels. *Nat. Chem.* **2015**, *7*, 848.
(251) Du, X.; Zhou, J.; Shi, J.; Xu, B. Supramolecular Hydrogelators and Hydrogels: From Soft Matter to Molecular Biomaterials. *Chem. Rev.* **2015**, *115*, 13165−13307.
(252) Yang, Z.; Liang, G.; Guo, Z.; Guo, Z.; Xu, B. Intracellular Hydrogelation of Small Molecules Inhibits Bacterial Growth. *Angew. Chem., Int. Ed.* **2007**, *46*, 8216−8219.
(253) Kuang, Y.; Shi, J.; Li, J.; Yuan, D.; Alberti, K. A.; Xu, Q.; Xu, B. Pericellular Hydrogel/Nanonets Inhibit Cancer Cells. *Angew. Chem., Int. Ed.* **2014**, *53*, 8104−8107.
(254) Feng, Z.; Wang, H.; Zhou, R.; Li, J.; Xu, B. Enzyme-Instructed Assembly and Disassembly Processes for Targeting Downregulation in Cancer Cells. *J. Am. Chem. Soc.* **2017**, *139*, 3950−3953.
(255) Zhou, J.; Du, X.; Berciu, C.; He, H.; Shi, J.; Nicastro, D.; Xu, B. Enzyme-Instructed Self-Assembly for Spatiotemporal Profiling of the Activities of Alkaline Phosphatases on Live Cells. *Chem.* **2016**, *1*, 246−263.
(256) Kalafatovic, D.; Nobis, M.; Son, J.; Anderson, K. I.; Ulijn, R. V. Mmp-9 Triggered Self-Assembly of Doxorubicin Nanofiber Depots Halts Tumor Growth. *Biomaterials* **2016**, *98*, 192−202.
(257) Monreal Santiago, G.; Liu, K.; Browne, W. R.; Otto, S. Emergence of Light-Driven Protometabolism on Recruitment of a Photocatalytic Cofactor by a Self-Replicator. *Nat. Chem.* **2020**, *12*, 603−607.
(258) Piotrowska, R.; Hesketh, T.; Wang, H.; Martin, A. R. G.; Bowering, D.; Zhang, C.; Hu, C. T.; McPhee, S. A.; Wang, T.; Park, Y.; et al. Mechanistic Insights of Evaporation-Induced Actuation in Supramolecular Crystals. *Nat. Mater.* **2021**, *20*, 403−409.
(259) Rabone, J.; Yue, Y.-F.; Chong, S. Y.; Stylianou, K. C.; Bacsa, J.; Bradshaw, D.; Darling, G. R.; Berry, N. G.; Khimyak, Y. Z.; Ganin, A. Y.; et al. An Adaptable Peptide-Based Porous Material. *Science* **2010**, *329*, 1053−1057.
(260) Martí-Gastaldo, C.; Antypov, D.; Warren, J. E.; Briggs, M. E.; Chater, P. A.; Wiper, P. V.; Miller, G. J.; Khimyak, Y. Z.; Darling, G. R.; Berry, N. G.; et al. Side-Chain Control of Porosity Closure in Single- and Multiple-Peptide-Based Porous Materials by Cooperative Folding. *Nat. Chem.* **2014**, *6*, 343−351.
(261) Katsoulidis, A. P.; Antypov, D.; Whitehead, G. F. S.; Carrington, E. J.; Adams, D. J.; Berry, N. G.; Darling, G. R.; Dyer, M. S.; Rosseinsky, M. J. Chemical Control of Structure and Guest Uptake by a Conformationally Mobile Porous Material. *Nature* **2019**, *565*, 213−217.
(262) Yan, Y.; Carrington, E. J.; Pétuya, R.; Whitehead, G. F. S.; Verma, A.; Hylton, R. K.; Tang, C. C.; Berry, N. G.; Darling, G. R.; Dyer, M. S.; et al. Amino Acid Residues Determine the Response of Flexible Metal-Organic Frameworks to Guests. *J. Am. Chem. Soc.* **2020**, *142*, 14903−14913.
(263) Pappas, C. G.; Mutasa, T.; Frederix, P. W. J. M.; Fleming, S.; Bai, S.; Debnath, S.; Kelly, S. M.; Gachagan, A.; Ulijn, R. V. Transient Supramolecular Reconfiguration of Peptide Nanostructures Using Ultrasound. *Mater. Horiz.* **2015**, *2*, 198−202.
(264) Inostroza-Brito, K. E.; Collin, E.; Siton-Mendelson, O.; Smith, K. H.; Monge-Marcet, A.; Ferreira, D. S.; Rodriguez, R. P.; Alonso, M.; Rodriguez-Cabello, J. C.; Reis, R. L.; et al. Co-Assembly, Spatiotemporal Control and Morphogenesis of a Hybrid Protein-Peptide System. *Nat. Chem.* **2015**, *7*, 897−904.
(265) Jones, C. D.; Steed, J. W. Gels with Sense: Supramolecular Materials That Respond to Heat, Light and Sound. *Chem. Soc. Rev.* **2016**, *45*, 6546−6596.
(266) Cravotto, G.; Cintas, P. Molecular Self-Assembly and Patterning Induced by Sound Waves. The Case of Gelation. *Chem. Soc. Rev.* **2009**, *38*, 2684−2697.
(267) Hakizimana, P.; Brownell, W. E.; Jacob, S.; Fridberger, A. Sound-Induced Length Changes in Outer Hair Cell Stereocilia. *Nat. Commun.* **2012**, *3*, 1094.
(268) Schwander, M.; Kachar, B.; Muller, U. Review Series: The Cell Biology of Hearing. *J. Cell Biol.* **2010**, *190*, 9−20.
(269) Cinar, H.; Cinar, S.; Chan, H. S.; Winter, R. Pressure-Induced Dissolution and Reentrant Formation of Condensed, Liquid-Liquid Phase-Separated Elastomeric Alpha-Elastin. *Chem. - Eur. J.* **2018**, *24*, 8286−8291.
(270) Pappas, C. G.; Mutasa, T.; Frederix, P. W. J. M.; Fleming, S.; Bai, S.; Debnath, S.; Kelly, S. M.; Gachagan, A.; Ulijn, R. V. Transient Supramolecular Reconfiguration of Peptide Nanostructures Using Ultrasound. *Mater. Horiz.* **2015**, *2*, 198−202.
(271) Conte, M. P.; Singh, N.; Sasselli, I. R.; Escuder, B.; Ulijn, R. V. Metastable Hydrogels from Aromatic Dipeptides. *Chem. Commun.* **2016**, *52*, 13889−13892.
(272) Kaur, H.; Jain, R.; Roy, S. Pathway-Dependent Preferential Selection and Amplification of Variable Self-Assembled Peptide Nanostructures and Their Biological Activities. *ACS Appl. Mater. Interfaces* **2020**, *12*, 52445−52456.
(273) Reddy M, A.; Srivastava, A. Mechano-Responsive Gelation of Water by a Short Alanine-Derivative. *Soft Matter* **2014**, *10*, 4863−4868.
(274) García-López, V.; Chen, F.; Nilewski, L. G.; Duret, G.; Aliyan, A.; Kolomeisky, A. B.; Robinson, J. T.; Wang, G.; Pal, R.; Tour, J. M. Molecular Machines Open Cell Membranes. *Nature* **2017**, *548*, 567−572.

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

(275) Watson, M. A.; Cockroft, S. L. Man-Made Molecular Machines: Membrane Bound. *Chem. Soc. Rev.* **2016**, *45*, 6118−6129.
(276) Sorrenti, A.; Leira-Iglesias, J.; Sato, A.; Hermans, T. M. Non-Equilibrium Steady States in Supramolecular Polymerization. *Nat. Commun.* **2017**, *8*, 15899.
(277) Donau, C.; Späth, F.; Sosson, M.; Kriebisch, B. A. K.; Schnitter, F.; Tena-Solsona, M.; Kang, H.-S.; Salibi, E.; Sattler, M.; Mutschler, H.; Boekhoven, J.; et al. Active Coacervate Droplets as a Model for Membraneless Organelles and Protocells. *Nat. Commun.* **2020**, *11*, 5167.
(278) Zhang, C.; Shafi, R.; Lampel, A.; MacPherson, D.; Pappas, C. G.; Narang, V.; Wang, T.; Maldarelli, C.; Ulijn, R. V. Switchable Hydrolase Based on Reversible Formation of Supramolecular Catalytic Site Using a Self-Assembling Peptide. *Angew. Chem., Int. Ed.* **2017**, *56*, 14511−14515.
(279) Marcus, R. A.; Sutin, N. Electron Transfers in Chemistry and Biology. *Biochim. Biophys. Acta, Rev. Bioenerg.* **1985**, *811*, 265−322.
(280) Panda, S. S.; Katz, H. E.; Tovar, J. D. Solid-State Electrical Applications of Protein and Peptide Based Nanomaterials. *Chem. Soc. Rev.* **2018**, *47*, 3640−3658.
(281) Amit, M.; Yuran, S.; Gazit, E.; Reches, M.; Ashkenasy, N. Tailor-Made Functional Peptide Self-Assembling Nanostructures. *Adv. Mater.* **2018**, *30*, 1707083.
(282) Tao, K.; Makam, P.; Aizen, R.; Gazit, E. Self-Assembling Peptide Semiconductors. *Science* **2017**, *358*, No. eaam9756.
(283) Ing, N. L.; El-Naggar, M. Y.; Hochbaum, A. I. Going the Distance: Long-Range Conductivity in Protein and Peptide Bioelectronic Materials. *J. Phys. Chem. B* **2018**, *122*, 10403−10423.
(284) Gray, H. B.; Winkler, J. R. Electron Transfer in Proteins. *Annu. Rev. Biochem.* **1996**, *65*, 537−561.
(285) Kumar, M.; Venkata Rao, K.; George, S. J. Supramolecular Charge Transfer Nanostructures. *Phys. Chem. Chem. Phys.* **2014**, *16*, 1300−1313.
(286) Reddy, S. M. M.; Raßlenberg, E.; Sloan-Dennison, S.; Hesketh, T.; Silberbush, O.; Tuttle, T.; Smith, E.; Graham, D.; Faulds, K.; Ulijn, R. V.; et al. Proton-Conductive Melanin-Like Fibers through Enzymatic Oxidation of a Self-Assembling Peptide. *Adv. Mater.* **2020**, *32*, 2003511.
(287) Tao, K.; Fan, Z.; Sun, L.; Makam, P.; Tian, Z.; Ruegsegger, M.; Shaham-Niv, S.; Hansford, D.; Aizen, R.; Pan, Z.; et al. Quantum Confined Peptide Assemblies with Tunable Visible to near-Infrared Spectral Range. *Nat. Commun.* **2018**, *9*, 3217.
(288) Sun, B.; Tao, K.; Jia, Y.; Yan, X.; Zou, Q.; Gazit, E.; Li, J. Photoactive Properties of Supramolecular Assembled Short Peptides. *Chem. Soc. Rev.* **2019**, *48*, 4387−4400.
(289) Amdursky, N.; Molotskii, M.; Aronov, D.; Adler-Abramovich, L.; Gazit, E.; Rosenman, G. Blue Luminescence Based on Quantum Confinement at Peptide Nanotubes. *Nano Lett.* **2009**, *9*, 3111−3115.
(290) Amdursky, N. Enhanced Solid-State Electron Transport Via Tryptophan Containing Peptide Networks. *Phys. Chem. Chem. Phys.* **2013**, *15*, 13479−13482.
(291) Sepunaru, L.; Refaely-Abramson, S.; Lovrinčić, R.; Gavrilov, Y.; Agrawal, P.; Levy, Y.; Kronik, L.; Pecht, I.; Sheves, M.; Cahen, D. Electronic Transport Via Homopeptides: The Role of Side Chains and Secondary Structure. *J. Am. Chem. Soc.* **2015**, *137*, 9617−9626.
(292) Guo, C.; Yu, X.; Refaely-Abramson, S.; Sepunaru, L.; Bendikov, T.; Pecht, I.; Kronik, L.; Vilan, A.; Sheves, M.; Cahen, D. Tuning Electronic Transport Via Hepta-Alanine Peptides Junction by Tryptophan Doping. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113*, 10785.
(293) Adler-Abramovich, L.; Aronov, D.; Beker, P.; Yevnin, M.; Stempler, S.; Buzhansky, L.; Rosenman, G.; Gazit, E. Self-Assembled Arrays of Peptide Nanotubes by Vapour Deposition. *Nat. Nanotechnol.* **2009**, *4*, 849−854.
(294) Lee, J. S.; Yoon, I.; Kim, J.; Ihee, H.; Kim, B.; Park, C. B. Self-Assembly of Semiconducting Photoluminescent Peptide Nanowires in the Vapor Phase. *Angew. Chem., Int. Ed.* **2011**, *50*, 1164−1167.
(295) Amit, M.; Cheng, G.; Hamley, I. W.; Ashkenasy, N. Conductance of Amyloid B Based Peptide Filaments: Structure-Function Relations. *Soft Matter* **2012**, *8*, 8690−8696.
(296) Ghadiri, M. R.; Granja, J. R.; Milligan, R. A.; McRee, D. E.; Khazanovich, N. Self-Assembling Organic Nanotubes Based on a Cyclic Peptide Architecture. *Nature* **1993**, *366*, 324−327.
(297) Lerner Yardeni, J.; Amit, M.; Ashkenasy, G.; Ashkenasy, N. Sequence Dependent Proton Conduction in Self-Assembled Peptide Nanostructures. *Nanoscale* **2016**, *8*, 2358−2366.
(298) Amit, M.; Appel, S.; Cohen, R.; Cheng, G.; Hamley, I. W.; Ashkenasy, N. Hybrid Proton and Electron Transport in Peptide Fibrils. *Adv. Funct. Mater.* **2014**, *24*, 5873−5880.
(299) Silberbush, O.; Amit, M.; Roy, S.; Ashkenasy, N. Significant Enhancement of Proton Transport in Bioinspired Peptide Fibrils by Single Acidic or Basic Amino Acid Mutation. *Adv. Funct. Mater.* **2017**, *27*, 1604624.
(300) Zimmer, M. Green Fluorescent Protein (Gfp): Applications, Structure, and Related Photophysical Behavior. *Chem. Rev.* **2002**, *102*, 759−782.
(301) Kumar, M.; Son, J.; Huang, R. H.; Sementa, D.; Lee, M.; O'Brien, S.; Ulijn, R. V. In Situ, Noncovalent Labeling and Stimulated Emission Depletion-Based Super-Resolution Imaging of Supramolecular Peptide Nanostructures. *ACS Nano* **2020**, *14*, 15056−15063.
(302) Shaham-Niv, S.; Arnon, Z. A.; Sade, D.; Lichtenstein, A.; Shirshin, E. A.; Kolusheva, S.; Gazit, E. Intrinsic Fluorescence of Metabolite Amyloids Allows Label-Free Monitoring of Their Formation and Dynamics in Live Cells. *Angew. Chem., Int. Ed.* **2018**, *57*, 12444−12447.
(303) Juszczak, L. J.; Eisenberg, A. S. The Color of Cation-Π Interactions: Subtleties of Amine-Tryptophan Interaction Energetics Allow for Radical-Like Visible Absorbance and Fluorescence. *J. Am. Chem. Soc.* **2017**, *139*, 8302−8311.
(304) Manchineella, S.; Govindaraju, T. Molecular Self-Assembly of Cyclic Dipeptide Derivatives and Their Applications. *ChemPlusChem* **2017**, *82*, 88−106.
(305) Chen, Y.; Orr, A. A.; Tao, K.; Wang, Z.; Ruggiero, A.; Shimon, L. J. W.; Schnaider, L.; Goodall, A.; Rencus-Lazar, S.; Gilead, S.; et al. High-Efficiency Fluorescence through Bioinspired Supramolecular Self-Assembly. *ACS Nano* **2020**, *14*, 2798−2807.
(306) Tao, K.; Hu, W.; Xue, B.; Chovan, D.; Brown, N.; Shimon, L. J. W.; Maraba, O.; Cao, Y.; Tofail, S. A. M.; Thompson, D.; et al. Bioinspired Stable and Photoluminescent Assemblies for Power Generation. *Adv. Mater.* **2019**, *31*, 1807481.
(307) Bystranowska, D.; Siejda, B.; Ożyhar, A.; Kochman, M. The Dityrosine Cross-Link as an Intrinsic Donor for Assembling Fret Pairs in the Study of Protein Structure. *Biophys. Chem.* **2012**, *170*, 1−8.
(308) Min, K.-I.; Kim, D.-H.; Lee, H.-J.; Lin, L.; Kim, D.-P. Direct Synthesis of a Covalently Self-Assembled Peptide Nanogel from a Tyrosine-Rich Peptide Monomer and Its Biomineralized Hybrids. *Angew. Chem., Int. Ed.* **2018**, *57*, 5630−5634.
(309) Whittaker, J. L.; Choudhury, N. R.; Dutta, N. K.; Zannettino, A. Facile and Rapid Ruthenium Mediated Photo-Crosslinking of Bombyx Mori Silk Fibroin. *J. Mater. Chem. B* **2014**, *2*, 6259−6270.
(310) Lampel, A.; McPhee, S. A.; Kassem, S.; Sementa, D.; Massarano, T.; Aramini, J. M.; He, Y.; Ulijn, R. V. Melanin-Inspired Chromophoric Microparticles Composed of Polymeric Peptide Pigments. *Angew. Chem., Int. Ed.* **2021**, *60*, 7564−7569.
(311) Kong, J.; Zhang, J.; Wang, Y.; Qi, W.; Rao, H.; Hu, L.; Su, R.; He, Z. Bioinspired Ph-Sensitive Fluorescent Peptidyl Nanoparticles for Cell Imaging. *ACS Appl. Mater. Interfaces* **2020**, *12*, 4212−4220.
(312) Ivnitski, D.; Amit, M.; Silberbush, O.; Atsmon-Raz, Y.; Nanda, J.; Cohen-Luria, R.; Miller, Y.; Ashkenasy, G.; Ashkenasy, N. The Strong Influence of Structure Polymorphism on the Conductivity of Peptide Fibrils. *Angew. Chem., Int. Ed.* **2016**, *55*, 9988−9992.
(313) Ardoña, H. A. M.; Draper, E. R.; Citossi, F.; Wallace, M.; Serpell, L. C.; Adams, D. J.; Tovar, J. D. Kinetically Controlled Coassembly of Multichromophoric Peptide Hydrogelators and the Impacts on Energy Transport. *J. Am. Chem. Soc.* **2017**, *139*, 8685−8692.
(314) Moulin, E.; Cid, J.-J.; Giuseppone, N. Advances in Supramolecular Electronics - from Randomly Self-Assembled

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

Nanostructures to Addressable Self-Organized Interconnects. *Adv. Mater.* **2013**, *25*, 477−487.
(315) Schenning, A. P. H. J.; Meijer, E. W. Supramolecular Electronics; Nanowires from Self-Assembled Π-Conjugated Systems. *Chem. Commun.* **2005**, *26*, 3245−3258.
(316) Diegelmann, S. R.; Gorham, J. M.; Tovar, J. D. One-Dimensional Optoelectronic Nanostructures Derived from the Aqueous Self-Assembly of Π-Conjugated Oligopeptides. *J. Am. Chem. Soc.* **2008**, *130*, 13840−13841.
(317) Wall, B. D.; Diegelmann, S. R.; Zhang, S.; Dawidczyk, T. J.; Wilson, W. L.; Katz, H. E.; Mao, H.-Q.; Tovar, J. D. Aligned Macroscopic Domains of Optoelectronic Nanostructures Prepared Via Shear-Flow Assembly of Peptide Hydrogels. *Adv. Mater.* **2011**, *23*, 5009−5014.
(318) Lee, T.; Panda, S. S.; Tovar, J. D.; Katz, H. E. Unusually Conductive Organic-Inorganic Hybrid Nanostructures Derived from Bio-Inspired Mineralization of Peptide/Pi-Electron Assemblies. *ACS Nano* **2020**, *14*, 1846−1855.
(319) Shmilovich, K.; Mansbach, R. A.; Sidky, H.; Dunne, O. E.; Panda, S. S.; Tovar, J. D.; Ferguson, A. L. Discovery of Self-Assembling Π-Conjugated Peptides by Active Learning-Directed Coarse-Grained Molecular Simulation. *J. Phys. Chem. B* **2020**, *124*, 3873−3891.
(320) Xu, H.; Das, A. K.; Horie, M.; Shaik, M. S.; Smith, A. M.; Luo, Y.; Lu, X.; Collins, R.; Liem, S. Y.; Song, A.; et al. An Investigation of the Conductivity of Peptide Nanotube Networks Prepared by Enzyme-Triggered Self-Assembly. *Nanoscale* **2010**, *2*, 960−966.
(321) Khalily, M. A.; Bakan, G.; Kucukoz, B.; Topal, A. E.; Karatay, A.; Yaglioglu, H. G.; Dana, A.; Guler, M. O. Fabrication of Supramolecular N/P-Nanowires Via Coassembly of Oppositely Charged Peptide-Chromophore Systems in Aqueous Media. *ACS Nano* **2017**, *11*, 6881−6892.
(322) Nalluri, S. K. M.; Shivarova, N.; Kanibolotsky, A. L.; Zelzer, M.; Gupta, S.; Frederix, P. W. J. M.; Skabara, P. J.; Gleskova, H.; Ulijn, R. V. Conducting Nanofibers and Organogels Derived from the Self-Assembly of Tetrathiafulvalene-Appended Dipeptides. *Langmuir* **2014**, *30*, 12429−12437.
(323) Kumar, M.; Ushie, O. A.; George, S. J. Supramolecular Clippers for Controlling Photophysical Processes through Preorganized Chromophores. *Chem. - Eur. J.* **2014**, *20*, 5141−5148.
(324) Roy, S.; Kumar Maiti, D.; Panigrahi, S.; Basak, D.; Banerjee, A. A New Hydrogel from an Amino Acid-Based Perylene Bisimide and Its Semiconducting, Photo-Switching Behaviour. *RSC Adv.* **2012**, *2*, 11053−11060.
(325) Draper, E. R.; Walsh, J. J.; McDonald, T. O.; Zwijnenburg, M. A.; Cameron, P. J.; Cowan, A. J.; Adams, D. J. Air-Stable Photoconductive Films Formed from Perylene Bisimide Gelators. *J. Mater. Chem. C* **2014**, *2*, 5570−5575.
(326) Draper, E. R.; Dietrich, B.; Adams, D. J. Self-Assembly, Self-Sorting, and Electronic Properties of a Diketopyrrolopyrrole Hydrogelator. *Chem. Commun.* **2017**, *53*, 1864−1867.
(327) Kim, J. H.; Lee, M.; Lee, J. S.; Park, C. B. Self-Assembled Light-Harvesting Peptide Nanotubes for Mimicking Natural Photosynthesis. *Angew. Chem., Int. Ed.* **2012**, *51*, 517−520.
(328) Liu, K.; Xing, R.; Chen, C.; Shen, G.; Yan, L.; Zou, Q.; Ma, G.; Möhwald, H.; Yan, X. Peptide-Induced Hierarchical Long-Range Order and Photocatalytic Activity of Porphyrin Assemblies. *Angew. Chem., Int. Ed.* **2014**, *54*, 500−505.
(329) Chen, L.; Revel, S.; Morris, K.; Adams, D. J. Energy Transfer in Self-Assembled Dipeptide Hydrogels. *Chem. Commun.* **2010**, *46*, 4267−4269.
(330) Albery, W. J.; Knowles, J. R. Evolution of Enzyme Function and Development of Catalytic Efficiency. *Biochemistry* **1976**, *15*, 5631−5640.
(331) Knowles, J. R. Enzyme Catalysis - Not Different, Just Better. *Nature* **1991**, *350*, 121−124.
(332) Behmoaras, T.; Toulmé, J.-J.; Hélène, C. A Tryptophan-Containing Peptide Recognizes and Cleaves DNA at Apurinic Sites. *Nature* **1981**, *292*, 858−858.
(333) Li, Y.; Zhao, Y.; Hatfield, S.; Wan, R.; Zhu, Q.; Li, X.; McMills, M.; Ma, Y.; Li, J.; Brown, K. L.; et al. Dipeptide Seryl-Histidine and Related Oligopeptides Cleave DNA, Protein, and a Carboxyl Ester. *Bioorg. Med. Chem.* **2000**, *8*, 2675−2680.
(334) Adamala, K.; Szostak, J. W. Competition between Model Protocells Driven by an Encapsulated Catalyst. *Nat. Chem.* **2013**, *5*, 495−501.
(335) MacDonald, M. J.; Lavis, L. D.; Hilvert, D.; Gellman, S. H. Evaluation of the Ser-His Dipeptide, a Putative Catalyst of Amide and Ester Hydrolysis. *Org. Lett.* **2016**, *18*, 3518−3521.
(336) Metrano, A. J.; Miller, S. J. Peptide-Based Catalysts Reach the Outer Sphere through Remote Desymmetrization and Atroposelectivity. *Acc. Chem. Res.* **2019**, *52*, 199−215.
(337) Duncan, K. L.; Ulijn, R. V. Short Peptides in Minimalistic Biocatalyst Design. *Biocatalysis* **2015**, *1*, 67−81.
(338) Zozulia, O.; Dolan, M. A.; Korendovych, I. V. Catalytic Peptide Assemblies. *Chem. Soc. Rev.* **2018**, *47*, 3621−3639.
(339) Guler, M. O.; Stupp, S. I. A Self-Assembled Nanofiber Catalyst for Ester Hydrolysis. *J. Am. Chem. Soc.* **2007**, *129*, 12082−12083.
(340) Solomon, L. A.; Kronenberg, J. B.; Fry, H. C. Control of Heme Coordination and Catalytic Activity by Conformational Changes in Peptide-Amphiphile Assemblies. *J. Am. Chem. Soc.* **2017**, *139*, 8497−8507.
(341) Gulseren, G.; Khalily, M. A.; Tekinay, A. B.; Guler, M. O. Catalytic Supramolecular Self-Assembled Peptide Nanostructures for Ester Hydrolysis. *J. Mater. Chem. B* **2016**, *4*, 4605−4611.
(342) Omosun, T. O.; Hsieh, M.-C.; Childers, W. S.; Das, D.; Mehta, A. K.; Anthony, N. R.; Pan, T.; Grover, M. A.; Berland, K. M.; Lynn, D. G. Catalytic Diversity in Self-Propagating Peptide Assemblies. *Nat. Chem.* **2017**, *9*, 805−805.
(343) Rufo, C. M.; Moroz, Y. S.; Moroz, O. V.; Stöhr, J.; Smith, T. A.; Hu, X.; DeGrado, W. F.; Korendovych, I. V. Short Peptides Self-Assemble to Produce Catalytic Amyloids. *Nat. Chem.* **2014**, *6*, 303−309.
(344) Lengyel, Z.; Rufo, C. M.; Moroz, Y. S.; Makhlynets, O. V.; Korendovych, I. V. Copper-Containing Catalytic Amyloids Promote Phosphoester Hydrolysis and Tandem Reactions. *ACS Catal.* **2018**, *8*, 59−62.
(345) Biswas, S.; Kumar, M.; Levine, A. M.; Jimenez, I.; Ulijn, R. V.; Braunschweig, A. B. Visible-Light Photooxidation in Water by 1o2-Generating Supramolecular Hydrogels. *Chem. Sci.* **2020**, *11*, 4239−4245.
(346) Singh, N.; Zhang, K.; Angulo-Pachón, C. A.; Mendes, E.; van Esch, J. H.; Escuder, B. Tandem Reactions in Self-Sorted Catalytic Molecular Hydrogels. *Chem. Sci.* **2016**, *7*, 5568−5572.
(347) Makam, P.; Yamijala, S. S. R. K. C.; Tao, K.; Shimon, L. J. W.; Eisenberg, D. S.; Sawaya, M. R.; Wong, B. M.; Gazit, E. Non-Proteinaceous Hydrolase Comprised of a Phenylalanine Metallo-Supramolecular Amyloid-Like Structure. *Nat. Catal.* **2019**, *2*, 977−985.
(348) Gayen, K.; Basu, K.; Bairagi, D.; Castelletto, V.; Hamley, I. W.; Banerjee, A. Amino-Acid-Based Metallo-Hydrogel That Acts Like an Esterase. *ACS Appl. Bio Mater.* **2018**, *1*, 1717−1724.
(349) Dolan, M. A.; Basa, P. N.; Zozulia, O.; Lengyel, Z.; Lebl, R.; Kohn, E. M.; Bhattacharya, S.; Korendovych, I. V. Catalytic Nanoassemblies Formed by Short Peptides Promote Highly Enantioselective Transfer Hydrogenation. *ACS Nano* **2019**, *13*, 9292−9297.
(350) Tena-Solsona, M.; Nanda, J.; Díaz-Oltra, S.; Chotera, A.; Ashkenasy, G.; Escuder, B. Emergent Catalytic Behavior of Self-Assembled Low Molecular Weight Peptide-Based Aggregates and Hydrogels. *Chem. - Eur. J.* **2016**, *22*, 6687−6694.
(351) Fischer, E. Einfluss Der Configuration Auf Die Wirkung Der Enzyme. *Ber. Dtsch. Chem. Ges.* **1894**, *27*, 2985−2993.
(352) Koshland, D. E. Application of a Theory of Enzyme Specificity to Protein Synthesis. *Proc. Natl. Acad. Sci. U. S. A.* **1958**, *44*, 98−104.
(353) Zhao, Y.; Lei, B.; Wang, M.; Wu, S.; Qi, W.; Su, R.; He, Z. A Supramolecular Approach to Construct a Hydrolase Mimic with

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914

Photo-Switchable Catalytic Activity. *J. Mater. Chem. B* **2018**, *6*, 2444−2449.
(354) Maeda, Y.; Javid, N.; Duncan, K.; Birchall, L.; Gibson, K. F.; Cannon, D.; Kanetsuki, Y.; Knapp, C.; Tuttle, T.; Ulijn, R. V.; et al. Discovery of Catalytic Phages by Biocatalytic Self-Assembly. *J. Am. Chem. Soc.* **2014**, *136*, 15893−15896.
(355) Kumar, M.; Ing, N. L.; Narang, V.; Wijerathne, N. K.; Hochbaum, A. I.; Ulijn, R. V. Amino-Acid-Encoded Biocatalytic Self-Assembly Enables the Formation of Transient Conducting Nanostructures. *Nat. Chem.* **2018**, *10*, 696−703.
(356) Levine, A. M.; Biswas, S.; Braunschweig, A. B. Photoactive Organic Material Discovery with Combinatorial Supramolecular Assembly. *Nanoscale Adv.* **2019**, *1*, 3858−3869.
(357) Whitesides, G. M.; Ismagilov, R. F. Complexity in Chemistry. *Science* **1999**, *284*, 89−92.
(358) Shmilovich, K.; Mansbach, R. A.; Sidky, H.; Dunne, O. E.; Panda, S. S.; Tovar, J. D.; Ferguson, A. L. Discovery of Self-Assembling Π-Conjugated Peptides by Active Learning-Directed Coarse-Grained Molecular Simulation. *J. Phys. Chem. B* **2020**, *124*, 3873−3891.
(359) Yoshida, M.; Hinkley, T.; Tsuda, S.; Abul-Haija, Y. M.; McBurney, R. T.; Kulikov, V.; Mathieson, J. S.; Galiñanes Reyes, S.; Castro, M. D.; Cronin, L. Using Evolutionary Algorithms and Machine Learning to Explore Sequence Space for The discovery of Antimicrobial Peptides. *Chem.* **2018**, *4*, 533−543.
(360) Senior, A. W.; Evans, R.; Jumper, J.; Kirkpatrick, J.; Sifre, L.; Green, T.; Qin, C.; Žídek, A.; Nelson, A. W. R.; Bridgland, A.; et al. Improved Protein Structure Prediction Using Potentials from Deep Learning. *Nature* **2020**, *577*, 706−710.

https://doi.org/10.1021/acs.chemrev.1c00089
Chem. Rev. 2021, 121, 13869−13914