Rational Design of Peptide-Based Biosupramolecular Systems

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1 INTRODUCTION

A highly attractive feature of peptides and proteins for use in what might be termed “biosupramolecular chemistry” is their ability to self-assemble in water with exquisite specificity and high affinities through noncovalent interactions. An aim of peptide design and engineering is to exploit this to create new supramolecular assemblies from the bottom up.1,2 Within this, one approach is to program simple peptide and protein building blocks (which might be referred to as “tectons”) to fold and self-associate in prescribed ways. This de novo design route contrasts with top-down approaches, which are concerned with protein engineering and the modification of already existing and even assembled natural proteins. The bottom-up approach has the potential advantage that it allows for full design and control over folding, assembly, size, and shape of the targets, and so opens up a wide range of structural space. It also tests our understanding of peptide and protein folding and assembly directly. However, at present, it has clear disadvantages over engineering natural systems, as we do not fully understand protein folding, and, therefore, the rational design of protein structure and function is in its infancy.3,4

This chapter focuses on supramolecular assemblies that are formed using a variety of de novo designed peptide-based tectons. A brief introduction to amino acids (the building blocks of peptides and proteins) is given, followed by a discussion of the basic structures that polypeptide chains of amino acids can adopt. These structures form the basis of the supramolecular assemblies that will be reviewed. The subsequent sections provide details of recent examples of repetitive, effectively “infinite,” and discrete supramolecular peptide-based assemblies, and also a discussion of their potential applications.

1.1 Amino acids

α-Amino acids are the fundamental building blocks of polypeptides. They consist of a central carbon atom (the α-carbon), which has both an amine and a carboxylic acid functionality attached. This central tetrahedral carbon additionally bears a hydrogen atom and an “R” group commonly referred to as the side chain. In natural proteins, there are 20 different common side chains (Figure 1). It is these that ultimately confer functionality onto polypeptides. The side chains can be classed according to their functional groups and are usually defined as being hydrophobic or polar, though a small number might best be referred to as “special,” as they do not fit easily into these brackets, for example, glycine, proline, and cysteine.

As the α-carbon has four different functional groups attached, α-amino acids are chiral (with the exception of...
glycine, where the side chain is hydrogen). It is the L-isomer that occurs naturally in ribosomally synthesized peptides and proteins (Figure 2a), although examples of both natural (non-ribosomally synthesized) and designed structures that contain both D- and L-amino acids are known.

In polypeptide chains, amino acids are joined together through the condensation of carboxylic acid and amine groups, leading to the formation of an amide or “peptide” bond (Figure 2b). A string of amino acids joined together in such a way is referred to as a polypeptide. Natural polypeptides are predominantly linear polymers and can range from a few (Figure 2c) to thousands of amino acids in length. All the information concerning the folding, assembly, and function of such polypeptides is usually contained within this primary sequence, which is the order of amino acids read from the amino (N-) to the carboxy (C-) terminus. The trick in rational peptide and protein design is to capture this information in short, chemically accessible (usually 30–100 amino acid) chains.

Contained within the folded tertiary structures of proteins are areas of regular local folding, known as secondary structures. The two most common secondary structures are the α-helix and the β-strand, which form the basis of most of the building blocks or tectons in peptide design.

1.2 α-Helices and β-strands

In the α-helix the main chain is wound up as a right-handed helix and the side chains point outwards from this central coil (Figure 3a). The structure is stabilized by hydrogen bonds parallel to the helix axis, formed between the carbonyl oxygen of amino acid “i” and the amide proton of residue “i + 4.” α-Helices have 3.6 residues per turn, which means that side chains that are three or four residues apart are brought together in space. This leads to one of the key design principles in the supramolecular assembly of α-helices, namely the use of patterns of hydrophobic (H) and polar (P) residues, spaced three or four residues apart, for example, (HPPHPPPP)$_n$, along the chain to make so-called amphiphilic structures that can assemble in water via association of their hydrophobic faces (Figure 3c).
β-Strands are the other common secondary structural element. Compared to the α-helix, the amino-acid residues in a β-strand are more extended, with the backbone hydrogen-bonding groups pointing orthogonal to the direction of the chain (Figure 3b) and side chains alternating between the two faces of the strand; effectively this gives the β-strand a two-residue repeat. As a result, construction of amphipathic β-strands requires alternating patterns of hydrophobic and polar amino acids, for example, \((HPPHPHP)\text{n}\).

Higher-order structures based on both α-helices and β-strands are common, with amphipathic α-helices coming together to form either helical bundles (Figure 3c) or coiled coils, in which hydrophobic faces are buried from the solvent to form hydrophobic cores, while β-strands associate via intermolecular hydrogen bonds to form β-sheets (Figure 3d), which again can be amphipathic and associate in layers.

1.3 Helical bundles, coiled coils, and β-sheets

Helical bundles and coiled coils are common examples of higher-order assemblies of α-helices. The two structures look similar in many respects: they consist of two or more α-helices that associate in either a parallel or an antiparallel fashion. A key difference is that coiled coils are based on a tight side-chain packing regime, known as “knobs-into-holes” (KIH) packing.5 The basis of this packing is that the hydrophobic residues that appear three and four residues apart on one helix act as “knobs” and pack into diamond-shaped “holes” formed by four residues from another helix (Figure 4a). This type of packing can be seen in crystal structures of coiled-coil peptides and proteins and can be identified using the program SOCKET.6 As a result of this regular, well-defined structural motif, clear sequence-to-structure relationships or rules that govern coiled-coil formation, association, and stability can be elucidated.7

Regularly repeated KIH packing leads to a repeating sequence pattern known as the heptad repeat, in which hydrophobic amino acids alternate every third and fourth residue giving rise to a \((HPPHPHP)\text{n}\) pattern. This pattern is the defining characteristic of coiled-coil sequences and is usually given the nomenclature \(abcd\text{efg}\), with the \(H\) residues falling at positions \(a\) and \(d\). As the number of residues per turn in the α-helix is 3.6 and the average spacing of the hydrophobic residues in the heptad repeat is 3.5, an amphipathic structure is formed with all the \(H\)-type amino acids on one face of the helix (Figure 4b). Two or more of these helices will then come together via their hydrophobic faces to form a coiled coil, driven by the hydrophobic effect in water. However, as 3.5 and 3.6 do not match precisely, the helices wind around each other ensuring maximal contact between the hydrophobic residues, resulting in a supercoil with a left-handed twist (Figure 4c and d). Note that variations on the heptad pattern are known and these lead to different helical supercoiling.8

The hydrophobic interactions, while being the driving force for coiled-coil formation, are not the only interactions that specify and stabilize these structures. Side chains found at the \(e\) and \(g\) positions of neighboring helices are also close in space and often have complementary charges, leading to electrostatic interactions across the hydrophobic interface and further stabilization and specification of the structures.

Extensive studies of coiled coils have elucidated sequence-to-structure relationships that direct oligomerization, orientation, and partnering of the helices found in Nature. These can be used with relative confidence in new designs. Generally, bulky and aromatic residues are excluded from the \(a\) and \(d\) sites, and glycine and proline tend not to be found or used in coiled coils as they break α-helices. More specifically, a combination of isoleucine and asparagine at \(a\) positions and leucine at \(d\) defines dimeric coiled coils; designs with isoleucine at both \(a\) and \(d\) positions form trimers; and tetramers are directed by leucine at \(a\) positions and isoleucine at \(d\).9 The \(e\) and \(g\) positions show preferences for certain charged and polar residues: for example, lysine, glutamic acid, and arginine can form electrostatic interactions across the helical interface, and in designed coiled-coil sequences these interactions can be exploited to direct the formation of coiled coils with a specific orientation (parallel or antiparallel) and association (homomeric or heteromeric). The remaining \(b\), \(c\), and \(f\) positions are less restricted as to which amino acids can be included and in design can often be

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substituted to introduce function. For design purposes, it is usually sensible to include residues with a high α-helical propensity, such as alanine, leucine, lysine, and glutamic acid, but if, for example, the coiled coils need to be functionalized, residues such as cysteine can be included.

β-Strands are the other common secondary structural element. While there are fewer rules for β-strand formation, certain sequence-to-structure relationships and fundamental principles are clear. For instance, it is well established that, for natural proteins, amino acids with β-branched (e.g., valine, isoleucine, and threonine) or aromatic (particularly, tyrosine, and phenylalanine) side chains are among those most frequently observed. In addition, though this is not necessarily the case in natural proteins,12 for design purposes alternating patterns of hydrophobic and polar residues \((HP)_n\) can produce self-assembling amphipathic strands. Such sequences may then associate side-by-side through interstrand hydrogen bonds to form amphipathic β-sheets, in which the different side-chain types protrude above and below the plane (Figure 3b and d). These sheets may associate further to form discrete β-sandwiches or indefinite β-tapes. The β-strands can combine in parallel or antiparallel fashions, or form mixed sheets; although, possibly due to better hydrogen bonding or simply that consecutive strands tend to be joined by short loops, antiparallel sheets are favored in proteins. Side chain–side chain pairings across both antiparallel and parallel β-sheets also show preferences, which have been successfully employed in peptide design, though full implementation of these in protein-structure design and prediction remains a challenge. Finally, it should be noted that most β-sheets are not flat, as the strands twist relative to one another, which has implications for understanding fundamental interactions in β-sheets and exploiting them in design.

To date, most β-structured designs have focused on antiparallel β-assemblies of either two-stranded β-hairpins, where contrary to the findings in natural proteins, tryptophan residues are found to be stabilizing and so are frequently used, or indefinite supramolecular assemblies of β-tapes and amyloid-like systems. Designed β-sheet assemblies have recently been the focus of much research, as β-sheets and the structures formed from their association readily lead to amyloid-like fibrous and gelling systems, which in Nature are implicated in many disease states such as Alzheimer’s and Parkinson’s. For these reasons, there is great interest in exploring their formation and behavior and also exploiting their potential as materials for use in bionanotechnology.

1.4 Collagen

Collagen is a naturally occurring protein found in the connective tissues of mammals, though examples are now clear in bacteria and viruses. It has a triple-helical structure, but in contrast to α-helical coiled-coil structures the individual collagen polypeptides form polyproline II (PPII) type helices, which are left-handed and associate via interchain hydrogen bonds to form right-handed supercoils (Figure 5). The sequences of fibrous collagens are defined by an \((X–Y-Gly)_n\) repeat. The glycine at every third residue is essential to allow tight packing of the three chains in the triple helix. Proline and hydroxyproline are prevalent at the X and Y position, respectively, and most of the recent \textit{de novo} designed collagen peptides use one (or both) of these residues at these positions. The superhelix can be either homotypic, meaning all three chains are identical (known as AAA homotrimers), or heterotypic. There are two types of heterotypic collagen, one where all three chains are different (an ABC heterotrimer), or a structure where two helices are the same and the remaining helix has a different primary sequence (an AAB heterotrimer). Examples of all three types of designer collagen will be discussed later in this chapter.

1.5 Peptide amphiphiles

Peptide amphiphiles (PAs) are another example of alternative tectons that can be used in the design of supramolecular systems. These non-natural, hybrid structures comprise a peptide, which is usually polar, and a nonpolar aliphatic region. They adopt structures similar to those formed
by lipids, which have polar head groups and non-polar fatty acid tails: in both cases, the hydrophobic tails pack together sequestered away from solvent, while the polar groups are solvent-exposed, leading to micelle-like structures. For the PAs, different functionalities can be conjugated via the peptide moieties. For example, long alkyl carbon chains can be attached, as can synthetic polymers such as polyethyleneglycol (PEG) or N-(2-hydroxypropyl)methacrylamide (HMPA). Similarly, amphiphatic peptides with suitable arrangements of hydrophobic and polar side chains have been designed to assemble in a similar fashion.22,23 The design of such amphiphiles has been the focus of several recent reviews; these cover in detail the different types of amphiphiles that can be synthesized and the resulting systems that can be programmed.24,25

2 REPETITIVE EXTENDED SUPRAMOLECULAR SYSTEMS

With the structural basis of the commonly used building blocks for the de novo design of biosupramolecular systems having been covered, specific examples of assemblies that can be formed are discussed. This section reviews types of repetitive and extended (effectively infinite) structures. These tend to contain many thousands of monomeric tectons self-assembled in one direction, hence the infinite (or at least indefinite) and repetitive description. Examples of these kinds of systems are predominantly fibrous assemblies and fibers capable of gelling. Many groups have made fibrous structures from various straightforward peptidic tectons over the past 15 years, resulting in a variety of systems of increasing sophistication. Owing to the large body of literature, including many review articles,26–28 this section is brief and covers archetypal examples of extended fibrous structures.

2.1 Fibrous systems with β-structured building blocks

The earliest examples of designed fibrous systems are based on β-structured tectons. The majority of these systems tend to adopt amyloid-like structures: that is, extended arrays of hydrogen-bonded β-strand forming sheets that pack into fibrils and then further assemble into fibers. In many cases, these fibers appear to be predisposed to form gels.

Some of the earliest work with β-structured tectons is by Zhang and colleagues. They examine a variety of simple repeating sequences and their fiber-forming abilities. The first peptide, EAK16, is based on a natural sequence found in the yeast protein zuotin.29 It forms stable membranes at high salt concentrations. Scanning electron microscopy (SEM) reveals interwoven filaments within the membranes, akin to amyloid fibrils. The group proposes that the fibers are formed by β-structured peptides interacting via both hydrogen-bonding and ionic interactions to give arrays of staggered peptides that twist around each other to form filaments which then associate and organize into membranes. More recent work demonstrates that EAK16 and a related de novo designed peptide RAD16 support the growth of mammalian cells.30

A second example of gels based on designed amyloid-like structures is presented by Aggeli et al.31 The group had previously studied natural β-structured peptides that gel in aqueous solution, and from these studies concluded that, along with the formation of intermolecular hydrogen bonds, attractive forces between side chains of adjacent strands, lateral recognition of the strands, and also the ability of solvent to adhere to the strands (to aid solubility) are all important criteria for gel formation. On the basis of these criteria, a design for an 11-residue β-strand-forming peptide with the sequence QQRFQWOQFEQ was developed. In aqueous solution, this assembles to form a gel consisting of “β-sheet polymer tapes.” The responses of the gel to changes in pH and shear forces have been studied. The group has expanded on this work and presented alternative designs that form similar “tape-like” structures as well as designs shown to form ribbons (essentially “double tapes” that form at higher peptide concentrations) and fibers.32 The group has also developed a useful, and potentially more broadly applicable, definitive statistical model for peptide assembly, which demonstrates the self-limiting nature of such higher order structures due to a competition between the free energy of attraction of the peptides for the assemblies, and an energy penalty associated with the elastic deformation of the peptides as they are incorporated into fibrils.

The Schneider and Pochan groups have outlined several different controllable designs for fibrous hydrogels based on β-hairpin tectons. One example exhibits thermally controlled and pH-dependent behavior33: MAX1, a peptide incorporating nine lysine residues is unfolded at pH 9 at room temperature, but adopts the β-hairpin conformation, self-assembles, and hydrogelates as the temperature is increased. The group has demonstrated that replacing lysine residues with glutamic acid (and thus reducing the net positive charge of the peptide) results in hydrogelation at lower pH values for a given temperature. This is because less thermal energy is required to overcome the repulsive electrostatic interactions that result from the lysine residues being on one face of the strand. An alternative heteromeric design from the group also exhibits
a temperature-induced transition from random coil to β-structure. At low temperatures, one of the peptides of the system, (strand-swapping peptide) SSP-1, is unstructured, but as the temperature is raised it adopts a β-hairpin conformation in which a β-strand extends from a β-turn region. This β-strand is designed to domain-swap with a second, similar β-strand domain (SSP-2), leading to the production of a strand-swapped dimer. This dimer then self-assembles into fibrils that gel (Figure 6). The group has other designs of β-structured hydrogels, many of which have been explored with regard to their controllability and materials properties. They have also demonstrated the potential for applications such as cell growth and delivery, as well as showing that one design has inherent antibacterial activity.

It is not just β-structured amyloid-like designs that are capable of forming fibrous systems. A notable example, and one of the first to demonstrate fiber formation, is Ghadiri’s extended nanotubes made from peptides containing alternating d- and l-amino acids, motivated by the antibiotic Gramicidin S. The design consists of a cyclic octapeptide with the sequence (D-AE2-AN)2. The rationale is that the rings formed should be near flat and stack in an antiparallel fashion via β-sheet-like intermolecular hydrogen bonds. In this conformation, the side chains all lie on the outside of the rings, due to the alternating d,l-configuration, and so give hollow tubes. Indeed, the peptides spontaneously assemble at acidic pH, to give fibrous nanotubes as viewed by transmission electron microscopy (TEM) (Figure 7), and the underlying β-sheet structure is confirmed by Fourier-transform infrared (FT-IR) spectroscopy. The designs have since been modified to produce further cylindrical assemblies, as well as antibacterial agents.

Related to the above work on β-structured systems, Gazit, Ulijn, and others have produced β-tubes, fibers, and

![Figure 6](image)

**Figure 6** Assembly of SSP-1 and SSP-2 peptides. (a) The proposed design and assembly mechanism detailing how fibril formation is achieved; (b) Transmission electron microscopy (TEM) image of the resulting fibrils. (Reproduced from Ref. 34. © American Chemical Society, 2008.)
2.2 Extended systems based on coiled coils

There are two broad kinds of extended systems based on coiled-coil frameworks: those designed using homomeric constructs, and those that use heteromeric designs; the latter carry advantages for control over assembly of the systems.

2.2.1 Homomeric fibrous systems

The first example of a fiber-forming system constructed from coiled-coil building blocks is from Kojima et al.\textsuperscript{47} The design $\alpha_3$ consists of a three-heptad peptide with the repeating sequence LETLAKA at the $abcdefg$ sites of the coiled-coil heptad repeat, respectively. Previous analysis of the peptide by sedimentation equilibrium analytical ultracentrifugation (AUC) showed that it forms tetrameric helical bundles. However, changing the buffer renders larger assemblies, which, when probed by TEM, are found to be fibrils with widths of 5–10 nm and lengths of several micrometers; the precise morphology alters with salt (Figure 8). The mechanism of fiber formation is not discussed in detail, but it is proposed that the hydrophobic effect provides the main driving force for fiber assembly. A more-recent paper describes the effect of reversing the sequence.\textsuperscript{48} This later design is more helical, has increased stability, and forms longer fibers, though with the same width as those produced by $\alpha_3$. In this case, increased salt produces irregular aggregates.

In 2001, Potekhin et al.\textsuperscript{49} presented an $\alpha$-helical fiber-forming peptide ($\alpha$-FFP) with the sequence QLAREL at $cdefga$, followed by (QLAREL)$^4$ at $bcdefga$. The peptide is designed to assemble into pentamers, with the rationale that fiber formation results from slippage of this repetitive sequence to give staggered ends that promote longitudinal assembly (Figure 9a).
Using a similar, but more sophisticated and rational approach, where staggered assembly is promoted by specific interactions between designed peptide sequences, Conticello et al. describe a construct intended to form fibrils from dimeric coiled-coil tectons.\(^2\) To achieve this, they introduce hydrogen-bonding polar residues into the hydrophobic core, and place charged residues to promote the desired staggered assembly and prevent the formation of the more usual, naturally observed, blunt-ended dimers in which the ends of the helices are fully in register, or flush, with respect to each other. Circular dichroism (CD) spectroscopy confirms that the peptides do indeed adopt an \(\alpha\)-helical structure, and TEM images reveal long fibers. The group has built on this initial design and made other fiber-forming systems, which, for example, are pH responsive.\(^3\)

A subtly different mode of fiber formation is proposed by Fairman et al.\(^4\) The designed peptide, \(\text{CpA}\), is based on the GCN4 leucine-zipper sequence, but has two alanine residues inserted between two identical two-heptad coiled-coil sequences. This insert results in the hydrophobic seam switching from one “face” of the helix to the other halfway through the sequence (Figure 10a). This positional shift drives a contiguous, longitudinal (as opposed to blunt-ended) coiled-coil assembly. The free ends generated allow additional peptides to add to the dimeric construct leading to fiber formation (Figure 10b). As each coiled-coil half is short, at only two heptads long, high salt concentration is needed to induce the helical structure of the peptides and trigger fiber formation. Ammonium sulfate is found to be the most effective salt for inducing helical structure and for forming longer and more stable fibers (Figure 10c).

Designed helix-turn-helix peptides that form fibrils have also been described.\(^5\) Two 18-residue \(\alpha\)-helical segments are joined by a variety of natural turn sequences from human apolipoprotein A–I to give a series of four peptides. All of these are \(\alpha\)-helical and three of the designs (those incorporating a proline residue in the turn region) demonstrate fibril-forming ability. One design has been
investigated in detail, to reveal that the α-helical axes are perpendicular to the long axis of the fibril; this is the first example of fibrils in which the α-helical tectons lie relative to the fiber axis in this way (Figure 11). Clearly, there is added complexity in moving to helix-turn-helix systems—for instance, turns are notoriously difficult to engineer in peptides and proteins—which makes such multi-helix-based design (or indeed any multisegondary structure system) more challenging. Such challenges should be addressed, however, if we are to succeed in engineering more ambitious, complex, and ultimately useful systems.56

Most recently, Hartgerink and coworkers have produced fibers from short, blunt-ended coiled coils.57 They use several subtly different three-heptad sequences. Assembly is concentration-dependent: at high concentrations, bundles of fibers are formed, leading to gelation (Figure 12a). In addition, fibril thickness can be controlled by varying amino acids at the b,c, and f positions of the heptad repeat; when positively charged residues are incorporated at these positions, fibrils with narrow diameters are observed, but in their absence fibers with much larger diameters form. A general mechanism is proposed for fibril formation, in which blunt-ended coiled-coil dimers spontaneously form; these then associate to offset pairs of coiled-coil dimers above a critical minimum concentration; and this association serves to nucleate fiber formation (Figure 12b).

### 2.2.2 Heteromeric fibrous systems

The next step in terms of complexity within coiled coil-based systems is to design two-component (or higher) fibrous systems. This has the added benefit of introducing control into self-assembly: assembly ensues only when all the necessary components are mixed.

Arguably, the best studied example of a two-component heteromeric coiled-coil fiber-forming system comes from our own lab. The self-assembling fibers (SAFs) consist of two different complementary sequences that are designed to assemble into an offset parallel coiled-coil dimer.58 This leads to a sticky-ended tecton for fiber assembly. This design is realized with two four-heptad peptides with sequences as follows:

**SAF-p1**: KIPPKLKP**IPPLKEIPPLEPENPLEP**

**SAF-p2**: KIPPKLKP**IPPLKEIPPLEP**

In these sequences, the hydrophobic residues at a and d promote parallel dimers, while the charge patterning at e and g is set for staggered assembly. However, the key design feature is the inclusion of offset asparagine residues at a positions, which specifies staggered assembly as the two asparagines residues most likely pair to form a hydrogen bond across the hydrophobic interface (Figure 13). The remaining P sites are filled primarily with residues with a high α-helical propensity in order to maximize the helical content of the peptides.

![Figure 11](image-url)

*Figure 11* Schematic of the two peptides employed by Lazar et al. in which α-helices stack perpendicular to the long fiber axis. (Reproduced from Ref. 55. © American Chemical Society, 2005.)

![Figure 12](image-url)

*Figure 12* Fibrous assembly using short coiled coils from Hartgerink et al. (a) Cryo-TEM image providing evidence for fiber formation; (b) The mechanism of fiber formation. (Reproduced from Ref. 57. © American Chemical Society, 2008.)
The α-helical structure of the peptides is confirmed by CD spectroscopy and X-ray fiber diffraction, and TEM confirms the presence of long, thickened fibers. The first drawbacks of this initial design is that the resulting fibers are relatively unstable and only form below room temperature. This led to a second-generation design, where positively charged arginine residues were incorporated at two consecutive c sites of SAF-p2—that is, outside the coiled-coil interface—to match and interact electrostatically with similarly placed aspartic acid residues in the original SAF-p1. The characterization of this design revealed more stable as well as better ordered and thickened fibers (Figure 14).

More recently, we have determined the kinetic pathway for peptide folding, and the designs have also been extensively modified and redesigned to generate examples of kinked, waved, and branched fibers. SAF variants that gel and support cell growth have also been developed. Gelation is achieved by replacing residues at the b, c, and f positions with either alanine (to promote interfiber hydrophobic interactions) or glutamine (promoting interfiber hydrogen bonding). These changes produce thinner, more-flexible fibers that interact as designed to form percolated networks and so physical hydrogels. Extensive biophysical, rheological, and microscopic characterization demonstrate robust hydrogels, particularly for the Ala-based peptides, that support mammalian cell growth and differentiation.

2.3 Fibrous systems based on collagens

Fiber-forming systems can also be designed using tectons based on the Pro-Hyp-Gly sequence repeat of fibrous collagens. These systems have only recently been widely investigated and, therefore, are less well represented in the literature than the above systems based on α-helical and β-structured components.

One of the first examples is from Kotch and Raines. They use two short collagen-based fragments rich in glycine and proline that self-assemble into an AAB-type triple helix. The three strands are joined by covalent disulfide bonds, which force the helices to assemble in a staggered fashion, promoting fibril formation. Work from Hartgerink’s lab a year later shows that designed nonfibrous homotrimers, AAB heterotrimers, and, for the first time, an ABC heterotrimer can be assembled and stabilized through noncovalent interactions alone. They use CD spectroscopy to demonstrate the correct folding of these structures and also to show high thermal stability. They conclude that the designed electrostatic interactions are the primary driving force for triple-helix formation, and that these interactions stabilize the structures efficiently. Similarly, Conticello et al. show that collagen-like fibrils with intriguing ultrastructure—that is, the detailed structure of the fibrils visible by electron microscopy, and above the underlying secondary and quaternary protein structure—that can be assembled from straightforward peptides.

Figure 14  TEM images of the second-generation SAFs. (a) Low-magnification image after maturation for 12 h; (b) High-magnification image showing regularly patterned striations on their surfaces. (Reproduced from Ref. 59. © Wiley-VCH, 2006.)
Their design, CPII, self-assembles into a homomeric triple helix and these helices are able to form collagen-like microfibers, which remarkably display \( \alpha \)-periodic-like patterns, an intrinsic feature of native fibrous collagens and one which many designed collagen-like fiber-forming systems lack. It is likely that this patterning results from the design containing hydrophobic and hydrophilic portions, leading to staggered assembly and the formation of regularly spaced electrostatic interactions. A final recent example of importance is from Krishna and Kiick.\(^6\)\(^9\) They show that synthetic collagen triple helices without hydroxyproline can be stable and can self-assemble into fibers as observed by TEM (Figure 15). In contrast to the more common GOP repeat, this design has GPP repeats at the \( N \)-terminus and near the \( C \)-terminus; a five amino acid sequence incorporating two cysteine residues at the \( C \)-terminus; and charged residues in the central portion to facilitate the stabilizing electrostatic interactions. Though their design contains cysteine residues, they show that self-assembly of the peptides is possible under both oxidizing and reducing conditions, so the formation of disulfide bonds is not essential to the stability of the self-assembled structures. This design is important, as it incorporates only natural unmodified amino acids, making it amenable to recombinant expression, thus expanding its future usefulness.

### 2.4 Peptide amphiphile fibrous systems

PA systems have perhaps the potential to be the most diverse of all the repeating fibrous assemblies due to the wide range of synthetic moieties that can be incorporated into the tectons. Owing to this variety, many examples of amphiphilic systems have been explored but a select few are highlighted here in order to present a taste of the potential systems that can be designed and synthesized, as well as the potential applications of such systems.

Work presented by Hartgerink and Stupp initiated this field. One of their notable designs consists of a PA containing a long alkyl tail and a peptidic head group functionalized with a variety of different moieties.\(^7\)\(^0\) The PA forms fibers (Figure 16a) but also gels at high concentrations in acidic solutions, and cryo-TEM reveals long ordered fibers which are assumed to be the PAs assembling into cylindrical micelles (Figure 16b). The assemblies are capable of nucleating hydroxyapatite on their surfaces (due to the presence of a phosphoserine residue incorporated into the peptidic head group). The hydroxyapatite mineralizes in a manner such that the hydroxyapatite crystals align with the fiber axis, which is similar to the hierarchical organization found in bone (Figure 16c).

Hartgerink’s own lab has built on this approach to self-assembly, with several new PA systems, specifically showing that the inhibition of cancer cell proliferation can be achieved by utilizing a designed PA system.\(^7\)\(^1\)

This field is vast and over recent years has spawned ever more intricate and sophisticated designs. Even though the area is well represented, it is envisaged that more designs will be presented in the coming years, especially those which lead to functional systems; for example, hydrogels that are capable of supporting cell growth and differentiation are currently the subject of intense research.

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**Figure 15** TEM images of the designed collagen-like fibrils from Krishna and Kiick. (a) TEM image of many collagen fibrils; (b) Higher magnification image of an individual fibril. (Reproduced from Ref. 69. \( \copyright \) American Chemical Society, 2009.)

**Figure 16** Images of the fibers formed from PAs designed by Hartgerink and Stupp. (a) TEM image of the self-assembled fibers arranged into ribbon-like arrays; (b) Cryo-TEM image of the fibers; (c) TEM image of a fiber covered in mature hydroxyapatite crystals (shown by the red arrows). (Reproduced with permission from Ref. 70. \( \copyright \) American Association for the Advancement of Science, 2001.)
3 DISCRETE SUPRAMOLECULAR SYSTEMS

While effectively “indefinite” or “infinite” fibrous structures have proven relatively straightforward to design, and are now advancing toward functional systems; the successful design of finite or discrete self-assembled peptidic systems is proving more challenging. These structures are much smaller than the repetitive fibrous systems and contain a finite and ideally defined number of tectons; hence the term discrete. While there are still relatively few examples of such assemblies, several systems have been designed successfully and are presented in this section. The design principles behind each system are covered, along with potential applications. As most of the examples are relatively unrelated in terms of their underlying design principles and function, they are given chronologically.

3.1 Belt-and-braces peptides

An early example of a discrete self-assembled system is our own “belt-and-braces.”72 The design comprises three coiled-coil peptides, one of which is twice the length of the others, and acts as a template for their assembly; the “belt” peptide is six heptads long, and the two “brace” peptides each have three heptads. The design is based on parallel dimeric assemblies, with the majority of the a sites in all three peptides occupied by isoleucine residues and all of the d sites leucine. Homo-assemblies are discouraged by giving the e and g sites on each peptide the same charge; the belt peptide has all glutamic acid residues at these positions, while the braces have lysines. To distinguish the two braces and direct them to different ends of the belt, one a site of the C-terminal brace is filled with asparagine to complement an asparagine at the a position in the belt. Together, these amino acid placements specify a ternary complex as demonstrated by biophysical and microscopic analyses.

The brace peptides are terminated by cysteine at their “free ends” in the complex to facilitate coupling to gold nanoparticles, the aim being to direct nanoparticle assembly. The resulting brace peptide–nanogold constructs interact only when the belt peptide is added; precipitation of aggregated particles is observed, accompanied by a color change, indicative of higher-order nanogold assembly. Assemblies are confirmed by TEM, which shows the assembly of both 2D and 3D networks of colloidal nanoparticles (Figure 17).

3.2 Formation of supramolecular assemblies using leucine-zipper displaying dendrimers

Ghosh and coworkers demonstrate that discrete supramolecular assemblies can be formed from leucine-zipper peptides tethered to a dendrimer core.73 These give fibrous structures when two complementary discrete dendrimer–peptide assemblies are combined; this is one of the first examples where both discrete and repeated assemblies can be formed using the same system (Figure 18a).

The group uses a poly(amido amine) (PAMAM) dendrimer core, functionalized with surface maleimide groups, chosen to react with cysteine-containing peptides. The coiled-coil peptides used are an acid–base pair, which assemble to form coiled-coil tetramers. Both have cysteine at the C-terminus to allow covalent attachment. Two molecules are described: one dendrimer with the acidic (EZ) peptide attached, and the other with the basic (KZ) peptide. The dendrimer–EZ molecule (D–EZ) combines with four equivalents of the free KZ peptide at pH 8.4 as judged by CD spectroscopy and AUC. Adding four equivalents of the free EZ peptide to the D–KZ construct at low pH gives similar results. Mixing the dendrimer–peptide assemblies (D–EZ and D–KZ) results in protofibrils and eventually fibers, as confirmed by TEM (Figure 18b and c).

In the following year, the group built on this work to show that the dendrimer core forms similar assemblies...
with the natural coiled-coil peptides Fos and Jun at neutral pH—making the design more biocompatible and amenable to further derivitization.\textsuperscript{74}

As Fos forms weaker homodimers,\textsuperscript{75} it is attached to the dendrimer hub. Even so, the dendrimer–Fos construct (D\textsubscript{0}–Fos\textsubscript{4}) is 85\% helical by CD spectroscopy. AUC confirmed that this folding is a result of intra- (as opposed to intermolecular) homodimer formation as no higher order assemblies are observed; that is, despite its low dimer content, Fos peptides interact because of their high effective concentration when attached to the dendrimer. An eightfold excess of Jun is required to form four Fos-Jun heterodimers when starting from the D\textsubscript{0}–Fos\textsubscript{4} construct. With 4 equivalents of Jun, only 2 appear to incorporate into the D\textsubscript{0}–Fos\textsubscript{4} assembly. It is suggested that the need for the excess could be attributed to either the energy penalty associated with breaking intramolecular Fos homo-pairings or for steric reasons. AUC analysis confirms that with an eightfold excess of the Jun peptide the D\textsubscript{0}–Fos\textsubscript{4}/4PJun complex is formed, albeit along with other homodimeric and monomeric Jun species. The group envisaged that this framework could be used as a basis for receptor targeting or DNA binding.

### 3.3 Self-assembly of regular polyhedral nanoparticles

The previous examples involve one coiled coil directing the assembly of a partner to form heterodimeric systems. The next case is slightly different in that two different coiled-coil sequences are contained in one building block. A coiled-coil construct comprising a sequence shown to form homopentamers is linked (via a two-glycine spacer) to a homotrimeric sequence.\textsuperscript{76} It was anticipated that 15 of these monomeric tectons would self-assemble into a construct containing 3 pentamers and 5 trimers and that these units would self-assemble further to form polyhedral nanoparticles containing 60 monomeric units or 4 of the smaller self-assembled constructs (Figure 19a). Nanoparticle assembly is probed using both TEM and AUC. The former shows the appearance of regular monodisperse nanoparticles when specific folding regimes are used (Figure 19b), while AUC shows that assembly of discrete nanoparticles is highly concentration-dependent, with the desired assembly of 60 monomeric units being formed though under very specific conditions.

It is proposed that such a system could be used as a platform for antigen display, as it is highly symmetrical and repetitive and resembles a viral capsid. Such assemblies are known as synthetic virus-like particles (SVLPs) (see below). Indeed, other papers have followed from the group demonstrating varying levels of success in displaying antigenic actin determinants, as well as being utilized in the development of prototypic vaccines for malaria and severe acute respiratory syndrome (SARS).\textsuperscript{77–79}

### 3.4 Synthetic virus-like particles

Continuing the theme of self-assembled nanoparticles, Robinson and coworkers have implemented a slightly different approach to form SVLPs. Their building block consists of a lipid conjugated to a coiled-coil peptide that is designed to form trimers. The rationale is that the coiled coils will self-assemble into parallel trimeric bundles, and the lipids will then drive further self-assembly of these trimeric bundles into nanosized SVLPs with the lipids buried and the C-termini of the coiled coils exposed (Figure 20a and b). The presence of regularly
Supramolecular aspects of chemical biology

Figure 19 Synthetic peptide nanoparticles. (a) Computer model of the self-assembled nanoparticle incorporating 60 copies of the monomeric construct; (b) TEM image of the nanoparticles. (Reproduced from Ref. 76. © Elsevier, 2006.)

Sized nanoparticles was confirmed by TEM (Figure 20c). Antigens have been added to the C-terminal end of the peptides to demonstrate the utility of the system to present novel immunogens. Further experiments confirm that these SVLPs do indeed generate antigen-specific antibodies in animal models.80

3.5 A self-assembling peptide polynanoreactor

Ryadnov describes a coiled-coil design that self-assembles to form a polynanoreactor.81 The design consists of two complementary peptide supradendrimers (noncovalent peptidic dendrimers) designed to self-assemble and form a polynanoreactor with various sized cavities. Peptide supradendrimer 1 (SD-1) is designed around a homodimeric sequence. However, polar charged residues are placed to encourage intermolecular electrostatic interactions and promote associations between dimers, leading to self-assembled, ordered noncovalent networks (Figure 21a). The cavities within the networks are too small to be of any practical use. To address this, a second peptide supradendrimer sequence (supradendrimer 2, SD-2) is designed to form heterodimers with SD-1. In addition, cysteine residues on the outside promote trimers of heterodimers, resulting in a starburst arrangement and self-assembly of a network with larger cavities (Figure 21b).

A metal redox reaction is described to determine whether the polynanoreactors are functional; the coiled-coil sequences incorporate cysteine residues so that encapsulated colloidal silver can be generated, and TEM confirms the presence of regularly dispersed nanoparticles.
3.6 A reduced SNARE model for membrane fusion

In an attempt to mimic membrane fusion events, the Kros lab has constructed a simplified peptide-based model of the SNARE (soluble NSF (N-ethylmaleimide sensitive factor) attachment protein receptor) proteins intrinsic to this process (Figure 22a and b). They describe two triblock copolymers consisting of a lipid domain, a central PEG spacer, and either an acidic or a basic designed coiled coil (Figure 22c). The concept is that the lipid domain should incorporate into vesicles, leaving the coiled coils on the vesicle surface. Two vesicle populations, functionalized with either acidic or basic coiled coils, can then be mixed, allowing the coiled coils to heterodimerize and cause vesicle fusion as the vesicles are brought into close proximity (Figure 22d). They test the design by performing a FRET (Forster resonance energy transfer) assay. A fluorescent complex is encapsulated in one vesicle population and a nonfluorescent ligand in the other. If fusion occurs, the contents of the two vesicle populations will mix and an increase in fluorescence will occur, which is exactly what is observed in practice.

3.7 Shape and release control of peptide decorated vesicles

A second example from the Kros lab is particularly interesting, as it uses a nonpeptidic scaffold to template the formation of β-structured peptides. A β-cyclodextrin vesicle (CDV) is employed as a scaffold to template the assembly of an octapeptide, (LE)_4, that is N-terminally modified with adamantane. The adamantane moiety forms an inclusion complex on the surface of the vesicles. The group uses CD spectroscopy to show that the peptide is unstructured.
Supramolecular aspects of chemical biology

Figure 23 Cryo-TEM images showing the stages in the peptide–vesicle interaction process from Kros et al. (a) The addition of peptide to CDVs at physiological pH does not affect vesicle morphology; (b) Fibers are seen 1 h after acidifying the solution; (c) The disappearance of fibers is observed when the pH is returned to physiological pH. (Scale bar in all images = 100 nm.) (Reproduced from Ref. 83. © American Chemical Society, 2009.)

and remains so when added to CDVs at physiological pH (Figure 23a). However, upon acidification of the solution the peptides adopt a β-structure. This structural change is conferred to the CDVs and a transition from vesicles to fibers is observed by cryo-TEM (Figure 23b). These transitions are reversible upon altering the pH to 7.4 (Figure 23c).

The group also demonstrates, through encapsulation of a fluorescent dye, that this system can be used as responsive capsules, allowing pH-triggered release of encapsulated cargo, opening possibilities for drug delivery.

3.8 Supramolecular replication of peptide and DNA patterned arrays

Stellacci and Stevens outline how ordered peptide arrays can be printed onto a surface. They have implemented a soft stamping technique known as liquid supramolecular nanostamping (LiSuNS), which they used previously to replicate DNA features onto surfaces. In this new work, they extend the scope of the technique to printing peptides. They use a thiol-modified version of the heterodimeric

Figure 24 The LiSuNS process. (a) The printing process; (b) Fluorescence micrograph of the printed peptide array; (c) Fluorescence micrograph showing the DNA and peptide array using false color overlay. (Reproduced from Ref. 84. © Royal Society of Chemistry, 2010.)
EK coiled coil introduced by Tripet and Hodges.\(^86\) The K peptide is incubated with a gold-printed silicon wafer and binds to its surface. This peptide-functionalized wafer is known as the “master” and is incubated with free E peptide, resulting in the formation of EK heterodimers at the surface. A poly(dimethyl siloxane) (PDMS) prepolymer solution is mixed with the master, cured, and removed, at which point the E peptide should be attached to the PDMS substrate (Figure 24a). To test this, they incubate the prepared PDMS surface with fluorescently labeled K peptide, and imaged by fluorescence microscopy (Figure 24b). In a final demonstration of the capabilities of the technique, the group shows that it is possible to print a mixed peptide–DNA array (Figure 24c).

4 TEMPLATED AND DIRECTED ASSEMBLIES

The work outlined in the section above demonstrates that assembly of discrete supramolecular nanostructures is achievable through directed hydrophobic and electrostatic interactions alone. While some of the examples employ metals, this is to illustrate that their assembled systems fulfill a particular function; they are not an integral part of the designs. The examples in this section employ metals as part of the assembly process. In many of the cases, assembly of metal ions or nanoparticles is directed by the peptides; in others, it is the metal that influences organization of the peptides. Examples of peptide assemblies constructed from coiled-coil, collagen-like, and \(\beta\)-structured tectons are all highlighted.

4.1 Peptide and metal hybrid systems

The majority of examples of hybrid systems are based on \(\beta\)-structured tectons and have been used as fibrous scaffolds for nanowires; assemblies that use other tectons have a wider scope, and have been used to produce a range of interestingly shaped discrete objects.

4.1.1 Assemblies utilizing \(\beta\)-structured tectons

The Lindquist group was among the first to demonstrate that amyloid-like fibrils could template nanowire formation.\(^87\) They use the so-called NM region of a yeast prion, which self-assembles into \(\beta\)-structured fibers, modifying the peptide building blocks to contain a chemically accessible cysteine residue for subsequent covalent attachment of nanogold particles. TEM demonstrates that nanogold is evenly distributed along the fibers. To make the fibers conducting, they enhance the gold-decorated nanofibers (Figure 25a), resulting in fibers displaying closely packed metal nanoparticles (Figure 25b).

Similarly, Reches and Gazit use a straightforward diphenylalanine building block as a basis for amyloid-like fibril formation and templating.\(^88\) In this case, the dipeptide forms regularly sized hollow nanotubes, visualized by SEM (Figure 26a and b); they propose that the aromatic diphenylalanine residues stack to form the higher order assemblies. To exploit the tubes, ionic silver is added to the peptide nanotubes, resulting in silver nanoparticles and, hence, silver nanowires within the tubes after reduction to silver metal. The nanotubes could then be degraded with protease, leaving only the assembled silver nanowires (Figure 26c and d). These are observed by TEM, and the chemical identity of the nanowires confirmed by energy dispersive X-ray (EDX) analysis.

A more recent example demonstrates how amyloid fibrils can be used to display cytochromes. A tandem repeat of a fibril-forming SH3 domain is fused to a cytochrome via a short peptidic linking sequence and it is demonstrated that fibers (albeit with different morphologies to those formed with the SH3 domains alone) are formed in the presence of both apo- and holoproteins.\(^89\) Such a system has the potential to be used as conducting nanowires, and also as a basis for understanding charge transfer in biological systems.

4.1.2 Coiled-coil assemblies

Several papers detail how coiled coils can be used to direct the assembly of nanoparticles; in particular gold nanoparticles are widely used. For instance, following on from the aforementioned belt-and-branches system,\(^72\) Stevens et al. use an acid–base heterodimeric coiled coil to direct the assembly of different sized gold nanoparticles. They also demonstrate that control can be exerted over assembly
and disassembly by altering pH. Studies with the gold-functionalized acid peptide at basic pH show a disperse population of nanoparticles, whereas at acidic pH aggregation occurs, indicative of coiled-coil homodimer formation. Mixing the acid and the base peptide–nanoparticle conjugates at neutral pH gives higher order nanoparticle assemblies. These assemblies are imaged using TEM, which reveals a central 53 nm gold nanoparticle (attached to the base peptide) surrounded by a layer of smaller 8.5 nm particles (conjugated to the acid peptides) (Figure 27a). The nanoparticles are presumably brought together by the formation of acid–base heterodimers. On lowering the pH, the assemblies appear more random, presumably because the acidic peptide–nanoparticle conjugates form homodimers (Figure 27b).

Wagner et al. describe something similar, but using a slightly different approach. They employ a de novo designed homodimeric coiled coil, VW05, with arginine residues at the surface-exposed $f$ positions of the heptad repeat. Under basic conditions, the arginine side chain is protonated, facilitating the formation of electrostatic interactions with mercaptoundecanoic acid (MUA) coated gold nanoparticles. The group uses UV–vis spectroscopy to monitor the surface plasmon resonance (SPR) band of the Au–MUA nanoparticles. Coiled-coil driven assembly causes red shift and broadening of the SPR band as the interparticle distance decreases. By monitoring the assembly process in this way, it is found that not only does pH have an effect on assembly but peptide concentration and incubation time do also. The group visualizes the nanoparticle assemblies by cryo-TEM and confirms that, at pH 9, ordered networks of nanoparticle assemblies are formed. Very thin fibers are also seen at high magnifications but not for the peptide alone, leading to the conclusion that high local peptide concentrations at the surface of the nanoparticle triggers nucleation and fibril formation. Finally, cycling the pH between 9 and 12 can be used to control the assembly of the system.

In a final example, Slocik et al. demonstrate that coiled coils can be used to direct the assembly of both gold nanoshells (NSs) and quantum dots (QDs), to produce two types of assembly: extended NS–NS assemblies and discrete NS–QD complexes. Two designed peptides, E5 and K5, form an antiparallel heterodimer, and both peptides are functionalized with a cysteine residue to allow for covalent attachment. To form extended NS–NS assemblies, NSs are incubated with either the E5 or the K5 peptide. Confirmation of an interaction between the NS and the peptide is obtained by FT-IR spectroscopy. Mixing the two conjugates (NS-E5 and NS-K5) results in higher order assembly.
assemblies as shown by TEM (Figure 28a–c). The images obtained confirm that the NSs are indeed brought into close proximity, presumably through the formation of the heterodimeric coiled-coil dimer. However, the assemblies have a tendency to aggregate and form extended structures. They demonstrate triggered dissociation of the assembled NS–NS complexes by irradiation with IR light. This process is reversible: once the temperature decreases, the coiled coils recombine and the extended assemblies reform (Figure 28d and e). To form discrete assemblies, the K5 peptide is incubated with cysteine-functionalized QDs and mixed with the E5–NS conjugates (Figure 28f). The QDs organize into uniform layers on the NS surface (Figure 28g). Unlike the NS–NS complexes, the NS–QD complexes cannot be photothermally dissembled.

4.1.3 Collagen-based metal–peptide assemblies

Collagen-based fibrous structures are the most recent additions to the field of de novo designed peptidic biomaterials; so it is not surprising that very few examples of collagen–metal assemblies exist. However, Chmielewski and coworkers have begun to explore possibilities for such assemblies. Their first example outlines the design of a peptide that assembles into an extended system of collagen triple helices upon the addition of divalent metal ions.\(^9\) The design is based on a standard (POG)\(_{9}\) sequence, where “O” is the modified amino acid hydroxyproline. The peptide is C-terminally modified with dihistidine and N-terminally modified with nitrilotriacetic acid (NTA). CD spectroscopy shows the presence of the extended collagen PPII helix. Addition of Ni(II), Zn(II), Co(II), or Cu(II) to the peptide results in the solution becoming turbid, and dynamic light scattering (DLS) reveals large aggregates. The formation of these is probed further to show that the morphology of the species formed depends on the ratio of the peptide to the metal. SEM reveals a wide range of species, ranging from florettes to open tubes, composed of layered sheets and irregularly shaped flakes (Figure 29a–d). Incubation of the peptide with Cu(II) and Co(II) also results in the formation of spherical microflorettes. However, with Ni(II) the structures formed are much smaller, and atomic force microscopy (AFM) analysis shows them to be composed of interconnected irregularly nanosized spheres. Assembly is reversible upon the addition of ethylenediaminetetraacetic acid (EDTA). If the collagen helices are functionalized only at one end, then higher order assemblies do not form.
Figure 29 SEM images indicating the range of structures that can be produced with the collagen-based designed peptides after incubation with varying concentrations of ZnCl₂. (a) 200 µM ZnCl₂; (b) 400 µM ZnCl₂; (c) 600 µM ZnCl₂; (d) 800 µM ZnCl₂. Scale bars = 5 µm. (Reproduced from Ref. 93. © American Chemical Society, 2009.)

To illustrate potential functions of these assemblies, the group has modified the initial design and demonstrated its ability as a 3D cell culture scaffold. The same basic building block is used, but with a bipyridyl (bipy) moiety incorporated at a central position in the peptide. Incubation of this peptide with Zn(II), Co(II), Cu(II), and Ni(II) again gives turbid solutions, and SEM reveals networks of cross-linked strands (Figure 30a–d). In this case, the metal-to-peptide ratio has no effect on the morphology of the structures formed, although there is some variation in the morphologies of the fibrous networks depending on the metal used. Two metals can also be incorporated into the peptide framework: incubating the peptide with rhuthenium binding to the bipyridyl moiety and then subsequent addition of a divalent metal ion binding the NTA and His functionalities. To demonstrate that the scaffold can be used to support cell growth, another peptide incorporating a fluorophore is mixed with the bipyridyl-functionalized peptide and metal. HeLa cells are added and incubated in a cell culture medium. Fluorescence microscopy shows that the HeLa cells are encapsulated by the 3D network, and the cells are shown to have similar viability to those grown on normal cell culture plates.

This demonstrates that peptidic collagen-based materials can support cell growth and potentially be used as scaffolds in regenerative medicine.

4.1.4 Assemblies using PAs

There are a few reported examples of nanoparticles driving the assembly of PA-based systems. One is from Li and Stupp, who demonstrate a system where β-structured PAs form linear arrays with gold nanoparticles (Figure 31a). Two PAs, one modified with thymine in order to bind diaminopyridine (DAP) modified gold nanoparticles, are used, and upon mixing the two a gel forms. Addition of DAP-modified nanoparticles leads to aggregated structures...
which are shown by TEM to be long linear arrays of gold nanoparticles forming along the fibers (Figure 31b).

4.2 Conformational state switching

In addition to driving the assembly of peptide-based systems, metals can be employed to induce folding or to cause a change of conformational state within a system. Ogawa’s lab primarily investigates metal coordination to coiled-coil peptides and presents several examples of metals inducing folding or conformational changes of designed peptides. One details a designed four-heptad coiled coil that binds metal complexes. To facilitate metal binding, an $f$ position incorporates 4-pyridyl alanine (Pal) and a $d$ position contains cysteine, allowing for disulfide bond formation. The disulfide-bonded coiled-coil dimers react with a rhenium compound resulting in assemblies where the dimers act as bridging ligands between $\text{fac-}[\text{Re(CO)}_2]$ cores. Matrix-assisted laser desorption ionization (MALDI) mass spectrometry shows assemblies incorporating one, two, and three ruthenium units.

In a second example, the group uses a similar Pal-containing coiled coil to form noncovalent complexes.

Figure 30  Fibrous scaffolds produced by Pires et al. through incubation of the designed peptides with metal. (a) With Zn(II); (b) With Cu(II); (c) With Co(II); (d) With Ni(II); (e) A bright-field image of the network when the biotin-labeled peptide is incorporated and bound to fluorescently labeled streptavidin; (f) A fluorescence microscopy image of (e). (Scale bars in images (a–d) = 5 µm and (e, f) = 200 µm.) (Reproduced from Ref. 94. © Wiley-VCH, 2009.)
Figure 31  Fibers formed from PAs and gold nanoparticles by Li and Stupp. (a) A schematic representation of the formation of gold-coated amphiphilic fibrils; (b) TEM image showing the regular ordering of gold nanoparticles along the fibrils. (Reproduced from Ref. 95. © Wiley-VCH, 2005.)

Two coiled coils coordinate to a platinum center and these monomeric units spontaneously self-assemble as the coiled coils form dimers. The morphology of these assemblies is probed by TEM and AFM to show predominantly nanospheres with dimensions of 30–50 nm; in addition, fibrils with lengths of several 100 nm and widths ranging from 4 to 10 nm are observed (Figure 32a and b).

A different example of metal coordination from the same group details the de novo design of a coiled coil based on a rubredoxin model; the Cys-X–X-Cys binding motif from rubredoxin is incorporated into a coiled coil.98 Interestingly, the group observes that the peptide adopts a random-coil conformation when no metal is present. They assumed that the peptide would spontaneously fold into a coiled coil as the sequence follows the heptad repeat, but they conclude that incorporation of two cysteine residues into the hydrophobic core disrupts folding. Upon addition of CdCl₂, however, an α-helical structure is observed and the peptide is shown to bind the metal in 2:1 ratio indicating that a metal-bridged coiled-coil dimer is formed.

Figure 32  AFM images of coiled-coil-metal complexes from Tsurkan et al. AFM image of (a) nanospheres and (b) nanofibrils. (Reproduced from Ref. 97. © American Chemical Society, 2007.)

A more recent paper gives design principles from both types of example. A coiled coil containing two Pal residues coordinates to a platinum center.99 A coiled-coil dimer is formed when metal is absent but, on reacting the peptide with Pt(en)(NO₃)₂, a metal-bridged coiled-coil tetramer is observed; this is proven by reversed-phase HPLC (high-performance liquid chromatography), SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis), and MALS (multiangle light scattering) analysis.

Tanaka’s lab has also produced assemblies comprising coiled coils and metals. They had previously designed a parallel trimer IZ,100 which is modified by incorporating histidines at a and d positions to produce IZ-3adH. In the absence of metal, the peptide adopts a random-coil conformation, but on addition of Co(II), Ni(II), or Zn(II) forms α-helical parallel coiled-coil trimers. Further investigation of the Ni(II)–peptide complex shows that Ni(II) coordinates to the histidine residues with an octahedral geometry.

In a later paper, they again use IZ, but change one isoleucine to alanine and a second to cysteine. They show the resulting peptide once again has a random-coil structure in the absence of metal, but upon addition of either Cd(II) or Hg(II) adopts a trimeric coiled-coil structure.101

Dublin and Conticello use the trimeric histidine-containing coiled-coil TZ1H, which is previously shown to form long fibers at pH values above the pKₐ of histidine, to bind metals.53,102 They postulate that the histidine residues could be arranged in a trigonal planar geometry that allows metal binding. They add silver triflate to the peptide and show that it changes conformation from random coil to α-helix. Further analysis by TEM shows that, in the absence of Ag(II) and at pH 5.6, no higher order assemblies are observed but addition of Ag(I) results in long fibers indicating metal coordination is driving fiber formation (Figure 33).

There are also examples from our own lab of peptides designed to switch conformational state. One describes
Template-α, which is a designed coiled coil. To encourage a conformational switch, residues at f positions were changed from glutamine to threonine to produce Template-αT. Below 70 °C, the peptide is α-helical and above this temperature it forms β-structured amyloid-like fibrils. Cross-linking the peptides to achieve a β-hairpin-like conformation increases amyloid fibril formation.

A later paper details the de novo design of peptides that switch between a coiled-coil and a helical-hairpin conformation. The parent peptide (coiled-coil switch peptide) CSP-1 is a parallel dimeric coiled coil, but oxidation of cysteine residues in the peptide causes the switch to a monomer. It remains α-helical, although the α-helical content is low. Increasing the loop length gives CSP-3, which is a peptide with a higher helical content. CSP-6, an anagram of CSP-3, is more helical still and can be switched between the helical-hairpin and coiled-coil conformations.

A final example from our lab details how ZiCo, another de novo designed peptide, can switch conformational state upon binding zinc. In the absence of zinc, the peptide adopts a coiled-coil structure, but upon addition of zinc, absorption bands indicative of β-sheet are observed by FT-IR spectroscopy. Zinc binding is reversible and occurs in a 1 : 1 ratio.

Similarly, Ambroggio and Kuhlman demonstrate the de novo design of a peptide able to switch between a 2Cys-2His zinc finger-like fold and a trimeric coiled coil (Figure 34). They use the sequences of the zinc-finger Zif268 and the coiled-coil region of hemagglutinin in a computer algorithm designed to find the lowest energy combination of these sequences. Their design, Sw2, is folded as a trimeric coiled coil in the absence of zinc. Upon the addition of zinc, the peptide adopts a conformation similar to that of other zinc fingers. The peptide and metal bind in a 1 : 1 ratio, regardless of whether zinc or cobalt is used; however, binding is reversible only with cobalt, indicating zinc binds strongly and the off rates are slow.

5 CONCLUSIONS AND OUTLOOK

It is evident from the large number of working examples that the design and engineering principles for effectively infinite, fibrous, peptide-based assemblies are now well established, and research in this area is burgeoning. This is particularly true for systems based on α-helical, β-structured, and PA tectons. Largely because of difficulties with peptide synthesis and slow folding kinetics, similar fibrous assemblies based on collagen-like building blocks are less-well developed, but recent work on these is extremely encouraging. Challenges that remain for such peptide-based assemblies in general include taking precise control over assembly kinetics, and of fiber morphology, functionalization, and dynamics; again, we note good progress on all of these fronts. Thus, in our view, polypeptide-based materials are now at a stage to compete with more traditional fibrous systems based on synthetic polymers and small molecules: if not in respect of cost and bulk preparation, at least in terms of rational design.
This improved ability to design peptide-based fibers from the bottom up should pave the way to real-life applications of such materials in bionanotechnology, particularly with regard to templating inorganic and other functional materials, and as soft biomimetic scaffolds in 3D cell culture and tissue engineering.

In contrast, the rational design of discrete supramolecular assemblies based on peptides is more elusive. True, the generation of simple discrete oligomers has been possible for some time using certain peptide-folding motifs—for example, the $\alpha$-helical coiled coil. However, with notable exceptions, the next steps to more complex multicomponent systems of defined stoichiometry and topology are proving more difficult. Again, the issue seems to be one of control: how can peptidic tectons be encouraged to assemble, but at the same time how can such associations be limited and directed precisely to give discrete rather than infinite objects (i.e., fibers and aggregates)? Of course, this is exciting in itself, and it is encouraging that ambitious de novo designs and engineered systems are already emerging. Moreover, the prospects for bottom-up assembly (tiling), drug delivery, and biosensing, to name but a few examples, with such assemblies in hand provide strong motivation for exploring this avenue of research.

Therefore, on both fronts—functional soft fibrous assemblies and discrete nanoscale objects—the rational design of peptide-based systems in what might be termed more broadly as biosupramolecular chemistry, is alive, kicking, and screaming to be nurtured, developed, and applied.

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