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Peptides as novel smart materials

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Important challenges in biomaterials design include predicting the formation of large-scale self-assembled structures based on local atomic-level interactions and then endowing such structures with the ability to respond sensitively to environmental cues. This responsiveness is referred to as smartness. With the advent of key technological advances in imaging, peptides have recently begun to be exploited for their potential use as biomaterials, such as filaments and fibrils, hydrogels, surfactants and peptide hybrids. Peptides offer attractive features, principally because of our detailed understanding of their ability to fold into specific structures, and the rich chemistry with which their structure and function can be manipulated for environmental response.

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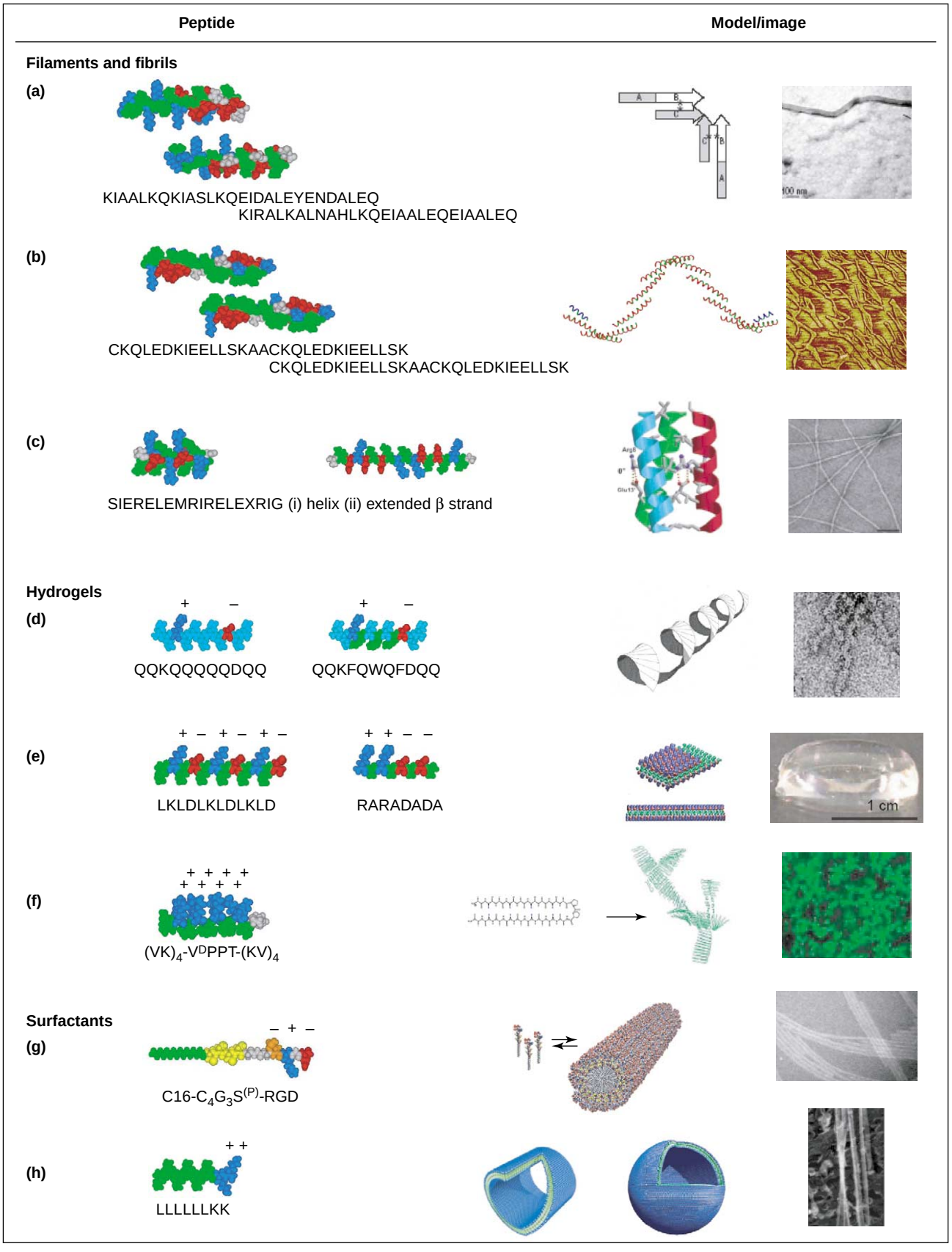
Introduction

Using traditional methods, the synthesis and rigorous structural characterization of nanostructures (with dimensions of nanometers to micrometers) is a daunting proposition. New synthetic strategies have therefore emerged, inspired by biological systems that build on the concepts of self-organization and self-assembly [1–3], whereby building blocks self-associate in particular patterns to form higher order organized complexes. The key advantage of using self-assembly is that it capitalizes on the formation of non-covalent and reversible interactions, including electrostatic, hydrophobic, van der Waals and metal–ligand interactions, hydrogen bonds and aromatic π-stacking [1–3]. Collectively, if sufficient in number, these weak interactions can yield highly stable assemblies. The main challenge involves the formation of structures that are homogeneous and structurally well defined. During the past decade, many examples of biomaterials based on peptides have been created, using well-defined strategies or simply by serendipitous discovery.

Modern synthetic methodologies offer ready access to virtually any amino acid sequence 5–50 residues long. Most systems discussed in this review use small and relatively simple amphiphilic building blocks in which one face of the unit is hydrophobic and the other is hydrophilic. Pairing of hydrophobic faces or hydrophilic faces can drive peptide assembly, resulting in layered structures with alternating polar character. The spacing of the hydrophobic residues in a sequence can be manipulated to drive either β-sheet formation (i, $i + 2$) or α-helix formation (i, $i + 3$ and i, $i + 4$) [4]. Peptides also offer the ability to incorporate non-natural amino acids or non-peptidic moieties, which is a particularly valuable feature for the inclusion of function and/or smartness in the scaffold (i.e. a photoswitch or non-peptidic ligands). Moreover, peptides are chiral, which can lead to the generation of materials of a particular handedness, which in turn can add another level of recognition. Given the richness of chemical functionality that is available in peptide biomaterials, orthogonal chemistries can be developed for smartness that do not interfere with the typical non-covalent chemistries that are responsible for peptide structure and stability.

Scientists are now beginning to turn to testing the ability of peptide-based biomaterials to respond to external cues; this responsiveness has been collectively referred to as 'smart behavior'. Responsiveness can be defined at either the structural level or the functional level [5•]. Particularly interesting are systems in which the response is reversible. For structural smartness, assemblies built from α-helical units tend to be more readily reversible than structures dominated by β-strand units. This difference arises principally from the relative importance of hydrogen-bonding and hydrophobic contributions to structural stability. β-Sheet structures have extensive interpeptide or interchain hydrogen bonds, which add great rigidity and irreversibility to such structures (i.e. the rigidity of cellulose is derived in a large part from hydrogen bonding). Because the hydrophobic effect largely dictates stability through exclusion of water, burial of non-polar groups does not require the strong geometrical constraints imposed by hydrogen bonding. Thus, structures tend to be more fluid and dynamic (i.e. saturated lipids in membranes are highly dynamic). Coiled coils, made up of α-helical pairing interactions, are often highly dynamic (and readily reversible in their folding) and thus offer obvious advantages as a smart material. Types of smartness that

Figure 1


Peptide
Model/image
Filaments and fibrils
(a)
KIAALKQKIASLKQEIDALEYENDALEQ
KIRALKALNAHLKQEIAALEQEIAALEQ
(b)
CKQLEDKIEELLSKAACKQLEDKIEELLSK
CKQLEDKIEELLSKAACKQLEDKIEELLSK
(c)
SIERELEMRIRELEXRIG (i) helix (ii) extended β strand
Hydrogels
(d)
QQKQQQQQDQQ
QQKFQWQFDQQ
(e)
LKLDLKLDLKLD
RARADADA
1 cm
(f)
(VK)4-VDPPT-(KV)4
Surfactants
(g)
C16-C4G3S(P)-RGD
(h)
LLLLLLKK

have been explored include sensitivity to solvent conditions (i.e. pH, salt), temperature, electronic or photonic energy, metals and other more complex ligands.

This review will highlight some recent advances in the field of self-assembling peptides used in biomaterials design. Emphasis will be placed on work that either implicitly or explicitly incorporates concepts of smartness. Specific examples are discussed of peptides that play an important structural and modulatory role in materials such as filaments and fibrils, hydrogels and surfactants. Recent innovations incorporate smart peptides into hybrid materials for additional tuning of structure and properties.

Filaments and fibrils

There are several recent reviews of the extensive literature on the structural analysis of peptides that use cross-β-structures to form filaments and fibrils. Thus, we focus only on a few papers that seek to identify the simplest sequence motifs that can form such structures, as models of both amyloid fibers and silk fibers. Such approaches will most certainly impact future biomaterials design work. The study of self-assembling α-helical peptides as biomaterials has a shorter history (also recently reviewed several times), but the goals have been more directly tied to the design of reversible biomaterials. We highlight work from Woolfson's laboratory and our own laboratory that aims to explore the properties of biomaterials based on α-helical modules. The design work described offers the promise of redesign using orthogonal chemical approaches to include functionality and responsiveness.

Filaments and fibrils using α-helices

Several laboratories, including our own, have designed fibrillar structures based on coiled-coil structural motifs, ranging from two-stranded to five-stranded coiled-coil structures [6–8,9•,10]. In each case, investigators have recognized that forming staggered pairing interactions between helical peptides can lead to larger scale self-assembly. Woolfson's laboratory has led such efforts. Their system takes advantage of electrostatic interactions to favor the formation of staggered arrangements of helices by two different 28-residue peptides (Figure 1a) [6]. To help stabilize the staggered interactions, they also take advantage of a buried asparagine residue in each of the two peptides, which can form structure-stabilizing hydrogen bonds with its partner on the opposite strand only in the staggered conformation. This breakthrough work has been described in several reviews [5•,11,12]. The filaments formed from this peptide system are microns in length and show a high degree of lateral association, resulting in fibril formation. The addition of salt inhibits polymer growth, demonstrating the importance of electrostatic interactions in the design and the ability to modulate the structure in a reversible fashion. More recently, the Woolfson laboratory has focused on controlling the morphology of the self-assembled polymers. They show that the morphology of the fibrils can be controlled using specifically designed peptides to introduce kinks (see image in Figure 1a), branches and cross-links [13,14•,15••,16]. In their 'belt and braces' design work [13], a peptide template was designed (the belt) to recruit two peptide fragments half its size (the braces), which are juxtaposed end-to-end with respect to one another upon binding the template (akin to the design used to create self-replicating peptide systems [17]). This assembly is stabilized by electrostatic interactions (as shown by a strong pH dependence) and is reversible (according to temperature studies). Upon deposition on a surface, braces modified with colloidal gold particles can form nanoscale particles only upon the specific addition of the belt peptide. Woolfson's laboratory has also described a clever synthetic method for introducing kinks and branches into fibrils using covalent attachment to the Nε group of specific lysine residues [14•,15••]. To introduce kinks and waves, a specialized peptide is synthesized on a resin functionalized with lysine in which both amino groups, Nα and Nε, are available for peptide chain extension. The resulting peptide has a unique branch-point near the C-terminus. This peptide, upon mixing with the straight-chain variants described above, introduces kinks in fibrils wherever they are present (Figure 1a). More recently, Woolfson's laboratory has taken advantage of lysine Nε groups for functionalization with biotin or FLAG tags (short peptide sequences that can be used to bind gold-modified anti-FLAG antibodies) in order to impart highly specific smart behavior [18].

(Figure 1 Legend) Structural building blocks for self-assembly. Amino acid residues in the CPK models are colored as follows: hydrophobic, green; acidic, red; basic, blue; glutamines, turquoise; phosphoserine, orange; cysteine, yellow; and others, gray. In addition, some structures indicate positive and negative charges to further highlight basic and acidic residues, respectively. **(a)** Schematic showing a branched peptide (labeled C) interacting with unbranched self-assembling peptides. The branched peptide induces kinks in fibrils, as shown in the uranyl-acetate-stained TEM image [15••]. **(b)** Schematic showing two views of the supramolecular assembly predicted to result from pairwise interactions of peptides; AFM image of a fibril prepared in ammonium sulfate and deposited on a mica surface [9•]. **(c)** Ribbon diagram showing the crystal structure of the coiled-coil form of the amyloid peptide; TEM image of negatively stained mature fibrils derived from this same peptide [21••]. **(d)** Schematic of a helical ribbon; negatively stained TEM image of left-handed helical tape comprising P_{11}-1 [42•]. **(e)** Schematic of self-complementary peptide Lego® assembly; hydrogel image [44•]. **(f)** Hydrogel consisting of cross-linked short fibers (green), with microstructure as determined by laser scanning confocal microscopy [5•]. **(g)** Schematic showing self-assembled nanofiber; negatively stained (phosphotungstic acid) TEM image of self-assembled nanofibers (before covalent capture) [51]. **(h)** Proposed molecular models of cut-away nanotube and nanovesicle, with positively charged residues in blue and hydrophobic residues in green; quick-freeze/deep-etch TEM image of L_6K_2 at ph 7 [59•].

During the past few years, our laboratory has also explored coiled coils as potential biomaterials. Similarly to Woolfson's conception, we imagined that forcing the stagger of helical interactions could foster self-assembly into one-dimensional polymers or filaments. Although we had considered using electrostatic interactions to drive the staggering of helices, akin to efforts by other groups, including Woolfson's, we felt that harnessing the power of the hydrophobic effect to drive folding and self-assembly might improve the robustness of the final biomaterial. We designed and synthesized a derivative of the first two heptads of the GCN4 sequence (CKQLEDKIEELLSK) as our modular unit, duplicated it and joined the two segments with a linker consisting of two alanines (Figure 1b). The consequence of this two-alanine insertion is that the hydrophobic faces of the two-heptad modules are rotated approximately 200° with respect to one another; thus, a peptide containing such a sequence could only assemble in a staggered conformation. This staggered unit would then drive self-assembly of one-dimensional polymers (Figure 1b). A manuscript describing the biophysical characterization of such polymers (including the analysis of polymer size both in solution and on surfaces, using analytical ultracentrifugation and atomic force microscopy [AFM], respectively) is currently in press [9•]. We show that axial and lateral assembly of our polymers is highly sensitive to salt and temperature. The addition of NaCl fosters axial growth of filaments, whereas addition of the divalent anion sulfate appears to foster lateral assembly, leading to fibril formation (see image in Figure 1b). We presume that the divalent anion can act to cross-link solvent-exposed lysines, fostering lateral association. Axial growth in response to added salt is due to the known effects of salt on the hydrophobic effect; we confirmed this by showing that other additives known to enhance the hydrophobic effect, such as glycerol, also foster axial assembly. In addition, we can regulate the size of our polymers by the addition of short peptides containing only the two-heptad module; this peptide serves to cap the available staggered ends in the dimer intermediate, thus limiting axial growth.

Filaments and fibrils using β-strands

The discovery of the cross-β-sheet aggregate form and its role in neurodegenerative disease has been a great boon to our understanding of the molecular basis of these diseases. Much work has focused on providing a detailed structural understanding of peptides and proteins that can form these structures, and important new paradigms in protein folding and misfolding have been elucidated [19,20]. This burst of activity in the study of amyloid fibrils has also prompted several laboratories to explore the possibility of using these types of structures as biomaterials. Towards this goal, recent efforts in the *de novo* design of peptide-based amyloid fibrils have aimed to identify simple sequences that minimally satisfy the requirements of fibril formation [21••,22]. In an elegant study, Lopez de la Paz *et al.* [22] laid out a carefully reasoned strategy for the design of short hexapeptide sequences (i.e. KTVIIE, STVIIE, KTVIIT, KTVLIE) in order to test sequence elements critical for the formation of cross-β-sheet structures and further test how polymeric β-sheets can mature into amyloid fibrils. The ability of these peptides to form polymeric β-sheets can be modulated by pH and salts in a manner that is dictated by the number and positioning of charged amino acid residues. More recently, the same laboratories also characterized how an alternative structure, using a coiled-coil sequence ($SIRELEEX_7RIRELEX_{14}RIG$; Figure 1c), can lead to the formation of amyloid fibrils. The two X positions are alanine or leucine, and mutation of these residues to methionine (which does not affect coiled-coil stability) is shown to foster cross-β-structure formation (Figure 1c). The authors also probed the environmental cues (such as temperature and salt) that can stimulate these two coiled-coil sequences to refold into a β-strand and seed the growth of cross-β-fibrils (Figure 1c) [21••]. These two papers set a high standard for careful structural analysis by combining structural probes (circular dichroism, IR spectroscopy, X-ray fiber diffraction and solid-state NMR spectroscopy) at the atomic scale to establish detailed local interactions, and structural probes at the nanoscale to microscale (electron microscopy, AFM and again X-ray fiber diffraction) to understand how such local interactions are promulgated at the mesoscale.

Other groups have also studied this structural duality [23–25]. For example, Zhang and co-workers [23] studied sequences that form helical and sheet structures by incorporating specific interactions within a peptide sequence that would stabilize both sheet and helix formation. In the design, the pattern of hydrophobic and hydrophilic residues alternates, promoting the formation of a β-strand. To offset the sheet-stabilizing sequence pattern, the hydrophilic residues near the C-terminal end are positively charged, whereas those near the N-terminus are negatively charged, as in $(DA)_4(RA)_4$. This charge arrangement was incorporated to stabilize the helix macrodipole. In these sequences, a preformed β-sheet could be induced to adopt an α-helix upon increasing temperature. Over time, the cooled structure went back to the sheet structure, although very slowly; this may then be assumed to be the lower energy state. Conformational plasticity in response to pH was also investigated.

The structural analysis of silks has been an active area of research for the past two decades [26]. More recently, peptide model systems have been developed to study sequence/structure relationships in the self-assembly of silk. Silk has been of interest in biomaterials research because of its unique mechanical properties in combining exceptional tensile strength with elasticity. Because of these favorable properties, it has been used for medical sutures and, more recently, as a scaffold for tissue

regeneration. Silk fibroin sequences have, for example, been covalently modified with RGD-containing peptides in an attempt to target osteoblast incorporation [27]. Silk-based peptides are highly polymorphic, resulting in interconversion among several different forms, although a primary conversion involves the silk I to silk II transition. This polymorphism can be regulated by wetting, redox conditions and phosphorylation, thus providing potential for smart behavior [28–30]. The sequences, particularly of fibroins A and C, are dominated by GA repeats and the most common motifs are GAGAGS (in fibroin C) and GAGAGY (in fibroin A); the ratio of these motifs in a given fibroin appears to play a role in whether the structures are crystalline or amorphous [31]. Several detailed structural analyses of simplified peptides, largely from Asakura's group [32•,33–35], have been carried out to explore sequence/structure relationships, with their most recent paper focusing on the importance of the total number of serines and tyrosines in peptides containing the GA motifs in regulating the silk I to silk II conversion [32•]. Peptides containing only a single serine or tyrosine favor the more amorphous silk I form, whereas multiple insertions of these two residues foster the conversion to the more crystalline or silk II form, which is dominated by β-sheets.

Some exciting examples of the functionalization of fibrils made up of cross-β-sheets have recently been published [36,37••]. Notably, metallation of peptide-based amyloid fibers has been described, involving diphenylalanine motifs known to form cross-β-sheets that self-assemble into nanotubes. These nanotubes were shown to be hollow by their ability to deposit ionic silver within the tubes. This exciting finding suggests that peptides may be used as casts for forming metal wires. The casts can be removed by using proteases that would degrade the peptides. It is interesting to imagine developing peptide sequences that can be selectively degraded to expose only certain portions of a nanowire, thus providing points of attachment for other wires in order to develop circuitry patterns. Such efforts will require the incorporation of smart behavior, as the selective incorporation of different peptide sequences will require strictly controlled self-assembly processes. In another metallation approach, a histidine-rich peptide, AHHAHHAAD, was immobilized on a heptane dicarboxylate nanowire surface [38]. Gold ions were then added and the assembly incubated, to provide a gold-coated wire after $NaBH_4$ reduction, as visualized by transmission electron microscopy (TEM).

Hydrogels and organogels

Gels as smart materials are of particular interest because of their responsiveness to a wide variety of chemical and physical triggers. The gel-to-fluid transition can be manipulated by the hydrogen bond donor strength of the solvent, pH, temperature and salt [39]. K24 (KLEALYVLGFFGFFTLGIMLSYIR), a peptide related to a naturally occurring transmembrane domain sequence derived from the protein IsK, forms gels in alcohol solvents. In methanol, K24 forms a transparent thermostable gel. It was shown by TEM that K24 consists of tape-like structures in which the width of the tape is comparable to that of the peptide in a β-strand conformation. In hexafluorisopropanol, however, K24 adopts a mixture of random coil and β-sheet, providing a low viscosity gel. A series of *de novo* designed glutamine-rich sequences was also explored for its gel-forming properties [40,41]. The 11-residue glutamine-rich sequences incorporate arginine and glutamic acid at positions 3 and 9, as in Ac-QQRQQQQQEQQ-$CONH_2$, known as P_{11}-1 (Figure 1d), forming antiparallel β-sheet tapes that are similar in structure to those formed by K24, in which the charged residues provide complementary electrostatic interactions between adjacent strands. At low concentrations, P_{11}-1 is predominantly random coil. At higher concentrations (~0.01 mM), it forms semi-flexible tapes with the width expected of an 11-mer in a β-strand conformation (Figure 1d). The tape adopts a left-handed twist as a consequence of the intrinsic chirality of the monomer building block [42•]. At higher concentrations (1 mM), the tapes self-associate to form ribbons (double tapes). There appears to be no further association of these ribbons into fibrils, even up to peptide concentrations of 25 mM. To promote interactions between tapes, the sequence was redesigned to include two phenylalanines and one tryptophan, generating Ac-QQRFQWQFEQQ-$CONH_2$, known as P_{11}-2 (Figure 1d). The hydrophobic residues form 'adhesive' stripes running along one face of the tape. This peptide forms ribbons at 0.1 mM concentration and fibrils at 0.6 mM. At yet higher concentrations, fibrils become entwined to form fibers. The degree of tape association and the specific properties of the gels generated by these and similar sequences can be tuned by the solvent used, and by manipulating the pH and ionic strength of the solvent [39,40]. Interestingly, a 'flattened' tape structure of P_{11}-2, dissolved in a mixture of 10% water in isopropanol at pH 5.5, was generated on a mica substrate [43•]. The tapes, with the hydrophobic phenylalanine and tryptophan residues facing the solvent, extend as monolayers across the surface. The tapes do not grow over each other, but instead come to a halt upon contact with another tape. Dried films generate different aggregate morphologies; one type consists of a closely packed monolayer of parallel-aligned tapes.

Tissue engineering

Zhang and co-workers have developed a series of peptides that form stable hydrogels at low peptide concentrations (0.1–1%) (reviewed in [44•,45•]). They are characterized by an alternating sequence of hydrophobic and hydrophilic residues, in which the hydrophilic residues, in turn, alternate between being positively and negatively charged, such as in (KLD)n, (EAKA)n and (RADA)n (Figure 1e). The alternation between polar and

non-polar residues promotes the formation of a β-strand building block with hydrophobic and hydrophilic faces. The blocks stack with the charged sidechains facing each other in a complementary pegs-into-holes ('Lego®-like') manner, as shown in the model in Figure 1e [44•,45•]. Variations have been made to the sequence to increase the size of the 'pegs' and the 'holes', as in RARADADA (Figure 1e) and RARARADADADA. The self-assembly process to produce a hydrogel can be triggered rapidly when the ionic strength exceeds a certain threshold or the pH is adjusted to provide a zero net charge on the peptide. These types of peptides have been shown to be non-cytotoxic and of potential use in the repair of cartilage tissue. Chondrocytes were encapsulated within the hydrogel scaffold produced by the peptide Ac-$(KLDL)_3$-$CONH_2$ [46]. The scaffold was shown to maintain differentiated chondrocytes, and to stimulate the synthesis and accumulation of extracellular matrix.

A 20-residue peptide that forms a pH-responsive self-assembling β-hairpin (Figure 1f) has been *de novo* designed by Schneider *et al.* [47]. The sequence consists of alternating hydrophobic (valine) and hydrophilic (lysine) residues of high β-sheet propensity flanking a four-residue segment incorporated to promote the formation of a type II′ turn, as in $(VK)_4$-V-^{D}PPT-$(KV)_4$. The folding of the β-hairpin is fully reversible. At lower pH, charge repulsion between the positively charged lysine residues provides a low-viscosity solution containing random-coil peptides. A change to basic pH (9) triggers the formation of a β-hairpin, which self-assembles to form a rigid gel [47]. The gel is composed of a network of ~3 nm wide (the width of the hairpin) and 10–200 nm long branched fibrils, which are proposed to consist of two layers of β-hairpins interacting face-to-face through hydrophobic interactions and further interacting in the fibril-growing direction through hydrogen bonding [5•,47]. The network is branched because of the presence of intersecting fibrils. The non-covalent nature of the hydrogel makes it responsive to mechanical stress, from which the gel strength quickly recovers after shearing [47]. Gel formation can also be triggered by the addition of salt [48]. At neutral pH and low ionic strength, a peptide solution (≤2 weight %) exhibits low viscosity. By raising the ionic strength of the medium, charge repulsion between the positively charged lysine residues is screened by chloride ions, leading to β-hairpin and gel formation. Additional elastic properties of the gel can be tuned by yet higher salt concentration, leading to faster gel formation and a stiffer gel, presumably due to the formation of a more highly cross-linked network. The rate of gel formation can also be tuned by temperature [48,49]. For example, at physiological conditions (pH 7.4 and 150 mM salt), β-hairpin folding is suppressed at 10 °C, slow at 20 °C and instantaneous at 37 °C, which is of particular interest for *in vivo* applications. The construct has been shown to be non-toxic and cytocompatible [50].

Surfactants

Biomineralization

Self-assembled peptide templates can be used to nucleate and guide the growth of minerals. Stupp and co-workers [51] designed a surfactant peptide hybrid that self-assembles to form cylindrical micelles, 6–8 nm in diameter, that are able to direct the mineralization of hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, a primary component of bone, onto its surface. The peptide monomer unit contains an N-terminal hydrophobic C16 hydrocarbon chain, a phosphoserine residue and an RGD cell-recognition tag (C16-C_4G_3-$S^{(P)}$-RGD; Figure 1g). The formation of the self-assembly is reversible. At low pH (4), the acidic peptide self-assembles and at higher pH it disassembles due to charge repulsion; thus, pH-sensitive self-assembly provides a means for controlled mineralization. Further control is incorporated into the peptide sequence through a segment of four adjacent cysteine residues, which can be used to covalently capture the self-assembled structure upon oxidation. The cross-linking is reversible, as the resulting disulfide bonds can be readily reduced.

Such cross-linked peptide amphiphiles provide highly robust and pH-stable micellar fibers (Figure 1g, model) that have been shown to be suitable for other mineralization reactions. In an interesting application, Cd^{2+} could be sequestered by the negatively charged cylindrical fibers, leading to the growth of CdS semiconductor nanocrystals [52•].

Tissue engineering

Self-assembling peptides are being developed as scaffolds for tissue regeneration purposes, including cartilage repair and promotion of nerve cell growth [53]. A major benefit of synthetic materials is that they minimize the risk of biological contamination. Self-assembling peptides also frequently show favorable properties concerning biocompatibility, immunogenicity and biodegradability, producing non-toxic waste products. The amphiphilic peptide construct discussed above, containing a long hydrophobic tail linked to a cell-recognizing tag, can be customized for specific cell response by tailoring the sequence of the tag. Laminin is an extracellular matrix protein that influences neurite outgrowth. A peptide amphiphile shown to promote the re-growth of nerve cells in rats was made by including a neurite-promoting laminin epitope tag, IKVAV (C16-G_3A_4-IKVAV) [54•]. Another construct, containing a heparin-binding site, shows very exciting preliminary results in being able to promote angiogenesis, the growth of blood vessels [55]. These types of peptide amphiphiles have been further modified with biotin [56] and a Gd^{3+} metal-chelating moiety suitable for detection by magnetic resonance imaging (MRI) [57].

Gene delivery

Synthetic carriers, including peptide-based delivery systems, are being developed in several laboratories as

promising alternatives to using inactivated viruses as gene vectors. Peptide-based vectors are particularly amenable to rational design and development in this field due to their exceptional adaptability [58]. Zhang *et al*. developed a series of surfactant peptides comprising a hydrophobic tail attached to a polar headgroup consisting of one to two positively charged residues at the C- or N-terminus, one example being LLLLLLKK (Figure 1h). These peptides self-assemble in water to produce nanovesicles and nanotubes [59•]. As reported in a *Science News* commentary [60] and in recent review articles [45•,61], these peptides have been used as DNA delivery vehicles. When placed in a solution of DNA, the positively charged peptides self-assembled into a tube, encapsulating the negatively charged DNA. This 'minivan' was then able, at least in some cases, to deliver the DNA to growing cells. These systems may be viewed as smart materials, as the minivan surface can be tagged with a marker that is specific to a particular cell type [60].

Patterning

Tagging of self-assembling peptides has many applications in surface engineering. Examples from Woolfson's laboratory have been described above. Tagged peptides that can recognize a particular cell type and that can also self-organize on a surface to form self-assembled monolayers (SAMs) have been used in pattern formation (reviewed in [62]). As an example, a series of peptides containing a cell adhesion motif, $(RADS)_n$, were anchored to a gold surface via an oligo(alanine) linker with a C-terminal cysteine residue. Cells recognizing the RADS sequence motif then adhered to the surface to provide unique patterns. The method employs microcontact printing, whereby a gold substrate is first 'ink stamped' with a different thiol-containing peptide (EG_6SH) to block portions of the surface [62]. The stamp provides a specific pattern because EG_6SH is only attached to the elevated portions of the stamp. The final pattern is then formed by applying the $(RADS)_n$-$(A)_m$-C peptide, which forms SAMs on the remaining areas of the gold surface, followed by binding of the RADS-recognizing cells.

Other peptide hybrid materials

The functionality imparted by dendrimer–peptide hybrids has been exploited for potential biomedical applications, such as diagnostics and drug delivery, particularly in anti-cancer therapies. For the most part, such peptides have been selected for sequence functionality only, with little consideration of structural aspects. For example, sequences rich in glutamates or lysines, or sequences recognized by antibodies have been incorporated for drug-targeting purposes; such applications have been reviewed [63]. Recently, peptides have been incorporated into dendrimers in which the structure of the peptide plays a critical role in regulating the heterogeneity, size and higher order shape of supramolecular structures involving many dendrimer units [64•,65]. One notable example of a dendrimer in which peptide structure is important comes from recent work in Ghosh's laboratory [64•]. They have found that sequences based on coiled coils originally made and studied by the Hodges [66] and Kim [67] groups could be modified with cysteines and subsequently attached to a PAMAM core functionalized with maleimides. These dendrimers can self-assemble into fibrillar structures, dependent upon coiled-coil heterodimer formation between oppositely charged peptides on different dendrimer units. The coiled-coil portions of these materials are pH and salt dependent, thus imparting semi-smart behavior.

Peptides have been used extensively in other hybrid structures, containing different types of synthetic polymers, to create hydrogels (as described above). Recent exciting work on PEG-ylated peptides, designed to form either β-sheets [68] or α-helices [69,70], recognizes the potential of incorporating peptides specifically for the purpose of directing structure. The groups involved in this work show that the peptides retain their ability to fold properly in the presence of PEG, and that the structure present in the peptide dominates the properties and responsiveness of the resultant hybrid biomaterial; this, and related, work has been recently reviewed [71].

Conclusions and perspectives

Peptides as self-assembling smart materials clearly have enormous potential. Sequence manipulations enable the specific fabrication of an astonishing number of different structures that can be developed for many important applications, including tissue repair, patterning, miniaturized solar cells, and optical and electronic devices. Although nascent efforts have begun to explore the question of how to build smartness into peptides, as described above, there is still much room for incorporating exquisite sensitivity and specificity into peptide biomaterials. Finding specific means to incorporate responsiveness into materials is a particularly exciting emerging area. Chemical strategies to build photosensitive switches into peptides as modulators of structure have been pioneered by Woolley *et al*. [72••,73–80]. They have shown that the inclusion of various azobenzene derivatives as cross-linkers of amino acid residues at well-defined positions can modulate helix formation upon irradiation with light of specific frequencies [75–77]. This concept has been applied to the regulation of tertiary structures, including coiled coils [80], and also provides a means for studying the kinetics of protein folding [72••,74]. Inclusion of such a switch in biomaterials designs would be useful in creating photoresponsive materials.

In our laboratory, a short 10-residue peptide has been modified with two porphyrin moieties, spaced i, $i + 4$, to provide a helical construct that forms extended organized arrays in which the chromophores engage in exciton coupling [81•]. The self-assembly can be controlled by

concentration, temperature and pH. We envision that these types of assemblies may be useful for the construction of conducting nanowires and light-harvesting devices. Inclusion of porphyrins, whether through covalent or non-covalent means, in peptide structures is not new [82–85], but we believe that our work is the first example of a peptide–porphyrin hybrid that directs the self-assembly of organized supramolecular structures, as indicated by the symmetrical couplet observed in CD spectra of the growing complex.

Self-assembly of peptides has been used as a driving force for chemical catalysis in amide bond formation, resulting in self-replication behavior [17,86]. This work has been pioneered by the laboratories of Chmielewski [87,88] and Ghadiri [89]. It is clear that self-replication will offer unique opportunities to modulate the properties of peptide biomaterials, as also envisioned by Woolfson [13].

Recent efforts also include the construction of chemical reactors, in which molecules are encapsulated within a cage to provide a platform for carrying out specific reactions [90••]. Encapsulation by a self-assembled peptide cage holds particular promise in the delivery of biological molecules, including DNA, as discussed above, water-insoluble drugs and tagged molecules for imaging. The non-covalently assembled vehicle could also contain a specific recognition tag for degradation or simply be able to degrade on its own through the manipulation of the surrounding environment.

Finally, an exciting emerging field is the development of molecular machines or robots that can be turned on and off in response to a signal. Most of this work has, thus far, been carried out on large proteins or small interlocked catenane- and rotaxane-based organic molecules (for a recent review, see [91••]).

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