Biomimetic peptide self-assembly for functional materials

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Abstract | Biomolecular systems have evolved to form a rich variety of supramolecular materials and machinery fundamental to cellular function. The assembly of these structures commonly involves interactions between specific molecular building blocks, a strategy that can also be replicated in an artificial setting to prepare functional materials. The self-assembly of synthetic biomimetic peptides allows us to explore chemical and sequence space beyond that used routinely by biology. In this Review, we discuss recent conceptual and experimental advances in self-assembly of artificial peptidic materials. In particular, we explore how naturally-occurring structures and phenomena have inspired the development of functional biomimetic materials that we can harness for potential interactions with biological systems. As our fundamental understanding of peptide self-assembly evolves, increasingly sophisticated materials and applications emerge and lead to the development of a new set of building blocks and assembly principles relevant to materials science, molecular biology, nanotechnology and precision medicine.

[H1] Introduction

Self-assembly in biological systems allows individual macromolecules to assemble into a wide set of supramolecular structures and architectures. In this manner, nature capitalizes on self-assembly to convert chemically-simple building blocks into sophisticated materials and structures that function cooperatively in living systems.[1-3] The molecular interactions

governing the formation of such systems are predominantly non-covalent, a key feature that determines their micro and macro-scale properties.[4] In particular, the reversibility of the interactions confers dynamism on the molecular architectures, which can modulate their properties and confer an ability to respond to external stimuli.[5] In nature, a particularly diverse class of self-assembling materials are formed from proteins.[6] For instance, cellular motility and traction to surfaces are largely controlled through the self-assembly of cytoskeletal proteins.[7] These proteins reversibly self-assemble to enable highly regulated extension and contraction of cells, thus allowing their movement. Moreover, networks of such protein assemblies generate force for a wide range of active processes, including cell migration, movement of endocytic vesicles and other membrane-bound organelles within cells along with intercellular transport of certain bacterial and viral pathogens.[1,8,9]

Much progress has been made in understanding the fundamental principles that govern native protein self-assembly processes by studying naturally-occurring building blocks.[10-15] A complementary approach is to use synthetic chemistry to explore the chemical space beyond that available to natural molecular building blocks. This has primarily been achieved by a bottom-up approach, whereby building blocks are designed to assemble into specific architectures with desired properties.[16] In natural systems, self-assembly benefits from the evolutionary processes that tune interactions to optimize the properties, morphology and functionality of the resulting biomaterials. Through evolution, nature exploits a narrow set of elementary motifs, including the α-helix and the β-sheet secondary structure of proteins and their complexes, to hierarchically assemble a remarkably complex set of structures.[17]

Even though nature commonly uses protein sequences of up to several hundred amino acid residues as building blocks of functional materials, substantially shorter sequences can also exhibit highly sophisticated self-assembly behaviour.[18,19] In this context, biomimetic peptide-based motifs, including peptide amphiphiles, lipopeptides and conjugates with other organic and inorganic molecules, exemplify how design and chemistry can be successfully employed to generate multifunctional molecules that assemble predictably and interact with specific biological ligands. The recent emergence of the field of peptide biomimetics, which combines principles from disciplines including biology, chemistry and engineering, allows the preparation of synthetic materials with functions similar to or surpassing those of natural products.[20,21,247]

Bio-inspired peptides, especially short peptide building blocks, are minimal recognition modules used to mediate and facilitate processes of molecular recognition and self-assembly.[22,23] The synthesis and chemical modification of these building blocks are facile and they can assemble with remarkable efficiency into biocompatible and controllably biodegradable materials. Further, the extraordinary and often surprising chemical, physical and mechanical properties of these structures make them ideal for a wide range of applications while opening new facets of molecular recognition, self-assembly and phase organization of these nanostructures.

In this Review, we explore recent advances in biomimetic peptide self-assembly and discuss the properties and applications of the resulting materials, all the while comparing these to their more established protein-based counterparts.[24-26] We describe diverse applications of peptide-based systems and nanostructures, including: surface modification, production of scaffolds for inorganic ultra-structures, generation of 3D hydrogel scaffolds for tissue engineering, drug delivery, production of new antimicrobial agents and active materials, and mimicking hierarchical self-assembly in protein misfolding disorders (Fig. 1).

[H1] Peptide assembly into amyloid-like nanofibrils

Linear fibrils are common units from which supramolecular materials can be formed. Many of these β-sheet rich units play functional or pathological roles in nature. This fibrillar self-assembly behaviour has been reproduced abiotically with diphenylalanine (FF), an archetypical model for elementary self-assembling units (Box 1). Studying this di-homopeptide and its analogues (such as the *tert*-butoxycarbonyl (Boc) derivative) has generated key insights into the nucleation and oligomerization pathways, as well as the physical properties of the resulting amyloid and amyloid-like fibrils.[27,28] Furthermore, through their dynamic self-assembly behaviour, fibrils derived from FF have been used to generate forces of a similar order of magnitude as those of complex biological systems and synthetic polymers.[29]

Short peptides that assemble into amyloid-like supramolecular structures can be used as drug delivery systems either in the form of drugs that can themselves form fibrils,[30] or by conjugating the drug molecules to a sequence that forms β-sheets.[31] In this way, the monomeric drug units are slowly released as the ordered fibrils dissassemble.[32] Another

promising delivery strategy uses short peptides as gel matrices to encapsulate drug molecules and then enable their sustained release.[33,34] One such molecule is the dipeptide FΔF, which contains an α,β-dehydrophenylalanine (ΔF) residue and assembles into hydrogels consisting of a network of amyloid-like fibrils[34] that traps and releases various structurally unrelated drug-like molecules. The potential of the FF motif in the formation of drug-releasing hydrogels has been further studied using the 9-fluorenylmethyloxycarbonyl (Fmoc)-protected monomer Fmoc-FF-KGM.[35] Here, self-assembly is driven by the Fmoc-FF motif, which forms peptide nanofibres interpenetrating and interwoven with KGM chains. This product has greater stability and mechanical strength than the hydrogel formed from Fmoc-FF alone.

Longer amino acid sequences can exhibit higher complexity in their self-assembly behaviour. Thus, a peptide FFKLVFF, inspired by the amphiphilic KLVFF core section of Aβ(16–20) fibrils,[36] self-assembles in MeOH.[37] This process is likely to be influenced by interactions between the Ph sidechains of F residues, which are also responsible for its low solubility in H_2O. This solubility can be increased by appending FFKLVFF with polyethyleneglycol (PEG), whence cylindrical fibrils containing a peptide core and PEG corona can form in aqueous solution.[38] As longer PEG chains are used, hydration of these hydrophilic groups appears to influence self-assembly to a greater degree than the hydrophobic/aromatic stacking interactions of the F residues.[39,40]

Along with aromatic peptides, a class of tri- to hexapeptides with a characteristic sequential motif simulate the process of fibre assembly and further condense to give amyloid fibrils.[41] These peptides consist of an aliphatic amino acid tail capped by a polar head that self-assemble first into an α-helical intermediate before converting into cross-β amyloid fibrils. This class of aliphatic peptides have further been compared with natural amyloid core sequences including Aβ, human amylin and calcitonin.[42] The designed aliphatic peptides self-assemble in a similar way to several natural sequences: through α-helical intermediates. Peptides containing the FF motif directly form β-sheet aggregates without going through α-helical intermediates.

In addition to the key role of the peptide sequence in driving self-assembly, the environment in which it takes place can also affect the final structure. Even small changes in humidity[43] or O_2 levels[44] during assembly can have a distinct influence on the structures formed. The self-assembly of FF dipeptides under various solution conditions has been particularly well

explored. Although FF self-assembles into fibrillar structures in both H_2O and MeOH, the crystal structures and properties of the products differ greatly.[45] The FF-NH_2 peptide, which exists in its cationic ammonium form in solution, self-assembles into fibrillar structures that reversibly transition into spheres on dilution.[46] These spheres have been shown to facilitate nucleotide delivery.[47]

A particularly promising aspect of peptide self-assembly is our ability to control the formation of supramolecular structures by changing environmental conditions such as pH. For example, by adjusting the pH and concentration of aqueous peptide P_{11}-4 (Ac-QQEFQWQFRQQ-NH_2), one can manipulate an equilibrium between a nematic gel and an isotropic fluid phase[48]. This responsive behaviour is governed by the choice of amino acid side chains that enable hierarchical assembly of β-sheets through chemical and structural complementarity. Similarly, the role of electric charge in peptide self-assembly has been probed by designing and synthesising the oppositely-charged amyloid-inspired sequences Ac-EFFAAE-NH_2 (AIP-1) and Ac-KFFAAK-NH_2 (AIP-2), both of which self-assemble into amyloid-like nanofibrils at neutral pH.[49] Surfaces can also play a role in directing peptide self-assembly. Studies with QQEFQWQFRQQ (P_{11}) conducted in the presence and absence of mica/highly-oriented pyrolytic graphite substrates show differences in self-assembly kinetics and product morphologies.[49,50] The properties of the short peptides described in this section are summarized in Table 1.

[H1] 3D peptide matrices as cell culture scaffolds

The biological extracellular matrix (ECM) serves as the main inspiration of engineered tissue scaffolds that can support and sustain cells within a 3D matrix (Box 2). Such biomimetic scaffolds enable cell binding and provide mechanical support by featuring a cell adhesion peptide (CAP), a minimal amino acid motif that promotes cell migration, differentiation and organisation through the interactions of cells with the matrix.[58] CAPs are key enablers of cell–matrix interactions, while the 3D nature of the material provides mechanical support for cell proliferation. Such peptide-based matrices must resist tensile forces acting on tissue and are thus required to mimic the properties of fibrillar assemblies of proteins such as collagens and glycosaminoglycans. These supramolecular fibrillar networks must further be able to be deposited, remodelled and degraded as cells grow into ordered tissues, thus presenting additional challenges to their generation.[59]

The needs for both biocompatibility and structural stability have motivated two decades of investigations into polypeptide matrices and gels that allow cell proliferation.[23,24] However, the self-assembly of short peptides can afford more diverse scaffolds that may offer optimal environments for different cell types. Control of composition, scaffold porosity and rigidity, along with the incorporation of growth factors, have now allowed further advances in cell culture viability and improved tissue regeneration. Furthermore, self-assembled β-sheet matrices are stable across wide temperature and pH ranges and can resist high concentrations of denaturing agents such as urea and guanidium hydrochloride.[23,60]

One route to robust hydrogels uses structurally well-defined peptides coupled to carbohydrate moieties.[61] These can be prepared through in vitro peptide glycosylation reactions, which enable systematic modifications to produce supramolecular hydrogels with diverse self-assembly behaviours. The glycopeptide-derived gels exhibit greater thermostability and biostability relative to the parent peptide gels.[62] In this way, the glycopeptide-derived gels can have high H_2O content and similar structural morphology and composition to the ECM in tissue, all the while exhibiting great potential as new biomimetic scaffolds for mammalian cell growth (Fig. 2b,c).[63]

The major component of the ECM is collagen, whose multi-scale hierarchical self-assembly we wish to replicate because of its potential biomedical applications in tissue engineering. Although many approaches to mimicking collagen self-assembly with synthetic peptide systems exist, until recently none of these systems simultaneously demonstrated all the different levels of structural assembly. This issue has been resolved using a peptide featuring collagen's characteristic Pro-hydroxyproline-Gly repeating unit, as well as salt bridges and H-bonds between Lys and Asp residues,[64] which can assemble into hierarchical nanofibres of several hundred nanometres in length with characteristic triple-helical packing.[65]

Amyloid-like peptide fibrils have recently been used to generate nanoscale biomaterials promoting cell adhesion and differentiation in vitro. The well-established cell adhesion motif Arg-Gly-Asp (RGD) can be conjugated to an 11-residue peptide corresponding to residues 105–115 of the amyloidogenic protein transthyretin (TTR1) to promote specific cell–fibril interactions.[66] Similarly, the hen eggwhite lysozyme peptide containing the tripeptide DGR, which is analogous to the integrin-binding RGD sequence, self-assembles into fibrillar networks that communicate force and signals between the ECM and cells.[67] More recently,

other synthetic β-sheet-containing fibrous meshes have been shown to promote cell adhesion and proliferation.[68] In a similar manner, Fmoc-protected α-synuclein[69] and β-amyloid-derived short peptides[70] self-assemble into hydrogels composed of nanofibrils that promote stem cell adhesion and differentiation. These results strongly suggest that functionalized amyloid-derived fibrils have real potential as components in novel biomimetic materials or as tools to probe and exploit fundamental biological processes and cell behaviour.

As with the glycopeptides describe above, incorporating peptide amphiphiles into hydrogel-forming networks can both promote cell viability and allow release of growth factors and be used for therapeutic applications(Fig. 2d–f). Nanofibrous matrices composed of two different self-assembling peptide amphiphiles have been designed to serve as a coating for cardiovascular implants.[71] The nanofibrous matrix exhibits initial adhesion and proliferation of endothelial cells, while limiting the proliferation of smooth muscle cells and the adhesion of platelets. These characteristics are essential in promoting re-endothelialization, thus increasing the potential of this matrix for cardiovascular applications. Similarly, a nanofibrous network prepared from a heparin mimetic peptide amphiphile (HM-PA) is a promising platform for pancreatic islet transplantation as a potential treatment for type 1 diabetes.[72]

In related work, a biomimetic peptide amphiphile derived from the extracellular glycoprotein tenascin-C promotes neurite outgrowth[73] by self-assembling into highly aligned supramolecular nanofibrils. Such peptide amphiphiles also increase the length and number of neurites extending from neurons differentiated from encapsulated cells. These bioactive gels could serve as artificial matrices that are delivered to regions of neuronal loss to guide neural stem cells and promote, through biochemical cues, neurite extension after differentiation. More recently, peptide amphiphile–DNA conjugates have been shown to reversibly self-assemble into hydrogels.[74] By controlling this dynamic supramolecular system's stiffness, changes in the architecture of the fibrous hydrogel networks can modulate important phenotypic transformations of neural cells in contact with these materials.

FF motifs have led to promising results in tissue engineering when incorporated with the RGD motif to facilitate cell growth and proliferation.[75] More complex cultures with multiple cell lines have further been studied with a scaffold assembled from the longer peptide Ac-ILVAGK-NH_2.[76] Incubated on this scaffold, human H1 embryonic stem cells proliferate into 3D spheroids while continuing to express various pluripotent nuclear transcription factors and

surface biomarkers. Furthermore, multicellular constructs with human umbilical vein endothelial cells, fibroblasts and keratinocytes can be used as a skin model.

The propensity of short Fmoc-protected peptides to produce rigid biocompatible gels has been studied in detail with varying degrees of success[77-79]. The two dipeptides Fmoc-3F-Phe-Arg and Fmoc-3F-Phe-Asp co-assemble into nanofibril hydrogels. The display of Arg and Asp residues at the nanofibril surface effectively mimics the integrin-binding RGD peptide of fibronectin without the need for covalent interactions, thereby supporting the viability and growth of fibroblasts.[80] This system forms a gel remarkably quickly and promote adhesion of fibroblasts through specific RGD–integrin binding, thus providing a model 3D scaffold enabling culturing with anchor points for cell spreading and proliferation.[81]

Artificial scaffolds, even those based on biopolymers, can still sometime offer only a sub-optimal adhesion and proliferation environment for all cell types. The scaffold needs to exhibit the necessary physicochemical properties — porosity, rigidity and elasticity — at the composition required to promote the viability of specific cells. Peptide-based scaffolds can mimic microenvironments in the ECM to organize cells into different types of tissues. There is growing interest in minimal self-assembled peptides and amino acids because they can afford hydrogel networks for tissue engineering and surgical applications due to their ability to undergo controlled sol-gel transitions, making them ideal injectable materials.[266] Indeed, we described above how the hierarchical self-assembly of basic peptide building blocks into final β-sheet-rich matrices affords 3D hydrogels with a fibrillar network that serves as a scaffold for cell growth. We now end our discussion on ECM models (Table 2) and describe the use of peptides to stabilize interfaces.

[H1] Peptides and their assemblies stabilize interfaces

A biological membrane composed of a lipid bilayer acts as a barrier to separate and protect a cell and its components from extracellular conditions and components, including ions, metabolites and pathogens. Along with the lipids forming the interface between the intra- and extracellular environments, specific proteins are incorporated into the membrane, thus controlling permeability and interactions between the cell and its environment. These proteins manage a wide range of biological processes such as active transport, signalling and energy

dissipation, thereby allowing for controlled compartmentalization, which contributes to the proper function of their cellular machinery.

Over the past few years, new approaches to mimic cell surfaces have emerged, in part motivated by the prospect of biocompatible and bioactive drug delivery systems, as well as for directed targeting (Fig. 3a,b). For example, self-assembling surfactant-like peptides are new alternatives to synthetic surfactants obtained from petrochemical sources.[88] Other applications of surfactant-like peptides stem from their antimicrobial activity based on micellar concentration and balanced amphiphilicity, consistent with their propensity for self-assembly and membrane lysis (Fig. 3c).[89] Furthermore, these peptides can self-assemble at fluid interfaces to give cohesive films that stabilize foams and emulsions in applications where renewability, biocompatibility or added functionality may be desired. Sinapultide is the HOAc salt of KLLLLKLLLLKLLLLKLLLLK (KL4) and represents the first peptide-based replacement for the human lung surfactant protein B in pulmonary surfactant therapies approved for clinical use.[90] The penta-residue repeat of KL4 leads to adaptive peptide helicity and variation with partitioning depth, and its effectiveness suggests that structural plasticity may represent an important mechanism for differential lipid trafficking at air–H_2O interfaces. More recently, a minimalistic approach to the design and synthesis of rigid helical peptides has afforded materials with the highest long-term stability among known peptide-based emulsifiers.[91] These peptide emulsifiers are composed of seven residues that mimic the rigid conformation of hydrophobins to afford stable oil–H_2O emulsions[92], the viscoelasticity of which can be high at relatively high peptide concentrations.

Related to our discussion on surfactants is the recent development of polymeric systems, not least amphiphilic block copolymers, that mimic biological membranes (Fig. 3d).[93] Thus, copolymerization of natural and modified N-carboxy anhydrides (NCAs) alone or coupled with synthetic monomers enables the synthesis of an almost unlimited number of supramolecular structures.[94] In particular, separate studies considered how poly(Glu)[95] and poly(Leu)[96] diblock copolypeptides self-assemble in aqueous solution into vesicles known as peptosomes. In these systems, the hydrophilic block can form a well-defined α-helix whose hydrodynamic radius can be modified through varying the solution pH.

Bacterial lipopeptides are cyclic peptides containing a single fatty acyl chain. Such lipopeptides are secreted into growth media by a number of different microorganisms and are

thought to play a role in bacterial swarming motility on semisolid surfaces, as well as in the formation of structured biofilms on solid surfaces.[97] Lipopeptide amphiphiles are an important class of biomimetic surfactants readily synthesized from commercially-available organics such as natural fatty and amino acids. In many cases, these amphiphiles can increase the rigidity of not only common organic solvents but also waxes, H_2O and ionic liquids, and can thus form hydrogels.[98]

Aside from the polymeric peptides described above, amphiphilic behaviour is also observed for short sequences in which a head group features charged residues and the tail group neutral ones. These surfactants are facially amphiphilic molecules that self-assemble at fluid interfaces to give cohesive films that stabilize foams and emulsions. Hydrophobic interactions between the amphiphilic peptides, along with interstrand H-bonds, are the main driving forces for self-assembly.[99] These interactions afford high-aspect-ratio structures such as ribbons, nanotubes, nanofibres and nanorods. Yet, a change in solution conditions can destabilize the interfacial film, leading to rapid foam or emulsion collapse.[99] Surfactant-like peptides composed of Ala residues as the tail group tend to form the most stable structures because it engages in very strong hydrophobic interactions.[100] The self-assembly of a cationic peptide A_6R that consists of six consecutive hydrophobic Ala residues as a tail group with a cationic Arg head group affords ultrathin sheets at low concentrations. At higher concentrations, the sheets first form helical ribbons that mature into nanotubes with an antiparallel arrangement of β-sheets that minimises electrostatic repulsion between the Arg head groups.[101] In contrast, the oligopeptide $A_{12}R_2$ is double as long and instead self-assembles into twisted fibres[102]. A similar system, A_6K, forms lipid-like peptide nanovesicles enabling drug delivery.[103] Furthermore, a simple amphiphilic decapeptide, with a phosphorylated Ser head located within a β-hairpin segment and linked to two hydrophobic tails has recently been described.[104] This phospholipid-inspired peptide self-assembles into semi-elliptical nanosheets incorporating the FF motif, known to facilitate self-assembly and structure stability, as well as a β-hairpin for forming a hydrophilic phosphorylated head. The resulting bilayer crystal structure features interactions along all three axes: aromatic π–π interactions, H-bonding and β-sheet formation.[105] Thus, this demonstrates the capacity of peptides to mimic self-assembly in nature and gives us more information to help predict the intermolecular interactions in future oligopeptide designs.

Biomimetic peptides have yet to be widely used as membranes and surfactants thus far but recent developments may facilitate the incorporation of these molecules into industrial and consumer products in the near future. This approach has recently allowed the conjugation of peptides onto stem cell membranes without affecting cell viability, proliferation or multipotency.[106] The systematic exploration of synthetic, genetically-engineered peptides produced by conventional methodologies may afford a class of biomolecules that are superior to polymer-based materials.

[H1] Self-assembled peptide antimicrobial agents

In the previous section, we discussed several mechanisms by which peptide-based assemblies self-organize at surfaces and stabilize interfaces. However, the phenomenon of peptide self-assembly and the resulting structures can also destabilize interfaces, including those forming biological membranes. This has increasingly been explored in the context of the development of new antimicrobial agents to combat the rise of multidrug-resistant bacteria.[107] Antimicrobial peptides (AMPs), a growing class of natural and synthetic peptides active towards a large spectrum of microorganisms, provide a potential source of such agents.[108-110]

Endogenous AMPs represent the innate immune system's first line of defence against pathogenic microbes. Produced by organisms found among all classes of life,[111,112] such peptides comprise a unique and diverse group of molecules formed by sequences generally shorter than 50 amino acids, sharing a net positive charge and containing a high fraction of hydrophobic residues. [113,114] This amino acid sequence contributes to the amphipathicity and cationic nature of AMPs that allow them to partition into the anionic bacterial lipid bilayer membranes. This important feature of antimicrobial peptides can enable membrane permeation, depolarization and destabilization (Fig. 4a).[113-115] This characteristic mechanism of action, mediated through non-membrane-dependent mechanisms, enables AMPs to avoid the common resistance mechanisms observed for classical antibiotics.[116,117]

The development of natural AMPs into therapeutically-relevant antibiotics has suffered from several problems, including their susceptibility to proteolysis, reduced efficacy, relatively high expense of manufacturing and limited tissue distribution and cell selectivity. Great strides have been made to overcome these limitations, both by rational and computer-aided design of enhanced functional biomimetics of the peptide sequences, which range from the optimization of natural amino acids to the development of synthetic mimics.[118-121] Because

the interaction of antimicrobial peptides with bacterial membranes depends primarily on the physicochemical properties of the peptides, and in particular the ordered structures formed upon their self-assembly rather than their specific amino acid sequences, many of these biomimetic sequences are much simpler than the innate AMPs evolved in nature.[118]

Biomimetic AMPs have been developed to harness self-organization to form hydrogels and nanostructures with intrinsic antimicrobial properties. The assembly process introduces relevant physicochemical features that are mostly absent from natural antibiotics. Indeed, one can readily modify the peptide sequence to tune the interactions between building blocks and the resulting supramolecular assemblies. Along with their antimicrobial functionalities, the resulting hydrogels and nanostructures can be highly dynamic and can demonstrate a wide range of structural properties, such as stimuli-responsiveness, improved stability and selectivity, injectability and sustained drug release.[122-124]

Antimicrobial hydrogels are formed by self-assembly of peptide building blocks on exposure to environmental stimuli such as changes in pH and the ionic composition of the surrounding solution. This induces interactions between the hydrophobic residues not commonly exposed to the environment, allowing for antimicrobial activity when it is most needed. One of the most prominent families of supramolecular macroscopic entities are the MAX peptides, which fold into an amphiphilic β-hairpin conformation to give hydrogels composed of fibril networks (Fig. 4b).[125-131] These hydrogels can be used as coatings and/or injectable agents, assemble on specific external stimuli and exhibit antibacterial activity against multidrug-resistant Gram-positive and Gram-negative bacteria by disrupting inner and outer membranes. Additional self-assembling antimicrobial hydrogels include variants of the KLD-12 self-assembling peptide that enable rapid fracture healing and antimicrobial activity.[132] Further, naphthalene- or Fmoc-based ultrashort peptide gelators display broad-spectrum antimicrobial activity due to the electrostatic interactions between the hydrogel and the anionic bacterial membrane.[133-135] The intrabacterial enzymatic triggering of self-assembly and subsequent hydrogelation of peptide amphiphiles also afford growth-inhibiting hydrogels in *Escherichia coli*.[136,137] A synergistic enhancement of the antibacterial activity of self-assembling hydrogels has also been achieved by incorporating classical antimicrobial agents[138] and metals[139,140] in these gels, with many additional strategies explored for the use of self-assembling antimicrobial-mimetic peptide-based hydrogels.[141,142]

Cyclic self-assembling antimicrobial peptides are among the first examples of non-hydrogel-forming self-assembling antimicrobial functional structures. These antimicrobial agents were first introduced in the development of cyclic D,L-α-peptides exhibiting proteolytic stability and rapid nanotube formation in lipid membranes. These agents cause bacterial cell death and display potent activity against a wide range of bacteria and exhibit a near order-of-magnitude increase in antibacterial activity compared to their linear peptide counterparts.[143] Parameters such as the size and sequence of the peptides are important, with the octameric peptides generally displaying higher antimicrobial potency than their hexameric counterparts.[143] This strategy has been further expanded to the development of antiviral cyclic D,L-α-peptides[144,145] that are substantially less toxic to mammalian cells while maintaining potent activities against multidrug-resistant bacteria. Cyclic lipodepsipeptides, as well as additional cyclic-peptide-based moieties, have been similarly developed and possess advanced antibacterial and antibiofilm activities.[146-148]

Although many different core–shell nanoparticles have been used as vehicles for drug delivery, those derived from the self-assembly of amphiphilic peptides further demonstrate strong antimicrobial properties against a broad spectrum of bacteria, yeast and fungi in vitro and in vivo.[149,150] These self-assembled nanoparticles are more potent than their free peptide counterparts and have a high therapeutic effect in abolishing *Staphylococcus aureus* infections in mice while presenting reduced cytotoxicity. Furthermore, the peptide nanoparticles can cross the blood–brain barrier to suppress bacterial growth in *S. aureus*-infected brains of meningitis rabbits and suppress yeast growth.[149,150] Importantly, the nanostructures do not interfere with the balance of electrolytes in the blood or cause substantial damage to the liver and kidney functions.

Additional developments in self-assembling antimicrobial mimetics have been achieved in the design of antimicrobial lipopolypeptides and lipidomimetic peptides. Conjugating palmitic acid to the N-terminus of very short cationic di- and tripeptides composed of all L- and D,L-amino acids affords a diverse range of morphologically distinct potent antimicrobial agents in vitro and in vivo.[151] Success has also been had with amphiphilic self-assembling antimicrobial lipidomimetics based on peptides comprised of consecutive hydrophobic Ala residues linked to a hydrophilic charged Lys head group. There is a strong correlation between the propensity of the peptides to self-assemble, their membrane-penetration capabilities and their antimicrobial activity.[152]

Peptide-based nanofibres and nanorods have recently been developed as antimicrobial agents. Indeed, peptide amphiphiles featuring cationic peptide sequences can self-assemble into nanofibres to affect a broad spectrum of bacteria. These nanofibres have significantly higher antibacterial properties than those of soluble peptide molecules with identical sequences (Fig. 4c).[153] Nanofibres and nanorods with substantial antibacterial activity can be generated from simple sequences, and indeed it is not complexity but rather the propensity to self-assemble that is most important. For example, FF forms nanostructures and has emerged as a minimal model for self-assembling, membrane-active, antimicrobial peptides (Fig. 4d).[154] Similarly, truncated nanofibre-forming versions of natural self-assembling peptides also have impressive antibacterial capabilities.[155] The peptides described in our discussion are collated in Table 3.

[H1] Supramolecular peptides in cancer diagnosis and therapy

The membranes of cancer cells can in many cases be enriched in anionic components in much the same way as bacterial outer membranes.[156-158] These anionic moieties include phosphatidylserines, glycosaminoglycans and glycoproteins. Thus, the cationic and amphipathic features of peptides useful against bacteria sees them selectively bind cancerous cells through electrostatic interactions and effect cytotoxicity. Indeed, several antimicrobial peptide mimetics have been recognized as novel targeted cancer therapeutics because of their ability to disrupt cellular and organelle membranes. [156-158]

The majority of anti-tumour peptide therapeutics act in their monomeric form, yet their bio-availability and stability are often limited. Self-assembling peptide nanostructures show greater durability under physiological conditions. The ability of the monomeric peptides to adhere and disrupt cancer cell membranes while undergoing controlled dissociation into monomeric subunits allows them to avoid unfavourable pharmacokinetic parameters that limit therapeutic efficacy and clinical translation.[159,160] Other strategies use self-assembled peptidic nanostructures to target cancer cells by binding receptors on cell surfaces[161] and exposing specific epitopes related to cancer cells and angiogenesis inhibition.[162] An additional important application for such self-assembling peptide nanostructures is their use in drug delivery, in which they are able to penetrate cell membranes and deposit their cargo intracellularly. Thus, the release of Boc-FF spheres through an oil–H_2O interface exemplifies how colloidal particles can encapsulate small hydrophobic and hydrophilic molecules, such

as rhodamine and fluorescein, and transfer them through interfaces in a jet-like manner.[163] The above properties have seen peptide-based microcapsules and ordered structures recently find use in gene delivery for immunomodulation[164,165] and chemotherapeutic agents. [166-171]

The examples we have described showcase peptide-based self-assembled nanostructures in a variety of therapeutic strategies. These developments have motivated many researchers to employ self-assembling nanostructures that themselves have specific membrane-disruption properties rather than having to find both a delivery agent and a bioactive species, or simply using soluble monomeric peptides. Recently, the peptide $(KLAKLAK)_2$, known for its antitumour properties in its monomeric form, has been combined with elastin-like polypeptide (ELP) and the AP1 peptide to give polymer nanoparticles that target interleukin-4 receptors.[172] The polymer nanoparticles form at physiological temperatures while stabilizing their helical conformations, leading to membrane disruption of cancerous cells selectively. Similarly, a combination of hyaluronic acid and $(KLAKLAK)_2$ peptide amphiphiles self-assemble into robust hybrid membranes to produce surface-bound cytotoxic agents or act as reservoirs for sustained release of such agents while avoiding their enzymatic degradation.[173] Furthermore, a different strategy has enabled $(KLAKLAK)_2$ to assemble into nanoparticles that can be internalized and accumulate within cells. In this way, there is a 400-fold increase in the peptide's antitumour activity as the nanoparticles enable efficient disruption of mitochondrial membranes, causing excessive production of reactive oxygen species in cells.[169] Another strategy exploiting the specific properties of emerging self-assembled building blocks uses the peptide FLGALFKALSHLL (commonly denoted PTP-7b), which undergoes concentration-dependent self-assembly on cell surfaces.[170] Following self-assembly into exosome-like aggregates at specific locations on cell membranes, PTP-7b induces cell tissue damage through cell lysis. This occurs because the assemblies can extract lipids from cell membranes and transport them into the cytoplasm.

We have described how self-assembled peptides can have anticancer effects on their own, but they can also show effects when triggered by external stimuli. Thus, short peptide sequences such as the FF motif can form ordered structures for photodynamic[174] and photothermal[175,176] therapies either on their own or when conjugated to active chromophores such as porphyrins and metal ions. Moreover, such peptide–metal ion assemblies allow the development of new cancer cell imaging techniques. For example, the red-shift observed in the yellow fluorescent protein, which results from π–π stacking, inspired the assembly of the TrpPhe dipeptide into

emissive nanoparticles.[177] These nanoparticles can be further functionalized with the MUC1 aptamer and doxorubicin payload, and the entire system can target cancer cells and image drug release in real-time. These results exemplify the therapeutic possibilities emerging from peptide-based nanostructures. By harnessing the properties of these ordered self-assembling nanostructures we can envisage novel anticancer and antibacterial mechanisms that allow for enhanced stability and cell selectivity of bioactive peptides for wide biomedical applications.

[H1] Peptides in liquid–liquid phase separation

Membrane-bound compartments provide spatial control over the localization of biomolecules in living cells. However, it has recently become apparent that many biomolecules can also spontaneously form spatially well-defined biological compartments as a result of liquid–liquid phase separation (LLPS, Fig. 5A), also referred to as coacervation or liquid phase condensation. [178-180,248-250] This phase transition involves the demixing of protein, RNA, and other biomolecules from a homogeneous solution within the cytoplasm of a cell into dense soft colloidal liquid droplets that coexists as membraneless organelles, or biomolecular condensates.[178] Liquid–liquid and liquid–solid phase transitions of such proteinaceous condensates are increasingly recognized to be at the heart of both biological function and malfunction,[251,252] motivating efforts towards understanding the physical principles that define these transitions in a biological context (Fig. 5B).[179,253,254]

The majority of LLPS phenomena in cells have been attributed to the complex interactions between intrinsically disordered proteins themselves and other molecular species, such asRNA molecules,[255-257] which affords biomolecular condensates with liquid-like properties and membraneless organelles.[180] In a biophysical context, the structures formed through LLPS are of particular interest as they, despite not being enclosed by a membrane, have persistent sizes and shapes even though the molecular building blocks exhibit dynamic exchange over timescales of minutes.[181] Moreover, the formation of such responsive condensate structures, either through precise control of protein mixing in bulk solution[182] or using microfluidic approaches to generate condensates from Gly-rich RGG domain peptides,[183] has given rise to a wide range of materials science applications.[258,259] The formation of such synthetic organelles allows one to further generate confined membraneless organelles by combining proteins and mRNA to perform orthogonal translation of desired sequences to introduce new chemical functionalities into mammalian cells in a site-specific manner.[184] Yet, the study of the protein–RNA interactions leading to such phenomena

542 remains challenging due to the high diversity and sequence complexity of these biologically-
543 relevant building blocks. As such, using simpler short peptide building blocks can help us to
544 more easily explore the chemical and physical determinants leading to LLPS.
545
546 Of key importance to LLPS is the presence of low-complexity (LC) protein domains, which
547 have been shown to interact with RNA to form liquid droplets.[185,260,261] Such LC domains
548 include repetitive polymers of Ser and Arg in many proteins involved in LLPS.[186,187] Based
549 on this, model polypeptides containing Ser-Arg repeats have been recently used to monitor
550 the formation of liquid droplets and hydrogels in vitro and in vivo. In specific, a
551 hexanucleotide repeat GGGGCC is the most common cause of amyotrophic lateral sclerosis
552 (ALS) and frontotemporal dementia (FTD). Thus, poly(Gly-Arg) (GR) and poly(Pro-Arg)
553 (PR) peptide repeats have been found to interact with RNA-binding proteins and proteins
554 with LC domains that often mediate the assembly of membraneless organelles.[188] LLPS
555 phenomena play a crucial role in the formation of disease-relevant disorders that are
556 challenging to study in vivo due to the complexity of processes involved. Yet, chemistry
557 comes to the fore because these complex systems can be modelled using short peptides to
558 yield a mechanistic understanding of these interactions.[197,199]
559
560 Capitalizing on the above findings, the role of polypeptide repeats in LLPS further depends
561 on the amino acid sequence and repeat length specificity. For example, repeats of the five
562 dipeptides GA, GP, GR, PA and PR have been shown to undergo LLPS both in vitro and in
563 vivo[189,190] with as little as 50 or 20 repeat units.Such peptides, foremost PR repeats, have
564 been shown to promote cellular toxicity by binding polymeric forms of the LC domains at the
565 amino termini of intermediate filament proteins, thereby promoting direct interactions with
566 RNA granules and further alter the properties of stress granules.[190,191] Indeed, RNA can cause
567 the formation of intracellular droplets by complex coacervation, a type of phase separation
568 that occurs due to electrostatic attraction between oppositely-charged macromolecules. For
569 example, the polycationic peptide RRASLRRASL, inspired by LRRASLG (Kemptide, a
570 model synthetic substrate for protein kinase), was used in combination with polyU as a model
571 for the regulation of intracellular droplet formation by post-translational modifications.[193]
572 Further, the polyU–RRASLRRASL system is extremely sensitive to peptide charge, and one
573 can switch the ability to form droplets on/off by removing/adding a single phosphate (Fig.
574 5C).[194]
575

Similarly, the effects of a variety of polymers and ion concentration on LLPS have recently been studied , exemplifying the role of coacervate interfacial tension and critical salt concentration in the formation of hierarchically organized multiphase droplets.[262] Similarly, oligonucleotide–peptide conjugates such as poly(L) peptides have been used to systematically explore nucleic acid hybridization during nucleic acid and cationic peptide complexation (Fig. 5D). The phase of the complexes formed is controlled by the hybridization of the nucleic acid — double-stranded nucleic acids form solid precipitates while single-stranded oligonucleotides have lower charge density and instead give liquid coacervates.[192] This charge sensitivity can be crucial for cellular regulation of compartment formation in response to external stimuli. Similarly, $I_3V_3A_3G_3K_3$, a surfactant-like peptide, can induce efficient DNA condensation into virus-mimicking structures in a two-step manner.[195] The peptide binds the DNA chain through electrostatic interactions, and then self-assembles into β-sheets under hydrophobic interactions and H-bonding, thus mimicking the nature of the virus capsid in helping to package DNA.

More recently, the mechanism by which liquid condensates form has been explored using carboxybenzyl (Cbz)-protected FF and even Fmoc-protected single amino acids.[196] In the case of phase separation of Z-FF, one obtains low-enthalpy solute-rich liquid droplets and high-entropy solute-poor phases. The solute-rich liquid droplets act as nucleation sites, allowing the formation of thermodynamically favourable nanofibrils following Ostwald's step rule, whereby metastable aggregates are converted to more ordered structures, thus, reducing the overall free energy of the system (Fig. 5E). This rule is exemplified here in that the nucleation barrier to self-assembled ordered structures is lowered when first transforming through a metastable liquid phase, as such droplets can serve as precursors in the formation of the thermodynamically more favourable supramolecular polymers.

[H1] Biomineralization and organic–inorganic hybrid materials

Evolutionary developments in biology have resulted in biomaterials with remarkable structural properties. The assembly involves cooperative but relatively weak molecular interactions that contrast with the covalent interactions in synthetic polymers. Crucially, however, the biological materials produced by peptide and protein self-assembly can be structurally reinforced by subsequent biomineralization (Fig. 6a). Thus, living organisms can build organic structures and then use ordered arrangements of inorganic materials to harden or stiffen existing tissues.[201] Although Ca^{2+} is the main cation in biogenic minerals,

610 biomineralization is widespread in various organisms and exploits a wide range of inorganic
611 components such as CO_3^{2-} and silicate anions. Bone, enamel and seashell nacre, for example,
612 have proteins and inorganic platelets as common constituents.[202]
613
614 Natural biomineralization has stimulated much research in the field of biomimetic systems
615 aimed at preparing complex materials with properties similar to those found in nature.[263,264]
616 These materials are critically important to regenerative medicine and studies on tissue
617 morphogenesis. In this regard, proteins and peptides are of interest in biomineralization
618 processes due to their high biocompatibility, structural stability, wide accessibility and
619 sequence diversity. Moreover, their aptitude to template 0D to 3D structures and affinity for
620 both hydrophobic and hydrophilic surfaces are very desirable.[202] Thus, the disadvantages of
621 synthetic polymers in biological setting are well-addressed by instead using peptides. In
622 particular, complex peptide-based fibrillar networks that bind inorganic components have
623 become attractive targets in materials design. One such system uses a supramolecular peptide
624 nanofibre that can emulate both the nanofibrous architecture of collagenous extracellular
625 matrix and the major chemical composition found on glycosaminoglycan (GAG) for bone
626 tissue regeneration. GAGs constitute a significant portion of the extracellular matrix and have
627 a substantial impact on regulating cellular behaviour, either directly or through encapsulation
628 and presentation of growth factors to cells (Fig. 6c).[63] This GAG and collagen-mimetic
629 peptide assembles into nanofibres that interact with bone morphogenetic protein-2, which is a
630 critical growth factor for osteogenic activity and mineralization by osteoblastic cells. The
631 resulting structures sustain and direct the growth of bone cells and hydroxyapatite
632 biominerals and thus can prove useful in the structural design of tissue-regenerating
633 materials.
634
635 The biomineralization of enamel is regulated by amelogenin proteins such Leu-rich
636 amelogenin peptide (LRAP), which self-assembles and stabilizes amorphous $Ca_3(PO_4)_2$ to
637 promote enamel formation.[203] Furthermore, phosphorylated LRAP not only stabilizes
638 amorphous $Ca_3(PO_4)_2$ but also prevents its transformation into $Ca_{10}(PO_4)_6(OH)_2$
639 (hydroxyapatite), aligned crystals of which form when non-phosphorylated LRAP is present.
640 Furthermore, the non-phosphorylated N- and C-terminal amelogenin domains are sufficient
641 to template amorphous $Ca_3(PO_4)_2$ transformation into ordered bundles of hydroxyapatite
642 crystals, making LRAP an excellent candidate for biomimetic enamel regeneration.[204] Indeed,
643 a peptidic amphiphile can self-assemble on a surface, thereby making it an amenable location

for hydroxyapatite growth.[205] The highly aligned nanofibrillar bundles guide hydroxyapatite nucleation by varying the overall charge and propensity for β-sheet H-bonding. These cylindrical bundles allow mineralization in a specific orientation relative to the principal axis of the fibres, as is found in mammalian bone structure (Fig. 6d). Thus, the controlled assembly of peptide amphiphiles and biomineralization at these sites can afford hierarchical structures that mimic bone. Similarly, the biomineralization of SiO_2 plays a central role in the formation of structural exoskeletons in marine species such as diatoms and sponges.[206] This process takes place through specific deposition of SiO_2 vesicles through interaction with a class of sillafin proteins at low pH. Inspired by this naturally-evolved system, a variety of sillafin-mimicking peptides have recently been used to control the formation of SiO_2 nanoparticles. The peptide SSKKSGSYSGSKGSKRRIL (R5) is of particular interest and promotes interactions with silicic acid through Lys residues, thereby leading to precipitation of SiO_2 nanoparticles.[206] This self-assembling peptide has thus enabled the formation of ordered nanostructures onto which SiO_2 shells can polymerize (Fig. 6e).

Another fascinating application of self-assembling systems is the ability to form organic–inorganic systems in which two types of building blocks are organized into intercalated layers to optimize mutual interactions and to combine their properties on greater length scales. Notable examples include protein-based self-assembling systems that incorporate nanoparticles into their structures.[265] In other studies, virus capsid-based peptides have been used as biotemplates to nucleate noble metal nanoparticles and photoactive materials. This enabled controlled formation,[207,208] which is desirable in the case of thin films in energy harvesting and storage applications. Similarly, amyloid-related peptides template the formation of C and Au nanoparticles.[202,209,210] The resulting hybrid materials can adopt membrane, platelet and fibrillary gel morphologies and thus have a diverse set of properties, such as high toughness and strength, tenable fluorescence, conductivity and sensing. Combining self-assembled peptide nanostructures with Au and Ag also has fruitful outcomes.[211,212] For example, the cyclic antimicrobial lipopeptide surfactin self-assembles on photoluminescent Au nanodots to give a hybrid material, in which synergism between the two components efficiently inhibits the growth of various bacterial strains in vitro.[211] Furthermore, Fmoc-protected peptides self-assemble into nanofibres decorated with carboxylic acid and thiol groups — ideal coordination sites to act as scaffolds for the mineralization of Ag nanoparticles.[212] These composite materials exhibit highly effective and

677 long-term antibacterial activity against both Gram-positive and Gram-negative bacteria and
678 can maintain their structures.
679
680 The incorporation of metal ions during peptide self-assembly modulates the structures formed
681 through coordination. Thus, adding Zn^{2+} ions to FF induces a structural transformation from
682 β-sheet to a superhelix at a 1:1 Zn^{2+}:FF ratio or a random coil at a 1:2 ratio, allowing specific
683 control over the nature of the resulting metallohydrogel.[213] Similarly, short cationic peptides
684 derived from FF spontaneously assemble into colloidal spheres in the presence of
685 $H_3[PW_{12}O_{40}]$ (phosphotungstic acid).[214] During the self-assembly of these spheres, they can
686 host a variety of charged or uncharged guest molecules along with hydrophobic and
687 hydrophilic nanoparticles.
688
689 Au and Ni nanoparticles can bind short peptides such as an amyloid fibril model peptide
690 containing a His_6 tag.[215] This surfactant-like peptide undergoes a remarkable two-step self-
691 assembly process at two distinct critical aggregation concentrations. When tagged with Au
692 nanoparticles bearing Ni-NTA groups (where NTA is a tri(2-acetato)amine chelating
693 derivative), one obtains functionalized amyloid fibrils as part of a peptide–nanoparticle
694 hybrid. Peptides that mimic the native coiled-coil structure have similarly been used in Au
695 nanoparticle functionalization,[216] as in the case of artificial Leu zipper-like peptides, which
696 perform specific biomolecular recognition to assemble Au nanoparticles.
697
698 A different synthetic approach to organic–inorganic composites involves the reduction of
699 metal ions in a controlled manner by self-assembled nanostructures. As demonstrated in the
700 context of self-assembled short peptides, the non-coded aromatic amino acid 3,4-dihydroxy-
701 L-phenylalanine (DOPA) can be introduced into peptides that self-assemble into a hydrogel
702 with remarkable adhesive properties.[217] The potential utility of these structures was further
703 explored in terms of spontaneously reducing metal cations into metal atoms. Thus, applying
704 Ag^{+} to the hydrogel resulted in efficient reduction into Ag nanoparticles that formed a
705 seamless metallic coating on the assemblies (Fig. 6b). Similarly, the T4P peptide from the
706 metal-reducing *Geobacter sulfurreducens* bacterium has recently inspired the design of
707 synthetic peptide building blocks that self-assemble into T4P-like nanofibres,[218] bind metal
708 oxide particles and reduce Au^{3+}. The resulting peptide–AuNP nanocomposites exhibit
709 enhanced thermal stability, electrical conductivity from the single-fibre level up, and
710 substrate-selective adhesion. Such nanoscale assemblies have unique properties and can serve

as multifunctional platforms for biotechnological applications by combining the inherent structural properties of the peptides with those of the metal-based nanoparticles.

Peptide-derived assemblies can template other structures aside from inorganic species and can, for example, be combined with molecular metal complexes and organic species. Thus, the co-assembly of a guanine-rich nucleic acid with a His-rich peptide and hemin affords catalytic nanoparticles that mimic the active site and peroxidase activity of haem proteins.[219] The His-rich peptide provides the activating groups and hemin the active site, while the guanine-rich DNA acts as a scaffold for hemin coordination and stabilization. Peptide–porphyrin co-assemblies can similarly afford activity, including for photocatalytic H_2 evolution. The peptides and porphyrins spontaneously self-organize into ordered hybrid fibres by molecular self-assembly and self-mineralization with the assistance of visible light.[220] Related peptide–porphyrin systems are catalysts for O_2 evolution, thereby mimicking cyanobacteria.[221] Here, DOPA, in combination with a metalloporphyrin and Co_3O_4 nanoparticles, affords hybrid fibres that absorb light and oxidize H_2O to O_2, with quinones serving as the electron acceptors. A similar approach uses photooxidase-mimicking nanovesicles formed from amphiphilic amino acids such as Fmoc-His and phthalocyanines to give a catalytic material.[222] Overall, these model systems showcase the potential utility of simple building blocks — peptides and even single amino acids — to give complex reactivity that mimics that found in nature.

[H1] Control of nanoscale morphology with hierarchical peptidic materials

We have discussed how peptides self-assemble into 1D fibrils or 2D structures. The assembly is hierarchical in that these structures can further pack into multiscale functional materials. Nature often uses structural units beyond linear and planar geometries, such that a variety of nanoscale shapes have functional roles. This has inspired the exploration of artificial peptide-based materials that derive their functionality from their complex nanoscale shapes. For instance, the formation of hierarchical structures by peptide dimers and trimers adopting a coiled-coil motif allows the generation of globular protein mimics with well-defined molecular morphology and function.[223,224] Similarly, such hierarchically-ordered structures have afforded peptide arrays used in bioelectronic, bioimaging and optical materials, as has recently been studied and reviewed.[225-232]

The formation of hierarchical peptide-based structures and materials has given rise to a field dubbed 'peptide tectonics'.[233] Through introducing complementary units with selective association, peptide tectons allow for programmable self-assembly through selective interactions across domains, facilitating the development of new materials. Similarly, using abiological folded oligomers (foldamers) affords supramolecular architectures with diverse functions that extend beyond those found in nature.[234-236]

Among the more striking examples of biomimetic hierarchical peptide self-assembly is the formation of artificial viruses. The self-assembly of peptides into distinct 3D hierarchical structures mimics the distinct packing observed in viral capsids and their controlled disassembly. While initial research in this area focussed on making peptide-based structures with dimensions similar to those of viruses, additional advances allowed the mimicry of linear viruses like the tobacco mosaic virus. Thus, the octapeptide lanreotide, synthesized as a growth hormone inhibitor, assembles into 20–30-nm-long nanotubes.[237] More recently, the β-annulus peptides from tomato bushy stunt virus have been observed to assemble into 30–50 nm viral-capsid-like nanocapsules.[238] These nanocapsules encapsulate various guest molecules and can be decorated with different molecules on their surface. In this way, one can prepare artificial viruses with human serum albumin or ribonuclease on their surfaces.[239,240]

A promising strategy in mimicking viral capsid surfaces is using short peptides that assemble into filamentous nanoribbons to form an outer coat that encapsulates DNA or RNA.[241] Using this strategy, plasmid DNA has been combined with the peptide K_3C_6SPD to generate cocoon□like viral mimics through peptide self-assembly.[242] The nanococoon morphology, stability and ability to encapsulate DNA molecules can be further tuned by regulating the inter-nanofibril hydrophobic interactions to afford a cellular delivery system. Such nanococoons can also be made from the H_4K_5-HC_{Bzl} peptide, which assembles into subunit components of a low aspect ratio, thereby forming β-sheet nanodiscs.[243] A similar system has been demonstrated using TR_4, a small molecule with four Arg residues with an N-terminus functionalized with a tetraphenylethene and a lipophilic tail. The species self-assembles and hosts plasmid DNA[244] in virus-mimicking nanoparticles that have low cytotoxicity, high stability and high transfection efficiency. The self-assembly process further induces bright fluorescence from tetraphenylethene groups packing together, allowing tracking of gene

delivery. Further details regarding the use of such virus-mimicking assembly for therapeutic applications can be found in other recently-published reviews.[245,246]

[H1] Conclusions and outlook

This Review has summarized new research into biomimicry that uses peptide self-assembly to afford ordered functional nanostructures with tunable physical, chemical and biological properties. Protein self-assembly is nature's powerful tool to produce structures of varying length scales and functions, and with unique physical properties. The range of protein structures in natural systems is vast — ranging from oligomers and nanospheres to tubes and hierarchical assemblies that play key roles in biological functions such as cargo transport, microbial defence and structural support. These structures are held together by interactions that are predominantly non-covalent, thus conferring dynamism and flexibility on the structures. Great effort has been devoted to exploring self-assembly of natural and synthetic proteins, which allows the formation of materials that are functional yet expensive and difficult to produce. New methods of studying these supramolecular structures, such as super-resolution microscopy and microfluidic platforms, have provided insights into the self-assembly process. But nature also uses short peptides composed of the minimal recognition modules, and these offer a unique platform for mimicking complex systems and phenomena with simple peptide-based model systems. These short peptide-based structures are more tractable and have shown great potential as materials for adhesives, cell scaffolds, drug-delivery systems, antimicrobial agents and surfaces, molecular machines and organic–inorganic matrices. The structural and functional diversity of such assemblies can be further expanded by incorporating inorganic molecules, such as inorganic materials and small molecules. Although simpler than protein derivatives, peptide-based biomimetic materials are still challenging to investigate and use. However, they present great promise for future research. We believe that exploring new modifications of short peptides will be the key to creating structures for new applications in even wider spread fields.

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[H1] Author contributions

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ToC blurb

The self-assembly of biomimetic peptides can mimic complex natural systems involving whole proteins. This Review describes how synthetic peptides afford tunable scaffolds for biomineralization, drug delivery and tissue growth.

Subject terms

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Box 1 | **Amyloid-like peptide nanofibrils.**

Linear assemblies of peptides and proteins serve as basic structural units for macroscopic materials in nature, such as collagen in skin and keratin in nails and hair. A particularly simple but common material forms when proteins or peptides assemble into β-sheets formed parallel to the fibril axis (see figure, top) to give highly ordered H-bonded networks. The self-assembly of peptide systems into such ordered structures with supramolecular fibril architectures is commonly associated with the amyloid state of proteins linked to misfolding diseases in humans.[51] Artificial peptidic systems capable of such linear assembly can afford insights into the fundamental principles governing the formation of ordered structures through nucleation-dependent mechanisms. Self-assembly begins with primary nucleation[52-54] and then growth of such structures by elongation and their replication by secondary nucleation on the surface of the initial fibrillar structures.[55] More recently, it has been found that in addition to their pathological role in a range of human diseases, nature uses these structures as the basis for a diverse set of functional materials including coatings and catalytic scaffolds.[26,56,57] The unique properties of fibrils composed of repeating sequences of short peptides are of considerable interest in nanotechnology and materials science, where they might serve as drug delivery systems, tissue engineering scaffolds, functionalized nanowires and bone mimetic composites.

The propensity of short peptides to adopt amyloid-like structures can be enhanced by including features that promote aggregation in nature through hydrophobic and π–π interactions (see figure, top[26]). Such interactions stabilize β-sheets involving one or more different component, as can be seen from the structures of specific aggregates, allowing them to form supramolecular systems structurally similar to amyloid fibrils (see figure, bottom left[5]). Of the many peptides explored in this context, short peptides of 2–5 residues adopt stable fibrillar amyloid-like supramolecular structures. Thus, FF fragments constituting the core of the Alzheimer's disease β-amyloid polypeptides (Aβ) self-assemble into supramolecular systems and form nanotubes, nanospheres, nanofibrils and hydrogels (see scanning electron micrograph, bottom right[27]).

Box 2 | **Natural and artificial extracellular matrices.**

The extracellular matrix (ECM) is composed of proteins, carbohydrates and minerals, in combination with a wide variety of cell adhesion molecules including integrins, cadherins and transmembrane proteoglycans. The ECM provides external support to individual cells and facilitates interactions between cells, allowing their assembly and organization into functional tissue.[82] Depending on the nature of the tissue, differences in the composition and organization of the component proteins define its physical properties such as elasticity, strength and influence on cell adhesion, all of which affects a cell's ability to proliferate. Artificial cell culture scaffolds and tissue-engineered constructs can enable improved cell viability and proliferation by mimicking the physicochemical conditions of the ECM. Self-assembly through non-covalent crosslinking can afford mouldable and injectable hydrogels as cell scaffolds. This approach has, however, so far largely used polymers such as alginate[83], poly(ethylene glycol)[84] and poly(glycerol sebacate)[85] in combination with a range of nanoparticles for controlled drug release applications.[86] Common biological scaffolds include peptide- and protein-based biopolymers, either in their natural forms, such as collagen, fibronectin and silk, or in related synthetic materials that can be used in various cell culture technologies. Indeed, biomimetic materials are finding increasing appeal in biomedical applications due to their ability to recapitulate the ECM both in architecture and in the capacity for cell signalling. In the case of self-assembled protein matrices, 3D fibrillar networks exhibit the potential to create scaffolds in tissue engineering. One notable commercially-available macroscaffold is the Matrigel matrix,[87] which is produced from

several proteins such as laminin, collagen IV and entactin, in combination with other growth factors and enzymes.

Fig. 1 | **Supramolecular chemical space accessible to biomimetic self-assembling peptides.** Chemically simple peptide sequences afford mechanistic understanding of molecular-level interactions in ordered supramolecular structures. Peptide building blocks have informed us about diverse phenomena, including the conversion of homogenous solutions of peptide building blocks into discrete biomolecular condensates (liquid–liquid phase separation) and ordered fibrillar structures such as amyloid fibrils. A subset of peptides can assemble at interfaces to generate biomimetic membranes of artificial cells and organelles, while others have been shown to disrupt the membranes of bacterial and cancer cells through pore formation, thus offering a wide range of therapeutic applications. The formation of ordered structures has given rise to the formation of biomimetic fibrils that can hierarchically assemble into complex structures, including 3D matrices used as scaffolds for cell growth and for forming organic–inorganic hybrid materials through incorporating peptide motifs known to be involved in biomineralization processes in nature.

Fig. 2 | **Biomimetic supramolecular peptide scaffolds enable cell adhesion and proliferation. a** | Peptides can self-assemble into biomimetic matrices that act as scaffolds to generate cell cultures. **b,c** | Scanning electron micrographs depict osteogenic cell viability and morphology when grown in glycosaminoglycan-mimetic peptide nanofibrils that promote biomineralization (scale bars represent 50 μm)[63]. **b** | Cells grown on sulfonated PA fibrils mimicking GAG sulfate. **b** | Cell proliferation is reduced when E-PA fibrils bearing carboxylate groups are used. This material mimics non-sulfated GAGs. **d** | The cells, falsely coloured here in cyan, adhere to the self-assembled peptide nanofibrils[68]. **e** | The biocompatibility is evident from the cells extending into the peptide matrix. **f** | The cells can also remodel the matrix to best suit them. Parts **b** and **c** reproduced with permission from Ref. 63, Elsevier. Parts **d**–**f** reproduced with permission from Ref. 68, Elsevier.

Fig. 3 | **Self-assembly of membrane and surfactant-like peptides at interfaces. a** | A peptide self-assembly can stabilize a liquid–liquid interface. **b** | Transmission electron micrographs of the surfactant peptides A_6D (left) and V_6D (right), which form a dense network several micrometres long[89]. **c** | On a smaller scale these materials form open-ended tubes (left), micelles and spherical vesicles budding off the nanotubes in H_2O (right). **d** | KL4

models built using backbone torsion angle restraints from solid-state NMR data. KL4 conformer from measurements with two different lipids, POPC and DPPC (top and bottom, correspondingly)[90]. **e** | Transmission electron micrographs of diblock copolypeptide-surfactant complexes, indicating a lamellar order of periodicity[94]. Parts **b** and **c** reproduced with permission from Ref. 89, US National Academy of Sciences. Part **d** reproduced with permission from Ref. 90, Elsevier. Part **e** reproduced with permission from Ref. 94, American Chemical Society.

Fig. 4 | **Self-assembling biomimetic peptide-based antimicrobial nanostructures. a** | Different peptide–membrane interactions are proposed to give rise to antibacterial functions. **b** | The MAX1 peptide undergoes environmentally-triggered folding, self-assembly and non-covalent fibril cross-linking processes to give a hydrogel[127]. **c** | The supramolecular nanofibres formed by self-assembling peptide amphiphiles present cationic peptide sequences that are essential to their proposed mode of action[153]. **d** | Scanning electron micrographs of *E. coli* with and without diphenylalanine. This dipeptide forms nanostructures that have clear effects on bacterial morphology[154]. Part **b** adapted with permission from Ref. 127, EBSA. Part **c** reproduced with permission from Ref. 153, American Chemical Society. Part **d** adapted with permission from Ref. 154, Springer Nature.

Fig. 5 | **Mechanisms of liquid–liquid phase separation and condensation. A** | A homogeneous peptide solution can undergo liquid–liquid phase separation (LLPS) to give metastable condensates. These, in turn, can undergo a phase transition to form thermodynamically favoured solid fibrils. **B** | LLPS involves several weak forces, including electrostatic, cation–π, dipole–dipole and π–π interactions[179]. **Ca** | Treating a solution of peptide RRASLRRASL with polyU RNA leads to complex coacervation on account of electrostatic forces, among other interactions[194]. **Cb** | Bright-field (left) and fluorescence images (right) highlight aggregation into coacervate phase droplets. **D** | Schematics and brightfield microscopy images presenting the effect of oligonucleotides hybridization, ion concentration and temperature on LLPS of poly(L) peptides[192]. **E** | Transmission electron micrographs of Fmoc-Ala undergoing LLPS and phase transition to form increasingly organized structures. The transition from the kinetically trapped nucleation precursors to the nanofibrils is accompanied by a decrease in Gibbs free energy[196]. Part **B** reproduced with permission from Ref. 179, Elsevier. Part **C** reproduced with permission from Ref. 194,

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Fig. 6 | **Peptides as biomineralization scaffolds and organic–inorganic composite agents.** **a** | Self-assembled peptides can serve as templates for the deposition of inorganic materials. **b** | For example, Fmoc-protected 3,4-dihydroxy-L-phenylalanine dipeptide affords a hydrogel that reduces Ag^+ ions over 3 days to give Ag crystals, as evidenced in transmission electron micrographs[217]. **c** | Scanning electron micrographs of mineralized bone-like nodules on nanofibres of a glycosaminoglycan-mimicking peptide.[64] **d** | Cryogenic transmission electron microscopy and selected area electron diffraction of hydroxyapatite mineralized at amphiphilic peptides. Arrows indicate the location of the organic template and arrowheads indicate the position of inorganic crystals[205]. **e** | Formation of SiO_2 nanoparticles directed by self-assembled silaffin R5 peptide structures[206]. Part **b** reproduced with permission from Ref. 217, American Chemical Society. Part **c** reproduced with permission from Ref. 64, Elsevier. Part **d** reproduced with permission from Ref. 205, Wiley-VCH. Part **e** reproduced with permission from Ref. 206, Wiley-VCH.

Table 1 **| Examples of peptides that formation amyloid-like fibrils and hydrogels.**

Peptide name	**Number of residues**	**Associated protein/system**	**Self-assembled structure**	**Ref.**
Diphenylalanine (FF)	2	Aβ peptide	Peptide nanotubes	27
α,β-Dehydrophenylalanine (ΔF)	2	Aβ peptide	Hydrogel-forming fibrillar networks	33,34
Fmoc-FF-konjac glucomannan (KGM)	2	Aβ peptide	Fibrillar hydrogels	35
FF-NH_2	2	Aβ peptide	Reversible peptide nanotubes/spheres	46,47
Ac-EFFAAE-NH_2 (AIP-1/2)	6	Aβ peptide	Amyloid fibrils	49
FFKLVFF	7	Aβ peptide	Amyloid fibrils	36–

				40
P_{11} (QQEFQWQFRQQ)	11	Aβ peptide	Amyloid anti-parallel β-sheet tapes	48,50

Table 2 | **3D peptidic matrices can allow cell adherence, growth and proliferation.**

Peptide name	**Number of residues**	**Associated protein/system**	**Self-assembled structure**	**Ref.**
Fmoc-3F-Phe-Arg Fmoc-3F-Phe-Asp	2	Fibronectin	Nanofibrillar hydrogels	77–79
P1-P8	2–3	β-Amyloid polypeptide	Nanofibre gels	72
A1-A7	5	α-Synuclein	Nanofibre gels	69
Diphenylalanine-RGD	5	β-Amyloid polypeptide	Nanofibrillar matrix	75
Ac-ILVAGK-NH_2	6	Lys-containing peptide	Nanofibrillar hydrogels	76
HM-PA	7	Heparin	Nanofibre gels	72
TTR1-cycloRGDfK	11	Transthyretin	Nanofibrillar matrix	66
PA-YIGSR	13	Endothelial cell-adhesive ligand	Nanofibrillar matrix	71
EAK16 (Ala-Glu-Ala-Glu-Ala-Lys-Ala-Lys)$_2$	16	Zuotin	β-Sheet-containing membranes	22,23
(Pro-Lys-Gly)$_4$(Pro-Hyp-Gly)$_4$(Asp-Hyp-Gly)$_4$	36	Collagen	Triple helix fibrils	64,65

Table 3 | **Peptides associated with antibacterial and anticancer activity through membrane disruption.**

Peptide	Number of residues	Associated protein/system	Self-assembled structure	Ref.
FF	2	β-Amyloid polypeptide	β-Sheet containing nanofibres	154
Cyclic D,L-α-peptides	6–8	Synthetic	Supramolecular peptide nanotubes	144, 145, 148
KLD	12	Synthetic	β-Sheet containing nanofibres	131
PTP-7b	13	Synthetic	β-Sheet containing nanofibres	171
$(KLAKLAK)_2$	14	Synthetic	α-Helix	172–174
MAX	20	Synthetic	β-Hairpin hydrogels	125–131

Table 4 | **Peptides template the formation of organic–inorganic hybrid materials.**

Peptide name	Number of residues	Associated protein/system	Self-assembled structure	Ref.
Fmoc-DOPA-DOPA	2	Synthetic	Nanofibrils	217
Fmoc-FFECG	5	Synthetic	Nanofibres	208
SO_3-PA	7	Glycosaminoglycan	Nanofibrillar network	63
Surfactin	7	Synthetic	Surface coating	212
PA	9	Synthetic	β-Sheet-	205

			containing nanofibrils	
$A_{10}H_6$	16	Synthetic	β-Sheet-containing nanofibrils	215
R5	19	Silaffin-$1A_1$	Micelle-like assemblies	206
Acidic/basic Leu zipper-like peptide	36	Synthetic	Left-handed coiled coil structure	216
LRAP	64	Amelogenin	Nanofibrillar bundles	203

1600