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Complete List of Authors:	de Santis, Emiliana; National Physical Laboratory, Ryadnov, Maxim; National Physical Laboratory,

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ARTICLE TYPE

Peptide self-assembly for nanomaterials: the old new kid on the block

Emiliana De Santis[a] and Maxim G. Ryadnov*[b]

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Peptide self-assembly is an increasingly attractive tool for nanomaterials. Perfected in biology peptide self-assembling systems have impacted on nearly any conceivable nanomaterial type. However, with all the information available to us commercialisation of peptide materials remains in its infancy. In an attempt to better understand the reasons behind this shortcoming we categorise peptide self-assembled materials in relation to their non-peptide counterparts. A particular emphasis is placed on the versatility of peptide self-assembly in terms of modularity, responsiveness and functional diversity, which enables direct comparisons with more traditional material chemistries.

Introduction

Substantial progress made over the last two decades in the design and synthesis of nanostructured materials has identified unique properties with a clear potential for commercialisation.[1] The desire here is for smaller and cheaper components with enhanced performance, which pre-selects strategies for the fabrication of more complex structures in a controlled fashion. In this vein, peptide self-assembly offers a matching solution for the challenge.[2]

Admittedly, our understanding of the phenomenon is not complete, and therefore often takes inspiration from biology. Virtually any naturally occurring material is a higher-order nanostructure self-assembled from the molecule up. Nature uses a relatively small number of peptide building blocks, which spontaneously arrange into functional forms or morphologies. These forms are ubiquitous for different molecular processes which they control precisely over the time and length scales required.[3]

For instance, water-filled hydrophobic nanotubes disrupt bacterial membranes within minutes,[4] extended fibrillar bundles support cell growth for days,[5,6] while virus-like nanoparticles instantaneously infect cells[7, 8] or elicit prolonged immune responses[9] (Fig. 1A). These forms are not exclusive to biological molecules, e.g. organic nanotubes and polymeric fibres find use in biology and medicine,[10] and are not confined to biological applications, e.g. protein cages template the synthesis of inorganic nanomaterials.[11] It hence can be argued that it is the form, not chemistry, which defines the function. Why self-assembly then? An answer to this question is not only *what* the form is but also *how* it is.

A multitude of non-covalent interactions supports subtle transitions in self-assembled systems in response to external stimuli which enrich material properties. A "breathing" virus can serve as an example (Fig. 1B) – while this form remains the same (what), the state of the material in response to external stimuli changes (how).[12] Thus, the rationale of biological assemblies can inform bio-inspired and, by association, "smart" materials for different, but not necessarily related applications. Indeed, new strategies of increasing complexity are constantly introduced for peptide materials, yet their widespread commercialisation remains in its infancy. Partly, this is due to the still emerging status of the field when compared to other more mature areas. Partly, because peptides are traditionally considered for applications which impose stricter criteria for enzymatic stability, potential immunogenicity and cost of production, all of which highlight the need for optimisation and scale-up methodologies. In this regard, a critical comparison with non-peptide materials is beneficial, but lacking.

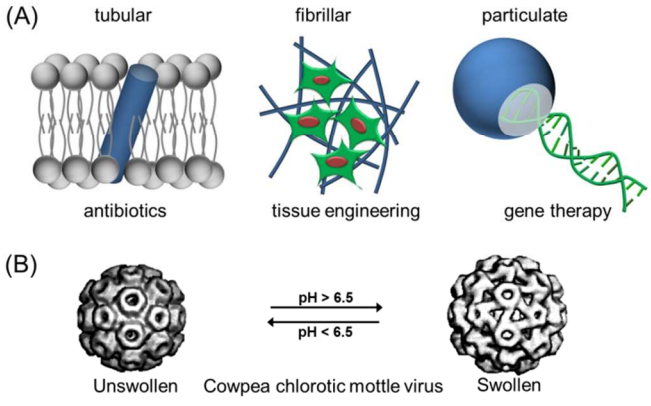


Fig. 1. Main forms of peptide nanomaterials. (A) Schematic representations of tubes, fibres and particles with related applications. (B) Responsiveness of cowpea chlorotic mottle virus to external stimuli (pH) accompanied by the opening and closing of capsid pores. Adapted by permission from Macmillan Publishers Ltd: Nature, copyright 1998.[12]

Here we review peptide materials in relation to their non-peptide counterparts. We build our comparison around major nanomaterial forms – fibrillar, tubular and particulate with more

complex variations including membranes and matrices (Fig. 1A) – and highlight their feasibility by design. A particular emphasis is placed on how peptide structure, self-assembly topology and nanomaterial form are linked in one hierarchical continuum.

Structural continuum in peptide materials

Peptide self-assembly offers a repertoire of unique properties ranging from modularity and biocompatibility to the ease of production and scale up.[13] The incorporation of unnatural amino acids into peptide sequences or the design of hybrid or poly-amino-acid materials provide additional advantages that can broaden exploitation routes for peptide-based materials. Nonetheless, the main strength of peptide materials is in the programmability of peptide structure. Peptides exploit a much narrower and more predictable sequence space compared to proteins, are more synthetically accessible and their structure permits substantial orthogonality in the chemistry of building blocks. For instance, branched and cyclic peptides can assemble on their own or co-assemble with linear sequences introducing different assembly pathways,[14] while as short sequences as dipeptides can furnish discrete nanostructures.[3] Collectively, these features constitute a hierarchical continuum of peptide sequence, folding and assembly which defines material properties.

Secondary structure elements as building blocks

Peptide materials assemble in water at the expense of hydrophobic interfaces formed between building blocks. The interfaces disfavour the highly polar peptide bond, but its regular repetition in peptides enables persistent hydrogen bonding, which neutralises its polar contributions. Different hydrogen-bond patterns support different secondary structure elements, two of which, α-helices and β-strands, have been predominantly used in peptide materials (Fig. 2).[15]

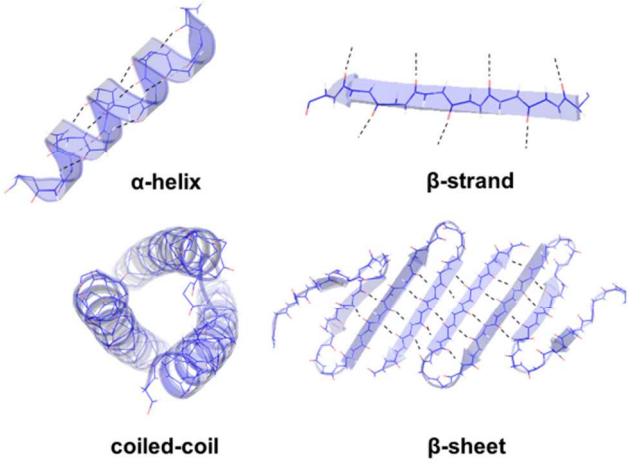


Fig 2. α-helix and β-strand protein folding elements and their oligomerisation states – coiled coils and β-sheets (PDB entries 1IJ3 and 1JY4 rendered with PyMol). Dotted lines indicate hydrogen bonds.

The α-helical type is arranged along the peptide backbone, i.e. it is *intra*-peptide. The bonds are maintained in *i, i+4* amino-acid pairs for conventional α-helices whose lengths can be unlimited. In contrast, β-strands adopt more extended conformations, which cannot be stabilised by the same type of hydrogen bonding, and *inter*-peptide bonds are formed instead. These induce the lateral assembly of β-strands into β-sheets (Fig. 2). β-strands need not be long and can be formed by sequences comprising <10 amino-acid residues.

Most peptide materials can be categorised as all-α and all-β materials using the analogy of protein topologies, which incorporate same or different elements in mutually specific orientations. Assemblies comprising both α and β-type structures have yet to be reported. A peptide material thus assembles from one or more types of building blocks (of the same secondary structure). Building blocks define the topology of the assembly, which according to its predominant propagation mode (longitudinal, lateral or both), determines a corresponding material form – linear or anisotropic (fibre or tube) and orthotropic, in which assembly occurs in all directions – x, y and z (particle, membrane and matrix) (Fig. 3).

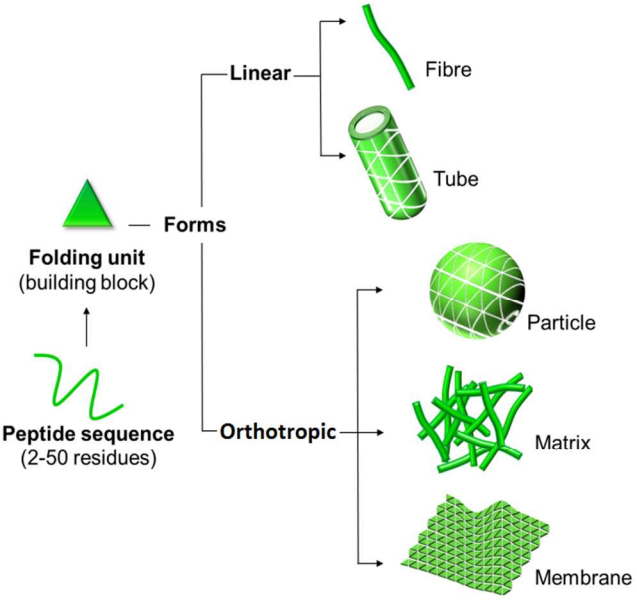


Fig 3. Schematic representation of self-assembly topologies (linear and orthotropic) in relation to peptide sequence, folding and material forms.

Linear versus orthotropic self-assembly topologies

Individual β-strands can be generated by simply alternating hydrophobic (H) and polar (P) residues, which are placed on the opposite sides of the peptide backbone. Introducing self-complementary charges into the polar face sets up directional assembly into microscopic membranes, which exemplify orthotropic topology.[16]

Specifically, β-strands stagger longitudinally by means of opposite charges, while expanding laterally through β-sheet hydrogen bonding and interactions between side chains facing each other (Fig. 4). The topology itself can pre-determine material properties. For example, sequences with alternating two negative and two positive charges (--++--++) give membranes stable to external stimuli.[17] With residue-specific alternations in this design, e.g. lysine-to-arginine mutations to set up more extensive hydrogen bonds, it is possible to generate polygonal fibrillar networks that gel in water. Gelation is a commercially valuable property and such materials already find use as cell-

supporting scaffolds for cell culture and tissue engineering.[18]

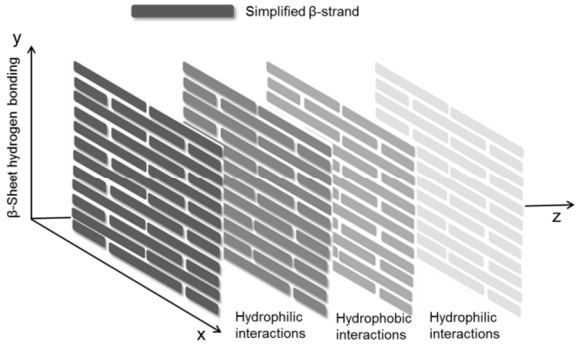


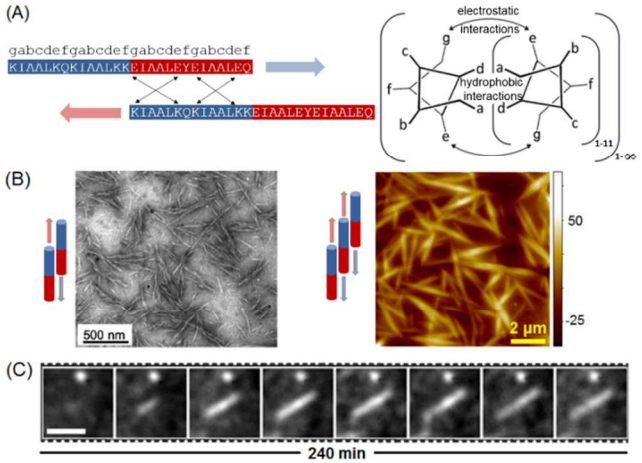
Fig. 4. An orthotropic all-β topology incorporating a pattern of staggered longitudinal assembly of β-strands and lateral β-sheet expansion through hydrogen bonding and side-chain interactions.

In contrast to β-strands, canonical monomeric α-helices do not typically oligomerize and have to be programmed to do so. The use of super-helical motifs, coiled coils, offers an optimal solution.[19] Coiled coils comprise two or more helices bundled together, each of which exhibits alternating *i, i+3* and *i, i+4* helical spacings or a $(HPPHPPP)_n$ repeat pattern, usually designated *abcdefg* (Fig. 2, 5A).[20] The patterns enable interdigitating contacts between side chains which direct *inter*-helical interactions. The number of helices in a bundle can be rationally programmed to assemble into different all-α topologies.[20] The same principles of complementarity that are used for all-β materials hold true here. Both linear and orthogonal types are possible using relatively short coiled-coil sequences (20-50-mers). In a similar fashion to β-membranes, for instance, α-helical staggers form extended fibres.[21]

Coiled-coil dimers, trimers and pentamers with complementary, "sticky", ends assemble into analogous fibrillar structures[21-23] whose lengths can correlate with the size of building blocks (Fig 5B).[24] Unlike β-membranes, which require three-dimensional assembly, all these fibres follow one *linear* pattern – longitudinal propagation of sticky-ended bundles. Most recently,[25] the fibres were shown to grow with uniform rates at both ends till maturation indicating a bi-directional assembly, while their thickening was arrested earlier in the assembly, which in some cases can be confined to the diameter of an individual bundle (Fig 5C). Similar to β-strands, helices in coiled coils are amphipathic. In contrast to β-strands, hydrophobic residues in coiled coils are buried in the helix-helix interface, with charged residues at the adjacent polar sites cementing it further (Fig 5A). In this case a non-linear, orthotropic topology is not obvious and requires to set up a main propagation pattern.

An efficient approach is an adaptation of the Crick's principles for interfacial hydrophobic packing[26] to the packing of polar faces. Namely, the polar face of a coiled coil split into two sub-faces can support a network of electrostatic interactions.[27] In most coiled coils opposite charges in complementary *g* and *e* sites bridge together.[19,20] If made of the same polarity (glutamates) the faces repel and destabilise the bundle. The repulsion can be neutralised by arginines (see orthotropic example for β-strands) at *c* (*i*) sites that can interact with glutamates at *g* (*i+4*) sites of the same helix and with *e* glutamates of a helix (*e'*) of another bundle.

Individual bundles are thus stabilised in a larger cluster with a repetitive unit of several bundles joined up in a starburst fashion (Fig. 6A).[27] Different coiled-coil oligomers – dimeric and trimeric[28] or trimeric and pentameric[29] – can also be employed to



direct the assembly in a similar symmetry-defined manner (Fig. 6B).

Fig. 5. Coiled-coil self-assembly motifs. (A) an exemplar linear sequence forming a homo-dimeric parallel coiled coil with two-heptad cationic (blue) and anionic (red) overhangs (left) and a coiled-coil homodimer configured into coiled-coil helical wheels (right). Double arrows indicate stabilising electrostatic interactions. (B) Dimeric and trimeric coiled-coil staggers (left) assembling into nanoscale fibres as seen in electron and atomic force micrographs (right).[24,25] (C) Time-lapse total internal reflection fluorescence images of a self-assembling peptide fibre. The bright round feature to the left of the centre is a fluorescent aggregate used for image registration. Scale bar is 2 μm. Adapted by permission from Macmillan Publishers Ltd: Scientific Reports,[25] copyright 2014.

Starburst topologies exploit classic coiled-coil interfaces and enable lateral and geometrically controlled propagation. This mode is cooperative and translates into the assembly of two-dimensional sheets, which, with increasing curvature, close to particulate aggregates.[27,28] Alternatively, coiled-coil domains of different oligomerisation states can be linked together to form an orthogonal building block that adopts a virus-like icosahedral assembly.[29]

In all these cases resultant nanoparticles have one or more cavities that can be used for the encapsulation of cargo such as genes or metal particles or as spatially confined vessels for simple redox reactions enabling the synthesis of smaller nanomaterials in situ (Fig. 6). Equally, owing to their large surface area and modularity in assembly the particles can present amplified antigenic epitopes to the immune systems thus providing prototypical platforms for vaccine designs.[9,30]

Alternative assembly forces

Other and more specialised folding motifs, notably collagen triple helices,[31,32] also exploit synergistic interplays between hydrophobic and electrostatic interactions and are covered in specialist reviews.[33] Complementary developments focus on different assembly forces that provide a somewhat broader application scope.[3] In this respect, interactions between aromatic residues (tryptophan, phenylalanine) can offer additional constraints that render material properties responsive. To give an

example, aromatic residues in the hydrophobic face of β-strands mediate the transition of water-soluble helical tapes into ribbons, which by having larger surface areas aggregate into tightly packed fibres that gel.[34] Aromatic residues appear to maintain sol-gel transition from early stages in the assembly thereby enabling responses to external stimuli. Placed in a specific environment the formed gels provide local control over physical phenomena that can be translated into useful properties. Indeed, by nucleating hydroxyapatite crystallisation – a capability commercialised as Curodont™ – such gels can regenerate tooth enamel in decay cavities eliminating the need for tooth filling.[35]

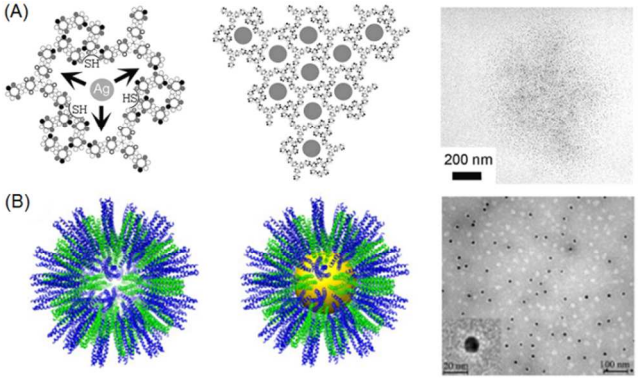


Fig 6. Translational assembly of α-helical coiled coils into nanoscale capsules. (A) Porous nanoparticles serving as nanoscale reactors for the conversion of ionic silver into uniformly sized silver nanoparticles. An electron micrograph (right) shows spherical spreads of silver nanoparticles after an enzymatic degradation of an individual nanoreactor (1-1.5 μm in diameter). Reprinted with permission from Ryadnov.[27] Copyright 2006, Wiley-VCH. (B) Icosahedral coiled-coil assemblies with and without encapsulated gold nanoparticle (yellow) in the central cavity. Blue and green indicate trimeric and pentameric coiled-coil domains, respectively. An electron micrograph (right) shows encapsulation of 15 nm gold nanoparticles in the peptide particles.[29]

All-aromatic assemblies are equally possible and are determined by π-π* stacking interactions, with an original design based on di-phenylalanine (FF).[3] Although N-protected hydrophobic amino-acids can also assemble,[36] these, strictly speaking, are not peptide materials, which makes FF the shortest self-assembling sequence reported. This peptide forms water-filled nanotubes[3] and its various modifications with other aromatic moieties or amino acids furnish fibrous hydrogels.[37] Importantly, short sequences used in these designs can afford only the β-type of hydrogen bonding, the continuity of which is maintained by π-stacked aromatic rings.[37] Such mode of packing presents a structurally permissive background that is amenable to the incorporation of different amino-acid derivatives or unnatural amino acids and is not restricted to L-enantiomers. For example, diastereomeric FF-based tripeptides were shown to successfully assemble into heterochiral gels that performed well in cell culture maintaining cell viability and proliferation in vitro.[38] Incorporating non-standard amino-acid blocks enriches the chemical space of peptide self-assembly and offers an efficient means to enhance the stability and biological selectivity of peptide materials under physiological conditions. Similarly, hybrid materials as co-polymers or conjugates with water-soluble polymers such as polyethylene glycol improve bioavailability without compromising peptide folding and self-assemby.[39, 40] The latter is important for expanding on design opportunities towards multi-component materials and somewhat cheaper poly-amino-acid materials, which are produced by polymer chemistry methods with much fewer reactions steps.[41] Yet again, sequence-prescribed peptide designs have not exhausted their unlimited potential and versatility.

Peptide topology defines nanomaterial form

Diversifying material forms is of an increasing demand in material design and nanotechnology.[2,3,15] The design of novel self-assembly topologies and the introduction of specialist periodic nanoscale features into known forms offer promising routes in this direction, with both exploiting possibilities of non-linearity in building blocks.

Non-linear sequences for specialist building blocks

Linear sequences underpin persistent assemblies at the mesoscopic length scale. An orthogonality enabled in their assembly gives that choice of additional topologies, which can support nanoscale modifications in bulk material forms. These topologies can be accessed via non-linear orthogonal constructs by modifying the amino-acid side chains of linear peptides. Different types of conjugates, dendrimers and branched structures have been attempted.[14] Some of these act as specialists by shaping existing forms into new versions or providing them with new functions. For example, microns long straight fibres assembled from linear standard coiled-coil sequences kink, split and branch when non-linear specialists are incorporated.[42,43] A caveat here is to ensure that specialists are complementary to standards: they may introduce discontinuities to nucleate specific features but should not disrupt or inhibit the assembly, in which case they would act as assembly terminators (Fig. 7A).[14]

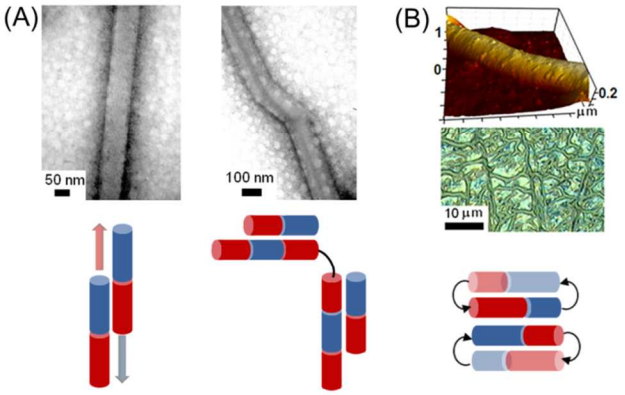


Fig. 7. From linear to orthotropic fibre assemblies. (A) A straight fibre (left) is converted into a kinked fibre (right) using a non-linear specialist peptide (two red domains linked via a linker) which diverts the linear assembly of standard peptides. Adapted by permission from Macmillan Publishers Ltd: Nature Mater,[43] copyright 2003. (B) Arbitrary assembly of two cyclised coiled-coil domains into microscopic fibres (upper) forming protein micro-nets (lower). Adapted with permission from Faruqui et al.[5] Copyright 2014, American Chemical Society.

Introduced as a concept of fibre-shaping[43] this strategy was successfully applied to template the synthesis of spatially defined

inorganic nanotubes. The silicification of fibres generated silica shells of controllable shapes and thicknesses.[44] After the removal of the encased peptide material, the shells can be developed as nanoscale containers or ampules for drug delivery, storage or biomarker capturing agents.

It is important to note that specialist designs play an auxiliary role and alone do not assemble. If made self-complementary these can give rise to structures similar to those produced by linear sequences.[45] In this case, the use of non-linear constructs may not be justified due to complex synthesis required for their production, while the resulting structures appear to be amorphous and less regular owing to a high degree of promiscuity in the assembly.[46] Alternative approaches to command nanomaterial shape and morphology are offered by arbitrary modes of assembly. These are deliberately promiscuous and directed to support a particular assembly scenario which is restricted at the nanoscale but expand at higher length scales, from micro- to millimetre.[47] A conspicuous topology to demonstrate this strategy is a cyclised peptide sequence assembling into fibrillar matrices (Fig. 7B). These matrices are free of directionality constraints, span microscopic dimensions and are biologically differential.[5] This design is based on two complementary coiled-coil heptad repeats, anionic and cationic, arranged into an asymmetric pattern of two domains. One domain (D1) comprises two anionic heptads and one cationic heptad, with the arrangement of the other domain (D2) being reversed. This gives a split of 2+1/1+2 pattern which sets up various heptad overlaps between domains.[5] Each overlap is probable in any direction with respect to the plane of the cyclic backbone, and the orientation of the domains in the block is antiparallel to ensure interactions between different blocks and not within the same block. The assembly is indiscriminate and encompasses several assembly patterns that can re-direct at any point, which introduces a cooperative knot-like propagation of the blocks into mesoscopic fibrillar nets (Fig. 7B). The nets were demonstrated to support human cell adhesion and proliferation while efficiently resisting bacterial colonisation over several days. The latter is a functional attribute of D2 domain. Its two-heptad cationic stretch has a binding affinity to bacterial membranes, which it is able to disrupt. The antimicrobial activity of individual D2 is negligible and become apparent only in the nets where the domain is persistently multiplied along assembled microscopic fibres, the activity also reported for native systems based on defensins.[48]

Since building blocks in the peptide nets possess no antimicrobial activity individually,[47,48] the concept of domain multiplication in fibres can be used to probe antimicrobial propensities of elementary peptide sequences that meet minimum requirements for potential activity. In this case, fibres serve as analytical displays of antimicrobial function and can be used as screening platforms for antimicrobial sequences of varied biological strengths.[49] In comparison, self-assembly can also enhance or enable the antimicrobial activity of polymeric materials. For example, micellar forms of di-block co-polymers exhibited greater activities against Gram-negative and Gram-positive bacteria compared to those of individual polymer chains as a result of locally amplified cationic charges.[50]

Admittedly, polymer-based materials with antimicrobial properties are mainly used as biocides without strict considerations for toxicity against mammalian cells.[51] Therefore, most common among them are biofilm-resistant surfaces and coatings which rely on physico-chemical modifications,[52] but can also extend to the use of supramolecular processes such as antimicrobial hydrogels.[53] However, the primary advantage of self-assembled peptide materials is that these can readily assemble from a single peptide, be used as such without specialist chemistry or modifications and can exhibit differential and selective responses against microorganisms without affecting mammalian cells.[5]

Non-linear building blocks from linear sequences

Orthogonal constructs and backbone cyclisation can be synthetically challenging and are not necessary for those sequences that fold into non-linear and cycle-like structures. A β-annulus is an obvious choice in this regard. The topology is a semi-cyclic arrangement of three β-strands that undergo a symmetrical assembly into ring-shaped supramolecular units with sticky ends. The ends direct the radial propagation of the units into larger two-dimensional sheets that bend and close with the formation of three-dimensional nanoscale particles (Fig. 8A).[54] This design follows established principles of domain swapping, successfully applied to engineered fibrillar, ribbon and membrane structures.[55] Domain swapping originates from a naturally occurring mechanism used by proteins as a selective means for oligomerisation. In this mechanism one domain of a monomeric subunit replaces the same domain from another copy of the same protein thus producing intertwined oligomers in which two subunits swap their identical domains.[55] An exchange domain can be a secondary structure element, α-helix or β-strand, or a fully folded protein. Domain swapping implies a directional reversal of a peptide chain that should result in a top-down topology.

In this regard, β-hairpins are intrinsic precursors of domain swapping which can be arranged by extending one of the hairpin ends that would stick to the same extension of another hairpin.[56]

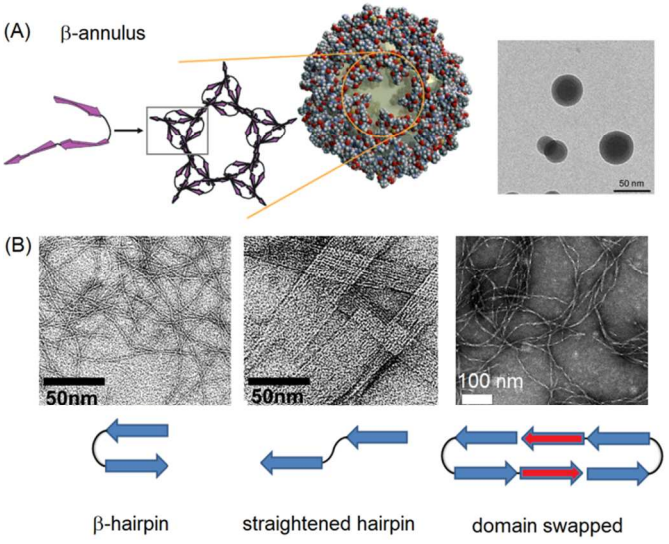


Fig 8. Non-linear folding units for orthotropic topologies. (A) Virus-like assembly of a β-annulus, schematic model (left) and an electron micrograph (right). Adapted with permission from Matsuura et al.[54] Copyright 2010, Wiley-VCH. (B) Schematics (lower) and electron micrographs (upper) of different β-hairpin topologies. Red strands indicate exchanged domains between two hairpins. Adapted with permission from

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Upon assembly extended hairpins form closed structures reminiscent of cyclic peptides and it is the relative orientation of the extension domain that defines the geometry of the assembly (Fig. 8B). Indeed, flat hairpins with or without extension tend to form twisted fibrils with varied gelation properties.[55,56] This is in contrast to the β-annulus which is assembled from a hairpin with its sticky domain being at an obtuse angle relative to a preceding domain (Fig 8A).[54] A variation of a β-hairpin arrangement can thus be envisaged to afford other material forms. For example, entangled fibrillar structures convert into laminated and extended membrane sheets by strengthening the same β-hairpin[57] (Fig. 8B).

The outlined develops infer that peptide topology drives assembly patterns and ultimately shapes the nanomaterial form. These also suggest that the role of a peptide sequence is limited to supporting peptide topology since major material forms are equally produced using different folding elements. This is not surprising given that material design serves the purpose of providing unique physical properties, which is different from more traditional protein design approaches that looks into sequence-prescribed biological functions. Biological nanomaterials are not sequence dependent, nor there strict a-priori requirements for the folding of building blocks. Nanomaterial architectures persist over mesoscopic length scales at which folding and assembly occur in synergy and within which a contiguous network of hydrogen bonds is maintained.[2] In fact, peptide nanotubes are long enough to support extended hydrogen-bonding networks, while the progressive stacking of ring-like building blocks appears to be an ample criterion for assembly. Short peptide sequences with alternating D- and L-amino acids that are constrained by backbone cyclisation provide just that.[4] These cyclopeptides fold into conformationally locked rings that stack forming hollow nanotubes. Hydrogen bonds run through the both sides of the rings with side chains placed on the outside surface of the assembled tube. The nanotubes persist hundreds of nanometres in length with a conserved diameter of 7-8 Å, and then pack into crystals of tens of microns in length and up to 500 nm in diameter. This type of assembly bears remarkable similarities with naturally occurring polypeptide antibiotics, gramicidins, which destroy bacterial cells by permeabilising their membranes.[58] Accordingly, the nanotubes proved to be able to insert into and span membrane bilayers in transmembrane orientations.[59] This mode of action was perfectly consistent with high antibacterial[5] and antiviral[60] activities of the nanotubes.

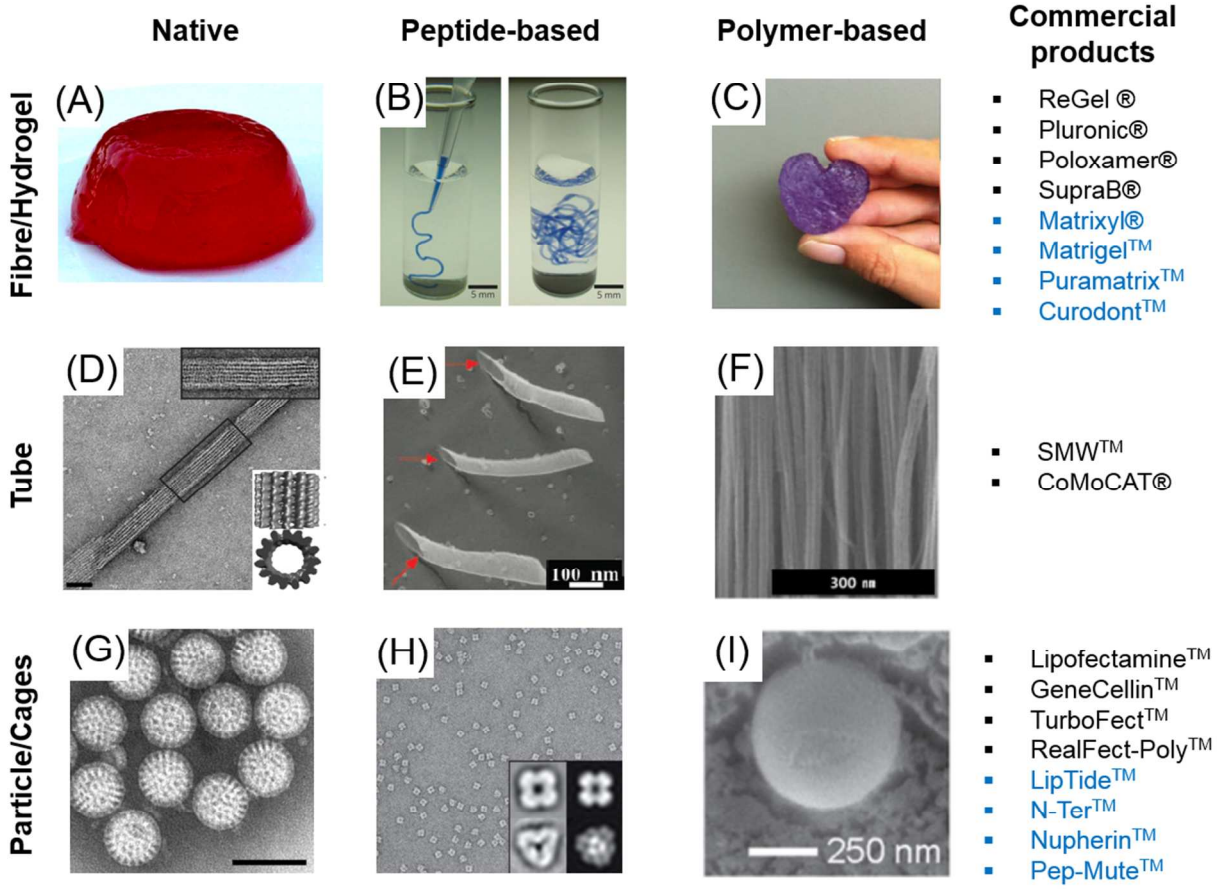

Fig. 9. Material forms of native, peptide and non-peptide origins with a representative list of commercial products based on peptide (blue) and non-peptide (black) materials. (a) Gelatine, (b) peptide amphiphile gel,[86] (c) ureidopyrimidinone (UPy) gel,[65] (d) tubulin microtubule,[6] (e) surfactant-like peptide nanotube,[106] (f) carbon nanotubes,[89] (g) viral particles,[8] (h) peptide nanoparticles,[113] (i) polymersome.[94] Adapted by permission from Macmillan Publishers Ltd: Nature Mater,[86] copyright 2010; adapted with permission from van Gemert et al.[65] copyright 2012 and Lee et al.[89] copyright 2008, Wiley-VCH; Adapted with permission from Vauthey et al.[106] . Copyright (2002) National Academy of Science, U.S.A; adapted by permission from Macmillan Publishers Ltd: Nature,[113] copyright 2014.

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ARTICLE TYPE

Peptide rationale for non-peptide materials

As outlined to this end, the topology of monomeric constituents determines the form, function and, ultimately, properties of a material. The chemistry of amino-acid side chains provides the topology, while secondary structure, being more sophisticated in comparison to lipids and polymers, can support more complex functions. However, exact relationships in the structure-function continuum are more difficult to envisage, let alone engineer. Encouragingly, design principles underpinning lipid, polysaccharide or polymer materials are generic enough to implement in peptides.[61-64]

Therefore, peptide materials are often designed retrosynthetically by transforming a desired form into a specific amino-acid sequence. Non-peptide materials identify useful properties which peptide designs can match and expand. Figure 9 represents three main categories of the material forms exemplified by commercial peptide and non-peptide products.

Fibrillogenesis and supramolecular networking in hydrogels

Hydrogels is perhaps the most advanced class of non-peptide materials with close relevance to peptide designs. Traditionally produced from polymers, hydrogels are physically cross-linked molecular networks.[64,65] These networks are defined as three-dimensional scaffolds that encapsulate water throughout the volume creating a non-fluid colloidal material. Because the networks constitute the continuous phase of the material their chemistry can be tailored to control gelation.[66] In polymers this is done by varying the degrees of polymerisation and cross-linking. The former denotes the number of monomers in a polymer chain, whereas the latter is the number of groups interconnecting polymer chains. Once a polymer is used to make a hydrogel its degree of polymerisation cannot be altered. By contrast, cross-linking can be applied at any stage thereby supporting a broad range of mechanical properties ranging from elastomer to resin. Granted that cell self-renewal and differentiation depend on immediate environments, with tensile strengths resembling those of developing tissues, hydrogels find use in surgery and regenerative medicine.[67] In these applications it is increasingly important to provide hydrogel materials with elastic properties matching tissue stiffness from $E \sim 1$ kPa (Young's modulus, E) for soft tissues (brain, fat) to 50 kPa for hard tissues (bone).[68] Tissue elasticity can dictate the controlled release of drugs, growth factors and nutrients that are readily encapsulated into hydrogels.[69,70]

Initial attempts in peptide hydrogels concerned reaching comparability with Matrigel™ – gelatinous extracts from tissue sources that are rich in the native extracellular matrix, matrix proteins and growth factors. Compositional heterogeneity characteristic of the material leading to batch-to-batch variations and poor utility for translational research prompted structural optimisation; that is at the nanoscale. The use of purified collagen, alginate and fibrin materials revealed similar tendencies of lacking reproducibility and control, but also exposed major factors necessary for proteinogenic hydrogels. One of these proved to be the formation of nano-to-microscale fibres that are structurally analogous to those of native collagen or fibrin.[71] Peptide fibres assume the role of polymeric chains in hydrogel scaffolds. However, covalent cross-linking is of little value since fibres are not individual, but bundled polypeptide chains.[72] Therefore, linking two chains in the bundle or between bundles does not guarantee stable interconnections. Fibrous networks provide an obvious solution. These can be random overlaps of individual fibres or branching and interconnected structures.[14] In all cases porosity or water-filled spaces between fibre chains appear to be an important factor and can be addressed using net-like architectures with pores expanding several microns in diameter.[73] Because fibre assembly results from the supramolecular polymerisation of polypeptide blocks the length or nature of peptide sequences is not prerequisite.[74] Both α-helices and β-strands, and more recently short synthetic collagen[33] and collagen mimetics,[75,76] are being used for the purpose. In most cases resultant materials are analogous and differ only in morphology and mechanical characteristics of component fibres.

Thus, the rationale of chemical polymerisation and cross-linking in polymers manifests in supramolecular polymerisation and networking in protein fibres. Defined peptide topologies provide both and are not constrained by specific sequence lengths or folding elements. Stability is critical for fibre-based materials and increasingly in conjunction with providing reversible responsiveness to external stimuli. Commercial co-polymer formulations such as ReGel®, Pluronic® or Poloxamer® exploit elevated temperatures as an external trigger to gel. Thermoreversible properties provide temperature-dependent gelation which upon injection can facilitate the local release of drugs as in drug delivery formulations of marketed gels. For example, ReGel® incorporating paclitaxel is used as OncoGel® for anti-cancer treatments.[77]

Responsive folding: material control and biology

Starting with the first thermostable peptide hydrogels reported back in 1990s,[78] most of the systems to date employ hydrophobic interactions balanced with polarity of amino-acid side chains to accommodate discontinued water phases. In all such systems peptide fibres are readily aligned to form nematic liquid-crystal gels, which may convert to fluid solutions upon cooling – the ability that can be tailored within narrow temperature ranges. For example, nanofibers that remain in solution at room temperature can be mixed with cells and then gel upon heating to physiological temperatures thus encapsulating cells in a fixed 3D scaffold-like environment. Subsequent cooling enables the

release of cells back into solution without compromising cell viability. Polarity of peptide chains is intrinsically pH dependent, which can drive sol-gel transitions through increases in pH from physiological values (pH 7-8) that disassemble gels or increases in ionic strength that induce gelation (see Curodont™ example).[34] Unlike polymers, peptides are also folding responsive which offers extra control over material properties at the level of individual amino-acid residues. In β-sheet designs single lysines can drive sol-gel transitions as a function of pH and ionic strength.[79] The trigger here is electrostatic switching which promotes or inhibits the self-assembly of fibrillar networks able to retain water.[80] Coiled-coil designs are characterised by more complex electrostatic interactions the disruption of which invariably leads to disassembly. In gels they instead rely on extending hydrophobic interactions that lead to similar response modes,[81] while minimalist hydrogelators comprising only hydrophobic residues and only a few (2-5) exhibit comparative responses to varying pH and ionic strength.[82] It becomes apparent therefore that the hydrophobic effect alone is sufficient to support responsive gel materials for as long as extensive hydrogen networks are maintained in the assembly. In this regard, primary peptide amphiphiles appear as the most cost-efficient materials.[73,74] A typical amphiphile is a short peptide that is covalently extended with an aliphatic domain.[83] The assembly of such an amphiphile is driven by the burial of aliphatic domains in the core of elongated micellar structures whose periphery displays peptides. The moieties can be rendered biologically active providing thus information-rich interfaces.[73] Indeed, amphiphile-based hydrogels tailored with strong binding affinities to growth factors or matrix proteins, individually or in complex, promote tissue growth and restoration ex vivo and in vivo.[84] It is an innate property of peptide materials to incorporate biology through, for example, modifications with short cell adhesion motifs (e.g. RGD, YIGSR) directly or as co-assembling components.[47,73,82] Puramatrix® is a representative peptide material with a consistent record of supporting in vitro cell culture applications. This material mimics the native extracellular matrix and is based on a RADA peptide motif reminiscent of RGD shown to mediate cell adhesion and proliferation.[18]

Close similarities in the structure, mechanical and physical properties of peptide gels regardless of topologies or sequences used suggest that their main strengths for commercialisation derive from the biology they can support. Nonetheless, there are few bespoke peptide hydrogels available in the market. The situation is gradually changing, but is largely dictated by the market trends in cell culture applications.

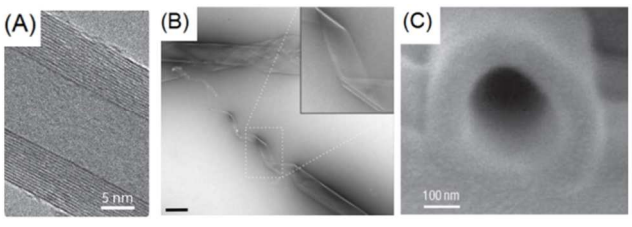
A general technological tendency is to use fibrous gels in cell culture to generate cell encapsulating formulations and induce selective substrate-supported cell growth and differentiation.[85] Approaches are being developed for self-assembly pathways that can lead to utilisable cellular aggregates in situ.[86] It was found, for instance, that by combining amphiphile assembly with a thermal pathway one can generate 2D plaques with a filamentous texture which can then spontaneously template long-range alignment of bundled fibres upon cooling. The obtained alignment can be extended over centimetres in noodle-shaped viscoelastic strings that, once mixed with live cells at physiological temperatures, form monodomain gels comprising aligned cells and filaments.[86]

Other approaches investigate commercial formulations to help improve existing products. A remarkable series of recent studies revealed that anti-wrinkle properties of Matrixyl® are enabled by a specific ingredient – a self-assembling peptide amphiphile which promotes native collagen production in a concentration-dependent manner.[87] The peptide assembles into tape-like structures that act as nano-thin skin adhesives which at critical aggregation concentration stimulate cell to produce excessive collagen.

Peptides offer ease of synthesis and scale up and a greater control over the homogeneity of the final product. However, the properties and performance of peptide gels are not unique and comparable with those of non-peptide analogues. Radically different developments may be needed to bring disruptive technologies to the market. These can clearly benefit from the versatility of peptide chemistry and biology which remains to be matched in material chemistry.[73]

Confined encapsulation at the nanoscale: tubes and cages

Encapsulation accommodates various applications in material science and biotechnology. Nanoscale tubes and hollow particles are the nanoscale expressions of the capability which provide well-defined cavities to host reactions, store cargo and facilitate drug transport. Classical non-peptide materials are carbon nanotubes (CNTs). These are 1-100 nm hollow cylinders of one (single-wall) or more (multi-wall) layers of graphene (Fig. 10A).[88,89] Outside of popular applications in molecular electronics and environmental nanofilter systems,[90] CNTs show promise as biosensors[91] or drug delivery systems.[92] However, their use imposes several side effects including inflammatory response, oxidative stress and toxicity, which need solving before widespread use can be possible. Hollow particles or cage-like forms prove more robust for biomaterial applications.[93,94] Liposome-based formulations are used as accepted materials for gene transfer (Lipofectamine®) and are particularly beneficial for macromolecular drug delivery (Myocet® for doxorubicin).[95] As a rule of thumb, surface functionalization can improve the targeting abilities of the materials, their solubility and extend circulation time. Concerns remain around cytotoxicity effects, polydispersity and relatively low loading capacities. An alternative is proposed in polymersomes.[93] These are based on high molecular weight block-copolymer amphiphiles that assemble into closed bilayer membranes showing high physical and chemical stability.[96] Main advantages include the systematic delivery of drugs and in combination allowing the simultaneous loading of molecules encapsulated in the lumen (macromolecular and hydrophilic) and in the bilayer (hydrophobic).[97,98] Although drug release is



possible both in vitro and in vivo, the exemplary stability of polymerosomes often carries a price of providing low membrane permeability which inhibits cargo release.

Fig. 10. Nanotube materials. Electron micrographs of (A) multi-wall carbon nanotubes,[90] (B) tape-like synuclein tube[100] and (C) FF peptide nanotube.[102] Adapted with permission from Morris et al. Copyright 2013, Wiley-VCH; adapted by permission from Macmillan Publishers Ltd: Nature Nanotech, copyright 2006.

Peptide materials are intrinsically biocompatible, biodegradable and underpin nearly all encapsulating systems in biology. They also share most of the useful features that made both carbon nanotubes and liposomes commercially promising. Peptide nanotubes have recently[74] been discussed and here we present only a few examples to make comparison. Taking the morphological properties of CNTs as an inspiration coiled-coil bundles were designed to produce 3-nm-wide nanotubes extending into several microns thus showing comparable dimensions to those of single-walled carbon nanotubes. The bundles comprise seven α-helices that assemble into a lock-washer structure with increased end to end association.[99] The coiled-coil bundle topology is supported by electrostatic interactions at the **b**, **c** and **f** positions of the coiled-coil helices whilst lateral association is inhibited. β-sheet bilayer membranes assembled from eight-residue fragments of α-synuclein is another archetypal example shown to form extended tubes of 200-330 nm in diameter by spiralling around the tube axis (Fig. 10B).[100] This design together with FF nanotubes (Fig. 10C) is consistent with water-filled nanotube structure of amyloid-like fibrils,[101] which implies that the contiguous discrete cavities of the tubes can be tailored to specific diameters enabling nanomaterial applications. Casting metal nanowires is one of these: ionic silver reduced in FF cavities followed by the enzymatic degradation of the FF shell gives silver nanowires with persistence lengths matching those of the nanotubes.[36] The tubes exhibit significant chemical and thermal stabilities as well as remarkable mechanical strengths.[102] The latter makes these assemblies suitable for applications that appear exclusively in the scope of CNTs. In general, non-biological applications of peptide materials become relevant with their enhanced mechanical properties. For instance, the elastic modulus of CNTs is in the range of 0.2-5 TPa, while for peptide hydrogels and nanotubes these are in kPa and GPa ranges, respectively. These differences specify applications. Further, peptide materials can be endowed with new properties through covalent or non-covalent functionalization. Peptide nanotubes assembled in the presence of magnetic nanoparticles align along the direction of applied magnetic field, while tubes incorporating lanthanides provide structural platforms for enhanced photoluminescence supporting cascade energy transfer.[103] These are sought after properties for nanoscale sensors and electronic devices.

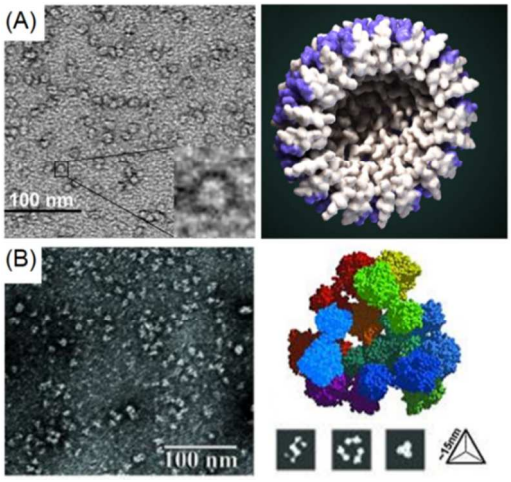


Fig. 11. Nanocage structures from naturally occurring non-cage peptides. Electron micrographs (right) and stereo models (left) of (A) peptide nanoparticles assembled from transmembrane peptide domains[111] and (B) a tetrahedral protein cage with the edge length of 15 nm shown with three simulated images calculated from the atomic coordinates of the cage in three orientations.[112]

Subtle alterations in nanotube designs were shown to drive particle-like assembly or shortened nanotubes with closed ends. For example, by removing one phenylalanine ring in FF chemically stable hollow nanoparticles were obtained.[104] Particles of similar sizes (100 nm) can also derive from the same FF motif incorporating thiols which via disulphide formation direct the assembly of a more compact structure. Similar to the tape-like synuclein assembly[100] or tube-cage transitions in CNTs,[105] FF-related designs are proposed to form two-dimensional membranes closing into cavity-defined structures.[104]

Although difficult to control such transitions are increasingly attractive in peptide materials and stimulate the search for complementary or more generic strategies. Surfactant-like peptides that consist of a hydrophobic amino-acid domain and a polar residue head draw certain analogies with phospholipid-based liposomes.[106] Indeed, the peptides exhibit a comparable behaviour by assembling into closed bilayer membranes that wrap into 50-nm particles or 300-nm nanotubes.[107] Nonetheless, in these and related designs particulate and tubular forms are often present in the same preparations without obvious relationships regarding their relative ratios and size distributions.

Discrete encapsulation for discrete biology

Discrete materials and a means for their directed assembly remain to be an apparent priority for material applications. One particular area of close relevance is drug and gene delivery. Most commercial developments are transfection agents for DNA and RNA transfer including peptide-enabled lipoplexes (LipTide™) and peptide nanoparticle complexes (N-Ter™) (Fig. 9).[108,109] Such formulations use nuclear-localisation sequences, DNA-binding motifs (Nupherin™) and cell-penetrating peptides – transduction domains used by viruses to facilitate own entry into the cytoplasm (PepMute™). The domains are of particular interest and can be rationally designed to complex with nucleic acids and promote their active intracellular transport.[110] A far

more attractive incentive in this direction is to mimic the most efficient gene-transfer reagents – viruses.[8,93] Viruses are rigid hollow nanoparticles or protein cages that are monodisperse, stimuli-responsive and capable of self-assembly with or without their main cargo – nucleic acids. A number of undesired attributes however limit their systemic use to in vitro experiments. Therefore, artificial cage-like structures that can function as viruses but lack their shortcomings attract strong commercial interest.

Apart from the examples outlined so far, two main approaches are applied. One is the continuous search for naturally occurring peptide motifs with an ability to form capsule-like structures. Such motifs do not necessarily derive from related assemblies and can have unexpected origins. An elegant design is the modification of transmembrane domains of membrane proteins.[111] These are hydrophobic sequences that without the support of their lipid environment fold into compact and remarkably uniform spherical nanoparticles. Because the sequences derive from a native protein the nanoparticles possess innate biological activity by inhibiting tumour metastasis associated with the protein and additionally could encapsulate hydrophobic drugs for intracellular delivery thus demonstrating dual biological function.[111]

The other approach concerns the re-purpose of known folded proteins as building blocks for cage-like assembly. An early design stems out from the conjugation of two protein domains with different oligomerisation states (dimer and trimer) into a fusion chimera, 12 copies of which assemble into cage-like tetrahedrons with an edge length of 12 nm.[112] Later designs apply computational engines to diversify cage-like structures, with each cage having a defined number of subunits following distinct tetrahedron architectures.[113] Well-packed complementary cores of hydrophobic amino-acid side chains between the subunits give hydrophobic interfaces, while polar side chains formed the periphery of the cores. Square- or triangle-shaped structures with individual cavities confirmed the design rationale. Analogous to DNA designs culminating in the DNA origami rationale, tetrahedron geometry is taken as a guide for more discrete structures assembled from a single polypeptide chain. Most recently, 12 coiled-coil segments combined into one contiguous peptide backbone was reported to fold into individually discrete tetrahedrons.[114] This design is unique in that it introduces an interfacial field in peptide design by bringing together de novo protein folds with qualitatively novel material applications.

Conclusions

We highlighted or rather touched current trends effecting union between design and commercialisation of peptide materials. The overview aimed to stimulate interest in this exciting and intellectually satisfying field. The outlined designs exemplify many approaches which are likely to expand with emerging technologies and quite possibly with new material forms. However, challenges facing the field today target the promise peptide materials offer in what we call real-life applications – commercial products. For this reason, we attempted a critical comparison of peptide materials with non-peptide materials that have already found their niche in the market. Healthcare, cosmeceutics, medicine and even defence are the areas where peptide self-assembly can impact substantially. Possibilities for exploitation are indeed vast, but ultimately depend on the success of commercialisation which is just starting to take place for self-assembling formulations – a perfect timing for newcomers.

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[a]*National Physical Laboratory, Hampton Road, Teddington, Middlesex, TW11 0LW, United Kingdom. E-mail: max.ryadnov@npl.co.uk*

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