REVIEW

Peptide nanotubes: molecular organisations, self-assembly mechanisms and applications

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Peptide nanotubes are promising bio-inspired self-assemblies with a wide range of envisioned applications. The present review addresses the recent advances in their fundamental comprehension and mechanistic aspects of their latest downstream uses. Through well-documented examples, including the Lanreotide peptide monodisperse nanotubes, the molecular organisations and interactions underlying such well-defined hierarchical nanoarchitectures are in particular examined. The kinetic and thermodynamic aspects of the corresponding self-assembly processes are also considered, especially the intriguing mechanism of nanotube wall closure. The recently unravelled Lanreotide self-assembly mechanisms have revealed, for instance, the limiting role of electrostatic repulsion in this critical step. Within the numerous applications currently explored, particular attention is given to promising inorganic deposition processes using peptide nanotubes as scaffolds. In exceptional cases, inorganic nanotubes with tunable diameters could be synthesised *via* peptide-based

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template-directed synthesis combined with peptide chemical design. Such examples highlight the importance of advanced molecular and mechanistic understanding of peptide nanotubes, particularly for bottom-up chemical design strategies and downstream applications. Although incomplete, the current fundamental comprehension of peptide nanotubes has already shown its potential by opening up new valuable routes in the field of biomimetic soft matter.

Introduction

From organised biological matter to supramolecular synthetic chemistry, molecular self-assembly is the ubiquitous strategy for the construction of architectures of controlled morphology with 1–100 nm dimensions and single-nanometre precision.[1–3] Kinetically biased and thermodynamically stable structures can be obtained by controlling the corresponding self-assembly equilibria, leading to defect-free and even self-healing supramolecular architectures. Exquisite biological examples include tubulin self-assembly into the cytoskeleton microtubules,[4,5] the formation of large monodisperse nanotubes by the capsid proteins of the tobacco mosaic virus,[1,6] and the dynamic actin filaments from muscle tissues.[7] All of these illustrate well how extremely defined functional architectures can be naturally achieved through molecular self-assembly. In contrast, biomimetic approaches are difficult to implement when the building blocks themselves exhibit complexity, as in the case of proteins.

Among the alternative routes to biomimetic self-assembly, peptides can act as excellent simple building blocks, due to their chemical variety, biocompatibility and ability to associate spontaneously. Over the past two decades, the diversity of design strategies explored has resulted in an extended library of nanoscale morphologies, including peptide nanofibrils, nanotapes, nanoribbons, nanospheres, nanobelts and nanotubes.[8–14] Following the principles of supramolecular chemistry, numerous and specific non-covalent interactions ensure the structural cohesion and stability of the peptide intermolecular networks.[3,15] For example, one or more specific side-chain interactions such as

ionic bonds or aromatic interactions, hydrophobic effects, van der Waals forces, and α-helix- or β-sheet-based extended hydrogen bond networks between the peptide backbones can contribute to the supramolecular cohesion of peptide assemblies. Such sets of weak interactions also foster the construction of dynamic, reversible and responsive nanoarchitectures. The opportunity for nanoscale responsiveness has been demonstrated through elegant designs of pH-triggered self-assembling peptide-based systems.[16–18] Well-balanced amphiphilicity of the peptide building blocks can further lead to nanoarchitectures with noteworthy hydrogelation properties, resulting in nanostructured matrices, possibly responsive.[17,19] The high potential of peptide-based nanoarchitectures, including nanoscale-precise morphological control, biocompatibility and versatile properties has opened the way to numerous applications. These have inspired outcomes ranging from soft materials in biology and pharmaceutical sciences, to nano-optics, electronics and templates for nanotechnology.[20–25]

As an overview of the contemplated biological soft materials, peptide nanofibre- and nanotube-based matrices have shown promising utility as nanoscaffolds for 3D cell culture and tissue engineering,[26,27] enzyme immobilization and mimics,[28,29] and drug delivery.[30–32] It is worth noting that peptide nanotubes have already been commercialised in the latter domain.[31,33] Peptide nanoarchitectures offer surfaces that can be chemically functionalised for the building of biosensors, and/or can serve as organic templates for the growth of ordered inorganic phases, towards leading to applications in nanotechnology.

The well-defined morphology of peptide nanotubes has particularly attracted such surface chemistry.[34,35] Biotinylated or antibody-functionalised peptide nanotubes[36,37] have, for example, been reported. Peptide-based template-directed syntheses of gold nanowires[38] or mineralised nanotubes[39] could prove possible. Such novel inorganic nanomaterials could contribute to nanoscale physics, in areas such as nano-optics or electronics.[34,35,40] The processes of bioinorganic synthesis are of fundamental interest, as well as of biological relevance. Natural biomineral constructions built from organic templates, such as diatoms, shells, bones or teeth, exhibit defined nanoscale to mesoscale hierarchies that cannot be attained by synthetic chemistry.[41,42] Biomineralisation of peptide architectures could provide a good model to gain knowledge in the molecular mechanisms governing such bioinorganic morphogenesis.[39,43]

The elucidation of biological assemblies has constantly fostered significant advances in peptide supramolecular chemistry. The strategies of peptide nanotube design, for instance, were bioinspired by pathological protein aggregation phenomena, especially amyloidoses.[44–46] Self-assembling peptides, as fragments of amyloid-forming proteins, have served as useful models in the structural elucidation of the generic amyloid nanofibres involved in numerous neurodegenerative diseases, and of the corresponding pathological mechanisms.[47–53]

Maité Paternostre

Dr Paternostre obtained her Master's degree in physical chemistry and Biophysics in 1981 and earned her PhD in Biophysics in 1983, both from the University P. & M. Curie (Paris, France). Her general interest is focused on biological and/or biomimetic self-assemblies. If she has been particularly involved in the understanding of biological membranes, her activity is now essentially focused on the self-assembly of amphiphilic peptides. Her aim is to understand the molecular determinants together with the physicochemical forces that drive the self-assembly processes of biological or biomimetic peptides and proteins. One of the challenges in this field is to code the shape and size of a supramolecular architecture from the structure of the building block, i.e. *the peptide sequence and conformation.*

The structural and general knowledge gained in this field, such as the axiom of β-sheet extended assembly being a generic feature of the polypeptide chain under appropriate physicochemical conditions,[54,55] and the discovery of functional amyloid-like nanofibrils,[56–58] provides important foundations for modern peptide supramolecular chemistry.[53,59] Bottom-up strategies, also successfully applied to peptide molecular self-assembly, have in return brought novel insights into structural biology. Nanotubes by designer cyclic peptides could, for instance, mimic trans-membrane ion channels.[60]

Over their wide range of envisioned applications, peptide nanotubes represent fundamental model systems for biology and soft matter. Nevertheless, despite decades of research, our generic understanding of these structures remains in its early stages. The concomitant achievement of shape, size and property control still requires better insights in the relationship between morphology, molecular organisations, and the kinetic and thermodynamic aspects of the self-assembly mechanisms. Nanotube monodispersity and diameter tuning have been for instance rarely attained. On the basis of the well-documented example of the Lanreotide nanotubes, we here review the current comprehension of these systems by addressing (i) the current library of peptide sequences self-assembling into nanotubes and their corresponding range of characteristic sizes, with a specific focus on monodisperse nanotubes; (ii) the few examples of solved molecular organisations underlying the structure of peptide nanotubes; (iii) the kinetic and thermodynamic aspects of nanotube formation, and the intriguing mechanism of wall closure; (iv) the critical issue of nanotube dimensions control and tuning; and (v) the current comprehension of inorganic deposition mechanisms on peptide nanotubes, and their applications.

Library of nanotube-forming peptide sequences, corresponding diameters and applications

In addition to the problem of attaining dimensions comparable to biological assemblies, biomimetic peptide self-assembly in solution faces other critical concerns. These mainly include sequence simplicity, peptide solubility, and the strict control and eventual tuning of the size parameters of the resulting assemblies, particularly diameter monodispersity in the case of nanotubes. As discussed in a following section, several micron lengths are generally obtained for peptide nanotubes, but cannot be easily controlled through molecular design. Given the extended library of nanotube-forming peptides,[23,61–65] an overview of the distinct approaches to sequence design is offered, focusing on the above key issues, indeed reached by very few remarkable examples.

Amyloid-inspired peptides

Hydrophobic dipeptides, including the aromatic diphenylalanine peptide, have been reported to form pore size hydrophilic nanochannels when crystallised.[66] The diphenylalanine sequence has been further shown to form large nanotubes in solution, upon dilution from organic solvents into aqueous solution, or via a heating–cooling cycle applied to the peptide aqueous solutions.[38,45,67,68] The resulting micron length polydisperse rigid nanotubes exhibit a substantial range of broadly distributed diameters, up to 300 nm or 2000 nm depending on the conditions

applied.[38,67,69] This remarkable self-assembling dipeptide, in fact the minimal sequence ever reported to form nanotubes in solution, was originally designed based on the hypothesis of a core aromatic motif triggering amyloid fibrillogenesis via π-stacking.[70] A family of aromatic dipeptides self-assembling into nanotubes and other nanoarchitectures has been developed from this initial example.[13,19,23] Although still under debate, it was proposed that the molecular organisation underlying the structure of the diphenylalanine nanotube walls relies on a laminated porous construction, stabilised by tri-dimensional aromatic stacking together with hydrogen bond networks.[69] A wide range of potential applications has been investigated for this family of self-assembling aromatic dipeptides, especially template-directed syntheses with various inorganic depositions.[19,23,35,38,67]

The Ac-KLVFFAE-NH$_2$ peptide (Fig. 1, sequence 1), or Aβ (16–22) peptide, corresponds to the central fragment of the Alzheimer disease-related amyloid peptide Aβ. This heptapeptide was initially synthesised for the investigation of amyloid fibrillogenesis and fibril lamination. Unexpectedly, Aβ(16–22) was shown to self-assemble into β-sheet-based nanotubes in solution under appropriate conditions, especially acidic ones, chosen to increase the peptide solubility and to foster the expression of amphiphilicity.[71] Structural investigations have revealed that the Aβ(16–22) nanotubes are monodisperse, with an outer diameter of 52 nm and a wall thickness of 4 nm. The molecular organisation proposed relies on a peptide bilayer, stabilised via specific cross-strand side-chains pairing through hydrophobic effects and electrostatic interactions.[71,72] The related hydrophobic sequence AAKLVFF has been reported to similarly self-assemble in methanol into large nanotubes, with a slightly polydisperse outer diameter, centred on 72 nm.[73] This peptide was further shown to undergo solvent-induced
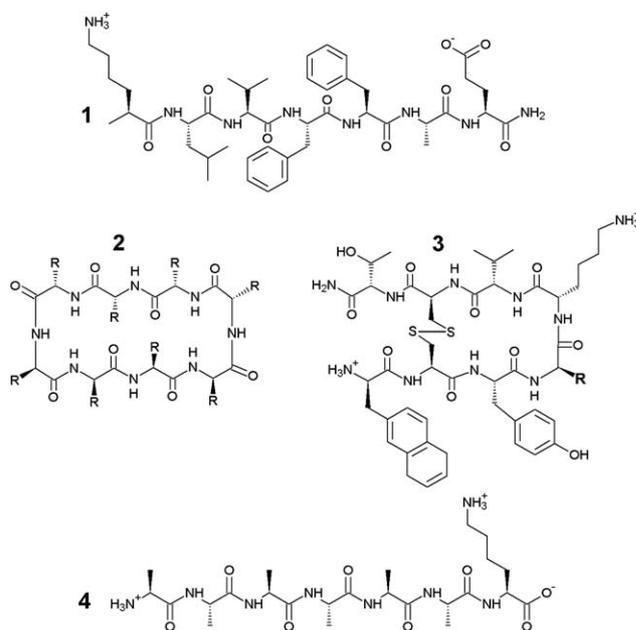

Fig. 1 Examples of peptide sequences forming monodisperse nanotubes. **1**: Aβ(16–22) peptide; **2**: cyclic D,L-peptides; **3**: Lanreotide peptide (R = W) and 4-D-Lanreotide peptides (R variation); **4**: A$_6$K surfactant-like peptide.

morphological transitions between β-sheet-based nanotapes, nanofibrils and nanotubes in water/methanol mixtures, thus providing a good model for the investigation of the non-covalent interactions interplay and related structural consequences.[74] Interestingly, both these self-assembling amyloid-derived heptapeptides contain the fibrillogenic and nanotube-forming diphenylalanine motif previously described.

Cyclic peptides

Bioinspired by the β-helical pores formed by the natural antibiotic gramicidin A,[75] and conceptually related to the β-helices by linear D,L-peptides,[76] the first designed peptide nanotubes relied on the one-dimensional directed assembly of cyclic D,L-peptides (Fig. 1, sequence 2).[8,61] The peptide rings were engineered to adopt a planar conformation through their even number of alternating D- and L-residues, which enabled further unidirectional stacking *via* intermolecular β-sheet hydrogen bonds between the peptide backbones. The diameters of the resulting monodisperse nanotubes are hence directly defined by the size of the peptide cycle, and remained around one nanometre. Cyclic octapeptides assemble into nanotubes with an internal van der Waals diameter of 0.7 nm, *versus* 1 nm and 1.3 nm respectively for cyclic decapeptides and dodecapeptides.[61] A remarkable feature of the D,L-cyclic peptide design is the opportunity for directly controlling the chemical groups that radiate from the nanotube outer surface, through chemical design of the peptide side-chains. Variations of these gave rise to a library of small peptide nanotubes with tunable properties, especially affinity for diverse environments, such as peptides forming nanotubes in the crystal state, in solution or within lipid bilayers and bacterial membranes. Applications of the cyclic D,L-peptides include transmembrane channel mimics,[60] antibacterial activity for cyclic D,L-α-glycopeptides[77] and molecular electronics, with the demonstration of charge transfer phenomena along the nanotubes, through incorporating unnatural conductive side-chains (1,4,5,8-naphthalenetetracarboxylic acid diimide).[21] Relatively in line with the cyclic D,L-peptides, β-helical protein fragments have been proposed as building blocks for pore-size equivalent nanotubes, from molecular simulations.[78]

Bi-dimensional assembly overcomes peptide molecular size to enable assembly into nanotubes of large diameters, as in the remarkable case of the cyclic octapeptide Lanreotide of sequence (D-2-naphthyl)A-cyclo(CY(D)WKVC)T-NH$_2$ (Fig. 1, sequence 3 with R = W). Besides its small size, this synthetic peptide has been shown to undergo self-assembly into monodisperse nanotubes with a discrete diameter of 24.4 nm.[79] Bi-dimensional assembly originates from the exquisite Lanreotide β-hairpin sequence, which combines not only antiparallel β-sheet propensity, cyclisation *via* a disulfide bridge and amphiphilicity through the respectively hydrophobic and hydrophilic faces of the β-hairpin, but also segregation on each β-strand of the aromatic residues from the aliphatic side-chains. Fine structural analysis of the corresponding molecular organisations showed that all these features were expressed in the nanotube inner structure (detailed in the following section). The nanotube walls were shown to rely on an original 1.8 nm wide peptide bilayer, built from head-to-tail Lanreotide amphiphilic dimers as basic building blocks, and stabilised by hydrophobic effect, extended

β-sheet hydrogen bond networks, aromatic interactions and electrostatic repulsions.[79,80] The mechanisms of assembly were shown to involve the formation of bi-dimensional helical nano-ribbons before nanotube closure (detailed in a following section).[80] Through a mutational approach combined with fine structural analysis, a family of Lanreotide self-assembling derivatives was developed.[81–83] In particular, the key structural role of the Lanreotide 4-D-aromatic side-chain in governing the nanotube diameter has been elucidated. This fine analysis led to the rational design of an elegant library of nanotube-forming 4-D-Lanreotide-derivatives, with discrete diameters ranging from 9.8 nm to 36 nm (Fig. 1, sequence 3 with R variation).[83]

As for applications, and as an analogue of natural Somatostatin-14, Lanreotide is a synthetic growth hormone inhibitor, with established therapeutic activity in the treatments of acromegaly and neuroendocrine tumors.[84,85] The nanostructured hydrogels resulting from Lanreotide self-assembly in water were the first commercialised controlled release "self-formulations", or "molecular therapeutic hydrogels" of the therapeutic agent itself (FDA approval 2007).[31,33,86] Lanreotide nanotubes also show utility in template-directed mineralization processes (detailed in a following section).[39]

Peptide amphiphiles

Inspired by the molecular structure of biological phospholipids, surfactant-like peptides have been designed to contain a charged hydrophilic head of one or two residues (aspartic acid or lysine), and a hydrophobic tail of at least four aliphatic residues (alanine, glycine or valine).[12,87] Within the extended library of self-assembling peptides thus created and extensively reviewed,[62,64,65] a few sequences have been shown to form large 30–50 nm wide polydisperse nanovesicles and nanotubes, *e.g.* Ac-A$_m$K-NH$_2$, Ac-V$_m$K$_n$-NH$_2$, Ac-G$_m$D$_n$-OH, Ac-A$_6$D-OH, Ac-V$_6$D-OH, Ac-A$_6$K-OH, and Ac-I$_3$K-NH$_2$. The corresponding molecular organisations were described as membrane-like peptide bilayers stabilised *via* hydrophobic interactions. Highly dynamic assembly–disassembly processes were observed between the nanotubes and nanovesicles.[12,87] However, the non-end-capped version of the A$_6$K peptide (Fig. 1, sequence 4) has been reported to form monodisperse nanotubes, with a large outer diameter of strictly 26 nm.[88] Structural insights suggested single-β-sheet thick nanotube walls, further stabilised by electrostatic interactions.[89] The A$_6$K peptide is the unique example of monodisperse nanotube-forming sequence in the surfactant-like peptide series.

Peptide amphiphiles inspired several bottom-up nano-architecture-forming variants, such as lipopeptides, peptide bola-amphiphiles and peptide–polymer conjugates. These have been reviewed elsewhere.[24,34,62,64] Although slightly outside the scope of the present review, the case of the α-helix peptide–poly-sarcosine conjugates is worth mentioning. These specific α-helical peptide-conjugates were recently reported to form well-defined 70 nm wide nanotubes. Interestingly, the complementary stereo-complexes of left-handed and right-handed α-helical peptide-conjugates also showed to co-assemble into nanotubes of varying dimensions.[90]

Despite the diversity of design strategies, the above overview highlights that only few peptide sequences have been reported to form strictly monodisperse nanotubes *via* molecular assembly,

namely the Aβ amyloid peptide Aβ(16–22) core fragment,[71,72] the family of cyclic D,L-peptides,[8,61] the Lanreotide peptide,[79] the engineered 4-D-Lanreotide derivatives,[83] and the surfactant-like A_6K peptide (Fig. 1).[88,89] The corresponding discrete diameters cover the order of magnitudes observed for biological assemblies (Fig. 2). Rational diameter tuning was only achieved in specific cases: in the diameter range 0.7–1.3 nm for the cyclic D,L-peptides through engineering sequence length,[61] and in the diameter range 9.8–36 nm for the 4-D-Lanreotide derivatives through rational chemical design of one specific residue in the Lanreotide sequence.[83]

Molecular organisations and wall curvature

Understanding molecular packing within the walls of peptide nanotubes can be a powerful tool for the further development of bottom-up strategies. The rationalisation of the inner close contact interactions enables potential control of the nanotube morphology through chemical design, including fine-tuning of the nanotube diameter for nanotechnology applications. Furthermore, such structural knowledge can be crucial to the understanding of macroscopic properties such as nanotube insolubility or slow release of the peptide building blocks from supramolecular assemblies. Despite the number of small molecules reported to self-assemble into nanotubes, very few organisations have been solved at molecular level to date.[79,91–93] Unravelling the molecular organisations of peptide nanotubes is indeed experimentally highly challenging due to their inability to undergo 3-dimensional crystallisation, thus preventing any structural resolution by single crystal X-ray diffraction. Solving the molecular organisations of peptide nanotubes therefore requires a multidisciplinary approach, as performed for the molecular-scale structure of biological self-assemblies, e.g. microtubules[4,5] or the Tobacco Mosaic Virus capsid.[1,6] Structural techniques generally used include electron microscopy, vibrational spectroscopy, NMR and X-ray scattering. A pivotal tool that has been previously used to investigate the molecular organisations within amyloid fibrils is X-ray fibre diffraction.[94–96]

Most peptide nanotubes are organised into laterally associated infinite β-sheet filaments. Two main types of molecular organisations can thus be observed upon the orientation of the peptide backbone—either out-plane or in-plane with regard to the

nanotube wall. In the first case (Fig. 3a), the β-sheet filaments are embedded between other filaments. Most of the lateral residues radiating from the filaments are in contact with another residue. Such organisation is well illustrated by amyloid-like nanotubes formed by linear octapeptides stacked into head-to-tail networks[81] (Fig. 3a). Both the inner and outer interfaces with water are chemically equivalent, resulting in a large radius of curvature. In the case of lipid nanotubes,[93] a symmetry breakage causes a slight packing difference of equivalent head groups at respectively the inner and outer interfaces, hence generating wall curvature. The mechanism of wall curvature is likely similar for large nanotubes by linear peptides.

The second type of molecular organisation (Fig. 3b) is a direct consequence of the amphiphilic nature of the peptide building block itself. Amphiphilicity induces the formation of a peptide bilayer, in which the confined hydrophobic residues are protected from water by the inner and outer β-sheet networks. This amphiphilic character is directly related to the peptide sequence, since odd and even residues are spatially separated by the peptide backbone in the β-sheet secondary structure. Such organisation has been observed in the case of linear Tau fragments,[91] amyloid model peptides,[92] or the cyclic Lanreotide peptide.[79] In the latter case, the β-hairpin planar conformation is stabilised by a disulfide bridge. The segregation of aromatic residues from both aliphatic residues and hydrophilic regions (green) over the three hierarchical levels of organisations, i.e. dimer, filament and nanotube is remarkable (Fig. 4). This molecular organisation is similar to the walls of the bacterial gas vesicles.[97] In this case, both the inner and outer β-sheet filaments exhibit slightly different hydrogen-bond lattices, thus resulting in wall curvature.

Peptide molecular packing within nanotubes strongly depends on the spatial repartition of amphiphilicity, either along the peptide backbone or between both peptide faces. The resulting organisations within nanotube walls can be interdigitated and/or bilayered. The physical origins of wall curvature are strongly dependent on this packing, and can offer the opportunity to modulate the nanotube diameter.

Mechanism of peptide nanotube self-assembly

In this section, the central question is the mechanism of peptide self-assembly focused on the particular case of nanotube formation. Indeed, understanding the molecular and supramolecular mechanism, together with the physical chemistry driving the process should lead to a better control of the morphology of the self-assembled architectures. As previously noted, Nature provides a variety of examples of self-organized tubular architectures based on proteins—virus capsids,[1] microtubules,[4] actin filaments[98] and even amyloid fibrils composed of pathological misfolded proteins.[47] However, the thorough understanding of the molecular processes and physicochemical parameters driving the nucleation and growth of these complex architectures is hampered by the scarcity of natural or synthetic systems known to date. The interplay between the dynamics, stability, and function of supramolecular complexes at the molecular level is a challenging issue, which has been elucidated for only a few systems, namely, the microtubules,[4,99] the actin filaments[7,98,100] and the TMV capsid.[1,101,102] Besides the importance for nanotechnology, this context generates high expectations for the
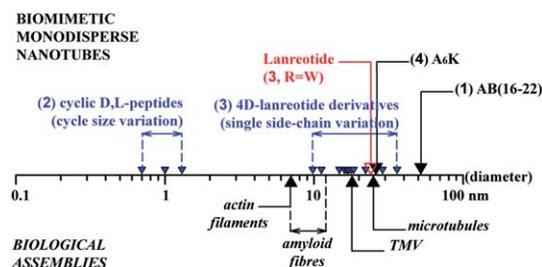

Fig. 2 Range of diameters by monodisperse nanotube-forming biomimetic peptides (sequences in Fig. 1) compared to biological assemblies, respectively Aβ(16–22) peptide (**1**), cyclic D,L-peptides (**2**), Lanreotide (**3** with R = W), 4-D-Lanreotide peptides (**3** with R variation) and A_6K peptide (**4**).
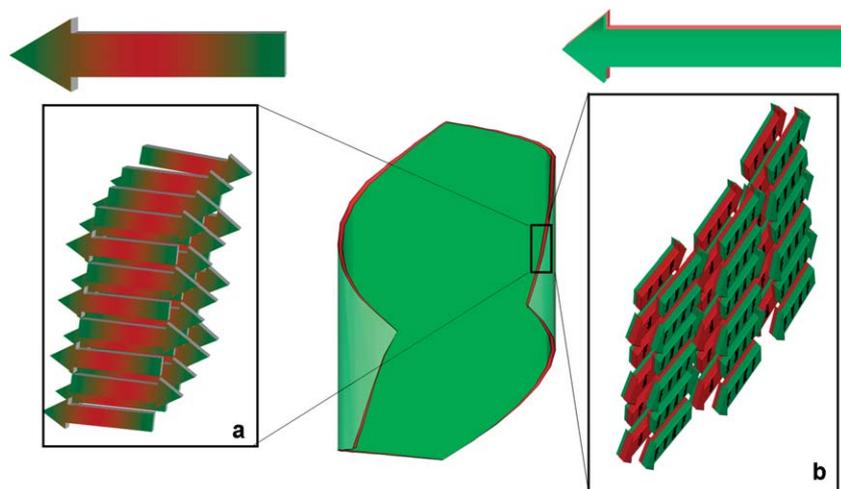
Fig. 3 Peptide backbone orientations within nanotube walls depending on the spatial repartition of amphiphilicity, *i.e.* localisation of hydrophilic side-chains (green) and hydrophobic side-chains (red). a: out-plane backbone orientation, b: in-plane backbone orientation, with regard to the nanotube wall.

development of model, yet realistic bioinspired systems, well suited for the study of elementary self-assembly mechanisms leading to complex architectures, that could allow experimental access to each intermediate molecular moiety. A partial answer to this challenge has been given in the specific cases of lipid-like peptides self-assembled into curved lamellae and nanotubes that mimic the spatial separation in cells,[27] and of β-amyloid peptides that reproduce the self-assembly properties of the entire protein.[103,104]

The most common architectures formed by peptides are amyloid fibres. Such assemblies result from the one-dimensional packing of peptides into fibrils, that further self-assemble by lamination to give the classical twisted amyloid fibres. The precise inner structures and mechanisms of formation of these fibres remain difficult to determine mainly for a few intrinsic reasons: (i) the *in vitro* formation of amyloid fibrils that results from a very slow nucleation stage followed by a very fast fibre

elongation, which makes observation of stable intermediates very difficult, and (ii) the existence of concomitant pathways that lead to different fibre morphologies, either experimentally observed[105] or predicted by molecular dynamics.[106–108] As a result, deciphering the molecular and supramolecular pathways leading towards complex self-assembly is exceedingly complex[109] and has been rarely elucidated by the observation of stable intermediates.[11,110]

For small lipidic molecules, morphological control mostly consists in the deformation of molecular sheets into either twisted, helical ribbons, or nanotubes (Fig. 5). Selinger and co-workers[111] proposed that among the different models explaining the formation of such architectures, the one based on chiral elastic properties provides the most likely explanation of current experimental results. For example, their model could theoretically explain why long chain and short chain charged lipids associated with chiral counterions either choose to self-assemble into helical (long chain) or twisted (short chain) ribbons.[112] Very recently, the different self-assembly steps of a dicationic amphiphile have been solved, indicating that (i) twisted ribbons are the
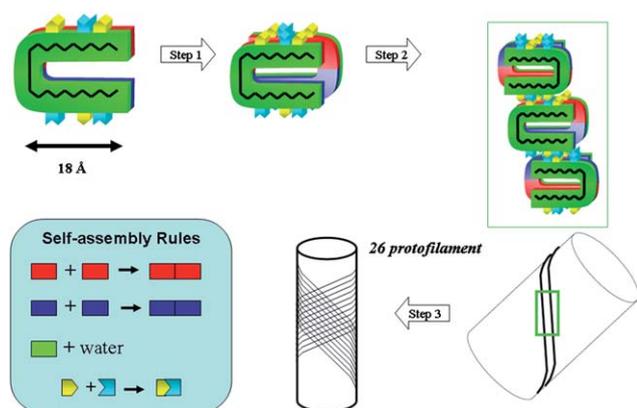

Fig. 4 Schematic view of the different hierarchical levels in Lanreotide self-assembly[79] and self-assembly interactions. The segregation of aromatic residues (red) from aliphatic residues (blue) and from hydrophilic region (green) is remarkable at the level of the molecule, as well as in the three hierarchical self-assembly levels: dimer, filament and nanotube. The filament structure involves extended intermolecular antiparallel β-sheet networks, *via* hydrogen-bonding between backbone H-bond donor (N–H, yellow) and acceptor (C=O, cyan) groups.
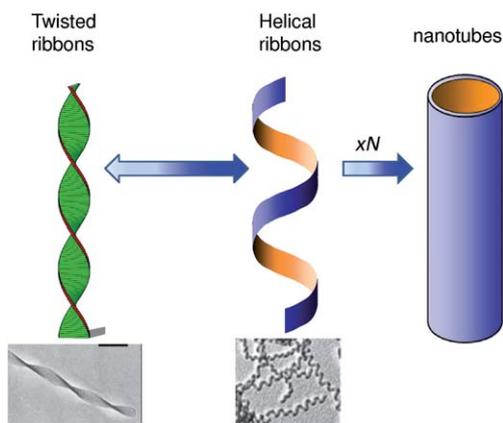

Fig. 5 Links between the different supramolecular architectures observed for lipids and peptides, adapted from: twisted and helical ribbons formed by cationic lipids with chiral counterions.[112]

precursors of helical ribbons, (ii) the latter structures give rise to nanotubes, and (iii) chirality is a key requirement for nanotube formation (Fig. 5).[113]

Peptides also self-assemble in solution to form twisted ribbons, helical ribbons and cylindrical tubules. However, although there are numerous examples of peptide twisted ribbons,[57,114] very few of them self-assemble into helical ribbons and/or monodisperse nanotubes.[79,81,83,88]

In this context, Lanreotide is an exquisite model because of its fast self-assembly kinetics that allow studies at equilibrium, which aid the understanding of its self-assembly mechanism.[80] The sequence of successive equilibria that occurs during Lanreotide nanotube formation has been investigated by observing the different intermediate oligomeric species (Fig. 6a and b): (i) monomer/dimer; (ii) dimer/open ribbons, and (iii) open ribbons/short nanotubes. Obviously, this sketch shows similarities to the mechanisms of nanotube formation by amphiphiles. Lanreotide nanotube self-assembly follows a three-stepped route marked by successive energy roadblocks, namely, peptide dimerisation, open ribbon growth up to the critical size (Fig. 6c), and nanotube closure (Fig. 6d). The first oligomeric species formed is the dimer building block. The high K_d (5 mM) of the monomer/dimer equilibrium indicates that its formation is not only regulated by attractive hydrophobic interactions, but also by repulsive electrostatic forces due to the two positive charges of the peptide. Higher oligomeric species, the open ribbons, are formed above a critical concentration, but remain stable only when their size

exceeds a value of about 19 nm. The open ribbons are rigid 2-D crystals with an intrinsic curvature that most probably results from the expression of the molecular chirality, and defines the nanotube diameter at the onset of the self-assembly process. Indeed, the nanotube walls are formed from two layers of peptides that are not identical in terms of packing. The non-equivalence of these two layers gives rise to the curvature radius of the walls. Open ribbons coexist in equilibrium with short nanotubes, through an unstable intermediate that has been identified as a helical ribbon that is subsequently seamlessly sealed into a nanotube. The nanotubes then undergo unrestricted longitudinal growth to attain lengths of a few hundred micrometres.

This mechanism tells us that two of the three size features of the nanotubes are determined by the earliest generated supramolecular species. The nanotube diameter is set by the intrinsic curvature of the 2D open ribbons, and the wall thickness results directly from the size of the dimer, i.e. the building block. This suggests that the funnel concept, which was introduced for protein folding[115] and subsequently used successfully to describe the binding behaviour in proteins,[116] seems to also apply to large macromolecular complex folding, such as ribosomes[117] or, in our case, to the 26-fold-axis symmetrical self-assembly of a short octapeptide. It thus appears that the spontaneous emergence of such well-defined complex and multi-scale supramolecular architectures is strongly enhanced when the formation route is punctuated with stable milestone states, each of them preparing
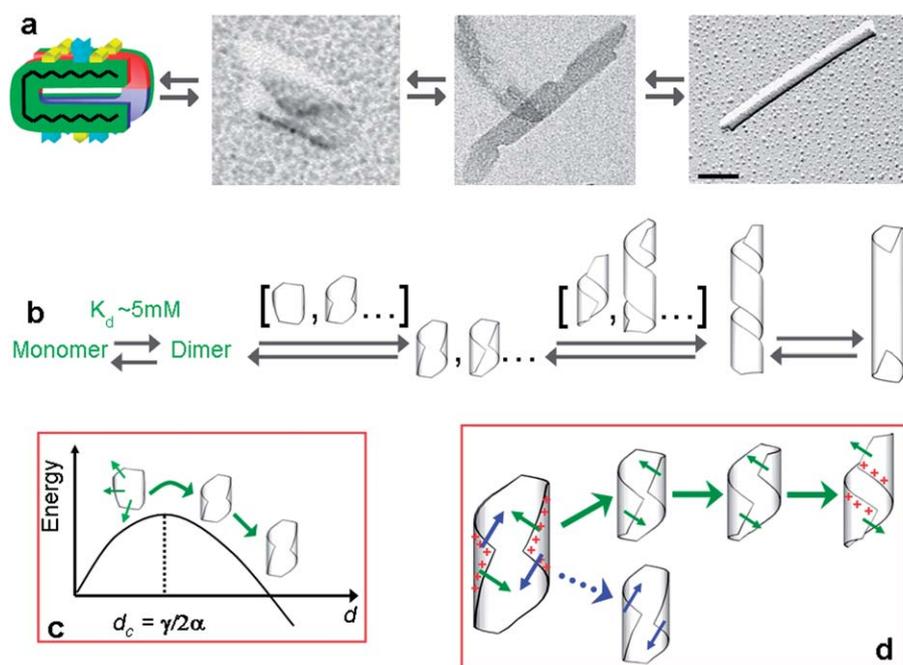

Fig. 6 Formation of Lanreotide nanotubes: roadblocks and intermediates. (a) Scheme and images of the nanotube intermediates. (b) Intermediates and sequence of the nanotube self-assembly process. The formation of Lanreotide nanotubes is described by a sequence of equilibriums between the different intermediate oligomeric species, i.e., monomer, dimer, open ribbons, helical ribbons, short nanotubes. (c) Nucleation and growth. 2-D crystal growth mechanism, i.e., nucleation and growth assembly and schematic evolution of the energy with the size of the nucleus that shows a typical "bell shape". The maximal energy is reached for $d = \lambda/2\gamma$, and smaller assemblies are not stable. γ and λ are the surface energy of the 2D crystal and the linear energy of the edges. (d) Helical ribbons growing with electrostatic repulsions. When the edge of the open ribbons get closer, the repulsive forces favour one growth direction by slowing down the one that brings the edges of the ribbons to healing and closure. Among the two possibilities (either blue or green arrows), the system systematically grows along the green direction, i.e., the direction of the antiparallel β-sheet network, as proven by the pitch length of the helical ribbons (see text for details). Figure reproduced with permission from ref. 80. Copyright 2010, American Chemical Society.

the next assembly step. Furthermore, this precise and unequivocal self-assembly process is driven by the subtle balance between van der Waals attractive and repulsive electrostatic forces. The unequivocal route followed by Lanreotide is the explanation of the monodispersity of the final architecture. We can further say that, the supramolecular process from the open ribbon to the nanotube, is controlled and regulated by electrostatic repulsion.

Control of the length and the diameter of peptide nanotubes

Precise morphological control is an important challenge in the field of molecular self-assembly. For nanotubular objects, the ultimate challenge is to control their length, diameter and wall thickness together. In reality, there are very few examples of rational control of these size parameters from a bottom-up approach (Table 1).

If we consider the three size parameters all together, *i.e.* length, diameter and wall thickness, a "macroscopic approach" using template-based nanofabrication has been developed to produce well-defined protein nanotubes. In this method, the diameter is fixed by the pore diameter in a membrane, the length by its thickness, and the nanotube wall thickness by the number of deposition cycles.[118] Interestingly, a molecular approach has been developed by Ueda and co-workers[90] for peptide–polymer conjugates. They showed that mixtures of right and left handed α-helical peptide–polymer conjugates form nanotubes, with the diameter and length depending on the molar ratio of right and left handed molecules.

Taking each size parameter individually, only few examples lead to not only peptide nanotube monodispersity, but also to size tuning. Concerning the length, peptide nanotubes are generally very long compared to their diameters. To control this parameter, a vapour phase deposition method has been adapted for diphenylalanine peptides, that allows the self-assembly of large arrays of aromatic peptide nanotubes of identical length. The length and density of the nanotubes is fine-tuned by carefully controlling the supply of the building blocks from the gas phase.[119] A very early example of molecular length control is the co-reconstitution of TMV capsid proteins with its RNA. In this case, the length of the protein nanotube is directly controlled by the length of the RNA molecule.[101,120]

With respect to diameter control, examples of molecular control are available, coming either from very small (*i.e.* a few Å) or large nanotubes (100 nm). The first case is well represented by the early work of Ghadiri *et al.*[8] on the 1-D assembly of cyclic peptides into nanotubes, the diameter of which strictly depends on the size of the peptide cycle. However, the diameter range achieved by this approach remains narrow and centred around 10 Å.[61] On the other hand, surfactant-like peptides self-assemble into peptide bilayers to form, as do polar lipids, flexible sheets that can either form vesicles or large (50–100 nm), but polydisperse, nanotubes.[87] Indeed, there is only one example of monodisperse nanotubes formed by a linear surfactant-like peptide, the A_6K peptide.[88] However, there is no example of diameter tuning by modification of the sequence of surfactant-like peptides.

In this context, Lanreotide nanotubes, once again represent a remarkable system. Thanks to the advanced knowledge we gained on their molecular and supramolecular structure, it was possible to predict and experimentally control the nanotube diameter within a range of 10 to 35 nm, by chemical modification of an amino acid at a precise position in the peptide sequence. The choice of the chemically modified amino acid in this study directly results from the supramolecular structure of the Lanreotide nanotube. The structure of the nanotube walls, together with further evidence from studies on derivatives,[81,82] suggested that the amino acids involved in close contact between peptides could play a role in setting the nanotube diameter. Moreover, the recently elucidated mechanism of formation of these nanotubes showed that the nanotube curvature radius is fixed at a very early stage of the assembly process, *i.e.* before nanotube closure, therefore upholding the idea that molecular determinants control the final curvature radius.[80] It was therefore hypothesised that the size of the side-chains involved in the close contact between peptides, within the crystalline walls of the nanotubes, directly controls the nanotube diameter. In Fig. 7 the Lanreotide nanotube wall was schematised, showing it is formed by a peptide bilayer, since the building block of these architectures is a head-to-tail dimer. Therefore, the two layers forming the wall are not equivalent, and in particular, the side chains responsible for

Table 1 Existing literature on the peptide nanotube size control and approaches

Tuned dimensions	Macroscopic approaches	Molecular approaches	
		Kinetic control	Chemical approaches
Length, diameter and thickness	Protein and templating[a]		
Length		Diphenyl peptides and vapour deposition[b]	Mixtures of right and left handed α-helical peptides[c]TMV nanotube length control by a co-reconstitution with RNA[de]
Diameter			Cyclic peptides: $0.7 < \phi < 1.3$ nm[fg]Surfactant like peptides: $50 < \phi < 100$ nm (polydispersed)[h]Lanreotide analogues: $9.5 < \phi < 35$ nm[i]

[a] Dougherty *et al.*, *Langmuir*, 2009. [b] Adler-Abramovich *et al.*, *Nat. Nanotechnol.*, 2009. [c] Ueda *et al.*, *J. Pept. Sci.*, 2011. [d] Fraenkel-Conrat and Williams, *Proc. Natl. Acad. Sci. U. S. A.*, 1955. [e] Fukuda and Okada, *Proc. Natl. Acad. Sci. U. S. A.*, 1987. [f] Bong *et al.*, *Angew. Chem., Int. Ed. Engl.*, 2001. [g] Ghadiri *et al.*, *Nature*, 1993. [h] Santoso *et al.*, *Nano Lett.*, 2002. [i] Tarabout *et al.*, *Proc. Natl. Acad. Sci. U. S. A.*, 2011.
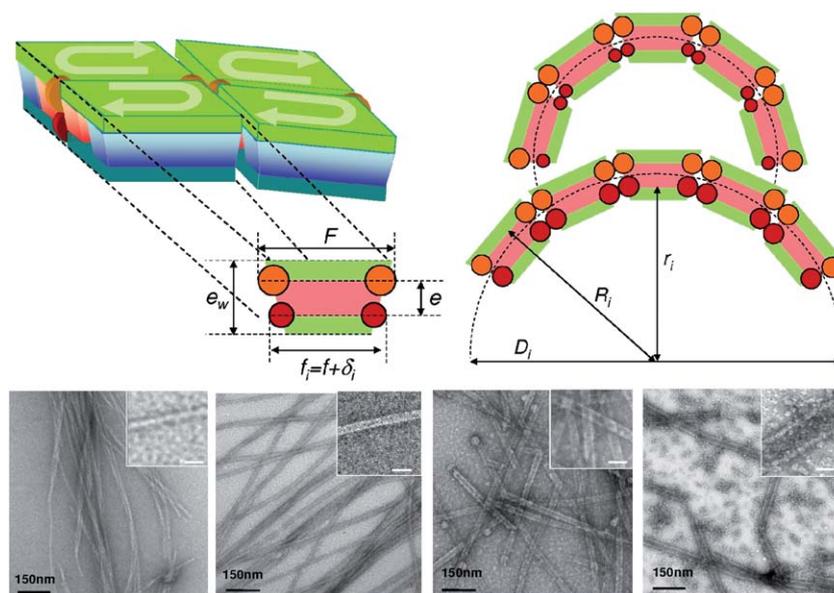
Fig. 7 Geometrical model that explains the control of the nanotube diameter by the close contacts.[83] Upper left panel: the nanotube wall is a bilayer that exposes the hydrophilic residues to water (green surfaces) and protects the hydrophobic aromatic (red) and the hydrophobic aliphatic (blue) residues from water. The red and orange circles show the internal and external aromatic close-contacts, respectively *i.e.*, the D-Trp and D-Nal residues. The unit cell of the bilayer is schematized as a trapezium. The green and red underline the hydrophilic nature of the surfaces of the walls and the aromatic core of the bilayer, respectively. Upper right panel: illustration of the influence of the size of the internal Trp close contact on the nanotube diameter. Lower panel: electron micrographs of nanotubes of different diameters. From the left to the right, nanotubes built with derivatives of Lanreotide for which the initial D-Trp in position 4 was replaced by D-Histidine (9.8 nm), β-2-thienyl-D-alanine (11.2 nm), *m*-bromo-D-phenylalanine (18.4 nm) and β-(1-naphthyl)-D-alanine (35 nm). The black scale on the micrographs is 150 nm and the white scale in the insets is 25 nm. This figure was inspired from Tarabout *et al.*, *Proc. Natl. Acad. Sci. U. S. A.*, 2011.[83]

molecular contacts between peptides are different on the external (Fig. 7, orange circles) and internal (Fig. 7, red circles) layers. The simple drawing in the right panel of Fig. 7 further shows that, if the size of the side chain involved in the close contact within the external layer increases, the nanotube diameter should decrease, whereas for the internal close contacts, the diameter should increase. To test this hypothesis, 17 peptides that differed in the side chain in position 4 of the Lanreotide sequence, *i.e.* originally a Trp residue, were synthesized. All the side chains substituted to Trp were aromatic. All 17 peptides self-assembled into monodisperse nanotubes of different diameters ranging from 9.5 to 35 nm. Based on this hypothesis and knowing the structural parameters of the nanotube wall, a simple geometrical model was built that directly correlated the size of the side chain δ_i with the inverse of the nanotube diameter $1/D_i$ by the equation $\delta_i = F - f - (2Fe/D_i)$, in which e is the transbilayer distance between internal and external close contacts, F is a constant, as it is the length between two close contacts on the external layer, and $f = f_i - \delta_i$, f_i being the length between two close contacts on the internal layer (Fig. 7, schematic representation of the structural parameters used in the model). The experimental results fitted the geometrical model, thus underlining the importance of the close contact geometry and hindrance in the curvature radius of the self-assembled nanotubes. This was the first time that the diameter of nanotubes had been precisely tuned over such a wide range by chemical modification of the building blocks without affecting monodispersity, and while retaining the fascinating organisation of Lanreotide. These diameters can be predicted with an average accuracy of about 4% by a simple geometrical

model that explains how a size increase of about 2 Å on a side chain of a peptide sequence induces a diameter increase from 10 to 36 nm.
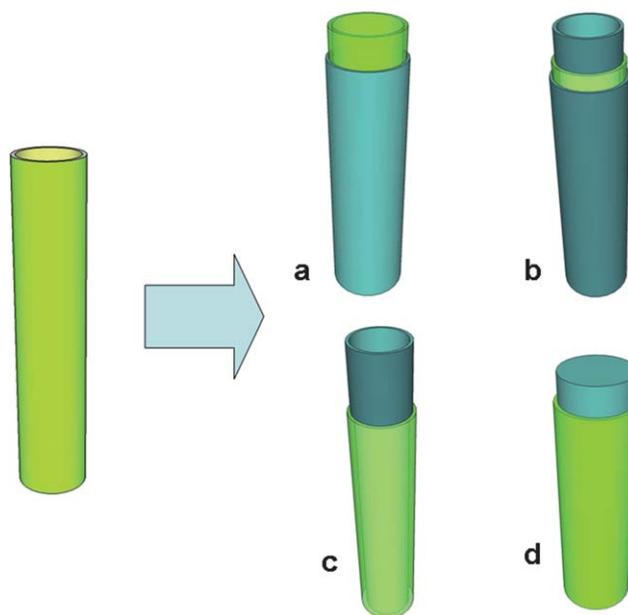

Fig. 8 Self-assembled nanotubes can be used as template to generate single wall nanotubes (a, c), double-walled nanotubes (b), or nanowires (d).
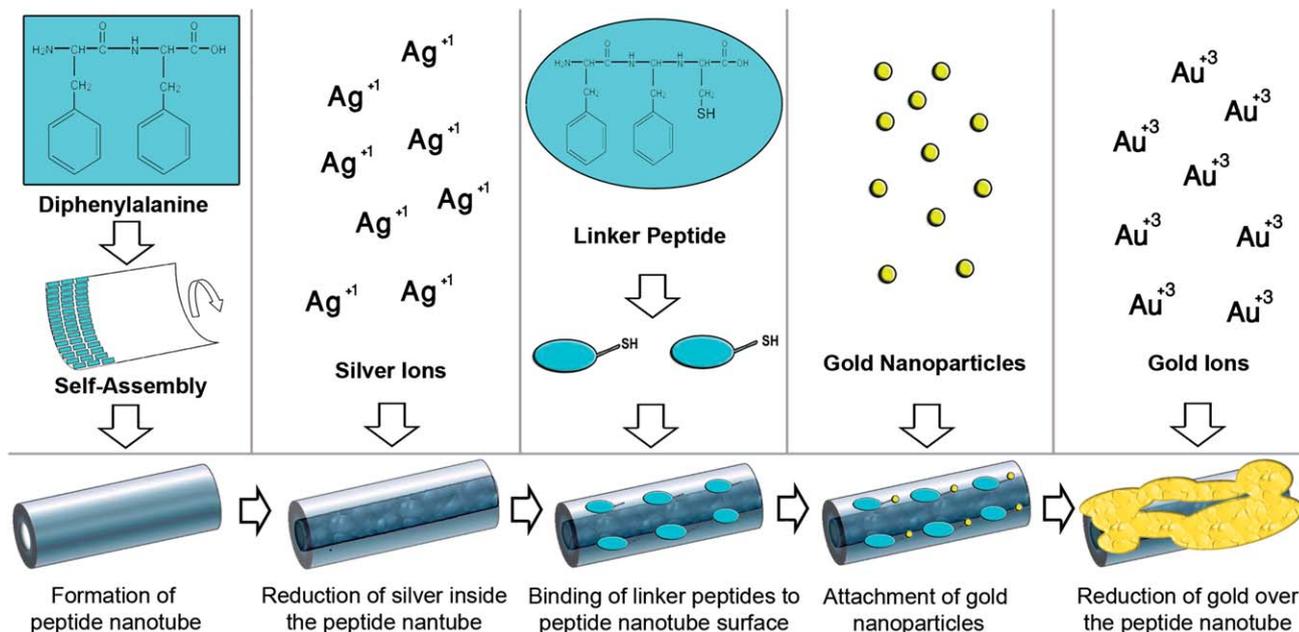
Fig. 9 Fabrication of coaxial metal nanocables using a self-assembled peptide nanotube. Figure reproduced with permission from ref. 124. Copyright 2006, American Chemical Society.

The achievement of diameter tuning by chemical modification comes from a few important features: (i) the structure of the Lanreotide nanotubes had been solved and allowed a rational approach for these chemical modifications, (ii) the supramolecular packing of Lanreotide is highly ordered and the nanotube walls are 2-D rigid crystals, (iii) the nanotubes are formed by two different peptide layers in terms of packing and structure, thus allowing the modification of the close contact on one layer while keeping the other one unaffected.

Applications in the synthesis of inorganic nanotubes

Peptide nanotubes have been extensively used for the synthesis of inorganic nanotubes.[23,34,121–123] Indeed, molecular self-assembly offers new routes for the fabrication of nanomaterials by bottom-up strategies. Peptide nanotubes can be decorated by a large variety of inorganic materials. Surface deposition can be further realised on the inner and/or outer wall of the peptide nanoscaffolds to generate thin inorganic nanotubes (Fig. 8). The confinement of the inorganic reagents within the nanotubes can also be used to create nanowires.

The most successful peptides to date for the fabrication of inorganic nanotubes are the diphenylalanine derivatives developed by E. Gazit and co-workers. The reduction of silver salts within the self-assembled peptide nanotubes was shown to result in metal nanowires with a monodisperse diameter of 20 nm.[38] Peptides bearing a sulfide function were then designed to coat this silver nanowire with gold, hence producing core–shell nanotubes made of two distinct metals (Fig. 9).[124] A few peptides have been designed to control the inorganic nucleation–growth processes onto the nanotube surface. For instance, the group of H. Matsui reported the coating of peptide nanotubes with various metals[125,126] or semiconductors,[127] mainly deposited as thin aggregates of nanoparticles. However, the preparation of thin and monodisperse metallic films has never been observed.

This is likely due to the catalytic properties of the corresponding nanotube surfaces, which induce many nucleation processes and, consequently, a large number of nanoparticles instead of a thin film. Crystalline organisations are further very difficult to accommodate with a strong wall curvature.

Amorphous oxides, for example silica, can be deposited by sol–gel chemistry through taking advantage of the presence of catalytic functions, including amine or ammonium groups.[128,129] A hierarchical peptide–silica composite organisation with unprecedented morphological control, from the nanometre to the centimetre, could be realised using a dynamical Lanreotide peptide-based template.[39] The corresponding hybrid peptide–silica nanotubes consist in a perfect helical assembly of the Lanreotide peptide in a 24 nm diameter nanotube, the internal and external surfaces of which are covered with two thin and
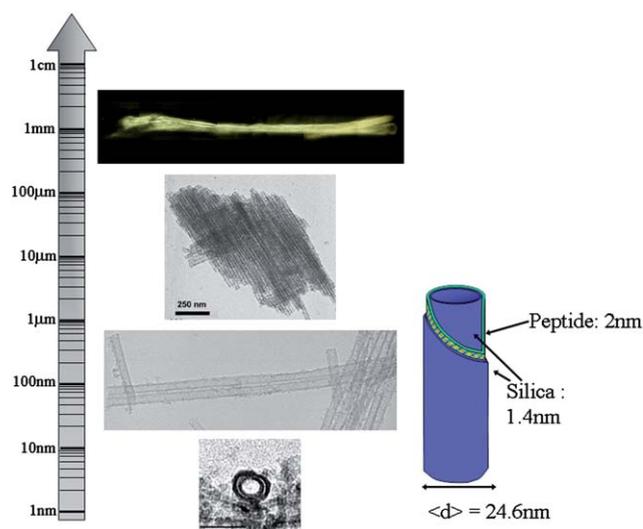

Fig. 10 Hierarchical structures of silica nanotubes.[39]

uniform layers of 1.4 nm silica. The composite nanotubes were shown to be several micrometres long and further aligned into a few millimetres wide fibres. Such organization is thus hierarchically controlled over more than 6 orders of magnitude (Fig. 10). The silica phase and the Lanreotide nanotube grow synergistically, almost in a concerted manner, by mutually neutralizing their charges (positive on the Lanreotide peptide and negative on the silica). Pouget and co-workers termed this phenomena 'dynamical templating' because of the requirement of kinetic coupling between two distinct chemical processes. The recurrence of this process ensured both the control of the organization at molecular scale, and the growth of an organic scaffold as the mineral phase is deposited.

Conclusion

Peptides perform as excellent simple building blocks to spontaneously generate nanostructures either with already envisioned applications or for future developments. Among the wide variety of peptide self-assemblies, nanotubes play a pivotal role due to their well-defined organisations that arise from the crystalline state of the molecules within the nanotube walls. A few detailed examples offer a unique opportunity to understand the corresponding molecular packing, intermolecular interactions, and self-assembly pathway. Such knowledge gives insights into pharmaceutical and biological applications, while paving the way towards the control of shape, size and properties. Peptide nanotubes can be chemically modified further and used as scaffolds for the deposition of an insulator, semiconductor or metal, towards applications in nanotechnology. The advanced understanding of the surface state has been used to investigate biomineralisation mechanisms. It could be further exploited to revisit some open issues related to the electrostatic interactions at the wall interface, including the competition phenomena with entropy known as Manning condensation.

Acknowledgements

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