Peptide Nanotubes and Beyond

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Abstract: Self-assembling nanotubes made from cyclic D,L-α-peptides and from cyclic β-peptides display a wide range of structural and functional capabilities that can be directed by design and that have enabled their application in biological as well as materials science, for example as ion channels and in novel composite materials. Recent advances in the field are described here.

Keywords: materials science · nanostructures · peptides · self-assembly · supramolecular chemistry

Introduction

Synthetic nanotubes have been the subject of intense studies lately due to their potential utility in chemical, biological, and materials science settings. Recent scientific literature abounds with reports of organic and inorganic tubular constructs such as graphite[1, 2] and related boron nitride[3] and tungsten disulfide nanotubes,[4] zeolites[5, 6] and similar mesoporous inorganics,[7–10] polymeric lipid-based tubules,[11] tubular mesophases,[12–14] carbohydrate-based nanotubes,[15–17] and other organic systems.[18–20] In particular, the class of self-assembling peptide nanotubes[21] highlighted herein has proven useful in the design of solid-state porous materials,[22–24] soluble cylindrical supramolecular structures,[25–28] biologically relevant ion channels and transmembrane pore assemblies,[29–32] solid surface-supported ion sensors,[33] and in the fabrication of inorganic nanocluster composites[34] (vide infra).

Conceptually, only a few fundamental approaches can be employed in the design of open-ended hollow tubular structures. Aggregation of rod- or stavelike subunits can be used to form hollow-core bundle- or barrel-shaped frameworks, respectively. Examples of this motif include membrane channel proteins such as the α-helical subunit B of cholera toxin,[35], the potassium channel[36] as well as the β-barrel structures of porins[37, 38] and α-hemolysin.[39] Another approach involves coiling of one or more linear molecule(s) into a helical conformation. This motif is illustrated by β-helical structures formed by the natural antibiotic gramicidin A[40] and related synthetic peptides.[41, 42] A tubular structure can also be made from a two-dimensional sheetlike starting material, either by rolling or by closing its opposing edges. Such processes have been noted in the formation of carbon nanotubes from graphite.[2] Mineralization or polymerization templated by aggregates of organic molecules constitutes the method of choice for preparation of mesoporous silicates and related materials.[7–10] Finally, extended tubular arrays can also be prepared from the stacking of toroidal or disk-shaped subunits. Self-assembly of the tobacco mosaic virus (TMV) coat protein is perhaps the best known biological instance of this motif.[43] Of these various approaches, the latter two have thus far offered the most design flexibility and synthetic convergence. As described here, certain cyclic peptides can adopt the required flat ring-shaped conformational states with self-complementary recognition surfaces that can be used to direct noncovalent stacking and self-assembly of tubular structures (Figure 1).

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Discussion

A tubular assembly based on cyclic peptide stacking was first suggested in 1972 by Hassal.[44] He proposed that cyclic tetramers of alternating α- and β-amino acids would assemble through backbone–backbone hydrogen bonding to form hollow cylindrical structures. This prediction was only partially validated by a 1974 crystallographic study of tetrapeptide cyclo[-(L-Ser(O*t*Bu)-β-Ala-Gly-β-Asp(OMe))-].[45] The peptide subunits were found to adopt a ring-shaped conformation and stack above one another in the crystal lattice, but only two of the four amide groups participated in the expected intersubunit hydrogen bonding. While the utility of mixed α- and β-amino acid cyclic peptides in the design of tubular assemblies awaits further scrutiny, cyclic D,L-α-peptides[22] and cyclic β-peptides[46, 47] have already been successfully employed in preparation of tubular constructs.

Tubular ensembles from cyclic D,L-α-peptides: Within the context of a theoretical analysis of regular enantiomeric peptide sequences, in 1974 De Santis et al. were the first to suggest that cyclic peptides composed of an even number of

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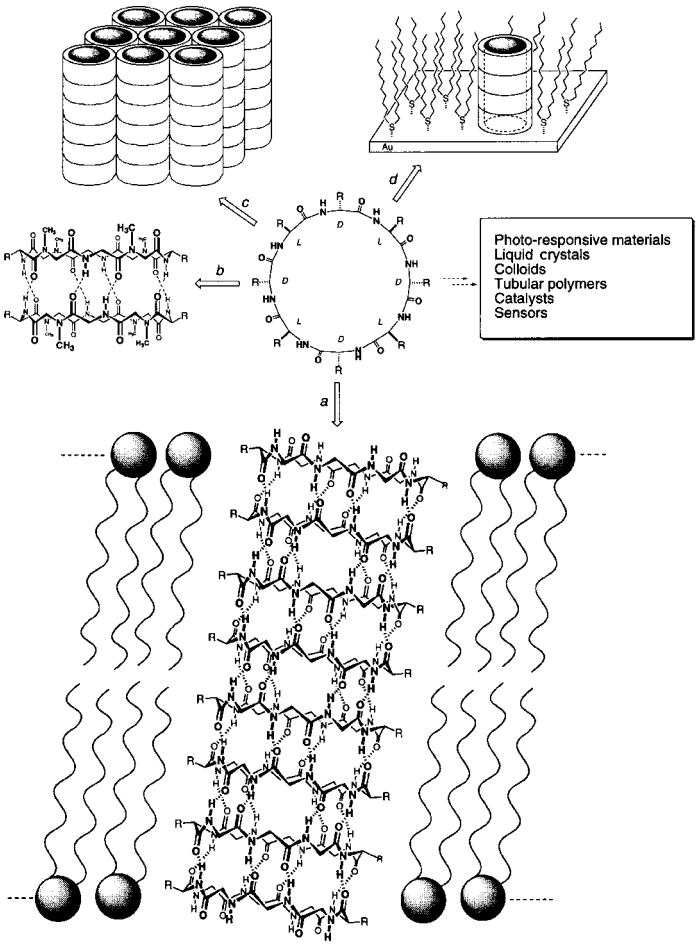

Figure 1. (center) Appropriately designed cyclic D,L-α-peptides and β-peptides can adopt a low-energy flat ring-shaped conformation in which the amide backbone moieties lie nearly perpendicular to the plane of the ring structure with side chains radiating around a central pore, the size of which is determined by the number of amino acids employed (for illustrative purposes only an eight-residue cyclic D,L-α-peptide is depicted). Depending on the peptide sequence and conditions employed, peptide subunits can be assembled into: a) transmembrane ion channels and pore structures, b) soluble cylindrical ensembles, c) solid-state tubular arrays, and d) surface-supported composites. Other plausible applications are denoted by the dashed arrow. The representations emphasize the antiparallel hydrogen- bond -directed stacking of the D,L-α-peptide nanotubes.

alternating D- and L-amino acids would stack through backbone – backbone hydrogen bonding to form extended cylindrical structures.[48] Initial attempts to verify this prediction experimentally proved inconclusive.[49] A 1989 X-ray crystallographic study by Lorenzi and co-workers of hexapeptides cyclo[-(L-Phe-D-Phe)$_3$-] and cyclo[-(L-Val-D-Val)$_3$-] revealed that the expected intersubunit associations were absent and each peptide was found instead to be tightly hydrogen-bonded to several cocrystallized solvent molecules.[50]

In 1993 our laboratory provided the first compelling evidence of nanotube formation by ring stacking of cyclic D,L-peptides (Figure 1c).[22] The sequence of octamer cyclo[-(L-Gln-D-Ala-L-Glu-D-Ala)$_2$-] was chosen to impart solubility in alkaline aqueous media and prevent subunit assembly through electrostatic repulsion. Controlled acidification gave rise to high aspect ratio microcrystalline aggregates, which were characterized by electron microscopy, electron diffraction, FT-IR and crystal structure modeling. These studies all pointed to the expected structure in which cyclic peptide subunits adopt flat, ring-shaped conformations and stack through an extensive antiparallel β-sheetlike hydrogen-bonding network to form nanotubes with an approximate 7 Å internal van der Waals diameter. Subsequent work in our laboratory has also demonstrated that the larger dodecapeptide cyclo[-(L-Gln-D-Ala-L-Glu-D-Ala)$_3$-] undergoes analogous proton-triggered assembly to give microcrystalline aggregates with an expanded 13 Å pore size.[23] Several uncharged cyclic octapeptides have also proven useful in construction of solid-state nanotubular assemblies.[24] In all cases cryoelectron microscopy and electron diffraction analyses have indicated unit cell parameters fully consistent with the expected tubular structures (Figure 2). In particular, observed intersubunit distances of 4.8 Å strongly support the anticipated β-sheet-type arrangements. Furthermore, FT-

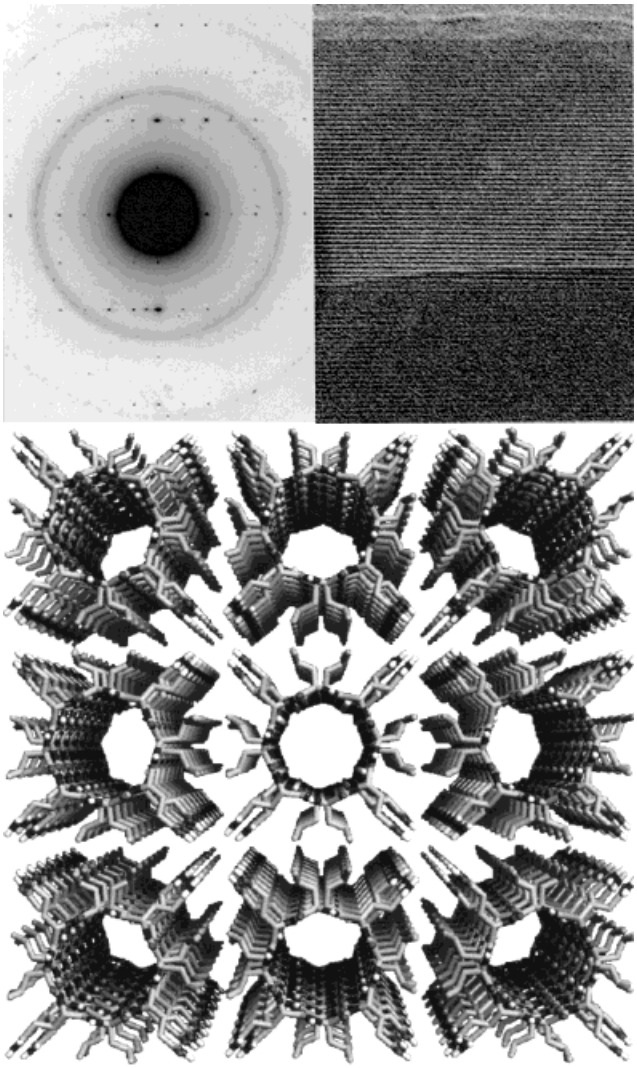

Figure 2. Calculated crystal structure model of a self-assembled cyclic peptide nanotube viewed along the *a* axis (bottom), its electron diffraction pattern (upper left) and a low-dose cryo-electron micrograph (upper right). Striations in the cryo-TEM image, which are approximately 18 Å apart, denote side-by-side packing of nanotubes in the crystal.

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IR analysis has revealed amide $I_{\perp}$, amide $I_{\parallel}$, amide $II_{\parallel}$, as well as hydrogen-bonded amide A bands characteristic of antiparallel β-sheet structures. Recently, Karlström et al. have explored dimerization of cyclic D,L-octapeptides in water using a fluorescence quenching assay. Their results suggest detectable association even in the strongly competing aqueous medium.[51]

Tubular ensembles from cyclic β-peptides: Seebach and coworkers have reported that cyclic tetrapeptides composed of chiral β^3-amino acids can adopt similar disklike conformations and stack to form hollow tubular structures.[46] Analyses of X-ray powder diffraction and molecular modeling of tetrapeptides cyclo[-(β^3-(*S*)-HAla)$_4$-], cyclo[-(β^3-(*S*)-HAla-β^3-(*R*)-HAla)$_2$-], and cyclo[-(β^3-(*S*)-HAla)$_2$-(β^3-(*R*)-HAla)$_2$-], have indicated that all exhibit tubular structures in the solid state. Most recently our laboratory has demonstrated that appropriately designed cyclic β^3-peptides self-assemble to form transmembrane ion channels (vide infra).[47] Cyclic β-peptides offer certain new structural and functional possibilities and thus are expected to augment the potential utility of self-assembling peptide nanotubes.

Self-assembling transmembrane ion channels and pore structures: Self-assembling peptide nanotubes possess two inherent design advantages: the outside surface properties and the tube's internal diameter can be controlled simply by the appropriate choice of the amino acid side chains and the number of the amino acids employed, respectively. In particular the ability to tailor surface characteristics of nanotubes has enabled their use in settings in which the physical properties of the media are of paramount importance. One such instance was encountered in the design of transmembrane channels. It was hypothesized that the low-dielectric-constant lipid bilayer would favor partitioning and self-assembly of cyclic D,L- as well as β^3-peptides bearing appropriate hydrophobic side chains. A 1994 study from our laboratory described the first self-assembling transmembrane ion channels based on the cyclic D,L-peptide nanotube framework (Figure 1a).[29] Since then several eight-residue peptides have been studied by liposome-based proton transport assays and single-channel conductance measurements.[52] These studies have revealed prodigious channel-mediated ion transport activities for K^+ and Na^+ ions in excess of 10^7 ions s^{-1}, rivaling the activity of the related natural product gramicidin A. In a recent study, polarized attenuated total reflectance (ATR), grazing-angle reflection–absorption, and transmission Fourier transform infrared (FT-IR) spectroscopy methods have demonstrated that the transmembrane peptide nanotubes adopt an orientation nearly parallel to lipid acyl chains, supporting our nanotube model of the active channel species.[32] It is also noteworthy that the class of self-assembling transmembrane peptide nanotubes exhibits significant in vitro antibacterial activity[53] and as such may open avenues for the discovery of antimicrobial and cytotoxic agents.

A recent report from our laboratory investigated ion channel formation by two cyclic β^3-tetrapeptides (Figure 3).[47] Liposome-based proton transport assays and single-channel conductance measurements with planar lipid bilayers indicate

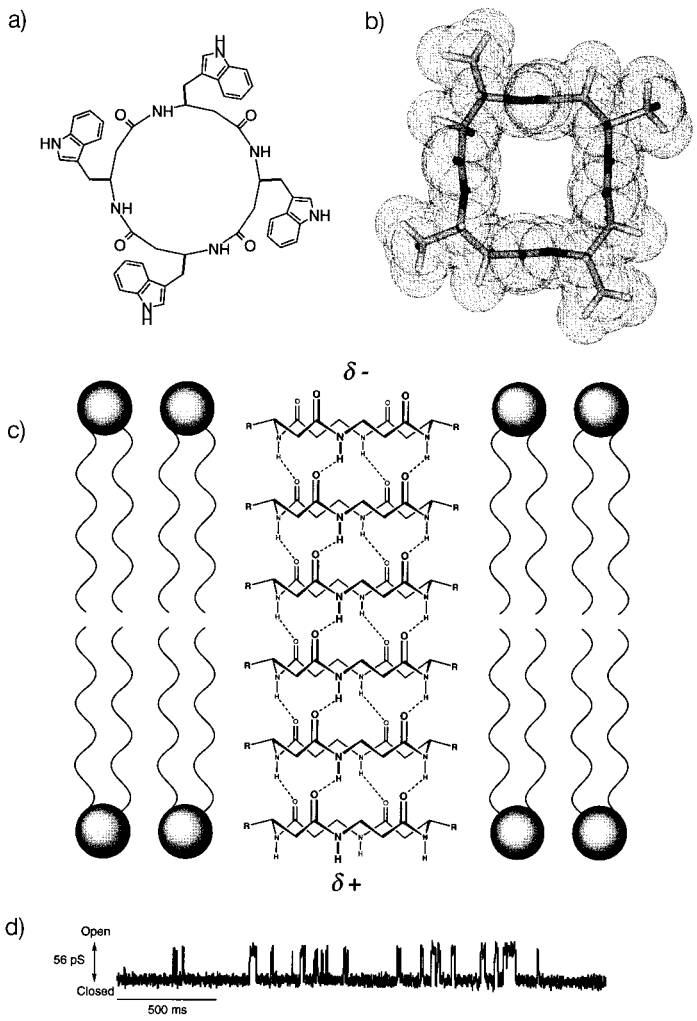

Figure 3. Appropriately designed cyclic β^3-peptides self-assemble inside lipid membranes to form active ion channels. a) The chemical structure of the peptide subunit cyclo[-(β^3-HTrp)$_4$-]; b) its molecular model in a flat-ring C4 symmetrical conformation emphasizing the open pore of 2.6–2.7 Å; c) the tubular transmembrane ensemble is represented with the expected parallel ring stacking. The alignment of backbone amides may create net dipoles at channel termini. d) K^+ single-channel conductance recorded at 60 mV. Sharp channel opening and closing events reflect rapid conformational changes or an assembly/disassembly process.

ion channel activities similar to those of cyclic D,L-α-peptides, with K^+ transport rates of 1.9×10^7 ions s^{-1}. However, channel-forming cyclic β^3-peptides are expected not only to complement these other systems but should also exhibit novel properties arising from the unnatural backbone structure. For instance, uniform alignment of amide groups in the proposed channel structures should give rise to a macrodipole moment reminiscent of an α-helix. This dipole is expected to exert interesting effects on channel conductance such as voltage gating and current rectification behavior.

As mentioned above, one of the unique advantages of self-assembling peptide nanotubes is that the internal diameter can be adjusted by varying the ring size of the peptide subunit employed. We hypothesized that larger transmembrane pore structures might be effective in mediating the transport of hydrophilic molecules across lipid bilayers. Indeed, decapep-

tide cyclo[-(L-Trp-D-Leu)$_4$-L-Gln-D-Leu-], possessing a 10 Å van der Waals internal diameter, has been shown to transport glucose efficiently, while the smaller octapeptide counterpart lacks such activity.[30] These findings suggest that even larger peptide macrocycles may be useful as drug delivery agents.

Solution-phase tubular assemblies: The minimal self-assembled repeat unit of cyclic D,L-peptide nanotubes consists of two stacked subunits hydrogen-bonded in an antiparallel fashion (Figure 1b). This basic motif was studied with the aid of selective backbone *N*-alkylation to block one face of the peptide ring.[25–28, 54, 55] The resulting peptides have been shown to self-assemble into soluble cylindrical dimers in nonpolar organic solvents. The thermodynamics of the self-assembly process and the structural details of eight-residue cylindrical structures have been studied by a variety of ^{1}H NMR techniques and X-ray crystallography (Figure 4). A recent study has examined scope and limitations of self-association in twenty cyclic D,L-α-peptides varying in ring size, location and identity of backbone alkyl substituents, and amino acid composition.[28] These studies indicate that cyclic octapeptides exhibit good rigidity and predisposition for nanotube assembly. The dimerization process was found to tolerate a variety of *N*-alkyl substituents including methyl, allyl, *n*-propyl, and pent-4-en-1-yl groups. Preliminary results suggest that γ-branched residues favor self-association, presumably by preorganizing the peptide backbone for dimerization-induced β-sheet formation.

Self-assembling peptide cylinders have also been used to provide the first experimental model system for the study of parallel and antiparallel β-sheet formation in a given peptide sequence.[26] Measurement of solution equilibrium constants revealed that the antiparallel orientation is favored over parallel by 0.8 kcal mol^{-1}. These findings corroborate results from computational studies.[56, 57]

In an effort toward design of polymeric tubular biomaterials, the feasibility of covalent capture of supramolecular cylindrical assemblies was studied (Figure 5). An olefin-bearing octapeptide cyclo[-(L-Phe-D-^{Me}*N*-Ala-L-Hag-D-^{Me}*N*-Ala)$_2$-] was shown to undergo selective hydrogen- bond mediated olefin metathesis to afford a covalently stabilized β-sheet peptide cylinder.[27] Subsequent work has extended

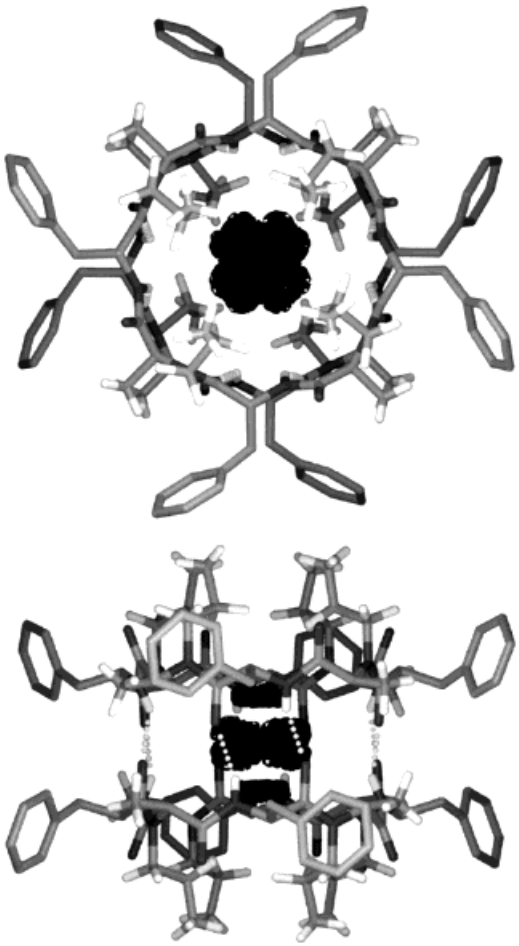

Figure 4. Top and side views of the X-ray crystal structure of a peptide cylinder prepared from the self-assembly of cyclo[-(L-Phe-D-^{Pr}*N*-Ala)$_4$-]. This structure indicates a tight hydrogen-bonded antiparallel β-sheet structure and a cylindrical cavity filled with partially disordered water molecules. *N*-Propyl moieties protect one face of the cylinder from participation in extended hydrogen-bonding interactions.

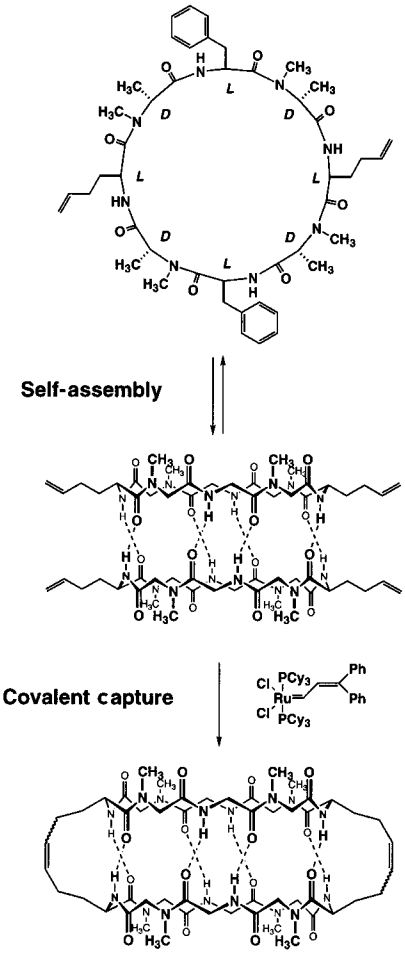

Figure 5. Schematic illustration of the covalent capture process in the stabilization of a noncovalent cylindrical assembly. The peptide subunit cyclo[-(L-Phe-D-^{Me}*N*-Ala-L-Hag-D-^{Me}*N*-Ala)$_2$-], bearing two homoallyl side chains, self-assembles in solution to give two equally populated interconverting cyclindrical dimers. Only the productive complex (shown) can undergo olefin metathesis to afford the covalent product shown.

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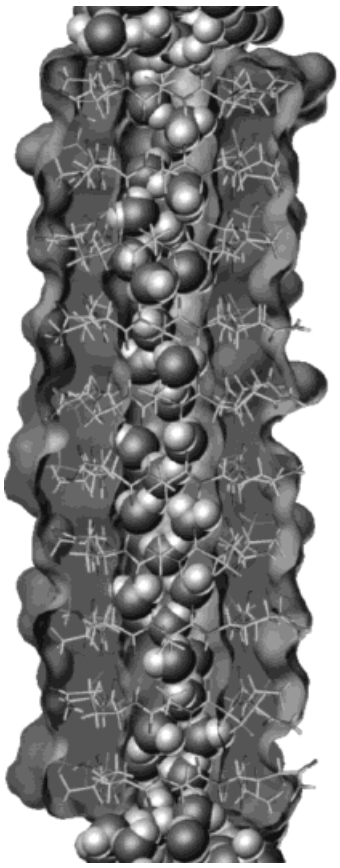

Figure 6. Water molecules organize inside peptide nanotube pores. In the course of the dynamic simulation four types of orchestrated water movements inside the peptide channel have been indicated that may be responsible for the observed fast rates of transport. The figure shows one snapshot of a 0.76 ns MD simulation with half the peptide channel sliced away to better show the channel interior.

this general strategy to include other covalent chemistries such as disulfide bond formation.[58]

Computational studies: Recent computational studies have explored various properties of D,L-α-peptide nanotubes. A transmembrane channel model structure was studied by means of molecular dynamics simulations which suggested that a particular ordering of water molecules inside the channel lumen may be responsible for the observed high rates of transport (Figure 6).[31] In another study, Carloni et al., using density functional theory with calculations employing gradient-corrected exchange-correlation potentials, have predicted a large gap in the low-energy electronic excitation spectrum, with both extended and localized states near the gap.[59] On the basis of ab initio calculations, Fukasaku et al. have suggested that intersubunit hydrogen bonding may delocalize electrons and holes toward the tube axis, so that band conduction might occur through the inter-ring hydrogen bonds.[60] Finally, the results of Lewis et al. indicate a wide ($\approx$5 eV) HOMO–LUMO gap for the nanotube structure, consistent with a transparent material and suggesting interesting bioelectronic device applications for peptide nanotube systems.[61]

Design of novel materials based on self-assembling peptide nanotubes: As an approach toward the design of diffusion-limited sensors, a cyclic eight-residue D,L-α-peptide has been assembled into oriented tubular structures and supported in organosulfur self-assembled monolayers (SAM) on gold films.[33] The structural properties of SAM-supported peptide nanotubes have been analyzed by grazing-angle FT-IR and their functional properties by cyclic voltammetry and impedance spectroscopy. These studies have demonstrated the feasibility of diffusion-limited size-selective ion sensing based on supported tubular biomaterials (Figure 1d).

We have also investigated the utility of peptide nanotube surfaces for the formation and stabilization of transition metal nanoclusters.[34] These studies were inspired by the natural biomineralization process, which is thought to be in part dependent on the display of oriented functionalities on protein and oligosaccharide matrices. In our initial work the crystalline surface of a peptide nanotube displaying an organized array of carboxylic acid functionalities has been used for nucleated deposition of $\approx$3 nm copper(I) oxide nanoclusters at room temperature (Figure 7). High-resolution transmission electron microscopy and electron energy-loss spectroscopy (EELS) have been used to characterize this novel nanocomposite material.

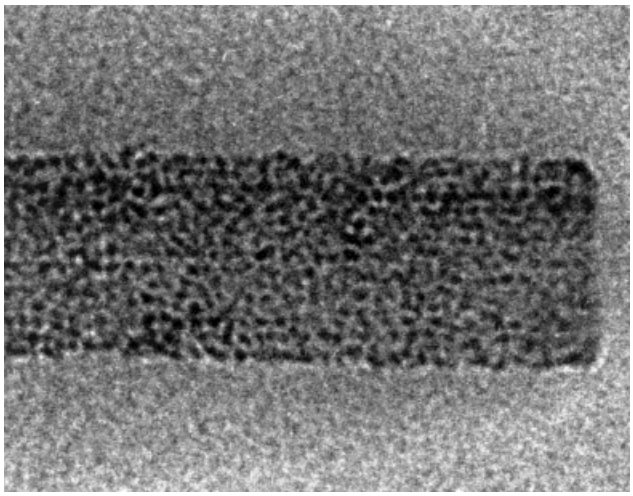

Figure 7. High-resolution cryo-transmission electron micrograph of copper oxide nanocomposite deposited on cyclo[-(L-Gln-D-Ala-L-Gln-D-Ala)$_2$-] self-assembled microcrystal. The copper(I) clusters have an average radius of 1.5 nm.

In summary, a number of peptide nanotubes have been constructed in recent years. Their possible applications range from preparation of novel antibacterial, cytotoxic, and drug delivery agents to catalytic and materials science applications. Future studies will likely focus on design of photoswitchable, polymeric, and liquid crystalline tubular biomaterials.

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