

Wrapping Peptide Tubes: Merging Biological Self-Assembly and Polymer Synthesis**

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Transmembrane channels and pores are among Nature's most admirable devices, especially with regard to their almost perfect selectivity, speed, and gating of transport processes. Their modular architectural design is often based on bundles of α -helices, best exemplified by the potassium channel,^[1] but alternative structures such as barrel proteins and β -helices have also evolved as illustrated by the gramicidin A channel.^[2] In addition to modifying existing biological pores,^[3] a wide variety of tubular structures based on synthetic backbones has been prepared.^[4] In particular, peptide-based tubular systems have received significant attention, and herein we highlight both general design concepts and major recent advances in this rapidly developing field.

The β -sheet motif present in various forms in α -peptides^[5] has proven to be a tremendously fruitful design element to mimic Nature's channel-forming structures. The parallel or antiparallel multiple-hydrogen-bonding array between building blocks with a varying degree of curvature leads to the formation of stable assemblies ranging from β -barrels, through hollow β -helices, to stacked macrocycles (Figure 1).

Natural β -barrel proteins such as α -hemolysin^[6] have inspired the construc-

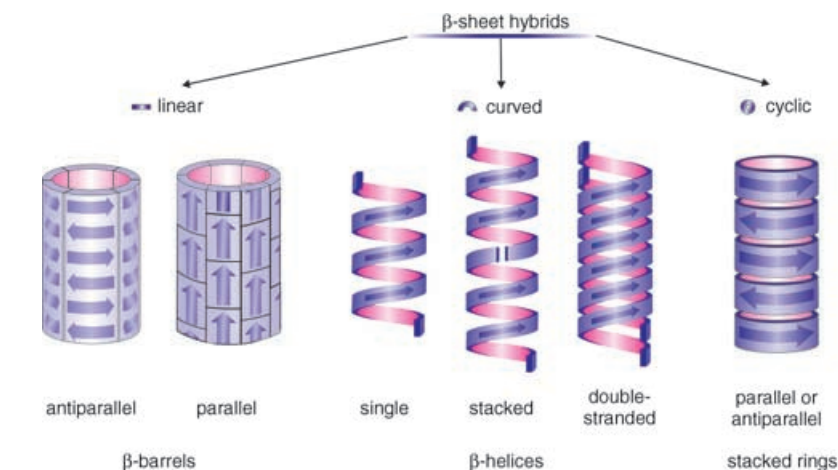


Figure 1. Tubular structures derived from the β -sheet hydrogen-bonding motif present in linear, curved, or cyclic form.

tion of β -barrels from staves consisting of β -sheets. On the one hand, the design of rigid-rod β -barrels by Matile and co-workers is based on the finite assembly of rigid octa(*p*-phenylene)s that carry short α -peptide side chains interdigitated with side chains of adjacent staves to form antiparallel β -sheets.^[7] On the other hand, Percec and co-workers created pores from amphiphilic dendritic α -dipeptides that self-assemble into a helical arrangement of short staves.^[8,9] Whereas in the first approach the internal diameter is tuned by the incorporation of a varying number of staves and α -peptide side chains of differing lengths, the latter pore-forming construct produces various diameters by changing the configuration of the dipeptide fragment. In both approaches, the length is controlled indirectly by assembly of the tube within the constraint of a lipid bilayer.

An alternative design used in channel construction is illustrated by the

gramicidin family.^[2] A α -peptide strand with an alternating sequence of D- and L- α -amino acids is twisted to form a single-stranded β -helix in which the intramolecular hydrogen bonding resembles that in parallel β -sheets. Further interactions between different strands allow either stacked or intertwined β -helices. The number of amino acid residues per turn (4.8–8.2 units) is greater than in α -helices which leads to a wider inner cavity with diameters between 2.3 and 4.7 Å. Whereas diameter control is largely dependent on the cationic guest species, length control is intimately connected to the length of the amino acid sequence.

In an elegant extension of the β -helix design, Ghadiri et al. pioneered peptide tubes based on stacked macrocycles consisting of an even number of alternating D- and L- α -amino acids.^[10] The high aspect ratio and the chemical stability of the tubular assembly originates from strong hydrogen-bonding

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interactions between the individual rings to yield an extended, circular two-dimensional β -sheet. An increase in the number of repeat units per cycle from cyclooctamer to cyclododecamer leads to an increase in the inner diameter from 7 to 13 Å with a constant periodicity of 4.7 Å between stacked rings.^[11] Control of the length of the tubes is again only possible by assembly within a lipid bilayer.^[12]

From this brief overview it is apparent that a multitude of approaches to peptide tubes exists. But some unsolved problems and future challenges still face this field:

- The control of the length of a self-assembled tubular object without the external constraint of a bilayer is still unconquered territory.^[13] One possible solution involves the addition of terminating agents to the supramolecular polymerization mixtures,^[14] for example, Janus macrocycles that engage in hydrogen bonding on only one face of the ring.^[15] Nevertheless, a broad distribution of lengths cannot be avoided. Perhaps the most promising approach to date towards finite self-assembly is that of Stupp et al. by means of crowded side chains, as exemplified by his nanomushrooms.^[16]

- Controlled functionalization of the interior and exterior surfaces is absolutely crucial to render the tubular structures functional. Whereas inner functionalization affects both the efficiency and selectivity of transport processes, functional groups present at the periphery of the tubes mediate integration into bilayers or other hierarchically organized materials. In particular, peripheral modification with polymers represents an attractive route to tune materials properties and control pattern formation on the nanometer scale.^[17] Peptide-polymer hybrids^[18] can be prepared conveniently through controlled radical-polymerization techniques^[19] that involve either post-functionalization by graft-from or attach-to routes or assembly from suitable macromonomers.^[20]
- It is essential that the tubular building blocks be isolated and integrated into hierarchically organized structures to harness their beneficial properties by constructing hybrid materials.

Very recently, the group of Biesalski reported an interesting approach to address some of these issues.^[21] They demonstrated the utility of a graft-from polymerization route initiated at the

outer surface of self-assembled peptide nanotubes to tune their properties (Figure 2). Octameric D,L-alternating cyclo- α -peptides that bear bromoisobutyramide initiators on the lysine side chains were assembled into peptide nanotubes. After subsequent addition of *N*-isopropylacrylamide monomer and polymerization catalyst mixture, Biesalski and co-workers took advantage of the controlled nature of atom-transfer radical polymerization (ATRP) to grow a poly(*N*-isopropylacrylamide) (PNIPAM) shell of homogeneous thickness around the intact cylindrical peptide core. With the aid of atomic force microscopy (AFM) imaging before and after post-polymerization, it was shown that bundles of peptide nanotubes ranging from 100–500 nm in length were broken up to distinct rod-shaped structures with dimensions of 80 ± 20 nm length and 12 ± 3 nm height. The observed shorter length suggests the possibility of using synthetic control over molecular weight of the polymer arms to tune local steric crowding and therefore influence the length of the tubular hybrid assembly.

In a related, however very different, approach, Francis and co-workers demonstrated polymer functionalization of the tobacco mosaic virus (TMV), a well-defined tubular self-assembled entity. They utilized various novel bioconju-

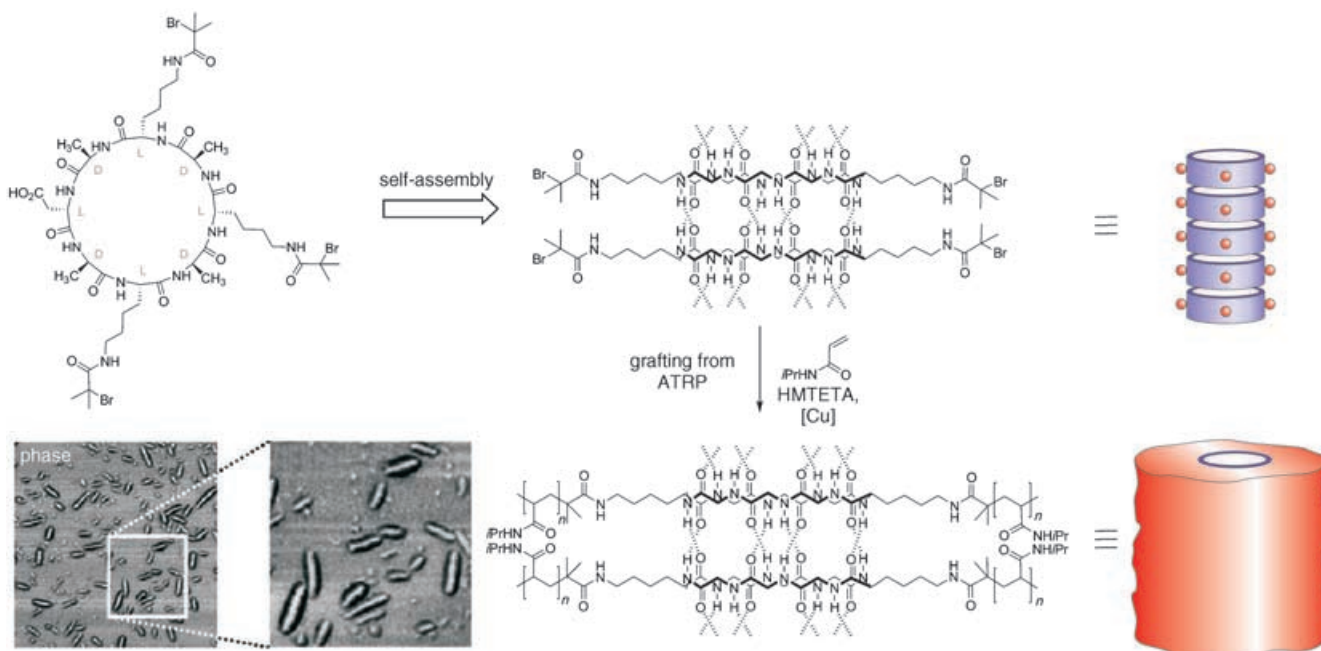


Figure 2. Grafting a polymer shell from preassembled peptide tubes:^[21] D,L-Cyclo- α -peptides carrying initiator groups stack to form tubular structures from which PNIPAM is grown by ATRP; HMTETA = 1,1,4,7,10,10-hexamethyltriethylene. Bottom left depicts AFM images of peptide-PNIPAM hybrids (scales: 2 μ m and 800 nm). Reproduced in part with permission from reference [21].

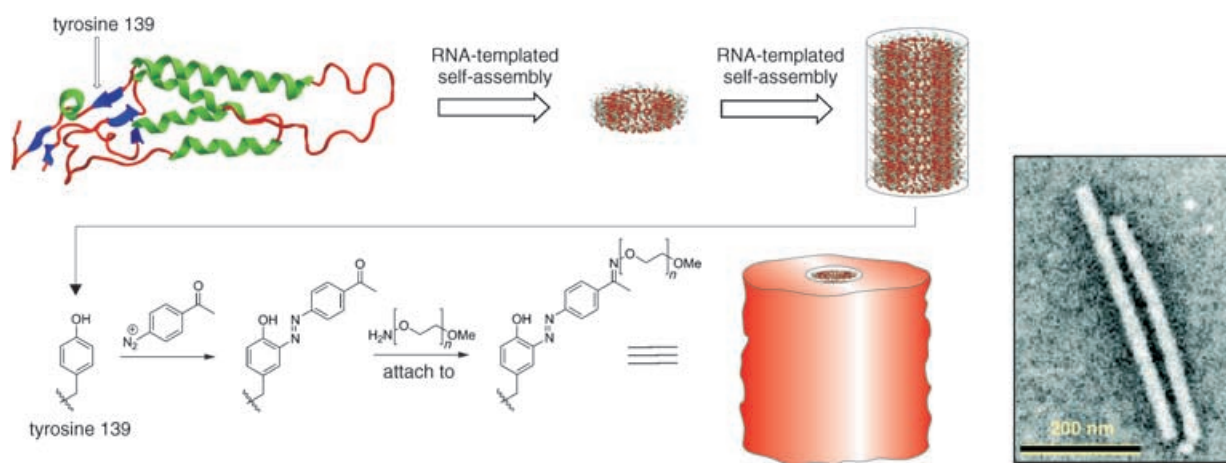


Figure 3. Attaching a polymer shell to preassembled TMV tubes.^[23] Tyr 139 at the periphery of the TMV coat protein (protein data bank code: 1ei7) is functionalized by means of diazonium coupling chemistry to yield a ketone platform to which PEG-based hydroxylamines are attached. Bottom right shows a TEM image of the PEG-TMV hybrid after transfer to an organic solvent, that is, CHCl_3 . Reproduced in part from reference [23].

gation approaches^[22] to decorate the outer and inner surfaces of the TMV coat protein.^[23] For instance, chemo- and regioselective modification of Tyr 139 located at the outer periphery of the TMV by means of electrophilic aromatic substitution was used to install ketone functionalities,^[24] which reacted efficiently with various hydroxylamines, including poly(ethylene glycol) (PEG) derivatives (Figure 3). This attach-to route affords intact tubes that are readily soluble in a variety of organic solvents and therefore allow the use of conventional processing steps for materials applications of the PEG-TMV hybrids. Notably, this system enables the unique possibility of length control owing to the discrete nature of the interior RNA strand, which templates and directs self-assembly of the TMV-coat protein.

The work described herein truly represents the continuing trend of merging classical disciplines namely biology, organic synthesis, and polymer chemistry. Meshing the characteristics of these often-complementary fields helps to surpass limitations and greatly expands the freedom of designing custom-tailored de novo hybrid materials. The controlled modification of peptides and peptide-based objects by modern polymerization techniques will continue to thrive, and the ability to tune properties promises interesting new applications in the emerging bionanotechnology area.

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