Design of nanostructured biological materials through self-assembly of peptides and proteins

Shuguang Zhang*, Davide M Marini, Wonmuk Hwang and Steve Santoso

Several self-assembling peptide and protein systems that form nanotubes, helical ribbons and fibrous scaffolds have recently emerged as biological materials. Peptides and proteins have also been selected to bind metals, semiconductors and ions, inspiring the design of new materials for a wide range of applications in nano-biotechnology.

Addresses
Center for Biomedical Engineering, 56-341 Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139-4307, USA
*e-mail: shuguang@mit.edu
http://web.mit.edu/lms/www

Current Opinion in Chemical Biology 2002, **6**:865–871

1367-5931/02/$ – see front matter

Introduction

Self-assembly can be defined as the spontaneous organization of individual components into an ordered structure without human intervention [1••]. The key elements of molecular self-assembly are complementarity in shape among the individual components and weak, non-covalent interactions. Molecular self-assembly as a fabrication tool will have a significant impact in the coming decades. Engineering principles for micro- and nano-fabrication can be learned by understanding molecular self-assembly phenomena in nature. Numerous self-assembling systems have already been developed, ranging from block copolymers and surfactant-like materials to scaffolds for three-dimensional (3D) cell culture, DNA-based structures and models to study protein folding and protein conformational disease.

The basis of molecular self-assembly

The challenge in molecular self-assembly is to design molecular building blocks that can undergo spontaneous organization into a well-defined and stable macroscopic structure using non-covalent bonds. These typically include hydrogen bonds, ionic bonds, water-mediated hydrogen bonds, hydrophobic and van der Waals interactions. Although each of these forces is rather weak, their collective interactions can produce very stable structures.

Amino acids and short peptides have not generally been considered to be useful for traditional materials science and engineering. The advent of genetic engineering and recent advances in peptide synthesis and molecular-engineered proteins have changed this view. Self-assembly of biomolecules is now emerging as a new route to produce novel materials and to complement other materials (i.e. ceramics, metals, alloys, synthetic polymers and other composite materials). Rapid developments in biotechnology have rekindled the field of materials science. Considerable advances have been made in the use of peptides and proteins as building blocks to produce a wide range of biological materials for diverse applications [2,3••–5••,6•,7••,8•,9••,10•–15•,16••,17•,18,19,20•].

Self-assembling peptide systems

In the past few years, we concentrated our research efforts on peptide self-assembling systems. Short peptides are easy to design and synthesize, making them an excellent model system for studying biological self-assembly. Several types of self-assembling peptides have been systematically studied [21]. This class of biological materials has considerable potential for a number of applications, including scaffolding for tissue repair in regenerative medicine, drug delivery and biological surface engineering.

Tirrell and colleagues [2] designed artificial proteins that undergo self-assembly to form hydrogels responsive to pH and other environmental changes. Ghadiri and colleagues [4••,5••] designed peptide nanotubes that allow ions to pass through and to insert themselves into lipid bilayers. Aggeli and colleagues [6•] showed that other β-sheet peptide systems can also undergo self-assembly into regular nanofiber structures. Although they share no sequence similarity, their nanofibers share remarkable structural similarity and physical properties with the ones we studied [7••,8•]. Several surfactant-like peptides have also been reported [9••,10•]. Furthermore, a number of biomimetic peptide and protein structures, such as helical coiled-coils and di-, tri- and tetra-helical bundles combined with heme groups have also been studied [11•,12•]. Belcher and colleagues [14•,15•] have selected specific peptides that can form complexes with metal and semiconducting elements. These collective efforts have emerged as a new field: molecular self-assembly for fabricating nanostructured materials; namely, designing materials using biological constituents and motifs.

Amphiphilic and surfactant peptides

It is generally known that small polymers which contain within them a hydrophobic region and a separate hydrophilic region will self-assemble in aqueous solution to form distinct structures such as micelles, vesicles and tubules. This is largely due to the hydrophobic effect, which drives the nonpolar region of each polymer molecule away from water and towards one another. The dimensions and shape of the supramolecular structures formed from such assemblies will then depend on different factors, such as the geometry of the polar head group and the shape of each molecule [22•]. In biology, the most

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
2002		**00-00-2002 to 00-00-2002**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Design of nanostructured biological materials through self-assembly of peptides and proteins	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Massachusetts Institute of Technology,Center for Biomedical Engineering,77 Massachusetts Avenue,Cambridge,MA,02139**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

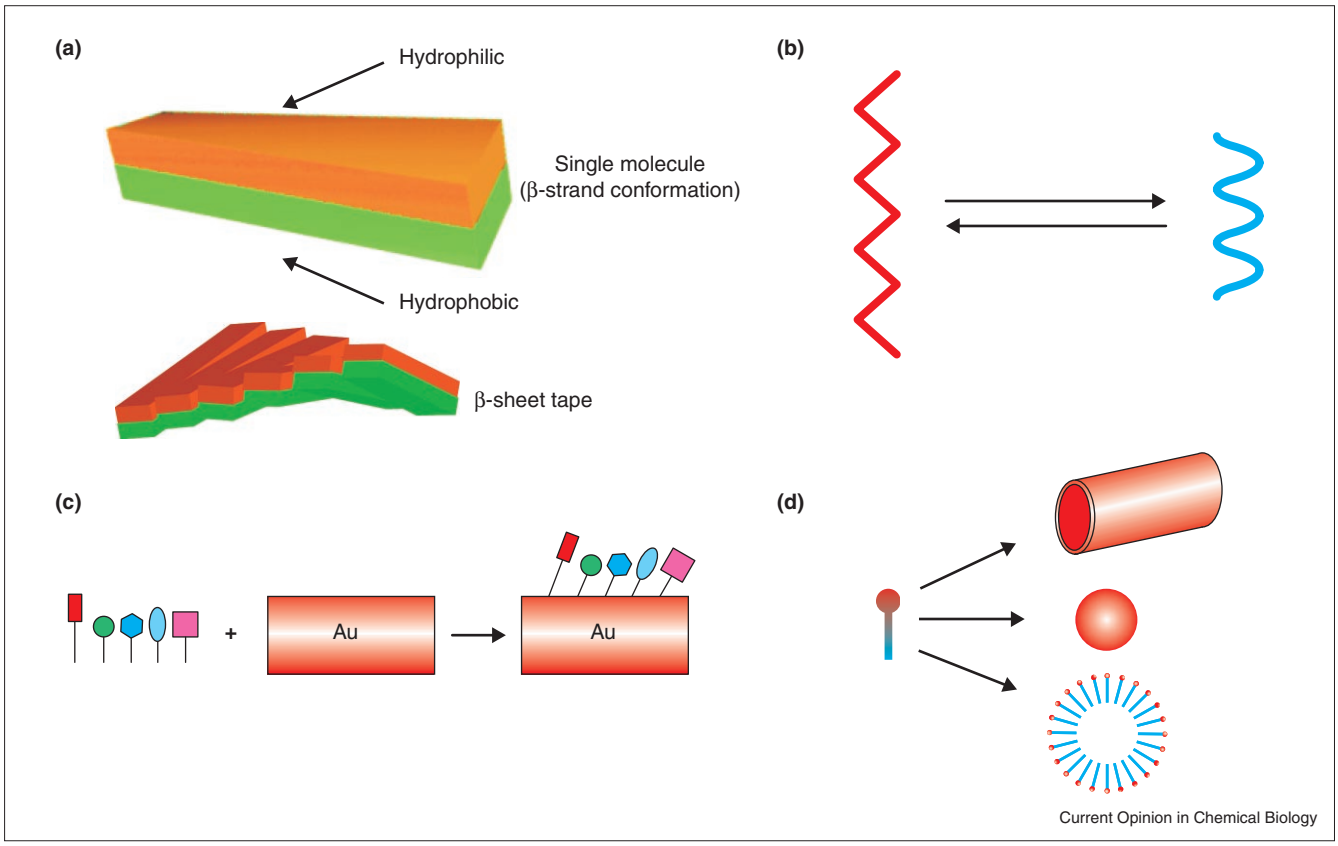
13. SUPPLEMENTARY NOTES
The original document contains color images.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**		**7**	

Figure 1



Various types of self-assembling peptide systems. **(a)** Amphiphilic peptides in β-strand conformation are chiral objects. As a consequence, they self-assemble into twisted tapes. **(b)** Helical dipolar peptides can undergo a conformational change between α-helix and β-sheet, much like a molecular switch [44•]. **(c)** Surface-binding peptides can form monolayers covalently bound to a surface [45•]. **(d)** Surfactant-like peptides can form vesicles and nanotubes [9••,10•].

common example of such amphiphilic molecules is the phospholipid, the predominant constituent of the cell membrane, which encapsulates and protects the cytoplasm from the environment.

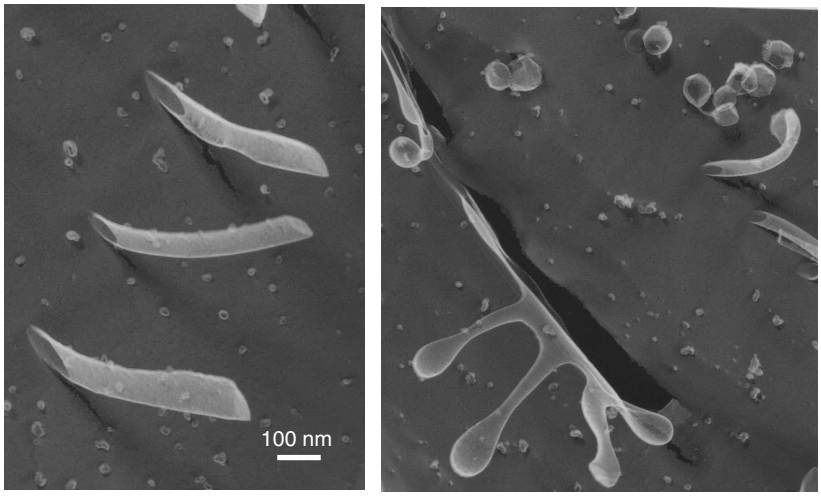
The growing interest in nanotechnology has stimulated the discovery and development of new materials that can self-assemble into well-ordered structures at the nanometer scale [23•]. Although such ordered and reproducible structures are very common in biology, they are a tremendous challenge for the material scientist. Therefore, much effort has been focused on investigating the use of biological molecules for nanotechnology applications [24•,25,26•].

Our laboratory has designed many simple amphiphilic peptides that consist exclusively of natural L-amino acids [3••,7••,8•,9••,10•] (Figure 1). One subset of these molecules is surfactant-like peptides ([9••,10•], von Maltzahn *et al.*, unpublished data). They share a common motif: the polar region of each molecule has one or two charged amino acids and the non-polar region is made from four or more consecutive hydrophobic amino acids (see figures in [9••,10•]). For example, the V_6D sequence has six hydrophobic valine residues from the N-terminus followed by a negatively charged aspartic acid residue, thus having two negative charges, one from the side chain and the other from the C-terminus [9••].

This peptide self-assembles in aqueous solution into 30–50 nm supramolecular structures, as detected by dynamic light scattering. Transmission electron microscopy (TEM) studies of the flash-frozen sample reveal the presence of a vast array of tubular structures having diameters approximately 30–50 nm (Figure 2) with lengths of several microns. Nanovesicles were also observed, suggesting that the dynamic behavior of the supramolecular assemblies may be tunable by changing the sequence of the monomer and the environment. The supramolecular structure of nanotubes formed by the surfactant peptide V_6D is proposed in Figure 3. These peptides can be tailored for added functionality using standard techniques in peptide chemistry. For example, biotinylation of a monomer will make it bind to a surface coated with streptavidin, or a string of histidines will allow it to bind to nickel for interfacing with inorganic materials.

Figure 2

Transmission electron microscope images of V_6D. The sample was flash frozen in liquid propane (–180°C) and surface-coated with a thin layer of platinum and carbon, yielding a replica. This technique preserves the structures formed in solution. The nanotube and vesicle structures are clearly seen in these images.



Current Opinion in Chemical Biology

Other similar protein amphiphiles were also reported by Tirrell and colleagues [2] as well as Deming and colleagues [13]. These amphiphiles have the familiar alternating polar–nonpolar regions but each polypeptide consists of 200 amino acids arranged as a di-block copolymer. In solution, these designed polypeptides self-assemble to form strong and fast-recovering hydrogels that respond to pH changes [2], or are heat resistant to 90°C [13•]. These biologically inspired materials may prove to be advantageous both in functionality and biodegradability, especially in biomedical applications.

The range of amphiphilic peptides is not limited to naturally occurring amino acids. Stupp and colleagues [16••,17•] have linked hydrophilic peptides to long alkyl chains, making the complex amphiphilic. The peptide end of the molecule is designed to be functional in biomineralization: a phosphorylated serine is incorporated within the peptide and used for attracting and organizing calcium ions to regulate the mineralization of hydroxyapatite [17•]. The C-terminus of the peptide is further functionalized with a cell-adhesion motif RGD (arginine-glycine-aspartic acid).

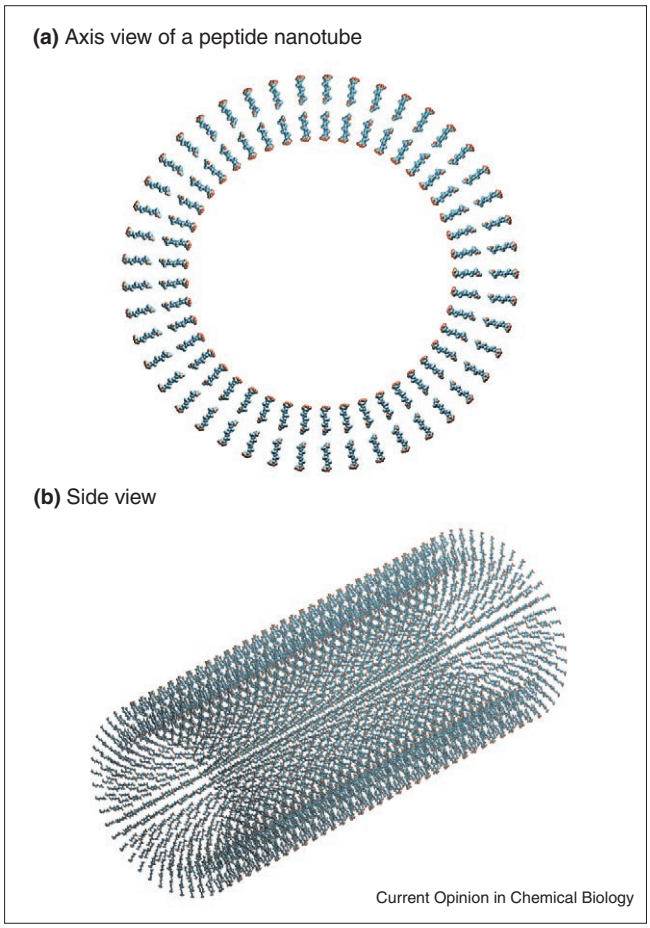
Besides their potential in biomaterials and medicine, these amphiphilic peptide materials may also serve as scaffolds in other areas of nanotechnology. Of particular interest is the fabrication of conducting nanowires. One may envisage that nanotubes made from these peptides can serve as templates for metallization [27•]. Once the organic scaffold is removed, a conducting wire can be formed and immobilized on a surface. It is of great interest to develop various methods to attach conducting metal nanocrystals to peptides for such purposes. Matsui and co-workers have made progress in functionalizing nanotubes formed by bolaamphiphile peptides [28]. They coated the nanotube with a metalloporphyrin compound [29] and showed that their nanotubes can also be coated with avidin, thus enabling them to specifically bind to gold surfaces that have been treated with biotinylated self-assembled monolayers (SAMs) [30].

To tailor the material for a specific need, researchers in material science, engineering and nanotechnology can indeed benefit from the knowledge of biochemistry, molecular biology and cell biology. The design flexibility offered by peptides may result in a wide variety of functions for the ordered structures formed by self-assembly. Further pursuits will focus on controlling the uniformity of the self-assembled nanostructures, a critical criterion for applications in nanotechnology.

Peptide self-assemble into 3D matrix scaffolds

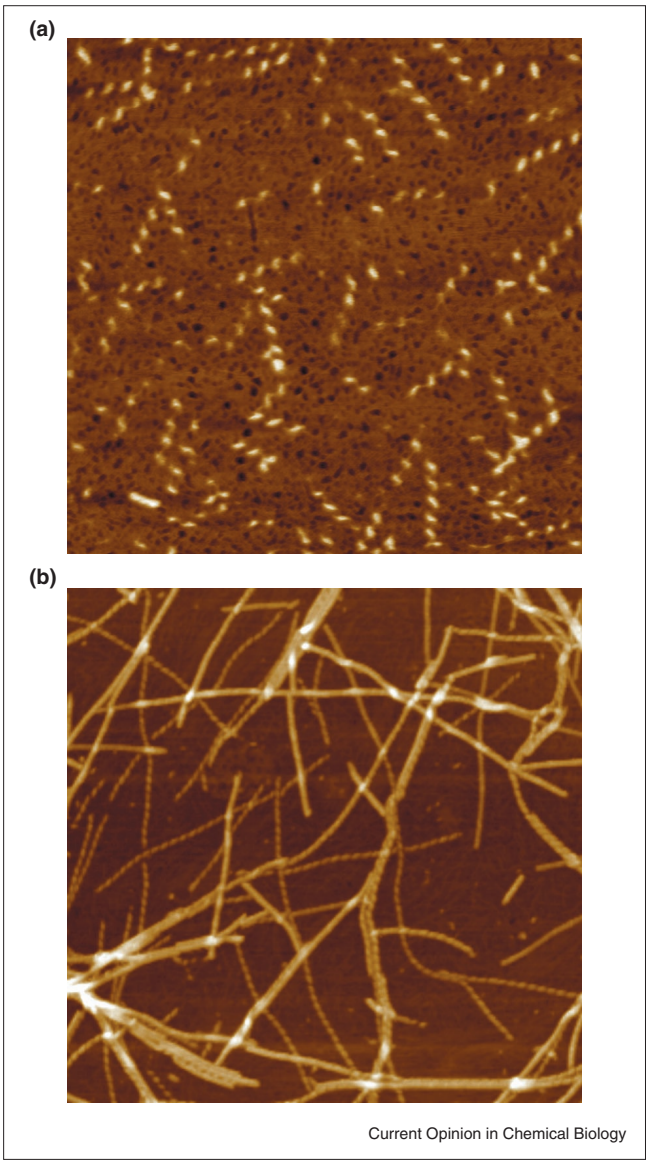
Self-assembly of peptides and proteins is a promising route to the fabrication of a variety of molecular materials including nanoscale fibers [3••,7••,8•,16••,17•] and fiber network scaffolds [2,3••,13•]. Efforts aimed at producing structured materials at the nanometer scale have already produced remarkable results. Chirality of the molecular building blocks plays an important role in such processes. An interesting example of how chirality at the molecular level influences the supramolecular structures is evident in the self-assembly of an eight-residue peptide, KFE8 [7••]. The right-handed twist of the peptide backbone [31] in β-strand conformation leads to the formation of left-handed helical ribbons of regular pitch at the nanometer scale (Figure 4). The molecular structure of these nanofibers is difficult to obtain, because they are not amenable to high-resolution X-ray diffraction nor solution NMR. Moreover, the precise mechanism of nucleation and growth of these fibers from free monomers still remains unclear.

Figure 3



Proposed supramolecular structure of the V_6D peptide nanotube. Each peptide monomer is 2 nm long and the diameter of the nanotube is 50 nm. The hydrophilic heads (red) are in contact with water, whereas the hydrophobic tails (blue) are segregated from it.

Figure 4



The peptide KFE8 (of sequence FKFEFKFE) self-assembles in aqueous solution into left-handed helical ribbons.**(a)** Atomic force microscopy image (500 nm × 500 nm) of a peptide solution deposited over mica 8 min after preparation. **(b)** Same sample, 4 days after preparation (1 μm × 1 μm).

Interestingly, researchers in designed peptide and protein materials are turning their attention toward studies of protein conformational diseases, and *vice versa*. Indeed, the non-covalent assembly of monomers to form ordered nanofibers is at the heart of a wide variety of physiological and pathological processes.

Theoretical models of self-assembled peptide fibers have been divided into three categories, depending on the level of scalings involved: first, a molecular model [32••]; second, a semi-continuum model, where the self-assembled structure is treated as an elastic tape composed of brick-like building blocks [4••,6•,33,34]; and third, a fully continuum, field-theoretic model [35]. Each contributes to part of the overall understanding. The molecular approach uses molecular dynamics simulation to explore the packing order between peptides in the nanofibers and the energy landscape associated with a deformation of the structure (Figure 5). Such an approach is useful in investigating the detailed structure of nanofibers, providing guidance in the design of peptide- and protein-based biological materials. The next level of coarse-graining simplifies monomers as bricks and postulates the existence of phenomenological interactions between them. Through thermodynamics considerations, the higher level ordering of nanofibers, such as bundling, can be analyzed. When the properties of single fibers are known, such an approach could help in controlling nanofiber organization. The fully continuum description uses order parameters to develop and analyze a field-theoretic expression of the free energy. Although originally developed for larger scale self-assembled lipid fibers (microns in diameter), this theory is applicable to

peptide nanofibers as long as the structural feature of interest is larger than the size of individual peptides. All of the above theories are focused on the already formed structure. Currently, very little is known about the dynamics of the self-assembly process. Likewise, surfactant or colloid self-assembly is relatively well studied at the molecular [36], mesoscopic [37,38] and thermodynamic [39•] levels. In the case of fiber formation, there are experimental reports that self-assembly is a multi-stage process with distinct intermediate structures [7••,40,41••,42]. Characterization of these intermediates and the structural transition between them is important for understanding and controlling peptide self-assembly.

Designed peptide-hydrogels for 3D cell culture and regenerative biology

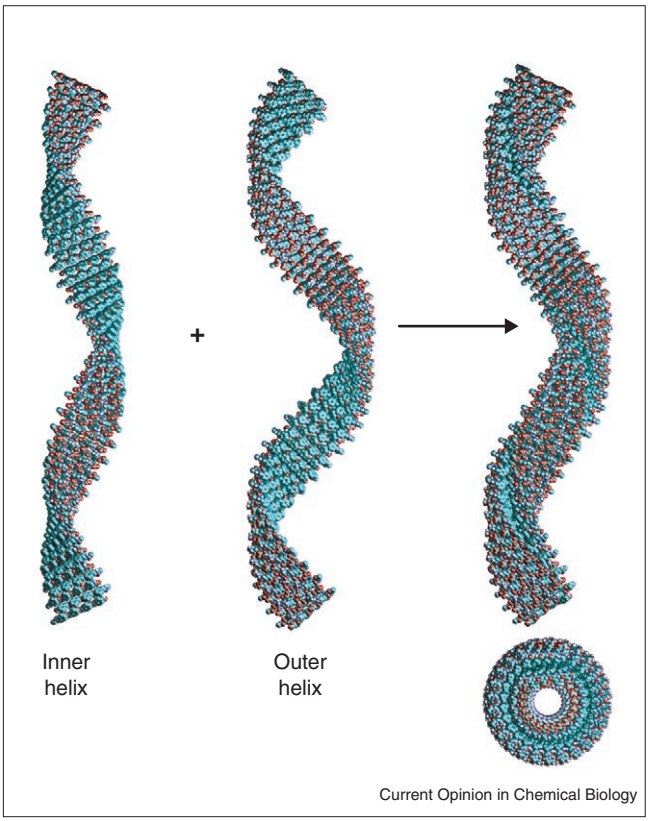
Upon addition of monovalent alkaline cations or introduction of the self-assembling peptide solutions into physiological media, these peptides spontaneously assemble to form macroscopic structures that can be fabricated into various geometric shapes [3••,43••]. Scanning electron microscopy and atomic force microscopy reveal that the matrices are made of interwoven nanofibers with a diameter of 10–20 nm and pores about 50–200 nm [3••,7••,8•]. If the alanines are changed to more hydrophobic residues, such as Val, Leu, Ile, Phe or Tyr, the molecules have a greater tendency to self-assemble and form peptide matrices [7••,8•].

Tests have been conducted on a number of mammalian cells and all have been found to form stable attachments with these peptide materials [3••,43••]. Several peptides have also been tested for their ability to support cell proliferation and differentiation ([3••,43••]; Semino C, unpublished results). These results suggest that not only can the peptide materials support various types of cell attachments, but they also enable their proliferation and differentiation. When primary mouse neuron cells were allowed to attach to these scaffolds, the neuron cells projected axons that followed the specific contours of the self-assembled peptide surface and made active and functional connections [3••]. When bovine chondrocytes were encapsulated in the peptide scaffold, they not only maintained their differentiated state but also produced large amount of Type II and Type XI collagen as well as glycosaminoglycan [43••].

Conclusions

Advancement in nanotechnology will require the ability to produce nanostructured materials and molecular self-assembly will undoubtedly play a fundamental role in this process. Moreover, the formation of structures by self-assembly is much more energy efficient than directed assembly. Amphiphilic peptides have been shown to be promising building blocks for biomolecular self-assembly. Current research has also shown that chemically tailoring individual monomers can confer a particular function to the supramolecular structure; for example, the ability to crystallize hydroxyapatite or to specifically immobilize patterned surfaces.

Figure 5



A molecular model of the helical ribbon intermediate formed by the peptide KFE8. The inner and outer β-sheets form a double-sheet helix with the hydrophobic side chains sandwiched between the two layers. The molecular packing and the energy landscape of this system was explored in [7••,32••].

Engineering through molecular design, self-assembly and programmed assembly will play an increasingly important role in research and industry. Investigating the self-assembly of peptides is also likely to provide new opportunities to unravel complex phenomena including amyloid fiber formation in protein conformational diseases.

Acknowledgements

We thank Kranthi Vistakula for helpful discussions and for suggesting some of the references. We gratefully acknowledge support from the US Army Research Office, Office of Naval Research, Defense Advanced Research Project Agency/Naval Research Laboratories, and NSF CCR-0122419 to MIT Media Lab's Center for Bits & Atoms, National Institute of Health, Du Pont-MIT Alliance and the Whitaker Foundation. We also gratefully acknowledge Intel Corporation for educational donation of high-speed computers.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. •• Whitesides GM, Boncheva M: **Beyond molecules: self-assembly of mesoscopic and macroscopic components.** *Proc Natl Acad Sci USA* 2002, **99**:4769-4774.

In addition to offering a general perspective on self-assembly, this excellent review envisions the potential of self-assembly as extending beyond molecules to the realm of macroscopic objects.

2. Petka WA, Harden JL, McGrath KP, Wirtz D, Tirrell DA: **Reversible hydrogels from self-assembling artificial proteins.** *Science* 1998, **281**:389-392.

3. •• Holmes TC, Delacalle S, Su X, Rich A, Zhang S: **Extensive neurite outgrowth and active neuronal synapses on peptide scaffolds.** *Proc Natl Acad Sci USA* 2000, **97**:6728-6733.

This is the first report of a primary neuron culture in a transportable peptide scaffold where neuronal networks do not adhere onto a solid Petri dish. It also showed full functional activity of neurons, which formed active connections: an important step for neuro-repair. This paper opened a new research avenue: production of movable scaffolds for functional cell culture.

4. •• Ghadiri MR, Tirrell DA: **Chemistry at the crossroads.** *Curr Opin Chem Biol* 2000, **4**:661-662.

Excellent, concise review of the design of new biological materials.

5. •• Fernandez-Lopez S, Kim HS, Choi EC, Delgado M, Granja JR, Khasanov A, Kraehenbuehl K, Long G, Weinberger DA, Wilcoxen KM, Ghadiri MR: **Antibacterial agents based on the cyclic D,L-alpha-peptide architecture**. *Nature* 2001, **412**:452-455.

This report shows that D-, L-amino acids form stacked nanotubes that can insert into membranes and change their properties. These nano porous structures have potential anti-bacterial activity.

6. • Aggeli A, Nyrkova IA, Bell M, Harding R, Carrick L, McLeish TCB, Semenov AN, Boden N: **Hierarchical self-assembly of chiral rod-like molecules as a model for peptide beta-sheet tapes, ribbons, fibrils, and fibers.** *Proc Natl Acad Sci USA* 2001, **98**:11857-11862.

By using an elastic tape model of the fiber, this paper provides theoretical framework for the organization (bundling) of these fibers.

7. •• Marini DM, Hwang W, Lauffenburger DA, Zhang S, Kamm RD: **Left handed helical ribbon intermediates in the self-assembly of a beta-sheet peptide.** *Nano Letters* 2002, **2**:295-299.

This paper reports the observation – via atomic force microscopy and transmission electron microscopy – of left-handed helical ribbons formed by self-assembly of a b-sheet peptide. The structure of such fibers was investigated using molecular dynamics simulation in concomitance with experimental measurements. Such a method may be applied to the design of other self-assembled materials.

8. • Caplan MR, Moore PN, Zhang S, Kamm RD, Lauffenburger DA: **Self-assembly of a beta-sheet protein governed by relief of electrostatic repulsion relative to van der Waals attraction.** *Biomacromolecules* 2000, **1**:627-631.

A systematic analysis of the rheological properties of gels formed by a self-assembling peptide shows that assembly happens upon relief of electrostatic repulsion, in agreement with the DLVO theory.

9. •• Vauthey S, Santoso S, Gong H, Watson N, Zhang S: **Molecular self assembly of surfactant-like peptides to form nanotubes and nanovesicles.** *Proc Natl Acad Sci USA* 2002, **99**:5355-5360.

This is the first reported design of a surfactant peptide. These molecularly designed peptides share many properties with phosphate lipids and non-peptidic surfactant molecules. They form well-ordered nanotubes and nanovesicles that could be useful for a number of applications.

10. • Santoso S, Hwang W, Hartman H, Zhang S: **Self-assembly of surfactant-like peptides with variable glycine tails to form nanotubes and nanovesicles.** *Nano Letters* 2002, **2**:687-691.

This report further shows that various lengths of glycines as the hydrophobic tail of surfactant peptides also permit the self-assembly of nanostructures.

11. • Marsh EN, DeGrado WF: **Noncovalent self-assembly of a heterotetrameric diiron protein.** *Proc Natl Acad Sci USA* 2002, **99**:5150-5154.

Four-helical bundle coiled-coil proteins that bind two ions are designed. On addition of ferrous ions and oxygen, the protein forms a complex with a UV-visible spectrum closely resembling that of peroxo-bridged diferric species in natural proteins and model compounds.

12. • Moffet DA, Case MA, House JC, Vogel K, Williams RD, Spiro TG, McLendon GL, Hecht MH: **Carbon monoxide binding by *de novo* heme proteins derived from designed combinatorial libraries.** *J Am Chem Soc* 2001, **123**:2109-2115.

The authors combinatorially selected heme proteins with biological function from a designed artificial protein library. This study has implications in selecting and evolving proteins for particular purposes. Hecht and colleagues have selected many novel artificial proteins in the past few years.

13. • Nowak AP, Breedveld V, Pakstis L, Ozbas B, Pine DJ, Pochan D, Deming TJ: **Rapidly recovering hydrogel scaffolds from self-assembling diblock copolypeptide amphiphiles.** *Nature* 2002, **417**:424-428.

The authors designed block co-polymer-type proteins with very interesting properties.

14. • Whaley SR, English DS, Hu EL, Barbara PF, Belcher AM: **Selection of peptides with semiconductor binding specificity for directed nanocrystal assembly.** *Nature* 2000, **405**:665-668.

This is the first report to use a bacterial phage library to select binding of several semiconducting elements. Phages can also organize these elements in a defined manner.

15. • Lee SW, Mao C, Flynn CE, Belcher AM: **Ordering of quantum dots using genetically engineered viruses.** *Science* 2002, **296**:892-895.

The authors show that bacterial phages can organize inorganic nanostructures on their surface. This work opened a new research avenue: interfacing biology with inorganic molecules.

16. •• Hartgerink JD, Beniash E, Stupp SI: **Self-assembly and mineralization of peptide-amphiphile nanofibers.** *Science* 2001, **294**:1684-1687.

By covalently linking polymers and peptide functional motifs, the authors first demonstrated that calcium atoms can be organized in a desired way, allowing the design of new composite biomaterials for bone repair.

17. • Hartgerink JD, Beniash E, Stupp SI: **Peptide-amphiphile nanofibers: a versatile scaffold for the preparation of self-assembling materials.** *Proc Natl Acad Sci USA* 2002, **99**:5133-5138.

The authors expand on the concepts developed in [16••] and demonstrate the high potential of their motif in the design of nanomaterials.

18. Szela S, Avtges P, Valluzzi R, Winkler S, Wilson D, Kirschner D, Kaplan DL: **Reduction-oxidation control of beta-sheet assembly in genetically engineered silk.** *Biomacromolecules* 2000, **1**:534-542.

19. Zhou Y, Wu S, Conticello VP: **Genetically directed synthesis and spectroscopic analysis of a protein polymer derived from a flagelliform silk sequence.** *Biomacromolecules* 2001, **2**:111-125.

20. • Zhou CZ, Confalonieri F, Jacquet M, Perasso R, Li ZG, Janin J: **Silk fibroin: structural implications of a remarkable amino acid sequence.** *Proteins* 2001, **44**:119-122.

This is a structural analysis of silk fibroin with many repeats. This structure has long eluded many high-resolution studies. For anyone interested in the design of new materials, this is an excellent paper to read.

21. Zhang S: **Molecular self-assembly.** In *Encyclopedia of Materials: Science & Technology*. Oxford, UK: Elsevier Science; 2001:5822-5829.

22. • Lee J, Macosko CW, Urry DW: **Elastomeric polypentapeptides cross-linked into matrixes and fibers.** *Biomacromolecules* 2001, **2**:170-179.

Urry and colleagues have spent decades engineering protein materials using selected repeating sequences. These biologically engineered materials have paved the way for many applications.

23. • Reinhoudt DN, Crego-Calama M: **Synthesis beyond the molecule.** *Science* 2002, **295**:2403-2407.

An excellent review of supramolecular self-assembly. The authors underline the importance of designing a specific chemical function in the formed structures

24. • Lowe CR: **Nanobiotechnology: the fabrication and applications of chemical and biological nanostructures.** *Curr Opin Struct Biol* 2000, **10**:428-434.

A nice review.

25. Drexler KE: **Building molecular machine systems.** *Trends Biotechnol* 1999, **17**:5-7.

26. • Merkle RC: **Biotechnology as a route to nanotechnology.** *Trends Biotechnol* 1999, **17**:271-274.

A nice review.

27. • Antonietti M: **Surfactants for novel templating applications.** *Curr Opin Coll Inter Sci* 2001, **6**:244-248.

A nice review.

28. Matsui H, Gologan B: **Crystalline glycylglycine bolaamphiphile tubules and their pH-sensitive structural transformation.** *J Phys Chem B* 2000, **104**:3383-3386.

29. Matsui H, MacCuspie R: **Metallophorphyrin nanotube fabrication using peptide nanotubes as templates.** *Nano Letters* 2001, **1**:671-675.

30. Matsui H, Porrata P, Douberly GE: **Protein tubule immobilization on self-assembled monolayers on Au substrates.** *Nano Letters* 2001, **1**:461-464.

31. Shamovsky I, Ross GM, Riopelle RJ: **Theoretical studies on the origin of β-sheet twisting.** *J Phys Chem B* 2000, **104**:11296-11307.

32. Hwang W, Marini DM, Kamm RD, Zhang S: **Supramolecular**
•• **structure of helical ribbons self-assembled from a beta-sheet peptide.** *J Phys Chem B* 2002, **106**:in press.
This paper quantitatively analyzes the structure of an intermediate in peptide self-assembly at atomic detail. The simulation approach developed here is applicable to the analysis of a variety of peptide nanofibers, other nanostructures and beyond.

33. Nyrkova IA, Semenov AN, Aggeli A, Boden N: **Fibril stability in solutions of twisted beta-sheet peptides: a new kind of micellization in chiral systems.** *Eur Phys J B* 2000, **17**:481-497.

34. Nyrkova IA, Semenov AN, Aggeli A, Bell M, Boden N, McLeish TCB: **Self-assembly and structure transformations in living polymers forming fibrils.** *Eur Phys J B* 2000, **17**:499-513.

35. Selinger JV, Spector MS, Schnur JM: **Theory of self-assembled tubules and helical ribbons.** *J Phys Chem B* 2001, **105**:7157-7169.

36. Bogusz S, Venable RM, Pastor RW: **Molecular dynamics simulations of octyl glucoside micelles: dynamic properties.** *J Phy Chem B* 2001, **105**:8312-8321.

37. Noguchi H, Takasu M: **Fusion pathways of vesicles: a Brownian dynamics simulation.** *J Chem Phys* 2001, **115**:9547-9551.

38. Tanaka H, Araki T: **Simulation method of colloidal suspension with hydrodynamic interactions: fluid particle dynamics.** *Phys Rev Lett* 2000, **85**:1338-1341.

39. Israelachvili J: *Intermolecular and surface forces* 2nd ed. London and
• New York: Academic Press; 1998.
This book reviews the fundamental forces involved in macromolecular systems. The last part deals with self-assembly of lipid systems. Many of the basic concepts necessary for understanding self-assembly are well explained.

40. Kowalewski T, Holtzman DM: ***In situ* atomic force microscopy study of Alzheimer's beta-amyloid peptide on different substrates: new insights into mechanism of beta-sheet formation.** *Proc Natl Acad Sci USA* 1999, **96**:3688-3693.

41. Lashuel HA, Hartley D, Petre BM, Walz T, Lansbury PT Jr: **Amyloid**
•• **pores from pathogenic mutations.** *Nature* 2002, **418**:291.
The authors report the intermediate structure in the self-assembly of alpha-synuclein. These ring-shaped structures might act as pores and puncture the cell membrane, causing neuronal death.

42. Lashuel HA, LaBrenz SR, Woo L, Serpell LC, Kelly JW: **Protofilaments, filaments, ribbons and fibrils from peptidomimetic self-assembly: implications for amyloid fibril formation and materials science.** *J Am Chem Soc* 2000, **122**:5262-5277.

43. Kisiday J, Jin M, Kurz B, Hung H, Semino C, Zhang S, Grodzinsky AJ:
•• **Self-assembling peptide hydrogel fosters chondrocyte extracellular matrix production and cell division: implications for cartilage tissue repair.** *Proc Natl Acad Sci USA* 2002, **99**:9996-10001.
This is the first report to use a peptide matrix to encapsulate primary chondrocytes to form a 3D piece cartilage tissue similar to isolated cartilage from animals. The peptide matrix is now used to culture cells in 3D and for tissue repair in regenerative medicine.

44. Altman M, Lee P, Rich A, Zhang S: **Conformational behavior of ionic**
• **self-complementary peptides.** *Protein Sci* 2000, **9**:1095-1105.
This paper reports the conformational plasticity of peptides with helical dipoles. They can undergo drastic structural changes between α-helices and β-sheets. Such peptides behave like a 'molecular switch'.

45. Zhang S, Yan L, Altman M, Lässle M, Nugent H, Frankel F,
• Lauffenburger DA, Whitesides GM, Rich A: **Biological surface engineering: a simple system for cell pattern formation.** *Biomaterials* 1999, **20**:1213-1220.
This paper describes a simple method to molecularly engineer functional peptides and to design patterns on surfaces for a number of applications, much like a carpet with designed patterns. Cells and molecules can be precisely organized on surfaces.