Designer Self-Assembling Peptide Materials

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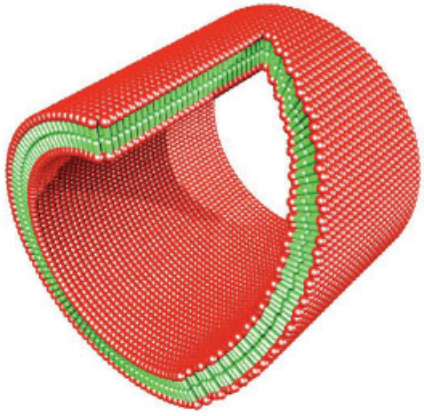
Understanding of macromolecular materials at the molecular level is becoming increasingly important for a new generation of nanomaterials for nanobiotechnology and other disciplines, namely, the design, synthesis, and fabrication of nanodevices at the molecular scale from bottom up. Basic engineering principles for microfabrication can be learned through fully grasping the molecular self-assembly and programmed assembly phenomena. Self- and programmed-assembly phenomena are ubiquitous in nature. Two key elements in molecular macrobiological material productions are chemical complementarity and structural compatibility, both of which require weak and non-covalent interactions that bring building blocks together during self-assembly. Significant advances have been made during the 1990s at the interface of materials chemistry and biology. They include the design of helical ribbons, peptide nanofiber scaffolds for three-dimensional cell cultures and tissue engineering, peptide surfactants for solubilizing and stabilizing diverse types of membrane proteins and their complexes, and molecular ink peptides for arbitrary printing and coating surfaces as well as coiled-coil helical peptides for multi- length scale fractal structures. These designer self-assembling peptides have far reaching implications in a broad spectrum of applications in biology, medicine, nanobiotechnology, and nanobiomedical technology, some of which are beyond our current imaginations.



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Introduction

Molecular self-assembly is ubiquitous in Nature, from lipids that form oil droplets in water, and surfactants that form micelles and other complex structures in water, to sophisticated multiunit ribosome and virus assemblies. Nature is a grand master who utilizes the strategy to bottom-up build hierarchical materials. These elegant molecular self-assembly systems lie at the interface between biochemistry, molecular biology, peptide and protein chemistry, macromolecular science, materials science, and engineering. The key elements are chemical

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complementarity and structural compatibility through numerous non-covalent weak interactions.

Learning from nature of the sophisticated fabrication and construction of diverse biological materials, which often is elegantly built one molecule and one atom at a time, we have developed many self-assembling systems, ranging from models for studying protein folding and protein conformational-related diseases, to molecular materials for producing peptide nanofibers, peptide scaffolds, peptide surfactants, and peptide ink (Figure 1).[1,2] Molecular self-assembly systems represent a significant advance in the molecular engineering of simple molecular building blocks for a wide range of material and device applications.[3–6] In this article we will only focus on designer Lego peptides and surfactant peptides. Other interesting work can be found from earlier reviews elsewhere.[3–6]

Lego TM Peptides

The first member of the Lego peptides was serendipitously discovered from a segment in a left-handed Z-DNA binding protein in yeast, Zuotin (*Zuo* means Left in Chinese, *tin* means protein in biology).[7] Inspired by its special structure, a class of 'Lego peptides' was designed. On the nanometer scale, these peptides resemble the Lego bricks that have both pegs and holes in a precisely determined manner. They can be programmed to assemble in well-formed structures. This class of Lego peptide undergoes spontaneous assembly into well-organized nanofibers.[8]

Various Modulus Peptides

Because of their beta-sheet structures in aqueous solution, modulus peptides have two distinct sides, one is hydrophilic and the other is hydrophobic, like the pegs and holes in Lego bricks. The hydrophobic sides shield themselves from water and thus facilitate their self-assembly in water, similar to that seen in the case of protein folding. In the case of protein folding, the driving force is intramolecular interactions where side chains of the amino acids form a hydrophobic core; but in the peptide self-assembly, the driving force is intermolecular interactions. The unique structural feature of these Lego peptides is that they form complementary ionic bonds with regular repeats on the hydrophilic surface. The complementary ionic sides have been classified into several moduli, i.e., modulus I, II, III, IV, etc., and mixed moduli. This classification is based on the hydrophilic surface of the molecules that have alternating positively (+) and negatively (–) charged amino acid residues, either alternating by 1, 2, 3, 4, and so on. For example, charge arrangements are for modulus I, $-+-+-+-+$; modulus II, $--++--++$; modulus III, $---+++$; and modulus IV, $----++++$. The charge orientation can also be designed in the reverse orientation to yield entirely different molecules. These structurally well-defined peptides undergo ordered assembly, and may resemble some polymer assemblies.[1,2]

Dynamic Behavior of the Peptide Re-Assemblies

The self-assembly process is reversible and dynamic (Figure 2)[9] since these peptides are short and simple,

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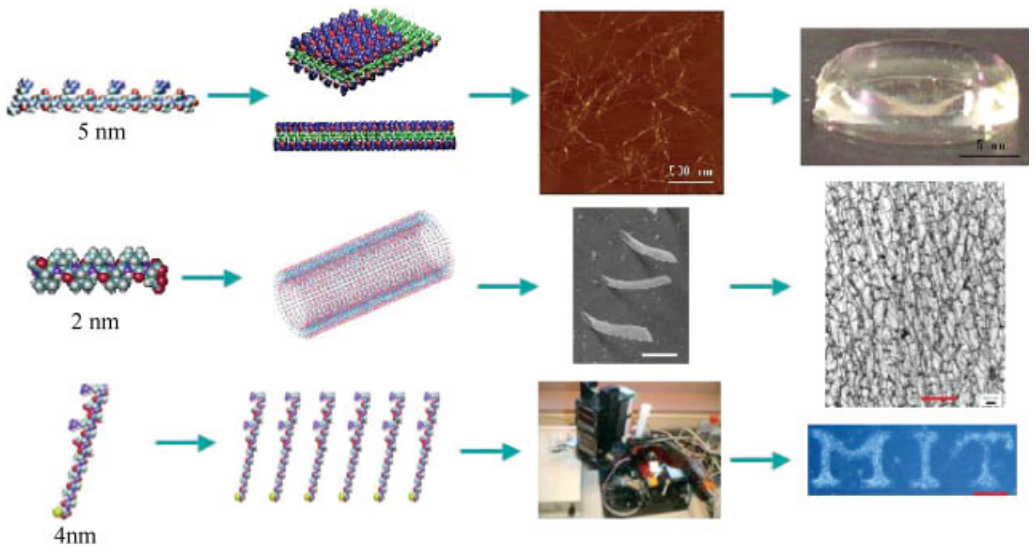

Figure 1. Three types of designer self-assembling peptide materials. a) Lego peptides, also called ionic self-complementary peptide, which has 16 amino acids, ≈5 nm in size, with an alternating polar and non-polar pattern. They form stable beta-strand and beta-sheet structures, thus the side chains partition into two sides, one polar and the other non-polar. They undergo self-assembling to form nanofibers with the non-polar residues inside (green), and the + (blue) and − (red) charged residues form complementary ionic interactions, like a checkerboard. These nanofibers form interwoven matrices that further form a scaffold hydrogel with very high water content, >99.5% water. b) Surfactant/detergent peptides, ≈2 nm in size, which have a distinct head group, either positively charged or negatively charged, and a hydrophobic tail that consists of six hydrophobic amino acids. They can self-assemble into nanotubes and nanovesicles with a diameter of ≈30–50 nm. These nanotubes go on to form an inter-connected network, which has similarly been observed in other nanotubes. c) Ink peptide: this type of peptide has three distinct segments: a functional segment where it interacts with other proteins and cells, a linker segment that can not only be flexible or stiff, but also sets the distance from the surface, and an anchor for covalent attachment to the surface. These peptides can be used as ink for an inkjet printer to directly print on a surface, instantly creating any arbitrary pattern, as shown here. Neural cells from rat hippocampal tissue form defined patterns.

thus numerous individual peptides can be readily self-organized through weak interactions that include hydrogen bonds, ionic bonds, hydrophobic and van der Waals interactions, as well as water-mediated hydrogen-bond formation. These nanofibers can be broken mechanically with sonication. However, they can undergo dynamic re-assembly repeatedly,[9] which is similar to a material self-healing process (Figure 3). Since the driving energy of the assembly in water is not only hydrophobic van der Waals forces but also the arrays of ionic interactions as well as the peptide backbone hydrogen bonds, this phenomenon can be further exploited for the production and fabrication of many such materials.

Unlike processed polymer microfibers in which the fragments of polymers cannot readily undergo re-assembly without addition of catalysts or through material processing, the supramolecular self-assembly and re-assembly event uncovered here is likely to be wide spread in many unrelated fibrous biological materials where there are numerous weak interactions involved. Self-assembly and re-assembly are very important properties for the fabrication of novel materials, and it is necessary to fully understand the detailed process in order to design better biological materials.

Peptide Scaffolds for Three-Dimensional (3D) Cell Cultures

In aqueous solutions, the Lego peptides spontaneously assemble to form well-ordered nanofibers of ≈10 nm in diameter. Numerous nanofibers further self-assemble to form scaffolds.[8,10–12] Since these nanofiber scaffolds with pores of about 5–200 nm have an extremely high water content, >99.9–99.5% or 1–5 $mg \cdot mL^{-1}$ (w/v), they have been used as 3D cell-culture scaffolds. These scaffolds closely mimic the porosity and structure of extra-cellular matrices, which allow tissue cells to reside and migrate in a 3D environment. Moreover, molecules, such as growth factors and nutrients diffuse in and out of the scaffold very slowly. These peptide scaffolds have been used for 3D cell culture, controlled cell differentiation, tissue engineering, and regenerative medicine applications (Figure 4).[10,11,13–18]

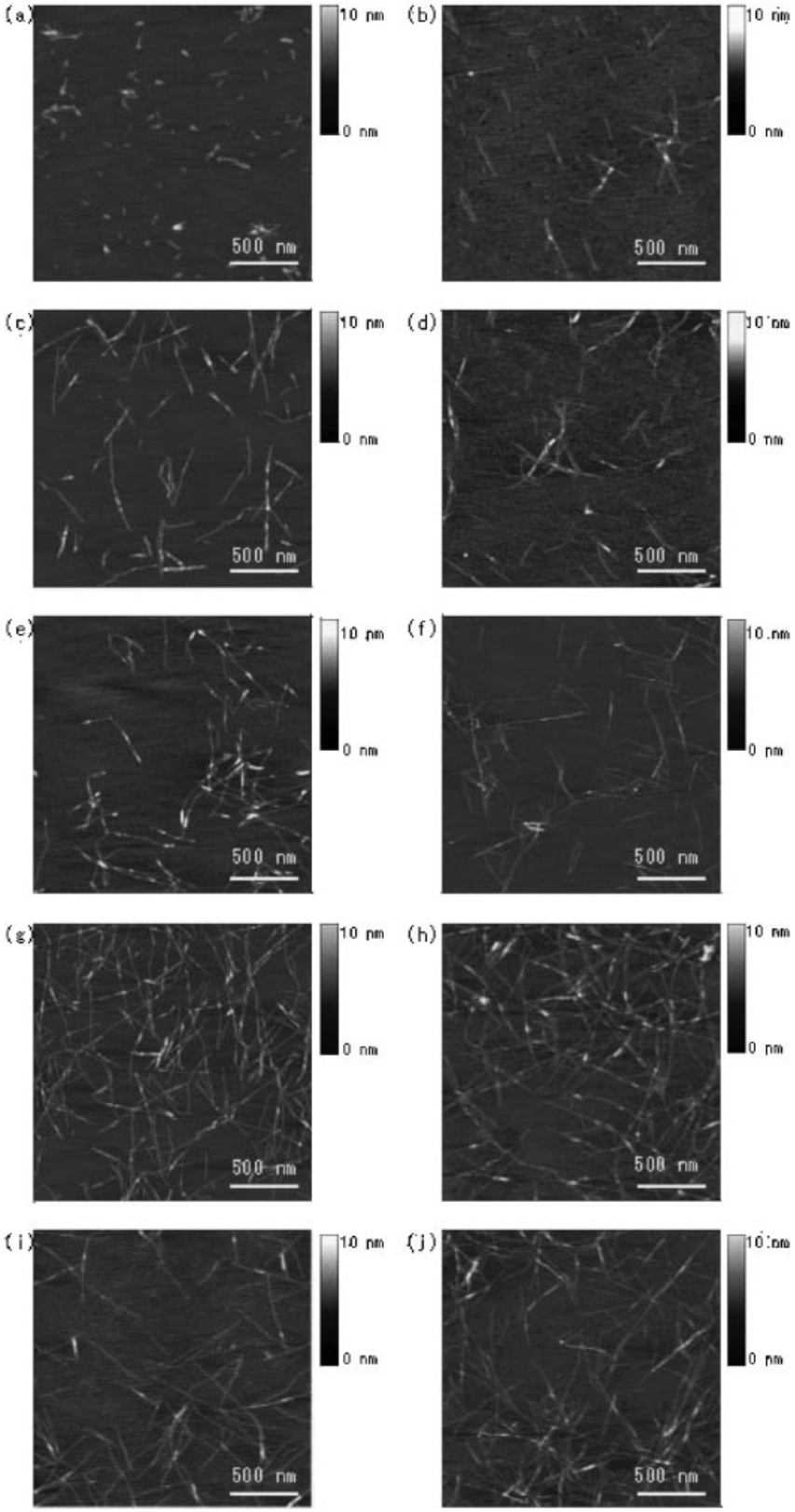

Figure 2. AFM images of a RADA16-I nanofiber at various time points after sonication. The observations were made using AFM immediately after sample preparation. a) 1 min after sonication, b) 2 min, c) 4 min, d) 8 min, e) 16 min, f) 32 min, g) 64 min, h) 2 h, i) 4 h, j) 24 h. Note the elongation and re-assembly of the peptide nanofibers over time.

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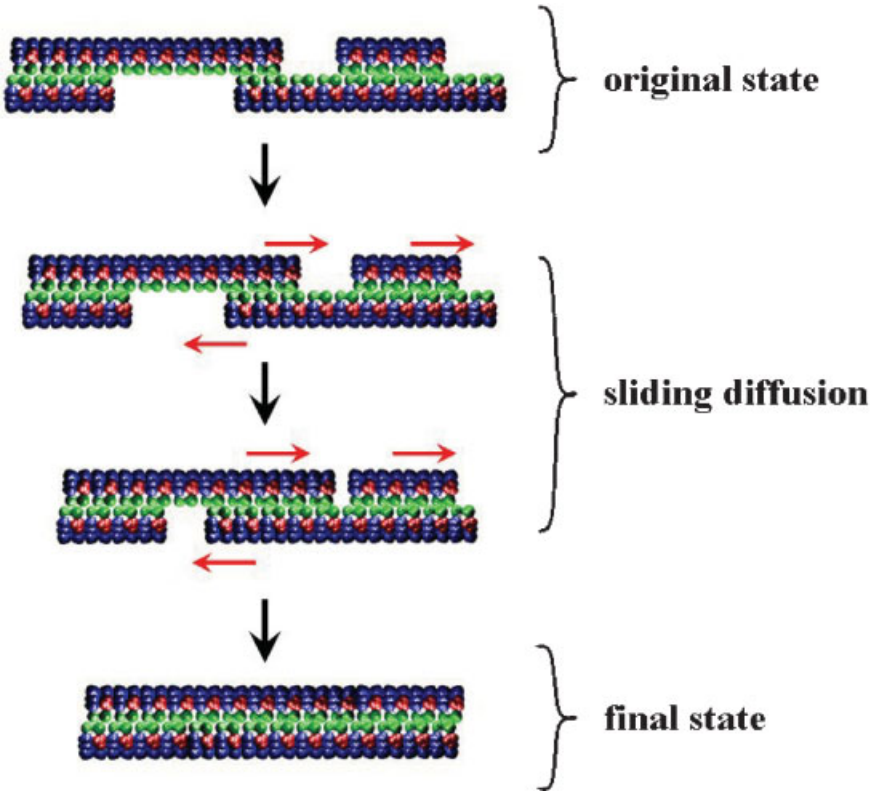

Figure 3. A proposed sliding diffusion molecular model for the dynamic reassembly of self-assembling peptides. When the peptides form stable β-sheets in water, they form intermolecular hydrogen bonds along the peptide backbones. The β-sheets have two distinctive sides, one hydrophobic with an array of alanines and the other with negatively charged aspartic acids and positively charged arginines. These peptides form anti-parallel β-sheet structures. The alanines form overlap packed hydrophobic interactions in water, a structure that is found in silk fibroin from silkworm and spiders. On the charged sides, both positive and negative charges are packed together through intermolecular ionic interactions in a checkerboard-like manner. When the fragments of the nanofiber first meet, the hydrophobic sides may not fit perfectly but with gaps. However, the non-specific hydrophobic interactions permit the nanofiber to slide by diffusion along the fiber in either direction, which minimizes the exposure of the hydrophobic alanines and eventually fills the gaps. The sliding diffusion phenomenon was also reported for nucleic acids of polyA and polyU. Color code: green, alanines; red, negatively charged aspartic acids; blue, positively charged arginines. For clarity, these β-sheets are not presented as twisted strands.

One of the Lego peptides, RADA16-I, now called PuraMatrix for its single component purity, is now commercially available for research (BD Bioscience). PuraMatrix has been used to culture diverse types of tissue cells including stem and progenitor cells, as well as differentiated cell types and tissues.[10,16] The examples of cell types cultured in PuraMatrix are listed in Table 1 and the list is rapidly expanding as more and more people use such scaffolds for their research and clinical tissue repair studies.

Lipid-Like Peptides

To further exploit the intrinsic self-assembling peptides as an avenue for emerging materials, two types of lipid-like peptides were designed.[19–21] These 7–8-residue peptides (Figure 5), each ≈2.4 nm in length, have properties very similar to those observed in biological lipid molecules. They have a hydrophilic head group of negatively charged aspartic acid at the C terminus (thus they contain two negative charges, one from the side chain carboxy group and the other from the C terminus), and a hydrophobic tail made of hydrophobic amino acids such as glycine, alanine, valine, isoleucine, or leucine (Figure 5). Similarly, cationic lipid-like peptides have been designed to mimic the properties of cationic lipid molecules. This class of surfactant peptides has a head group that consists of one or two positively charged amino acids at either the C or the N terminus, such as lysine or histidine, and a tail of hydrophobic amino acids at another terminus.[19–21] The length can also be varied by increasing or reducing the

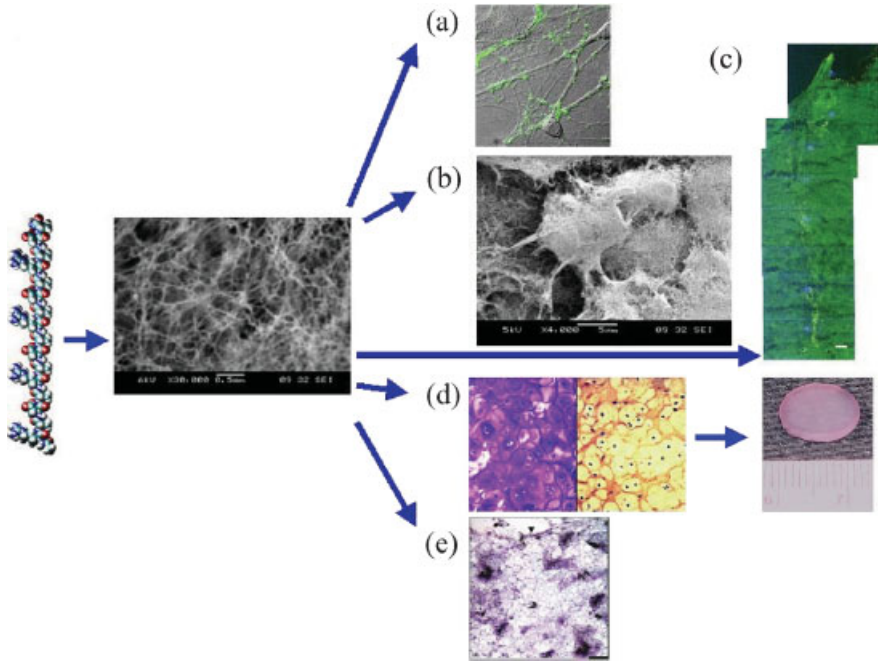

Figure 4. From designer self-assembling peptides to nanofiber scaffolds for tissues regenerations. a) Active synapses on the peptide surface. Primary rat hippocampal neurons form active synapses on peptide scaffolds. The confocal images shown bright discrete green dot labeling indicative of synaptically active membranes after incubation of neurons with the fluorescent lipophilic probe FM-143. FM-143 can selectively trace synaptic vesicle turnover during the process of synaptic transmission. The active synapses on the peptide scaffold are fully functional, indicating that the peptide scaffold is a permissible material for neurite outgrowth and active synapse formation. b) Adult mouse neural stem cells embedded in a 3D scaffold (image courtesy of F. Gelain). c) Brain damage repair in a hamster. The peptide scaffold was injected into the optical nerve area of brain that was first severed with a knife. The cut was sealed by the migrating cells after two days. A great number of neurons form synapses (image courtesy of R. Ellis-Behnke). d) Peptide KLD12 (KLDLKLDLKLDL), chondrocytes in the peptide scaffold and cartilage. The chondrocytes stained with TB show abundant GAG production (left panel) and antibody to type II collagen, which demonstrates abundant Type II collagen production (right panel). A piece of premolded cartilage with encapsulated chondrocytes in the peptide nanofiber scaffold. The cartilage formed over a 3–4 week period after the initial seeding of the chondrocytes (image courtesy of J. Kisiday). e) Von Kossa staining showing transverse sections of primary osteoblast cells on HA-PHP-RADA16-I self-assembling peptide nanofiber scaffold. Scale bar = 0.1 mm. The intensely stained black areas represent the formation of bone nodules (image courtesy of M. Bokhari).

number of amino acids, one at a time to a desired length.[20]

Nanotubes and Nanovesicles of Lipid-Like Peptides

When dissolved in water, these lipid-like peptides tend to self-assemble to isolate the hydrophobic tail from contact with water. The process of surfactant self-assembly is an enthalpy driven process of energy minimization in which the individual monomers pack together to sequester their hydrophobic tails from water. The peptide surfactants are demonstrated to form a curved bilayer. Within this curved bilayer, the peptides stack so that their hydrophilic heads are exposed to the water with their hydrophobic tails packed within. These proposed bilayers are approximately 5 nm in thickness because of a 2.4 nm length of the single peptides and, because of both the peptide shape and the electrostatic repulsion among the head groups, curve to form both nanotubes and nanovesicles having an average diameter of 30–50 nm. These nanostructures have been observed using quick-freeze/deep-etch transmission electron microscopy (TEM) (Figure 6).[19–21] This self-assembling phenomenon is very similar to well-studied events in lipids.[22–24]

Lipid-like Peptide Surfactants Stabilize Membrane Proteins

These lipid-like peptide surfactants have been found to be excellent materials not only for solubilizing and stabilizing several diverse membrane proteins and membrane protein complexes but for also crystallizing one membrane

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Table 1. A diversity of cells and tissues cultured on PuraMatrix. These cells include stable cell lines, primarily isolated cells from animals, progenitor, and stem cells.

Animal	Human
Mouse fibroblast	Human cervical carcinoma
Chicken embryo fibroblast	Human hepato-cellular carcinoma
Chinese hamster ovary	Human embryonic Kidney
Rat pheochromocytoma	Human epidermal keratinocytes
Rat neural stem cells	Human hepatocytes
Mouse embryonic stem cells	Human osteosarcoma
Mouse cerebellum granule cells	Human neuroblastoma
Bovine osteoblasts	Human foreskin fibroblast
Bovine calf & adult chondrocytes	Human neural stem cells
Bovine endothelial cells	Human embryonic stem (ES) cells
Rat adult liver progenitor cells	
Rat cardiac myocytes	
Rat hippocampal neural tissue slice	
Mouse neural colony stem cells	
Mouse & rat hippocampal cells	
Hamster pancreas cells	

protein so far. Although membrane proteins account for approximately one-third of the total cellular proteins, and carry out some of the most important functions in cells, from solar energy harvesting, signal transduction and processing, sensing the cellular environment, to cell-cell communication, only 225 membrane protein structures including 116 unique ones have been elucidated (Oct. 26, 2006). This is in sharp contrast to greater than 35 000 non-membrane protein structures that have been determined.[25,26] These simple designer lipid-like peptides[27–31] may now open a new avenue to overcome one of the biggest challenges in biology – not only to obtain large numbers of high-resolution structures of membrane proteins and but also to understand their important biological functions.

Photosynthetic system I (PS-I) is one of the first examples that clearly demonstrates how designer lipid-like peptides stabilize membrane protein complexes.[29] It is plausible that lipid- like peptides, similar to other surfactants, may directly interact with the hydrophobic domains of membrane proteins (Figure 7). It is likely that numerous peptide surfactant molecules, approximately 2.4 nm in size, like lipids, can effectively surround the hydrophobic trans-membrane domains of membrane proteins, thus sequestering them from directly interacting with water molecules and preventing them from undergoing self-aggregation. Some other membrane proteins, including a membrane protein enzyme glycerol-3- phosphate dehydrogenase and a G-protein coupled receptor bovine rhodopsin, have also been stabilized with various peptide surfactants.[29–31]

Applications of Lipid-Like Peptides

This new type of molecularly engineered peptide surfactant may have a broad range of applications, not only as surfactants, but also in growing biotechnologies and emerging nanobiotechnologies. Since the individual molecules can be designed and modified, they can be easily tailored for a variety of uses. These include encapsulation of water-insoluble molecules and delivery of drugs and other biological molecules. Because these surfactants are made of amino acids that can be bio-absorbed and reused, they may also be useful for cosmetic industries where other surfactants are used. Furthermore, for the positively charged peptide surfactants, they can compact and encase negatively charged DNA and RNA for gene delivery. Currently, gene therapy endeavors still lack optimal DNA delivery systems that are highly efficient, non-toxic, non-immunogenic, widely available through commercial synthesis vendors, and simple to produce in large scales. Preliminary experiments using the cationic surfactant peptides presented here showed promise to compact DNA and to deliver DNA into several types of cells in a cell

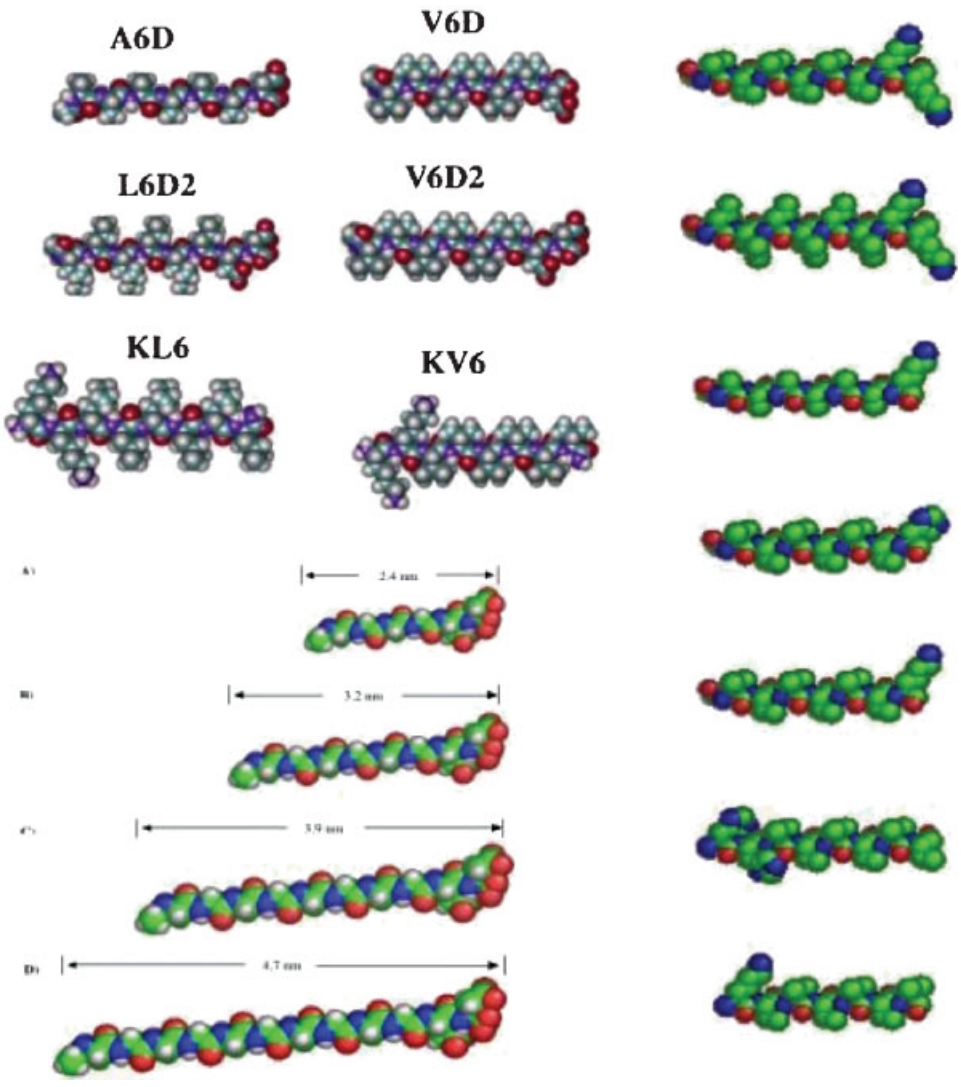

Figure 5. Molecular models of lipid-like peptide surfactants. A_6D, V_6D, L_6D_2. KL_6, KV_6. K_2L. D (Aspartic acid) bears negative charges and A (alanine), V (valine), and L (leucine) constitute the hydrophobic tails with increasing hydrophobicity. Each peptide is ≈2–3 nm in length, similar to biological phospholipids. K (lysine). Molecular structures of individual glycine tail-based surfactant peptides. $G_{10}D_2$, G_8D_2, G_6D_2 and G_4D_2. The tail length of the glycines varies depending on the number of glycine residues. The lengths of these molecules in the extended conformation range from 2.4 nm of G_4D_2 to 4.7 nm of $G_{10}D_2$. Color code: carbon, green; hydrogen, white; oxygen, red; and nitrogen; blue.

culture.[32] We should not be surprised if many unexpected findings and applications emerge in the coming years.

Perspective and Remarks

One of the emerging fields in macromolecular science is the development of new biologically inspired materials. These materials will often broaden the questions we can address and, therefore, deepen our understanding of seemingly intractable biological phenomena. The versatile self-assembling peptides discussed here will create many classes of molecular materials that will likely have a high impact in many fields.

We have encountered many surprises since we started our serendipitous journey of working on various self-assembling peptide systems: from developing a class of pure peptide nanofiber scaffolds for 3D tissue culture and for regenerative medicine,[10,11,13,14,33,34] the study of the model system of protein conformational diseases,[35–37] the design of peptide/protein inks for surface printing,[38,39] to finding peptide surfactants that solubilize and stabilize membrane proteins.[29–31]

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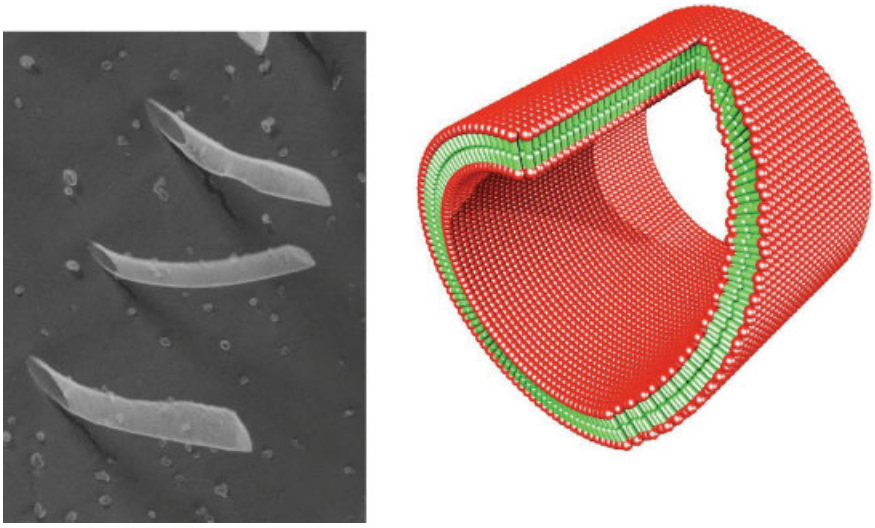

Figure 6. Nanostructures formed by lipid-like peptides. A) Nanotubes and nanovesicles revealed with quick-freeze/deep-etch TEM. The openings of the nanotubes are clearly visible. B) Molecular model of a cut-away structure formed from the peptides with negatively charged heads and hydrophobic tail.

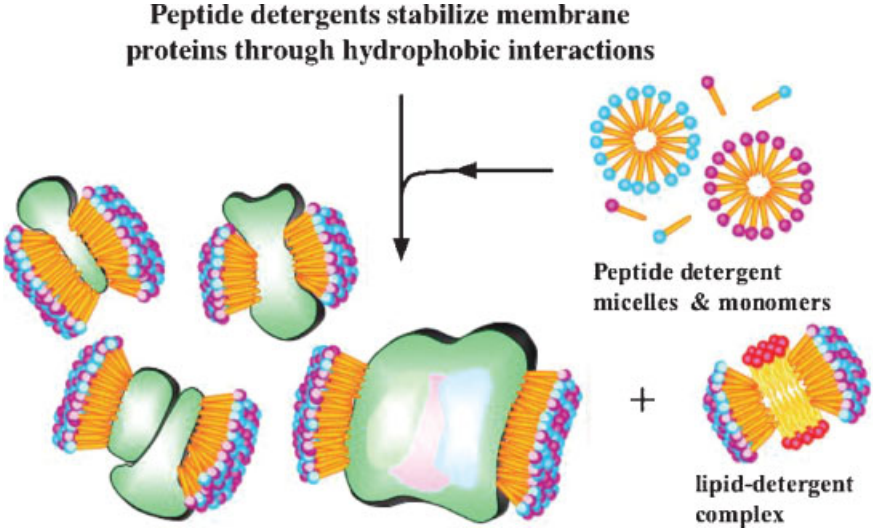

Figure 7. A proposed model for the lipid-like peptides to stabilize membrane proteins. These simple self-assembling lipid-like peptides have been used to solubilize, stabilize, and crystallize membrane proteins. These peptides have a hydrophilic head and a hydrophobic tail, much like other biological lipids. They use their tail to sequester the hydrophobic part of the membrane proteins, and the hydrophilic heads are exposed to water. Thus, they make membrane proteins soluble and stable outside of their native cellular lipid milieu. These lipid-like peptides are very important for accelerating determination of the high resolutions of molecular structure for challenging membrane proteins.

We believe that applications of these simple and versatile molecular self-assembly systems will provide us with new opportunities to study some complex and previously intractable biological phenomena. Molecular engineering through molecular design of self-assembling peptides is an enabling technology that will likely play an increasingly important role in future materials chemistry and will change our lives in the coming decades.

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[1] S. Zhang, *Biotechnol. Adv.* **2002**, *20,* 321.
[2] S. Zhang, *Nat. Biotechnol.* **2003**, *21,* 1171.
[3] Y. C. Yu, T. Pakalns, Y. Dori, J. B. McCarthy, M. Tirrell, G. B. Fields, *Meth. Enzymol.* **1997**, *289,* 571.
[4] G. B. Fields, *Bioorg. Med. Chem.* **1999**, *7,* 75.
[5] G. M. Whitesides, J. P. Mathias, C. T. Seto, *Science* **1991**, *254,* 1312.
[6] G. M. Whitesides, B. Grzybowski, *Science* **2002**, *295,* 2418.
[7] S. Zhang, C. Lockshin, A. Herbert, E. Winter, A. Rich, *EMBO J.* **1992**, *11,* 3787.
[8] S. Zhang, T. Holmes, C. Lockshin, A. Rich, *Proc. Natl. Acad. Sci. USA* **1993**, *90,* 3334.
[9] H. Yokoi, T. Kinoshita, S. Zhang, *Proc. Natl. Acad. Sci. USA* **2005**, *102,* 8414.
[10] S. Zhang, T. Holmes, C. Dipersio, R. Hynes, X. Su, A. Rich, *Biomaterials* **1995**, *16,* 1385.
[11] T. Holmes, S. Lacalle, X. Su, G. Liu, A. Rich, S. Zhang, *Proc. Natl. Acad. Sci. USA* **2000**, *97,* 6728.
[12] D. Marini, W. Hwang, D. A. Lauffenburger, S. Zhang, D. K. Roger, *Nano Lett.* **2002**, *2,* 295.
[13] J. Kisiday, M. Jin, B. Kurz, H. Hung, C. Semino, S. Zhang, A. J. Grodzinsky, *Proc. Natl. Acad. Sci. USA* **2002**, *99,* 9996.
[14] M. A. Bokhari, G. Akay, S. Zhang, M. A. Birch, *Biomaterials* **2005**, *26,* 5198.
[15] S. Zhang, F. Gelain, X. Zhao, *Seminars Cancer Biol.* **2005**, *15,* 413.
[16] S. Zhang, X. Zhao, L. Spirio, "*Scaffolding in Tissue Engineering*", P. Ma, J. Elisseeff, Eds., CRC Press, Boca Raton 2005, pp. 217–238.
[17] R. Ellis-Behnke, Y. Liang, S. You, D. K. C. Tay, S. Zhang, K. So, G. E. Schneider, *Proc. Natl. Acad. Sci. USA* **2006**, *103,* 5054.
[18] Y. Nagai, L. D. Unsworth, S. Koutsopoulos, S. Zhang, *J. Controlled Release* **2006**, *115,* 18.
[19] S. Vauthey, S. Santoso, H. Gong, N. Watson, S. Zhang, *Proc. Natl. Acad. Sci. USA* **2002**, *99,* 5355.
[20] S. Santoso, W. Hwang, H. Hartman, S. Zhang, *Nano Lett.* **2002**, *2,* 687.
[21] G. von Maltzahn, S. Vauthey, S. Santoso, S. Zhang, *Langmuir.* **2003**, *19,* 4332.
[22] J. N. Israelachvili, D. J. Mitchell, B. W. Ninham, *J. Chem. Soc.* **1976**, *72,* 1525.
[23] M. S. Spector, K. R. Easwaran, G. Jyothi, J. V. Selinger, A. Singh, J. M. Schnur, *Proc. Natl. Acad. Sci. USA* **1996**, *93,* 12943.
[24] J. V. Selinger, F. C. MacKintosh, J. M. Schnur, *Phys. Rev. E: Stat. Phys.* **1996**, *53,* 3804.
[25] E. Wallin, G. von Heijne, *Protein Sci.* **1998**, *7,* 1029.
[26] P. J. Loll, *J. Struct. Biol.* **2003**, *142,* 144.
[27] S. Santoso, S. Zhang, "*Handbook of Nanostructured Biomaterials and their Applications in Nanobiotechnology*", H. S. Nalwa, Ed., American Scientific Publishers, Stevenson Ranch, CA. 2004, pp. 459–471.
[28] S. Yang, S. Zhang, *Supramol. Chem.* **2006**, *18,* 389.
[29] P. Kiley, X. Zhao, M. Baldo, B. Bruce, S. Zhang, *PLoS Biol.* **2005**, *3,* 1181.
[30] J. I. Yeh, S. Du, A. Tordajada, J. Paulo, S. Zhang, *Biochemistry* **2005**, *44,* 16912.
[31] X. Zhao, Y. Nagai, P. Reeves, P. Kiley, H. Khorana, S. Zhang, *Proc. Natl. Acad. Sci. USA* **2006**, *103,* 11707.
[32] G. von Maltzhan, S. Zhang, unpublished results.
[33] S. Zhang, *Nat. Biotechnol.* **2004**, *22,* 151.
[34] M. E. Davis, P. C. H. Hsieh, T. Takahashi, Q. Song, S. Zhang, R. D. Kamm, A. J. Grodzinsky, P. Anversa, R. T. Lee, *Proc. Natl. Acad. Sci. USA* **2006**, *103,* 8155.
[35] S. Zhang, A. Rich, *Proc. Natl. Acad. Sci. USA* **1997**, *94,* 23.
[36] M. Altman, P. Lee, A. Rich, S. Zhang, *Protein Sci.* **2000**, *9,* 1095.
[37] S. Zhang, M. Altman, A. Rich, "Structural Plasticity of Peptides and Proteins", in: *Diseases of Conformation—A Compendium*, E. Katzir, B. Solomon, A. Taraboulos, Eds., Bialik Institute, N. Ben-Zvi Printing Enterprises Ltd., Jerusalem, Israel 2001, pp. 63–72.
[38] S. Zhang, L. Yan, M. Altman, M. Lässle, H. Nugent, F. Frankel, D. Lauffenburger, G. M. Whitesides, A. Rich, *Biomaterials* **1999**, *20,* 1213.
[39] N. Sanjana, S. B. Fuller, *J. Neurosci. Meth.* **2004**, *136,* 151.

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