Design of molecular biological materials using peptide motifs

Shuguang Zhang*[a] and Xiaojun Zhao[b]

[a]Center for Biomedical Engineering NE47-379 and Center for Bits & Atoms, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 2139-4307, USA. E-mail: shuguang@mit.edu; Fax: 617-258-5239; Tel: 617-258-7514
[b]Center for Biomedical Engineering NE47-376, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139-4307, USA. E-mail: xiaojunz@mit.edu; Fax: 617-258-5239; Tel: 617-253-2627

Two complementary fabrication technologies are employed in the production of materials and tools. In the 'top-down' approach, materials and tools are manufactured by stripping down an entity into its parts, *e.g.* carve a boat from a tree trunk. This contrasts sharply with the 'bottom-up' approach, in which materials and tools are assembled bit by bit to produce supra-structures and architectures, *e.g.* build a ship using wood strips. The bottom-up approach is likely to become an integral part of materials manufacture in the coming decades. This approach requires a deep understanding of individual molecular building blocks, their structures, assembling properties and dynamic behaviors. Two key elements in molecular materials manufacture are chemical complementarity and structural compatibility, both of which confer the weak and noncovalent interactions that bind building blocks together during self-assembly. Following Nature's leads, significant advances have been made at the interface of materials chemistry and biology, including the design of helical ribbons, peptide nanofiber scaffolds for three-dimensional cell cultures and tissue engineering, peptide surfactants, peptide detergents for solubilizing, stabilizing and crystallizing diverse types of membrane proteins and their complexes.

Introduction

Design of molecular biological materials requires detailed structural knowledge to build advanced materials and complex systems. Using basic biological building blocks and a large number of diverse peptide structural motifs,[1,2] it is possible to build new materials from the bottom up. One of the approaches is through molecular self-assembly using these construction units.[1–4] Molecular self-assembly is ubiquitous in Nature, from lipids forming oil droplets in water, and surfactants forming micelles and other complex structures in water, to sophisticated multi-unit ribosome and virus assemblies. Molecular self-assembly has recently emerged as a new approach in chemical synthesis and materials fabrication in polymer science, nanotechnology, nanobiotechnology, and various engineering pursuits. Molecular self-assembly systems lie at the interface of molecular and structural biology, protein science, chemistry, polymer science, materials science and engineering. Many

Shuguang Zhang is an Associate Director for the Center of Biomedical Engineering at Massachusetts Institute of Technology. He obtained his Ph.D. in Biochemistry, Genetics & Molecular Biology from the University of California at Santa Barbara, USA. He made a serendipitous discovery of self-assembling peptides from studying the yeast protein zuotin with Alexander Rich at MIT in 1990. He subsequently conceptualized, developed and commercialized diverse self-assembling peptide materials including peptide nanofibers, functional peptide ink, peptide molecular switches and antennae, and peptide surfactants/detergents. These self-assembling peptides materials have a broad spectrum of uses, ranging from nanofiber scaffold hydrogel for 3-D tissue cell culture, tissue repair, tissue engineering and regenerative medicine; biochips for direct printing, anchoring and patterning of molecules and cells; and peptides for solubilizing, stabilizing and crystallizing membrane proteins. Using a systematic and molecular engineering approach, he and his students, postdocs and colleagues opened a new avenue to fabricate novel nanobiological materials from bottom up through molecular self-assembly.

Shuguang Zhang

Dr Xiaojun Zhao is at the Center for Biomedical Engineering at the Massachusetts Institute of Technology. He received his Ph.D. in Biological Chemistry from the University of California at Los Angeles, where he studied the structure and functions of DNA recombination and the repair enzyme RecA protein. He then worked on mitochondrial disease at Caltech and studied the molecular and cellular mechanism of neuronal synaptogenesis at MIT. He is currently working with various self-assembling peptide systems to develop new classes of biological materials including peptide matrix scaffolds for tissue engineering, biological surface engineering, molecular switches, and peptide surfactant nanotubes for stabilizing membrane proteins and their complexes. He is also trying to gain understanding of a class of protein conformational diseases, including Alzheimer's, Parkinson's and the prion diseases (mad cow disease).

Xiaojun Zhao

self-assembling systems have been developed. These systems range from bi-, tri-block copolymers, and complex DNA structures, simple and complex proteins and peptides. 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.[5–8]

The basis of self-assembly and molecular self-assembly

Self-assembly is ubiquitous in Nature on both the macroscopic and microscopic scales, for example, from the assembly of schools of fish in the ocean, flocks of birds in the sky, herds of wild animals to oil droplets in water. Self-assembly describes the spontaneous association of numerous individual entities into a coherent organization and well-defined structures to maximize the benefit of the individual without external instruction.

Molecular self-assembly, by definition, is the spontaneous organization of molecules under thermodynamic and kinetic conditions into structurally well-defined and rather stable arrangements through a number of noncovalent interactions. These molecules undergo self-association forming hierarchical structures. The key engineering principle for molecular self-assembly is to artfully design the molecular building blocks that are able to undergo spontaneously stepwise fine-tuned interactions and assemblies through the formation of numerous noncovalent, weak chemical bonds. These weak interactions promote the assembly of molecules into units of well-defined and stable hierarchical macroscopic structures. Although each of the bonds or interactions is rather weak, the collective interactions can result in very stable structures and materials. The key elements in molecular self-assembly are chemical complementarity and structural compatibility. Like hands and gloves, both the size/shape and the correct orientation, *i.e.* chirality, are important in order to have a complementary and compatible fitting.

Molecular self-assembly in Nature

Biomimicry and designing Nature-inspired materials through molecular self-assembly will be an emerging field in the coming decades. Nature is a grand master at designing chemically complementary and structurally compatible constituents for molecular self-assembly through eons of molecular selection and evolution. Chemical evolution from the first groups of primitive molecules through countless iterations of molecular self-assembly and disassembly has ultimately produced more and more complex molecular systems.

There are numerous examples of molecular self-assembly in Nature. One of the well-known examples is silk assembly. The monomeric silk fibroin protein is approximately 1 μm in length but a single silk worm can spin fibroins into silk fiber materials over 2 km in length, two billion times longer! Such a marvellous engineering skill of Nature can only make us envious. Human ingenuity and current advanced technology is far behind the seemingly easy task of the silkworm. Likewise, spiders are grand master materials engineers who can produce many types of spider silks through self-assembly of building blocks in combinatorial ways, thus producing spider silk fiber that has both tremendous strength and flexibility. These building blocks are often on the nanometer scale. However, the resulting materials could be measured on meter- and kilometer-scales. Likewise, the size of individual phospholipid molecules is approximately 2.5 nm in length, but they can self-assemble into millimeter size lipid tubules with defined helical twist, many millions of times larger. Bioadhesives and biominerals follow a similar way. These fascinating materials are built with the units of protein scaffolds and other inorganic ions.

One approach to design new materials is through molecular self-assembly. Molecular self-assembly is ubiquitous in Nature.

Molecular self-assembly involves mostly the weak and non-covalent bonds. Individually they are quite insignificant. However, collectively these weak interactions, notably, hydrogen bonds, ionic bonds, hydrophobic interactions (electrostatic interactions), van der Waals interactions, hydrophobic interactions, and water-mediated hydrogen bonds, play an indispensable role in structures of all biological structures and their interactions. The water-mediated hydrogen bond is especially important for biological systems since all biological materials interact with water. Consider, for example, the structure of collagen. Water-mediated hydrogen bonds are absolutely crucial for holding the 3-stranded collagen helix together, through both intra- and inter-molecular means.

Peptides as building motifs

Similar to the construction of a house, doors, windows, and many other parts of the house can be prefabricated and program assembled according to architectural plans. If we shrink the construction units by many orders of magnitude into the nanoscale, we can apply similar principles to construct molecular materials and devices, through molecular self-assembly and programmed molecular assembly.

Given the growing trend and interest but limited space, only two self-assembling peptide construction units are summarized here (Fig. 1).

Two distinct classes of self-assembling peptide construction motifs are described. The first class [Fig. 1(1)] belongs to amphiphilic peptides that form well-ordered nanofibers. These peptides have two distinctive sides: one hydrophobic and the other hydrophilic. The hydrophobic side forms a double sheet inside of the fiber and the hydrophilic side forms the outside of the nanofibers that interact with water molecules such that they can form an extremely high water content hydrogel, containing as high as 99.9% water. At least three types of molecules can be made, with −, +, −/+ on the hydrophilic side. The second class of self-assembling peptides [Fig. 1(2)] belongs to a surfactant-like molecule. These peptides have a hydrophilic head and a hydrophobic tail, much like lipids or detergents. They sequester their hydrophobic tail inside of micelles, vesicles or nanotube structures and their hydrophilic heads are exposed to water. At least three kinds molecules can be made, with −, +, −/+ heads.

The first class includes 'peptide Lego' which forms well-ordered nanofiber scaffolds and can be used not only for 3-D tissue cell culture but also for regenerative medicine. The second class includes peptide surfactants and detergents that can be used not just for drug, protein and gene deliveries but also for solubilizing and stabilizing membrane proteins. These designed peptide construction motifs are structurally simple, and are versatile for a wide spectrum of applications.

Peptide Lego

Molecular-designed 'peptide Lego', on the nanometer scale, resembles the Lego bricks that have both pegs and holes in a precisely determined manner and can be programmed to assemble into well-formed structures. This class of 'peptide
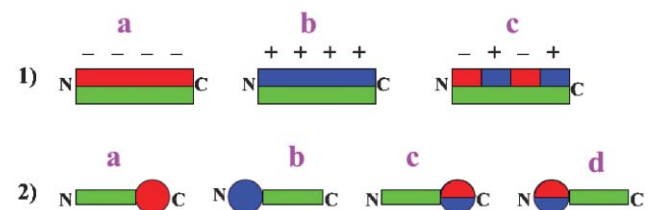

Fig. 1 Two distinct classes of self-assembling peptide construction motifs.

Lego' can spontaneously assemble into well-formed nano-structures at the molecular level.[9] The first member of the 'peptide Lego' was serendipitously discovered from a segment in a left-handed Z-DNA binding protein in yeast, Zuotin ('Zuo' means 'left' in Chinese, where Z is the Chinese character; 'tin' means 'protein' in biology).[10]

These peptides form stable beta-sheet structures in aqueous solution and thus they have two distinct surfaces, one hydrophilic, the other hydrophobic, like the pegs and holes in Lego bricks. The hydrophobic sides must shield themselves from water thus facilitating their self-assembly in water, similar to that seen in the case of protein folding. The unique structural feature of these 'peptide Lego' units is that they form complementary ionic bonds with regular repeats on the hydrophilic surface (Fig. 2). 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 surfaces of the molecules that have alternating + and − charged amino acid residues, alternating by either 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, which can yield entirely different molecules. These well-defined sequences allow them to undergo ordered self-assembly, resembling some situations found in well-studied polymer assemblies.[3,4,11]

The 'peptide Lego' molecules can undergo self-assembly in aqueous solutions to form well-ordered nanofibers that further associate to form nanofiber scaffolds.[9,12–14] One of them, RADA16-I, is called PuraMatrix, because of its purity as a designed biological scaffold in contrast to other biologically-derived scaffolds from animal collagen and Matrigel which contain unspecified components in addition to known materials.

Since these nanofiber scaffolds contain 5–200 nm pores and have an extremely high water content ($>99.5\%$ or 1–5 mg ml^{-1}), they are of utility in three-dimensional cell-culture media for expansion and controlled differentiation of many types of stem cells. The scaffolds closely mimic the porosity and gross structure of extracellular matrices, allowing cells to reside and migrate in a 3-D environment, and molecules such as growth factors, nutrients and waste to diffuse in and out very slowly. These peptide scaffolds have been used for 3-D cell culture, controlled cell differentiation, tissue engineering and regenerative medicine applications (Fig. 2).[12,13,15,16]

Peptide surfactants and detergents

We designed a new class of peptide surfactants/detergents with short hydrophobic tails and hydrophilic heads, taking advantage of their self-assembly properties in water.[17–19] Several peptide surfactants have been designed using the natural lipids as a guide. These peptides have a hydrophobic tail with various degrees of hydrophobicity and a hydrophilic head, either negatively-charged aspartic and glutamic acids or positively-charged lysine or histidine (Fig. 3). These peptide monomers contain 7-8 amino acid residues and have a hydrophilic head composed of aspartic acid and a tail of hydrophobic amino acids such as alanine, valine or leucine. The length of each peptide is approximately 2 nm, similar to that of biological phospholipids.[17–19] The length can also be varied by adding more amino acids, one at a time, to achieve a desired length as shown in Fig. 4.[18]

Although individually these peptide surfactants/detergents have completely different compositions and sequences, these peptides share a common feature: the hydrophilic heads have 1–2 charged amino acids and the hydrophobic tails have four
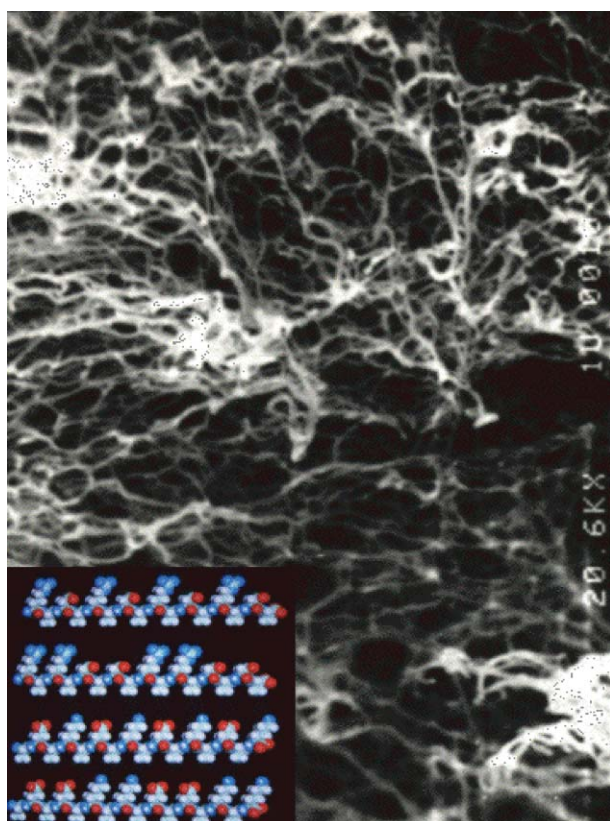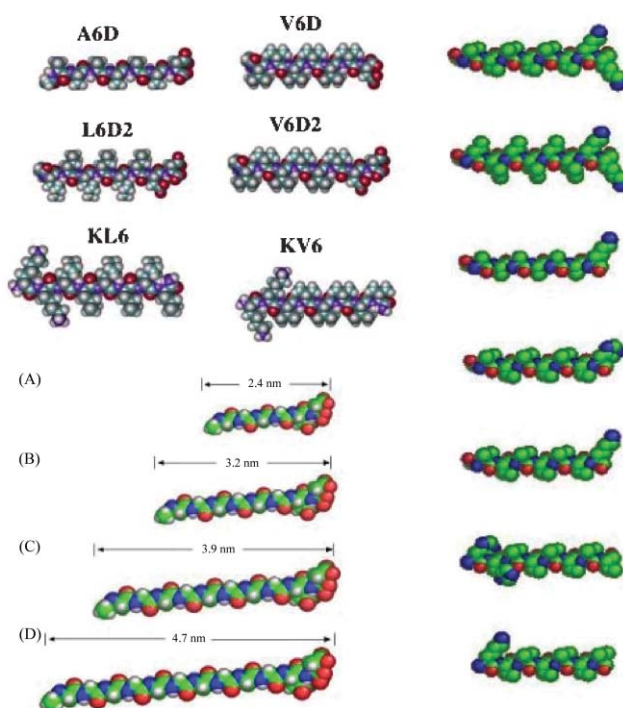
Fig. 2 Example of the first class of self-assembling peptides that form well-ordered nanofibers. The diameter of the nanofiber is *ca.* 10 nm with pores of *ca.* 5–200 nm. They form 3-D scaffolds that can be used for tissue cell culture and for tissue engineering and regenerative medicine. The insert shows a few members of a very large family. Note their Lego-like characteristics, where hydrophobic side alanine residues are on the lower side and the hydrophilic side with charged amino acids (red and blue) is on the other.

Fig. 3 A few selected self-assembling peptide surfactant/detergent molecules are modeled here. These peptides have a hydrophilic head and a hydrophobic tail, much like lipids or detergents. They sequester their hydrophobic tail inside of micelles, vesicles or nanotube structures and their hydrophilic heads are exposed to water. At least three kinds molecules can be made, with −, +, or −/+ heads. The charges can also be at both ends thus creating different types of detergents.
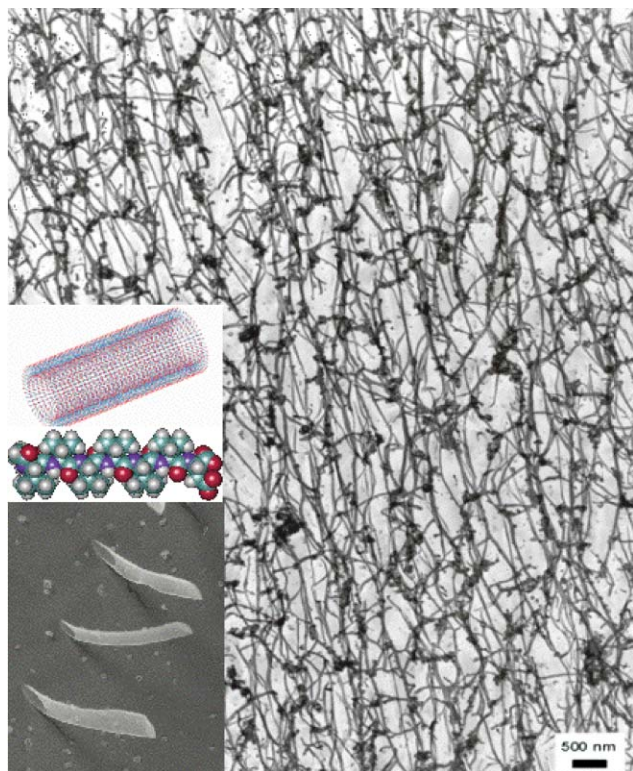
Fig. 4 Example of the second class of self-assembling peptide surfactant/detergents that form well-ordered structures. These include nanotubes with openings, nanovesicles and micelles. These structures are dynamic entities and can undergo self-assembly and disassembly.

or more consecutive hydrophobic amino acids. For example, A_6D (AAAAAAD), and V_6D (VVVVVVD) peptides (see Fig. 3) have six hydrophobic alanine or valine residues at 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; likewise, G_8DD (GGGGGGGGDD), has eight glycines followed by two asparatic acids with three negative charges. In contrast, KV_6 (KVVVVVV) and V_6K (VVVVVVK) have one positively-charged lysine as the hydrophilic head and six valines as the hydrophobic tail.[17–19]

These peptides undergo self-assembly in water to form nanotubes and nanovesicles, having an average diameter of 30–50 nm.[17–19] Tails consisting of alanine and valine residues produce more homogeneous and stable structures than those of glycines, isoleucine and leucine. This property may be due to their hydrophobic and hydrophilic ratios. These monomer surfactant peptides were used for molecular modeling, where the negatively-charged aspartic acid is modeled as red and positively-charged lysine is blue with green as the hydrophobic tails.

Quick-freeze/deep-etch sample preparation where the sample is instantly flash-frozen below −190 °C produced a 3-D structure with minimal structural disturbance. Using transmission electron microscopy it revealed a network of open-ended nanotubes with a three-way junction to connect the nanotubes (Fig. 4).[17–19] They seem to be dynamic molecular entities over time. Likewise, A_6K cationic peptide also exhibited similar nanotube structures with the opening ends clearly visible.

It is interesting that these simple peptide surfactants can produce remarkably complex and dynamic structures. This is another example for building materials from the bottom up.

One may ask how could these simple peptide surfactants form such well-ordered nanotubes and nanovesicles? The answer may lie in the molecular and chemical similarities between lipids and the peptides since both have a hydrophilic

head and a hydrophobic tail. Organic surfactants have been well studied over last few decades. The key lies in the molecular packing. However, the packing between lipids and peptides is likely to be quite different. In lipids, the hydrophobic tails pack tightly against each other to completely displace water, without formation of hydrogen bonds at all. On the other hand, in addition to hydrophobic tail packing between the amino acid side chains, surfactant peptides also interact through inter-molecular hydrogen bonds along the backbone. Some of these peptide surfactants displayed typical beta-sheet structures, implying the backbone extended. Thus, the tails are likely to pack in the beta-sheet form with certain curvature due to the repulsion of the charged heads.

Furthermore, several cationic peptide surfactants have been tested for their ability to encapsulate DNA and deliver DNA into cells (von Maltzahn & Zhang, unpublished results). These observations suggest the potential broad uses of this class of peptide surfactants for drug and gene delivery.

Peptide surfactants/detergents stabilize membrane proteins

Although membrane proteins are composed of at approximately one-third of total cellular proteins and carry out some of the most important functions in cells, only few dozen membrane protein structures have been elucidated. This is in sharp contrast to the 25 000 non-membrane protein structures that have been solved.[20,21] The main reason for this delay is due to the difficulty in purifying and crystallizing membrane proteins because the removal of lipids from the membrane proteins affects the protein solubility and conformation stability. Despite the fact that a variety of detergents and lipids as surfactants have been used to facilitate the solubilization, stabilization, purification, crystallization, manipulation of membrane proteins for several decades now, how surfactants interact with a membrane protein to impact its structure and functions and how to choose good surfactants for the right membrane proteins remain largely unknown. This is partly due to the complexity of membrane protein–detergent–lipid inter-actions and the lack of a 'magic material' surfactant. Therefore, the need to develop new materials is acute.

Recent experiments show that these peptide surfactants or peptide detergents are excellent materials for the solubilization, stabilization and crystallization of several diverse membrane proteins. These simply designed peptide detergents may now open a new avenue to overcome one of the biggest challenges in biology – to obtain large numbers of high resolution structures of membrane proteins (Fig. 5). Study of membrane proteins will not only enrich and deepen our knowledge of how cells communicate with their surroundings, since all living system respond to their environments, but these membrane proteins can also be used to fabricate the most advanced molecular devices, from energy harnessing devices, extremely sensitive sensors to medical detection devices which, at the moment, we cannot even imagine.

Perspectives in materials chemistry

One of the emerging fields in materials chemistry 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 peptide construction motifs will create new classes of molecular materials that will likely have a high impact in many fields.

One of the unexpected benefits of studying self-assembling peptide systems emerged recently. We found that the simple peptide surfactant/detergents (Fig. 5) may be excellent materials to solubilize and stabilize membrane proteins that have
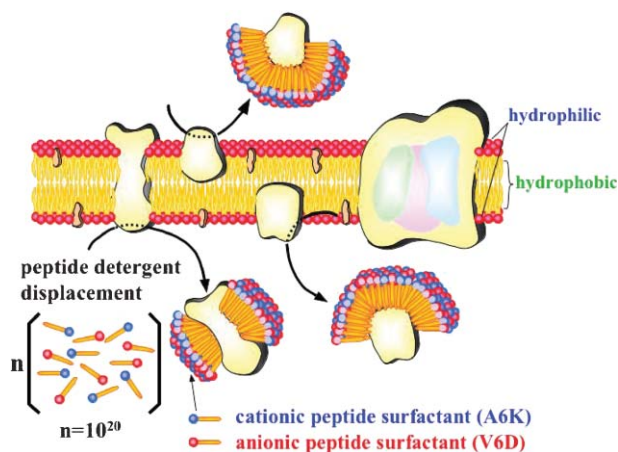
Fig. 5 These simple self-assembling peptide surfactant/detergents can be used to solubilize, stabilize and crystallize membrane proteins. These peptides have a hydrophilic head and a hydrophobic tail, much like lipids or detergents. They use their tail to sequester the hydrophobic part of membrane proteins, the hydrophilic heads exposed to water, thus making the membrane protein soluble and stable outside of the native lipid environment. These surfactant/detergents peptides are very important materials to overcome the barrier for solving the molecular structures of membrane proteins.

been extremely difficult to work with and thus the determination of their high-resolution structures lags far behind. In one case, a membrane protein was crystallized in a very short time. This surprising finding encouraged us to work on membrane proteins. Without working on the peptide surfactant/detergents, we would never have moved toward facing the big challenge.

We believe that application 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 the future of materials chemistry and will change our lives in the coming decades.

We have encountered many surprises since we started our serendipitous journey of working on various self-assembling peptide systems:[22] from developing a class of pure peptide nanofiber scaffolds for 3-D tissue culture and for tissue engineering,[11–16] studying the model system of protein conformational diseases,[23–25] designing peptide/protein inks for surface printing,[26,27] to finding peptide surfactant/detergents that solubilize and stabilize membrane proteins.[17–19]

New materials will undoubtedly come from study the diverse materials in Nature. As John Maddox best put it: "The most important discoveries of the next 50 years are likely to be ones of which we cannot now even conceive."[28]

Acknowledgements

References

1 C.-I. Branden and J. Tooze, *Introduction to Protein Structure*, 2nd edn., Garland Publishing, New York, 1999.
2 G. A. Petsko and D. Ringe, *Protein Structure and Function*, New Science Press Ltd, London, 2003.
3 S. Zhang, *Biotechnol. Adv.*, 2002, **20**, 321–339.
4 S. Zhang, *Nature Biotechnol.*, 2003, **21**, 1171–1178.
5 Y. C. Yu, T. Pakalns, Y. Dori, J. B. McCarthy, M. Tirrell and G. B. Fields, *Methods Enzymol.*, 1997, **289**, 571–587.
6 G. B. Fields, *Bioorg. Med. Chem.*, 1999, **7**, 75–81.
7 G. M. Whitesides, J. P. Mathias and C. T. Seto, *Science*, 1991, **254**, 1312–1319.
8 G. M. Whitesides and B. Grzybowski, *Science*, 2002, **295**, 2418–2421.
9 S. Zhang, T. Holmes, C. Lockshin and A. Rich, *Proc. Natl. Acad. Sci. USA*, 1993, **90**, 3334–3338.
10 S. Zhang, C. Lockshin, A. Herbert, E. Winter and A. Rich, *EMBO J.*, 1992, **11**, 3787–3796.
11 S. Zhang, D. Marini, W. Hwang and S. Santoso, *Curr. Opin. Chem. Biol.*, 2002, **6**, 865–871.
12 S. Zhang, T. Holmes, M. DiPersio, R. O. Hynes, X. Su and A. Rich, *Biomaterials*, 1995, **16**, 1385–1393.
13 T. Holmes, S. Delacalle, X. Su, A. Rich and S. Zhang, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 6728–6733.
14 D. Marini, W. Hwang, D. A. Lauffenburger, S. Zhang and R. D. Kamm, *Nano Lett.*, 2002, **2**, 295–299.
15 J. Kisiday, M. Jin, B. Kurz, H. Hung, C. Semino, S. Zhang and A. J. Grodzinsky, *Proc. Natl. Acad. Sci. USA*, 2002, **99**, 9996–10 001.
16 C. E Semino, J. R. Merok, G. Crane, G. Panagiotakos and S. Zhang, *Differentiation*, 2003, **71**, 262–270.
17 S. Vauthey, S. Santoso, H. Gong, N. Watson and S. Zhang, *Proc. Natl. Acad. Sci. USA*, 2002, **99**, 5355–5360.
18 S. Santoso, W. Hwang, H. Hartman and S. Zhang, *Nano Lett.*, 2002, **2**, 687–691.
19 G. von Maltzahn, S. Vauthey, S. Santoso and S. Zhang, *Langmuir*, 2003, **19**, 4332–4337.
20 E. Wallin and G. von Heijne, *Protein Sci.*, 1998, **7**, 1029–1038.
21 P. J. Loll, *J. Struct. Biol.*, 2003, **142**, 144–153.
22 S. Zhang, *Nature Biotechnol.*, 2004, **22**, 151–152.
23 S. Zhang and A. Rich, *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 23–28.
24 M. Altman, P. Lee, A. Rich and S. Zhang, *Protein Sci.*, 2000, **9**, 1095–1105.
25 S. Zhang, M. Altman and A. Rich, 'Structural plasticity of peptides and proteins', in *Diseases of Conformation – A Compendium*, E. Katzir, B. Solomon, A. Taraboulos, ed., Bialik Institute, N. Ben-Zvi Printing Enterprises Ltd., Jerusalem, Israel, 2001, pp. 63–72.
26 S. Zhang, L. Yan, M. Altman, M. Lässle, H. Nugent, F. Frankel, D. Lauffenburger, G. M. Whitesides and A. Rich, *Biomaterials*, 1999, **20**, 1213–1220.
27 N. Sanjana and S. B. Fuller, *J. Neurosci. Methods*, 2004, **136**(2), 151–163.
28 J. Maddox, *Sci. Am.*, December 1999.