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Self-Assembling Nanopeptides Become a New Type of Biomaterial

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Abstract Combining physics, engineering, chemistry and biology, we can now design, synthesize and fabricate biological nano-materials at the molecular scale using self-assembling peptide systems. These peptides have been used for fabrication of nanomaterials including nanofibers, nanotubes and vesicles, nanometer-thick surface coating and nanowires. Some of these peptides are used for stabilizing membrane proteins, and others provide a more permissive environment for cell growth, repair of tissues in regenerative medicine, and delivering genes and drugs. Self-assembling peptides are also useful for fabricating a wide spectrum of exquisitely fine architectures, new materials and nanodevices for nanobiotechnology and a variety of engineering. These systems lie at the interface between molecular biology, chemistry, materials science and engineering. Molecular self-assembly will harness nature's enormous power to benefit other disciplines and society.

Keywords Regenerative medicine · Polymers · Nanobiotechnology · Self-assembly peptide · Designer nanomaterials

1 Introduction

1.1 The Nature's Building Blocks at the Molecular Scale and Design, Synthesis and Fabrication

Nature is the grandmaster when it comes to building extraordinary materials and molecular machines—from the bottom up, one atom and one molecule at a time. Multifunctional macromolecular assemblies in living organisms, including hemoglobin, polymerases, ATP synthase, membrane channels, the spliceosome, the proteosome, ribosomes, and photosystems are all essentially exquisitely designed molecular machines acquired through billions of years of prebiotic molecular selection and evolution. Nature has produced a basic set of molecules that includes 20 amino acids, a few nucleotides, a dozen or so lipid molecules and few dozens of sugars as well as naturally modified building blocks or metabolic intermediates. With these seemingly simple molecules, natural processes are capable of fashioning an enormously diverse range of fabrication units, which can further self-organize into refined structures, materials and molecular machines that not only have high precision, flexibility and error correction, but also are self-sustaining and evolving. Indeed, nature favors bottom-up design, building up from molecular assemblies, bit by bit, more or less simultaneously in a well-defined manner.

1.2
A Fabrication Tool: Nanobiotechnology Through Molecular Self-Assembly

Design of molecular biological nanostructures 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. 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. These systems lie at the interface of molecular and structural biology, protein science, chemistry, polymer science, materials science and engineering. Many self-assembling systems have been developed, which range from organic supramolecular systems, bi-, tri-block copolymers [3], and complex DNA structures [4, 5], simple and complex proteins [6–8] to peptides [9–22].

1.3
Basic Engineering Principles for Micro- and Nano-Fabrication Based on Molecular Self-Assembly Phenomena

Programmed assembly and self-assembly are ubiquitous in nature at both macroscopic and microscopic scales. The Great Wall of China, the Pyramids of Egypt, the schools of fish in the ocean, flocks of birds in the sky, protein folding and oil droplets on water are all such examples. On the other hand, 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. If we shrink construction units by many orders of magnitude into nano-scale, such as structurally well-ordered protein fragments, or peptides [21], we can apply similar principles to construct molecular materials and devices, through molecular self-assembly and programmed molecular assembly.

1.4
Both Chemical Complementarity and Structural Compatibility for Bionanotechnology

The "bottom-up" approach, by which materials are assembled molecule by molecule (and in some cases even atom by atom) to produce novel supramolecular architectures is a powerful technology. This approach is likely to become an integral part of materials manufacture and requires a deep understanding of individual molecular building blocks and their structures, assembly properties and dynamic behaviors. Molecular self-assembly interactions typically include hydrogen bonds, electrostatic attractions, and Van der Waals interactions. Although these bonds are relatively insignificant in

isolation, when combined together as a whole, they govern the structural conformation of all biological macromolecules and influence their interaction with other molecules. The water-mediated hydrogen bond is especially important for living systems, as all biological materials interact with water. It is a powerful approach for fabricating novel supramolecular architectures, which is ubiquitous in nature and has now emerged as a new approach in chemical synthesis, nanotechnology, polymer science, materials and engineering.

To date, several self-assembling peptide systems have been studied, ranging from models for studying protein folding and protein conformational diseases, to molecular materials for producing peptide nanofibers, peptide scaffolds, peptide surfactants and peptide ink [9, 10] easy to produce at a large scale to drive the development of this new industry. These self-assembly peptide systems represent a significant advance in molecular engineering for diverse technological innovations. This field is growing at a rapid pace and it is impossible to summarize all aspects of the work being done by others in this limited space, and hence this review focuses on a few examples especially from our laboratory. We focus on our work from the past decade, but those who are interested in trends over a longer period of time are referred to earlier reviews [10, 11].

2
Self-Assembly Peptide Systems

A new class of oligopeptide-based biological materials was serendipitously discovered from the self-assembly of ionic self-complementary oligopeptides [3]. A number of peptide molecular self-assembly systems has been designed and developed. This new class of biological materials has considerable potential for a number of applications, including scaffolding for tissue repair and tissue engineering, drug delivery of molecular medicine and biological surface engineering. Molecular self-assembly relies on chemical complementarity and structural compatibility [23]. These fundamentals are keys to the design of the molecular units required for the fabrication of functional macrostructures, which in turn permit molecular self-assembly in nanotechnology and nanobiotechnology.

The complementary ionic sides have been classified into several moduli (modulus I, modulus II, modulus III, modulus IV, etc., and mixtures thereof). This classification is based on the hydrophilic surfaces of the molecules, which have alternating positively and negatively charged amino acids alternating by one residue, two residues, and three residues and so on. For example, charge arrangements for modulus I, modulus II, modulus III and modulus IV are –+–+–+–+, ––++––++, –––+++ and ––––++++, respectively. The charge orientation can also be designed in the reverse orientation,

which can yield entirely different molecules. These well-defined sequences allow the peptides to undergo ordered self-assembly, in a process resembling some situations found in well-studied polymer assemblies. A broad range of peptides and proteins have been shown to produce very stable nanofiber structures, also called amyloid fibers [24–34].

2.1
Peptides as Construction Motifs

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

2.2
Modulus I: "Peptide Lego"

Type I peptides, also called "molecular Lego" are the first member of the "peptide Lego", which was serendipitously discovered from a segment in a left-handed Z-DNA binding protein in yeast and named Zuotin [14]. Lego bricks have pegs and holes, which can be assembled into particular structures. In a similar way, these peptides can be assembled at the molecular level. The nanometer scale "peptide Lego" resembles Lego bricks that have both pegs and holes in a precisely determined organization and can be programmed to assemble into well-formed structures. This class of "peptide Lego" can spontaneously assemble into well-formed nanostructures at the molecular level [15].

The molecular structure and proposed complementary ionic pairings of the modulus I peptides between positively charged lysines and negatively charged glutamates in an overlapping arrangement are modeled in Fig. 1. This structure represents an example of this class of self-assembling β-sheet peptides that spontaneously undergo association under physiological conditions. If the charged residues are substituted, i.e. the positively charged lysines (Lys) are replaced by the positively charged arginines (Arg) and the negatively charged glutamates (Glu) were replaced by negatively charged aspartates (Asp), the peptide would still be able to undergo self-assembly into macroscopic materials. However, if the positively charged residues, Lys and Arg, were replaced by negatively charged residues, Asp and Glu, the peptide would not be able to undergo self-assembly and form macroscopic materials although β-sheet structures have been observed in the presence of salt. If the alanines (Ala) were changed to more hydrophobic residues, such as Leu, Ile, Phe or Tyr, the molecules had a greater tendency to self-assemble and formed

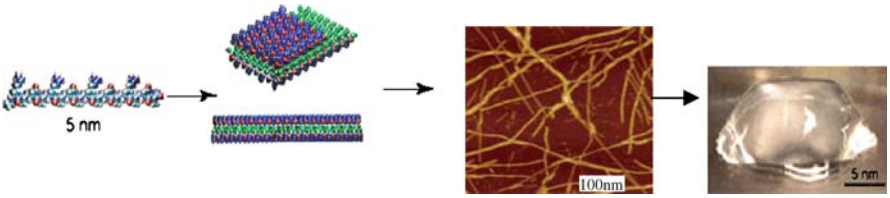

Fig. 1 Fabrication of various peptide materials. Peptide Lego, also called ionic self-complementary peptide has 16 amino acids, 5 nm in size, with an alternating polar and non-polar pattern. They form stable b-strand and b-sheet structures, thus the side chains partition into two sides, one polar and the other non-polar. They undergo self-assembly to form nanofibers with the non-polar residues inside (*green*) and positive (*blue*) and negative (*red*) charged residues forming complementary ionic interactions, like a checkerboard. These nanofibers form interwoven matrices that produce a scaffold hydrogel with very high water content, 99.5% water (images courtesy of Hidenori Yokoi)

peptide matrices with enhanced strength [35]. The fundamental design principles of such self-assembling peptide systems can be readily extended to polymers and polymer composites, where copolymers can be designed and produced.

2.3 Molecular Switches

Several peptides have been developed as "molecular switches" in which the peptides can drastically change their molecular structure. One of the peptides with 16 amino acids, DAR16-IV, has a β-sheet structure 5 nm in length at ambient temperature but can undergo an abrupt structural transition at high temperatures to form a stable α-helical structure 2.5 nm long [13]. Similar structural transformations can be induced by changes in pH. This suggests that secondary structures of some sequences, especially segments flanked by clusters of negative charges on the *N*-terminus and positive charges on the *C*-terminus, may undergo drastic conformational transformations under the appropriate conditions. These findings do not only provide insights into protein–protein interactions during protein folding and the pathogenesis of some protein conformational diseases, such as Alzheimer's disease, Gestmann–Straussler–Scheiker syndrome and/or kuru in humans and scrapie in sheep, cow, mink or elk, as well as certain types of cancer, all of which are examples of such conformational disorder [34–41], but can also be developed as molecular switches for a new generation of nanoactuators. Both peptides of DAR16-IV (DADADADARARARARA) and EAK12 (AEAEAEAEAKAK) have a cluster of negatively charged glutamate residues close to the *N*-terminus and a cluster of positively charged Arg residues near the *C*-terminus. It is well known that all α-helices have a helical dipole moment with a partially negative *C*-terminus toward a partially positive

N-terminus [42]. Because of the unique sequence of DAR16-IV and EAK12, their side chain charges balance the helical dipole moment, therefore favoring helical structure formation. However, they also have alternating hydrophilic and hydrophobic residues as well as ionic self-complementarity, which have been previously found to form stable β-sheets. Thus, the behavior of these Type II molecules is likely to be more complex and dynamic than other stable β-sheet peptides. Additional molecules with such dipoles have been designed and studied, and the results confirmed the initial findings. Others have also reported similar findings that proteins and peptides can undergo self-assembly and disassembly or change their conformations depending on the environmental influence, such as its location, pH change, and temperature, or crystal lattice packing [43–45].

2.4 Peptide Ink

"Peptide inks", undergo self-assembly on the surface rather than with themselves. They form monolayers on surfaces for a specific cell pattern formation or to interact with other molecules. These oligopeptides have three distinct features. The first feature is the terminal segment of ligands that incorporate a variety of functional groups for recognition by other molecules or cells. The second feature is the central linker where a variable spacer is not only used to allow freedom of interaction at a specified distance away from the surface but also controls the flexibility or rigidity. The third feature is the surface an-

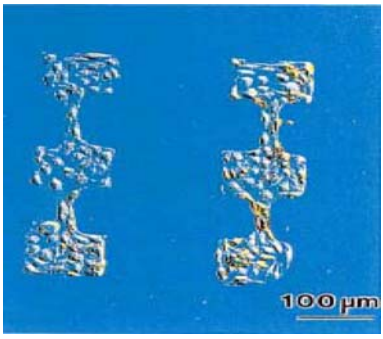

Fig. 2 Peptide ink. This type of peptide has three distinct segments: a functional segment where it interacts with other proteins and cells; a linker segment that is either flexible or stiff and sets the distance from the surface, and an anchor for covalent attachment to the surface. These peptides can be used as ink for an inkjet printer to directly print on a surface, instantly creating any arbitrary pattern, as shown here. Bovine aortic endothelial cells were confined to the patterns of squares connected with linear tracks. The patterns were made with an oxygen gas treated PDMS stamp to increase the surface hydrophilicity to facilitate EG6SH wetting

chor where a chemical group on the peptide can react with the surface to form a covalent bond [17].

Whitesides and coworkers developed a microcontact printing technology that combines semi-conducting industry fabrication, chemistry and polymer science to produce defined features on a surface down to the micrometer or nanometer scale [46–48]. Following microcontact printing, a surface can be functionalized with different molecules using a variety of methods which have now been modified with a variety of chemical compounds. Furthermore, peptides and proteins as inks have also been printed onto surfaces. This development has spurred new research into the control of molecular and cellular patterning, cell morphology and cellular interactions, and fueled new technology development. Peptide or protein inks have been directly printed on surfaces to allow adhesion molecules to interact with cells and adhere to the surface (Fig. 2) [49].

2.5 Peptide Surfactants/Detergents

Peptide surfactants or detergents stabilize membrane proteins, although membrane proteins make up approximately one-third of total cellular proteins and carry out some of the most important functions in cells, only several dozen membrane protein structures have been elucidated. This is in striking contrast to about 33 000 non-membrane protein structures that have been solved [50, 51].

The main reason for this delay is the difficulty in purifying and crystallizing membrane proteins because removal of lipids from membrane proteins

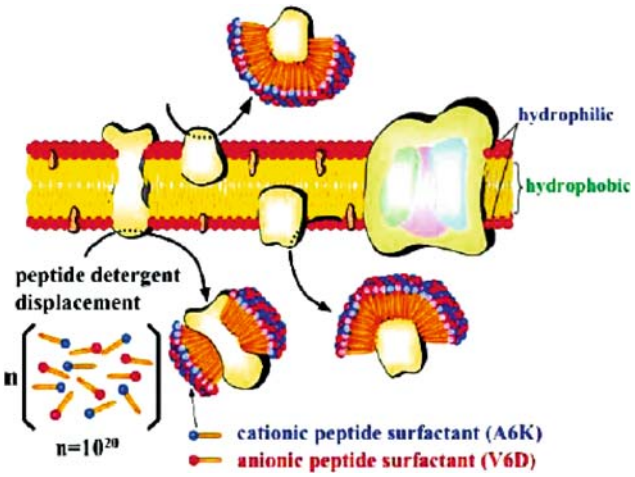

Fig. 3 Peptide surfactants of A6D and V6D. These simple self-assembling peptide surfactant/detergents can be used to solubilize, stabilize and crystallize membrane proteins

affects protein solubility and conformational stability. Although a variety of detergents and lipids as surfactants have been used to solubilize, stabilize and crystallize membrane proteins for several decades, these surfactants are still unable to significantly maintain structural stability of membrane proteins during experimental handling. In the other words, there is no "magic material" surfactant working on membrane proteins and there is an urgent need to develop new types of surfactants. We have used A6K (AAAAAAK) and V6D (VVVVVVD) to stabilize the photosynthetic protein-molecular complexes in solid-state devices and we showed that this new type of peptide detergents was very effective in stabilizing membrane protein functions, providing a powerful tool for membrane proteins research and application (Fig. 3) [10, 52–54].

2.6 Other Systems

Molecular self-assembly systems using nucleic acids on a chip have been developed. This new technology is based entirely on the principles of nucleic acid molecular self-assembly. Numerous new devices and technologies have been advanced. The most well-known example is the biochip technology "Lab on a Chip", "GeneChip", or "Microarray Technology" [55]. This microarray system is widely used in gene expression analysis, the human genome project, diagnostics, discovery of new functions of genes, and high-throughput drug discovery and screenings. In addition, people are now beginning to turn to testing the ability of peptide-based biomaterials to respond to external cues; this responsiveness has been collectively referred to as "smart behavior". Responsiveness can be defined at either the structural level or the functional level [56].

3 Fabrication of Nanomaterials Through Self-Assembling Systems

3.1 Nanofibers

The peptide Lego molecules can undergo self-assembly in aqueous solutions to form well-ordered nanofibers that further associate to form nanofiber scaffolds [15, 16, 57]. One of them, RADA16-I [58], is called PuraMatrix, because of its purity as a designed biological scaffold in contrast to other biologically derived scaffolds from animal collagen and Matrigel. Because these nanofiber scaffolds have 5–200 nm pores and have very high water content (99.5% or 5 mg/ml) (Fig. 4), they are useful in the preparation of 3D cell-culture media. The scaffolds closely mimic the porosity and gross structure

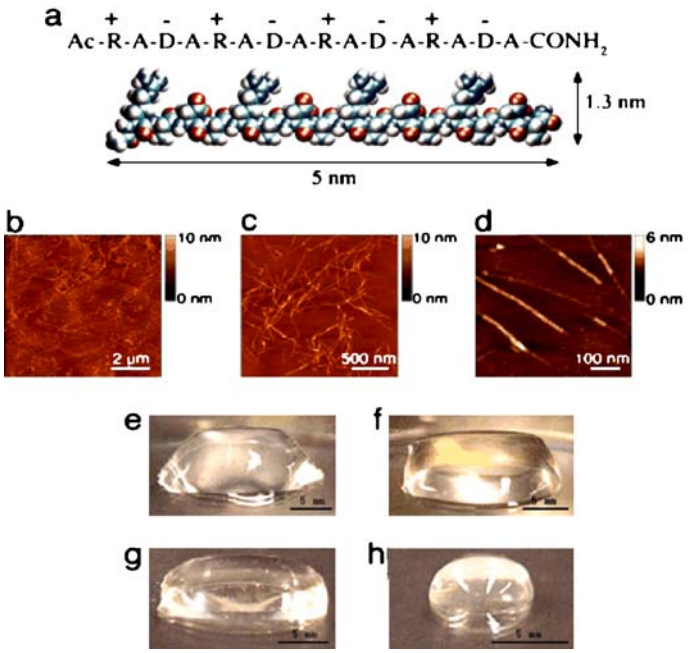

Fig. 4 Peptide RADA16-I. **a** Amino acid sequence and molecular model of RADA16-I. The dimensions are 5 nm long, 1.3 nm wide, and 0.8 nm thick; **b–d** AFM images of RADA16-I nanofiber scaffold. Note the different height of the nanofiber, ≈ 1.3 nm in **d**, suggesting a double-layer structure. **e–h** Photographs of RADA16-I hydrogel at various conditions: 0.5 wt % (pH 7.5) in **e**, 0.1 wt % (pH 7.5, Tris.HCl) in **f**, 0.1 wt % (pH 7.5, PBS) in **g** before sonication, and reassembled RADA16-I hydrogel after four rounds of sonication in **h**.

of extracellular matrices, allowing cells to reside and migrate in a 3D environment, and molecules, such as growth factors and nutrients, to diffuse in and out very slowly. These peptide scaffolds have been used for 3D cell culture, controlled cell differentiation, tissue engineering and regenerative medicine applications [59, 60].

3.1.1 Nanofibrils from α-helices

Several laboratories have designed fibrillar structures based on coiled-coil structural motifs, ranging from two-stranded to five-stranded coiled-coil structures [61–65]. In each case, investigators have recognized that peptides containing the coiled-coil motifs can self-assemble into a staggered interaction structure. Electrostatic interactions favor the formation of staggered arrangements of helices by two different 28-residue peptides. To help stabilize the staggered interactions, Woolfson's laboratory also took advantage of a buried asparagine residue in each of the two peptides, which can form structure sta-

bilizing hydrogen bonds with its partner on the opposite strand only in the staggered conformation [61]. In addition, they have also described a clever synthetic method for introducing kinks and branches into fibrils [66, 67].

3.1.2
Nanofibrils from β-strands

We have studied sequences that form helical and sheet structures by incorporating specific interactions within a peptide sequence that would stabilize both sheet and helix formation [18]. In these sequences, such as DADADADARARARARA, a preformed β-sheet could be induced to adopt a α-helix in response to temperature and pH changes. Other groups have also studied this structural plasticity [45, 68]. In addition, investigators laid out a carefully reasoned strategy for the design of short hexapeptide sequences (i.e. KTVIIE, STVIIE, KTVIIT and KTVLIE) in order to test sequence elements critical for the formation of cross β-sheet structures and further test how polymeric β-sheets can mature into amyloid fibrils [69]. Towards this goal it is important to know how the cross β-sheet aggregates form and its role in neurodegenerative disease; recent efforts in the de novo design of peptide-based amyloid fibrils have aimed to identify simple sequences that minimally satisfy the requirements of fibril formation [69, 70].

3.2
Bionanotubes and Vesicles

These amphiphilic molecules readily interact with water and form various semi-enclosed environments. One of the best examples are phospholipids, the predominant constituents of the plasma membrane, which encapsulate and protect the cellular contents from the environment and are an absolute prerequisite for almost all living systems. Phospholipids readily undergo self-assembly in aqueous solution to form distinct structures that include micelles, vesicles and tubules. This is largely a result of the hydrophobic forces that drive the non-polar region of each molecule away from water and toward one another.

3.2.1
Short Amphiphilic Peptides

Our laboratory has designed a simple peptide system with those properties [52, 53]. We made short peptides of around six to seven amino acids that had the properties of surfactant molecules in that each monomer contained a polar and a non-polar region. For example, a peptide called A6D, the peptide molecule looked like a phospholipid in that it had a polar head group and a non-polar tail.

The homogeneity and size of the supramolecular assembly were sequence-sensitive: peptides of the same length behaved differently when they had different polar head or hydrophobic tail sequences. Such phenomena have been described theoretically and experimentally in other amphiphilic systems. The shape and size of the assemblies are ultimately dependent on the size and geometry of their constituents [71]. In order to visualize the structures in solution, we utilized the transmission electron microscope with the quick-freeze/deep-etch method for sample preparation [72], to preserve the structures that formed in solution for electron microscopy. We observed discrete nanotubes and vesicles

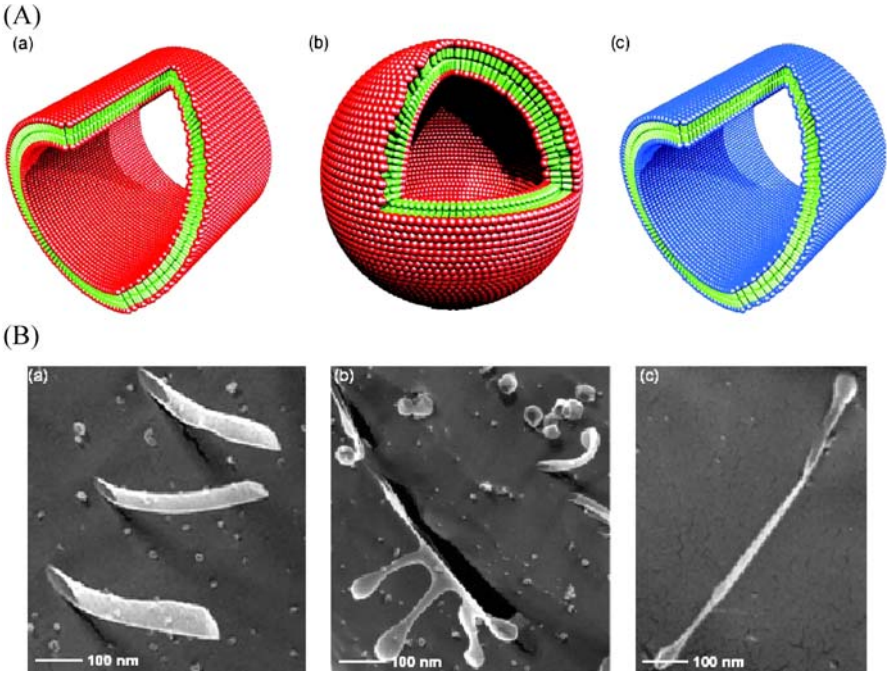

Fig. 5 **A** Molecular models of surfactant peptides V6D and K2V6. These peptides have hydrophilic heads; either negatively charged aspartic acid or positively charged lysine with hydrophobic valine tails [13–15]. **a** V6D in nanotube form. Billions of these molecules self-assemble to sequester the valine tails from water in **b** vesicle form or **c** nanotube form with positively charged heads. These nanostructures are rather dynamic undergoing assembly and disassembly. Color code: green-hydrophobic tails, red-aspartic acid, and blue-lysine. **B** Quick-freeze/deep-etch transmission electron micrographs of structures from surfactant peptides. **a** The nanotubes are clearly represented, with a diameter ~ 30–50 nm. **b** The nanotubes and vesicles are visible in the same frame suggesting that these structures are quite dynamic. It is plausible that the vesicles may be budded out from the nanotubes and/or they may fuse to form nanotubes in a reversible manner [13–15]. The diameter of these nanostructures is ~ 30–50 nm. **c** Phosphor-serine surfactant peptides form nano Q-tips

(Fig. 5) in the samples that gave homogeneous size distribution in the dynamic light scattering experiment. Those samples that were polydispersed tended to give irregular membranous layers. The nanotubes that formed had an average diameter of around 30 nanometers as examined by TEM, consistent with results obtained from the dynamic light scattering.

These nanotubes have the potential to act as templates for metallization and formation of nanowires. Furthermore, the nanovesicles may be useful as an encapsulating system for drug delivery. Chemical modification of the peptide monomer may expand the function of these structures. For example, a specific cell-surface ligand can be directly incorporated into a vesicle for targeted delivery of insoluble drugs to particular cells.

3.3 Nanometer-Thick

Molecular assembly can be targeted to alter the chemical and physical properties of a material's surface. Surface coatings instantly alter a material's texture, color, compatibility with and responsiveness to the environment. Conventional coatings are typically applied by painting or electroplating. Erosion is common mostly because the coatings are usually in the ten- and hundred micron size ranges and the interface is often not complementary at the molecular level [47, 73]. Peptides and proteins have also been printed onto surfaces which have now been modified with a vast family of chemical compounds; Mirkin and colleagues [74–76] have also developed dip-pen nanolithography to directly print micro- and nano-features onto surfaces. These developments have spurred new research into the control of molecular and cellular patterning, cell morphology, and cellular interactions [73, 77–79] and fueled new technology development.

Work in our laboratory has focused on designing a variety of peptides to self-assemble into a monolayer on surfaces and to allow adhesion molecules to interact with cells and adhere to the surface. Using proteins or peptides as ink, we have directly microprinted specific features onto the non-adhesive surface of polyethylene glycol to write any arbitrary patterns rapidly without preparing the mask or stamps (Fig. 6). This simple and rapid printing technology allowed us to design arbitrary patterns to address questions in neurobiology that would not have been possible before. Because understanding of correct complex neuronal connections is absolutely central to comprehension of our own consciousness, human beings are always interested in finding ways to further investigate this. However, the neuronal connections are exceedingly complex, and we must dissect the complex neuronal connections into smaller and more-manageable units to study them in a well-controlled manner through systematic biomedical engineering approaches. Therefore, nerve fiber guidance and connections can now be studied on special engineered pattern surfaces that are printed with protein and peptide materials [49].

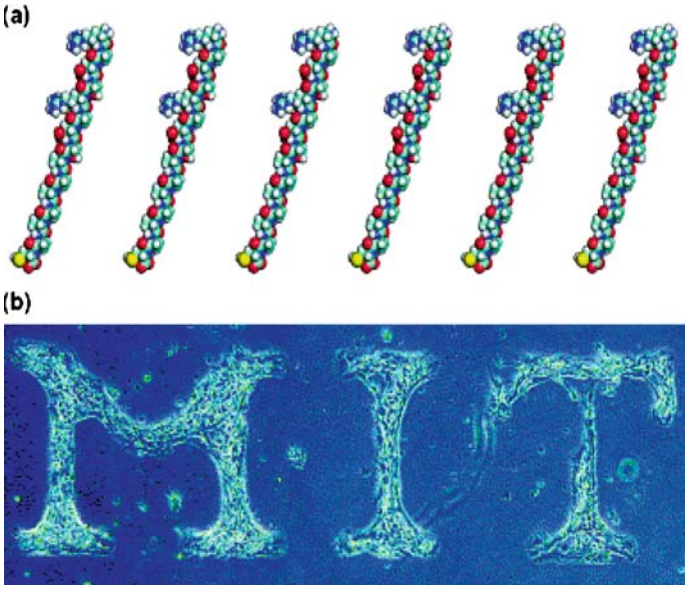

Fig. 6 Molecular structure of peptide ink. **a** This class of peptide ink has three general regions along their lengths: a ligand for specific cell recognition and attachment, a linker for physical separation from the surface, and an anchor for covalent attachment to the surface. Color code: carbon, *green*; hydrogen, *white*; oxygen, *red*; nitrogen, *blue*; thiol group, *yellow*. **b** Cells adhere to printed patterns. The protein was printed onto a uniform PEG inhibitory background. Cells adhered to patterns after 8–10 days in culture. (Images courtesy Sawyer Fuller and Neville Sanjana)

We are interested in studying the nerve fiber navigation on designed pattern surfaces in detail. Studies of nerve fiber navigation and nerve cell connections will undoubtedly enhance our general understanding of the fundamental aspects of neuronal activities in the human brain and brain–body connections. It will probably also have applications in screening neuropeptides and drugs that stimulate or inhibit nerve fiber navigation and nerve cell connections.

3.4 Nanowires

In the computing industry, the fabrication of nanowires using the "top-down" approach faces tremendous challenges. Thus, the possibility of fabricating conducting nanowires by molecular means using peptide scaffolds is of particular interest to the electronics industry. One can readily envision that nanotubes made from self-assembling peptides might serve as templates for metallization. Once the organic scaffold has been removed, a pure conducting wire is left behind and immobilized on a surface (Fig. 7). There is great inter-

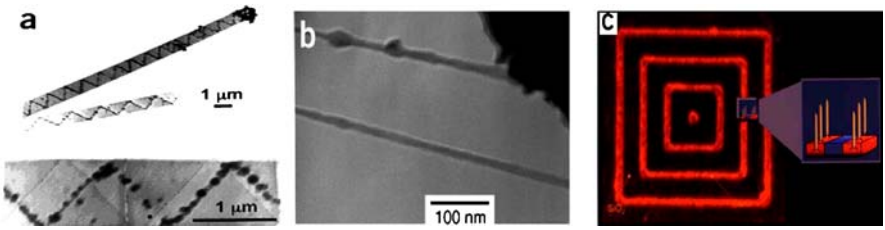

Fig. 7 Lipid, peptide and protein scaffold nanowires. **a** Lipid tubule–coated wire. Nanoparticles are coated on the left-handed helical lipid tubules. The nanoparticles are aligned inside the tubule along the regular helical pattern [66]. (Image courtesy of J. Schnur.) **b** Silver ions fill the nanotubes formed from a dipeptide, Phe-Phe, the shortest peptide possible. The silver alone formed a wire after removal of the Phe-Phe peptide scaffold [67]. (Image courtesy of M. Reches and E. Gazit.) **c** Discovery and selection of electronic materials using a bacteriophage display system. A combinatorial phage library was used to selectively bind to electronic materials. Selected recombinant phage peptide has a high affinity for GaAs. Fluorescently labeled phage has displaced the peptide specificity for GaAs and is capable of binding to the patterned GaAs nested in the square pattern on a wafer. The red line (1 μm in diameter) corresponds to GaAs and the black spaces (4 μm in diameter) are SiO_2. This peptide-specific binding could also potentially be used to deliver nanocrystals to specific locations [69]. (Image courtesy of A. Belcher.)

est in developing various methods for attaching conducting metal nanocrystals to a peptide for such a purpose.

3.4.1 From Nanotube to Nanowire

Matsui and colleagues [80] have reported success in functionalizing peptide nanotubes into nanowires. They not only coated the peptide nanotube with copper and nickel but also showed that their nanotubes can be coated with avidin, making them able to bind specifically to gold surfaces that have previously been treated with biotinylated self-assembled nanolayers. Lvov et al. [81] have fabricated nano- and microwires by coating the lipid tubules with silica and gold nanocrystals. They found that these nanocrystals are linked to the tubules according to the tubules' helical periodicity. These wires have been used for coating in a number of industrial applications. Reches and Gazit have demonstrated that a Phe-Phe dipeptide—the shortest peptide length possible, consisting of only two amino acids with a single amide bond—can form stable nanotubes. They then diffused silver ions into the defined tubes and were able to remove the peptide either enzymatically, chemically or through heat burning to reveal the silver wire [82].

In other recent work, amyloid protein nanofibers have been used as scaffold to align gold nanocrystals. Lindquist and colleagues [83] have reported how a bioengineered version of the prion-determining (NM) domain of the yeast prion protein Sup35 can provide a scaffold for fabricating nanowires,

and tested the conducting capability of the resulting wires. These efforts collectively open a new direction in the fabrication of electronic nanomaterials.

3.4.2 Templates for Nanowires: DNA for Nano-Electronics

The DNA molecule has been suggested as a template for making nanoscale wires for the emergent field of nano-electronics. This is due to the regularity of the width of the DNA double helix and its robust mechanical properties. Several groups have succeeded in coating DNA molecules with metallic particles and have shown data on the conductive properties of these biotemplated materials. Braun et al. non-covalently bound a stretch (16 μm) of bacteriophage λ-DNA between two gold electrodes by allowing it to hybridize with short DNA fragments that had been covalently attached to those surfaces [84]. Electrical measurements indicated that the wires were non-conducting at low voltage bias, with resistances greater than the experimentally measurable 10^{13}. Furthermore, the shape of the I–V curve obtained was dependent on the voltage scan direction. Richter et al. employed a similar strategy to produce DNA-templated nanowires that showed relatively low resistances under low-voltage bias [85]. They reduced palladium on λ-DNA and immobilized the nanowire on gold electrodes. The resistances obtained were lower than 1 kv, with the specific conductivity approximately one order of magnitude lower than bulk palladium. Subsequently, the resistances of these palladium nanowires [86] were studied at low temperatures which discovered that the palladium metals reduced on a DNA template showed the expected quantum mechanical behavior, with their resistances increasing at low temperatures. This behavior is similar to that of thin palladium films and shows that wires templated with DNA molecules behave normally.

Mertig et al. discovered conditions in which fine and regular platinum clusters formed on DNA molecules by using first-principle molecular dynamics (FPMD), which ultimately yielded a faster rate of growth and finer metal clusters on the template [87]. Another metal that has been investigated for surface templating of DNA is gold. Harnack et al. investigated the binding and reduction of tris(hydroxymethyl) phosphine derivatized gold particles on calf-thymus DNA [88]. The rapidly formed nanowires show electrical conductivities about 1/1000th that of gold, which the authors attributed to the graininess of the material.

Patolsky et al. modified *N*-hydroxysuccinimide-gold nanoparticles with a nucleic-acid intercalating agent, amino psoralen [89]. In addition, Belcher and colleagues [75, 90–92] took a very different approach toward not only discovering, but also fabricating, electronic and magnetic materials, departing sharply from traditional materials process technology. Such approaches for producing finer and finer features at the nanoscale, with increasing density and in finite areas, may prove complementary to the microcontact printing

process. Although the latter approach has become widely used and is rapidly being perfected, fabrication of the finest feature using microcontact printing is limited by the capabilities of lithography technologies currently used in the semiconductor industry.

3.5
Other Nanomaterials

The most advanced top-down technology for fabricating complex optical systems falls far short when compared with the accomplishments of living organisms at ambient temperature and low pressure (and without clean rooms) [93, 94]. Several groups have studied biomineralization in diverse marine organisms, notably the brittlestar Ophiocoma wendtii and the sponge Euplectella. Some remarkable living optical systems have been uncovered, such as the fiber-optical spicules from Euplectella that have the dimensions of a single human hair and can act as multimode waveguides. These discoveries have inspired Aizenberg et al. [95] to fabricate micropatterned single crystals and photonics with potential applications in communication technology. Christopher et al. report the synthesis and characterization of tethered PNA molecules (bisPNAs) designed to assemble two individual DNA molecules through Watson–Crick base pairing. The spacer regions linking the PNAs were varied in length and contained amino acids with different electrostatic properties [96, 97], their results indicate that the bisPNAs can be used for nanotechnology applications and that their favorable characteristics may lead to improved assemblies.

4
Application of Self-Assembling Systems

4.1
Simple Peptides Stabilize Mighty Membrane Proteins for Study

Cell membranes are largely made up of proteins, and membrane proteins account for about a third of all genes. Despite their importance, they are very hard to isolate and stabilize, which therefore prevents further understanding of membrane protein functions and related disease study. We have made a new type of peptide detergent and successfully stabilized the dauntingly large protein complex photosystem I (PS-I), an integral part of the photosynthetic machinery.

Two key technologies were employed to preserve the functionality of these photosynthetic complexes outside of their native environment. First, we added two peptide surfactants, one cationic A6K (AAAAAAK), and the other anionic V6D (VVVVVVD) into the photosynthetic complex fraction to

stabilize it during device fabrication. Secondly, we examined the stability of PS-I by testing its fluorescence after attaching the detergent-protein complex to a glass slide [53, 54]. The intact PS-I emits red light with a characteristic peak wavelength as it degrades. This peak subsides and is replaced by another bluer peak. Even the two best standard surfactants did poorly at maintaining the red peak. In contrast, the spectrum after A6K extraction was almost a perfect match for the normal one, indicating the complex was largely intact after drying. Furthermore, the complex appeared to remain stable for up to three weeks on the glass slide. PS-I itself remains to be fully characterized, and this stabilization technique offers new means to explore its properties [98].

In addition, photosynthetic complexes are archetypal molecular electronic devices, containing molecular optical and electronic circuitry organized by a protein scaffold. Conventional technology cannot equal the density of the molecular circuitry found in photosynthetic complexes. Thus, if integrated with solid-state electronics, photosynthetic complexes might offer an attractive architecture for future generations of circuitry where molecular components are organized by a macromolecular scaffold. For utilization in practical technological devices they must be stabilized and integrated with solid-state electronics. Our results suggest that photosynthetic complexes may be used as an interfacial material in photovoltaic devices. Evolved within a thin membrane interface, photosynthetic complexes sustain large open circuit voltages of 1.1 V without significant electron-hole recombination, and they may be self-assembled into an insulating membrane, further reducing recombination losses. Peptide surfactants have been shown to stabilize these complexes during and after device fabrication. It is expected that the power conversion efficiency of a peptide-stabilized solid-state photosynthetic device may approach or exceed 20%. Similar integration techniques may apply to other biological or synthetic protein-molecular complexes [99].

These simply designed peptide detergents may now open a new avenue to overcome one of the biggest challenges in biology—to obtain large number of high resolution structures of membrane proteins. Study of the membrane proteins will not only enrich and deepen our knowledge of how cells communicate with their surroundings since all living systems respond to their environments, but these membrane proteins can also be used to fabricate the most advanced molecular devices, from energy harnessing devices to extremely sensitive sensors and medical detection devices.

4.2 Tissue Engineering

A new type of self-assembling peptide nano-fibril that serves as a substrate for neurite outgrowth and synapse formation is described (Fig. 8). The self-assembling peptide scaffolds are formed through the spontaneous assembly of ionic self-complementary β-sheet peptides under physiological conditions,

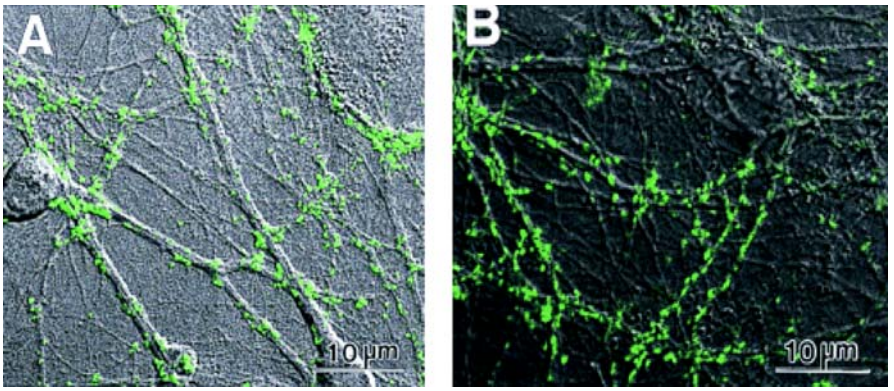

Fig. 8 Primary rat hippocampal neurons form active synapses on peptide scaffolds. The confocal images show bright discrete *green labeling* indicative of synaptically active membranes after FM1-43 incubation of neurons. **A** Active synapses on the peptide surface. **B** Active synapses on Matrigel. The active synapses on these different materials are not readily distinguishable, indicating that the peptide scaffold is a permissible substrate for synapse formation. *Bar* = 5–10 mm

producing a hydrogel material. The scaffolds can support neuronal cell attachment and differentiation as well as extensive neurite outgrowth. Furthermore, they are permissive substrates for functional synapse formation between the attached neurons. That primary rat neurons form active synapses on such scaffold surfaces in situ suggests these scaffolds could be useful for tissue engineering applications. The buoyant self-assembling peptide scaffolds with attached cells in culture can be transported readily from one environment to another. These biological materials created through molecular design and self-assembly may be developed as a biologically compatible scaffold for tissue repair and tissue engineering [100].

Self-assembling peptides are being developed as scaffolds for tissue regeneration purposes, including cartilage repair and the promotion of nerve cell growth [101]. A major benefit of synthetic materials is that they minimize the risk of biological contamination. Self-assembling peptides also frequently show favorable properties concerning biocompatibility, immunogenicity and biodegradability, producing non-toxic waste products. The amphiphilic peptide construct discussed above, containing a long hydrophobic tail linked to a cell-recognizing tag, can be customized for specific cell response by tailoring the sequence of the tag. Laminin is an extracellular matrix protein that influences neurite outgrowth. A peptide amphiphile shown to promote the re-growth of nerve cells in rats was made by including a neurite-promoting laminin epitope tag, IKVAV (C16-G3A4-IKVAV) [102]. Another construct, containing a heparin-binding site, shows very exciting preliminary results in being able to promote angiogenesis, the growth of blood vessels [103]. These types of peptide amphiphiles have been further modified with biotin [104]

and a Gd^{3+} metal-chelating moiety suitable for detection by magnetic resonance imaging (MRI) [105].

Because an adequate blood supply to and within tissues is an essential factor for successful tissue regeneration, promoting a functional microvasculature is a crucial factor for biomaterials.

In 2005, Lee et al. demonstrated that short self-assembling peptides form scaffolds that provide an angiogenic environment promoting long-term cell survival and capillary-like network formation in three-dimensional cultures of human microvascular endothelial cells. Data showed that, in contrast to collagen type I, the peptide scaffold inhibited endothelial cell apoptosis in the absence of added angiogenic factors, accompanied by enhanced gene expression of the angiogenic factor VEGF. In addition, the results suggest that the process of capillary-like network formation and the size and spatial organization of cell networks may be controlled through manipulation of the scaffold properties, with a more rigid scaffold promoting extended structures with a larger inter-structure distance, as compared with more dense structures of smaller size observed in a more compliant scaffold. These findings indicate that self-assembling peptide scaffolds have potential for engineering vascularized tissues with control over angiogenic processes. Since these peptides can be modified in many ways, they may be uniquely valuable in the regeneration of vascularized tissues [106].

Emerging medical technologies for effective and lasting repair of articular cartilage include delivery of cells or cell-seeded scaffolds to a defect site to initiate de novo tissue regeneration. Biocompatible scaffolds assist in providing a template for cell distribution and extracellular matrix (ECM) accumulation in a three-dimensional geometry. A major challenge in choosing an appropriate scaffold for cartilage repair is the identification of a material that can simultaneously stimulate high rates of cell division and high rates of cell synthesis of phenotypically specific ECM macromolecules until repair evolves into steady-state tissue maintenance.

In 2002, we made a self-assembling peptide hydrogel scaffold for cartilage repair and developed a method to encapsulate chondrocytes within the peptide hydrogel. During 4 weeks of culture in vitro, chondrocytes seeded within the peptide hydrogel retained their morphology and developed a cartilage-like ECM rich in proteoglycans and type II collagen, indicative of a stable chondrocyte phenotype. Time-dependent accumulation of this ECM was paralleled by increases in material stiffness, indicative of deposition of mechanically functional neo-tissue. Taken together, these results demonstrate the potential of a self-assembling peptide hydrogel as a scaffold for the synthesis and accumulation of a true cartilage-like ECM within a three-dimensional cell culture for cartilage tissue repair.

In 2005, Lee and colleagues demonstrated that self-assembling peptides can be injected and that the resulting nanofiber microenvironments are readily detectable within the myocardium. Furthermore, the self-assembling

peptide nanofiber microenvironments recruited progenitor cells that express endothelial markers, as determined by staining with isolectin and for the endothelial-specific protein platelet-endothelial cell adhesion molecule. Vascular smooth muscle cells were recruited to the microenvironment and appear to form functional vascular structures. After the endothelial cell population, cells that expressed sarcomeric actin and the transcription factor Nkx2.5 infiltrated the peptide microenvironment. When exogenous donor green fluorescent protein–positive neonatal cardiomyocytes were injected with the self-assembling peptides, transplanted cardiomyocytes in the peptide microenvironment survived and also augmented endogenous cell recruitment [107].

4.3 Gene and Drug Delivery

The lack of predictable safety and efficacy standards in somatic gene therapy systems, have brought the whole field to a crossroads. Replication-incompetent viruses, naked DNA injection and liposomal agents have been the predominant means of genetic transfer. To date, there has been little lasting impact in the typical practice of medicine conferred by these gene therapy technologies. The crux of today's gene therapy dilemma is still the same as it has always been: efficient, safe, targeted delivery and persistent gene expression [108, 109].

Peptide-based gene delivery agents are emerging as alternatives for safer in vivo delivery. The main attraction of these peptide systems is their versatility. Peptide-based delivery systems have the ability to deliver therapeutic proteins, bioactive peptides, small molecules and any size of nucleic acids. The use of these agents allows the researcher to intervene at multiple levels in the cells genetics and biochemistry and is a fundamental new technology in the gene therapy field [110, 111]. Peptide-delivery agents are more like traditional pharmacological drugs than gene therapy vectors. With the past to guide us, a critical re-evaluation of the best characteristics for an ideal delivery system is in order. The desirable features may include the items displayed in the paper [112].

We developed a series of surfactant peptides comprising a hydrophobic tail attached to a polar headgroup consisting of one to two positively charged residues at the *C*- or *N*-terminus, one example being LLLLLLKK. These peptides self-assemble in water to produce nanovesicles and nanotubes [54] as reported in a Science News commentary [113] and in recent reviews [114, 115]; these peptides have been used as DNA delivery vehicles. When placed in a solution of DNA, the positively charged peptides self-assembled into a nanotube or vessel, encapsulating the negatively charged DNA. This "minivan" was then able, at least in some cases, to deliver the DNA to growing cells as the minivan surface can be tagged with a marker that is specific to a particular cell

type [113]. We expect that out of this emerging field, self-assembling peptide systems will play an increasing role in targeted molecular therapeutics and gene therapy.

4.4 Other Applications

Since peptides that can specifically bind to inorganic surfaces for particular applications that have no known analog in biology, molecular design may not be an efficient route to pursue. Even though one can potentially test many different biomolecular species to perform a particular function, the sheer number of samples that must be screened makes such an endeavor prohibitive in cost. For binding to GaAs (100), peptides with a higher number of uncharged polar and Lewis base side-chains became more predominant with successive rounds of selection. This could be attributed to the interaction of these functional groups with the Lewis acid sites of the GaAs surface.

Using a similar selection strategy, Lee et al. identified a bacteriophage that had the propensity to bind to ZnS crystal surfaces [116]. These phages were then mixed with ZnS quantum dots, forming a liquid crystalline suspension of the complex. This will push forward the areas of nano-electronic, optical and magnetic sciences and engineering.

Artificial peptide and protein libraries have been constructed for selection of novel proteins and peptide motifs that Nature never made [117–119]. Many investigators completely designed the peptide and protein libraries de novo, without a pre-existing protein basis. Although Nature has selected and evolved many diverse proteins for all sorts of functions that support life, it has not ventured into the functions outside of life. The protein universe is enormous, in comparison with what we know today. There are, undoubtedly, a great number of proteins that can exist beyond what has been founded in living systems. Numerous new proteins and peptides with desired and novel properties have been selected for a particular application. This strategy permits us to purposely select and rapidly evolve non-natural materials, nano-scaffolds and nano-construction motifs for a growing demand in nanotechnology. The numbers of these biologically based scaffolds are limitless and they will likely play an increasingly important role for the design of molecular machines, nanodevices and countless other novel, unanticipated new tools and applications.

5 Conclusions and Perspectives

From physics and engineering to biology, molecular design of self-assembling peptides is an enabling technology that will likely play an increasingly im-

portant role in the future of bionanotechnology 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: from developing a class of pure peptide nanofiber scaffolds for cell engineering and for tissue repairing and tissue engineering, studying of the model system of protein conformational diseases, and designing peptide or protein inks for surface printing to finding peptide surfactants that solubilize and stabilize membrane proteins, bionanotubes and vesicles for delivering genes and drugs. Self-assembling peptide systems will create a new class of materials at the molecular scale and will have a high impact in many fields. We believe that application of these simple and versatile molecular self-assembly systems will provide us with new opportunities for studying complex and previously intractable biological phenomena.

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