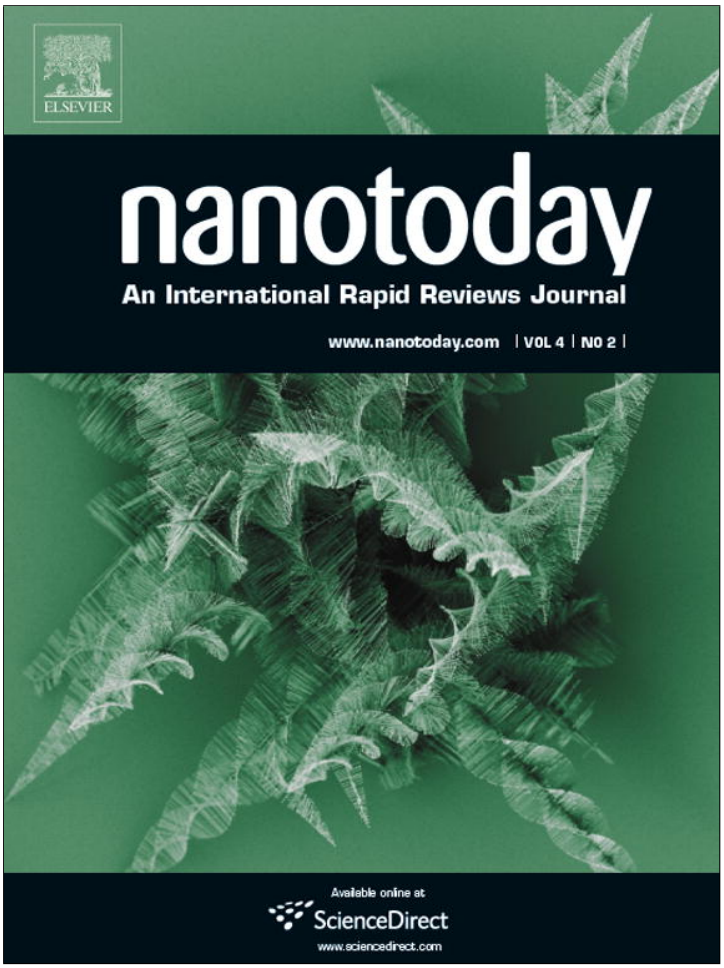

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REVIEW

Designer self-assembling peptide nanomaterials

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Summary Short peptides that are made of natural amino acids were never seriously considered as useful materials as recent as 16 years ago. However, the discovery of a class of self-assembling peptides that spontaneously undergo self-organization into well-ordered structures resulted in a conceptual change. Since then diverse classes of short peptides have been invented with broad applications including 3D tissue cell culture, reparative and regenerative medicine, tissue engineering, slow drug release, stabilization of membrane proteins to develop nanobiotechnology and molecular devices. Furthermore one of the self-assembling peptides has shown promise not only to slow down prion infection, but also to extend ~50% animal life. Molecular design using short peptides as new materials may play increasingly important role in nanoscience, nanotechnology, nanobiotechnology and nanomedicine.

Introduction

The late legendary and visionary Nobel laureate physicist Richard Feynman was already wondering loud about science at nanoscale in 1959. His lecture ''*There's plenty room at the bottom*'' for the American Physical Society annual banquet was published in January 1960 in Pasadena. During the lecture he said: ''*What I wanted to talk about is the problem of manipulating and controlling things on a small scale. In the year 2000, when they look back at this age, they would wonder why it was not until the year 1960 that anybody began seriously to move in this direction.*'' [1]. Precisely 40 years later, on 21 January 2000, former US President Bill Clinton at California Institute of Technology announced the formal governmental funding for the nanotechnology.

Nanoscience, nanotechnology and nanobiotechnology have been rapidly advancing in a breakneck pace in recent years. Enormous amount funding has been poured into this endeavor; numerous nanocenters, institutes and innovative nanotech start-ups have mushroomed world wide, countless journals focusing on nanoscience and nanotechnology have proliferated. Nano Today is one of the leading journals.

We have been conducting nanomaterial research since 1993 after one of us, Shuguang Zhang while working on yeast genetics and structural biology, made a serendipitous discovery of a self-assembling peptide EAK16 that self-organized into ordered nanofibers and further into scaffold.

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This discovery led a new thinking how to design a variety of materials from bottom up with exquisitely fine-tuning and manipulating one amino acid at a time to lay the foundations for fabrication of a wide spectrum of nanomaterials and nanodevices.

We here select a few of our research highlights over last 16 years. We will also summarize our other designer peptides and their applications. Since this field is advancing rapidly, it is impossible to cover the entire field with limited space; we therefore only focus on our own research in this article. Interested readers should consult other articles and reviews in this issue and literature to have a comprehensive view.

Discovery of the first self-assembling peptide EAK16 in yeast

In science, many discoveries are made unintentionally and serendipitously, it is sometimes referred as making unauthorized scientific discoveries. While one worked in area but accidentally discovered something in completely unrelated areas. The discovery of self-assembling peptides is no exception.

While working on yeast genetics and protein chemistry and trying to understand a left-handed Z-DNA structure in 1989, one of us, Shuguang Zhang, identified a protein called Zuotin for its ability to bind to left-handed Z-DNA in the presence of 400-fold excess of sheared salmon DNA that contains ubiquitous right-handed B-DNA and other random DNA structures [2]. Zuotin had an interesting repetitive 16-residue peptide sequence motif n-AEAEAKAKAEAEAKAK-c (EAK16-II) [3]. This peptide has been extensively developed to create a class of simple beta-sheet peptides. These peptides are ionic self-complementary as a result of the presence of both positive and negative side chains on one side of the beta-sheet and hydrophobic side chains on the other (Fig. 1). These peptides have two distinctive sides, one hydrophobic and the other hydrophilic. The hydrophobic side forms a double sheet inside of the fiber and hydrophilic side forms the outside of the nanofibers that interact with water molecules that they can form extremely high water content hydrogel, containing as high as 99.5—99.9% water (1—5 mg peptide/ml water). At least three types of molecules can be made, with −, +, −/+ on the hydrophilic side (Fig. 1) [4].

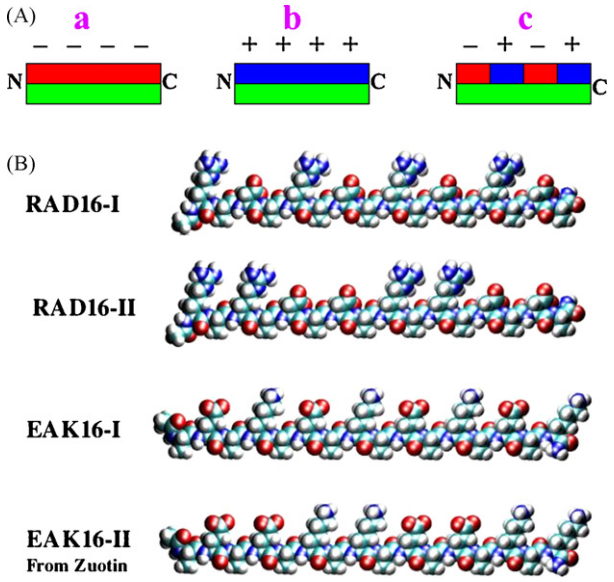

Figure 1 The designer amphiphilic self-assembling peptides that form well-ordered nanofibers. (A) These peptides have two distinctive sides, one hydrophobic and the other hydrophilic. The hydrophobic side forms a double sheet inside of the fiber and hydrophilic side forms the outside of the nanofibers that interact with water molecules that they can form 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. (B) The individual self-assembling peptide molecules are 5 nm long. The first such peptide, EAK16-II, was discovered from a yeast protein, zuotin. This peptide inspired us to design a large class of self-assembling peptide construction motifs. Upon dissolve in water in the presence of slat, they spontaneously assemble into well-ordered nanofibers, further into scaffolds.

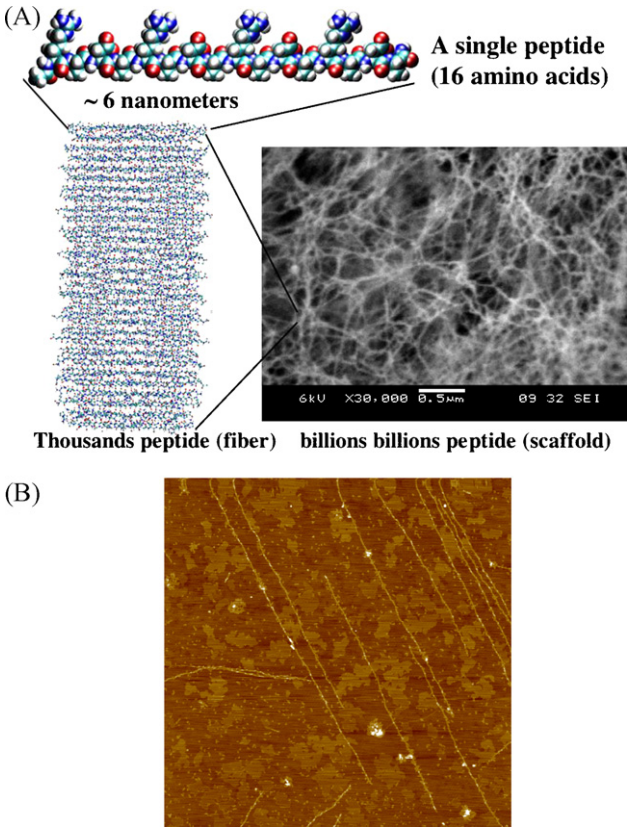

Figure 2 Self-assembling peptide RADA16-I nanofiber scaffold hydrogel. (A) Amino acid sequence of RADA16-I, molecular model of a single RADA16-I nanofiber, the dimensions are ~6 nm long, 1.3 nm wide and 0.8 nm thick; tens and hundred thousands of individual peptides self-assemble into a nanofiber, SEM images of RADA16-I nanofiber scaffold. Note the scale bar, 0.5 μm or 500 nm (SEM image courtesy of Fabrizio Gelain). (B) Alignment of peptide nanofibers using a microfluidic device. The peptides aligned by flow water through the device with tracks, a few microns wide and a few centimeters long (AFM image courtesy of Jessica Dai).

This serendipitous discovery of a self-complementary peptide inspired us to design many more members of this class of peptides, which form three-dimensional (3D) nanofiber scaffolds that have been used in 3D cell tissue cultures [5—8]. The four ionic self-complementary peptides (Fig. 1B) RDA16-I, RAD16-II, EAK-I and EAK16-II (the segment from yeast zuotin) form stable beta-sheet structures in water and undergo spontaneous assembly to form nanofiber scaffolds. The nanofiber scaffolds hold large volumes of water since water molecules can be organized to form clusters through surface tension and the nanofibers divide water clusters into compartments [3,9]. Tissue cells can be embedded in a 3D nanofiber scaffold [6—8] in which they can establish molecular gradients that often mimic the *in vivo* environment. Other related self-assembling peptide systems have also been designed, which range from 'molecular switch' peptides that undergo marked conformational changes [10,11] to 'molecular carpet' peptides for surface engineering [12] to peptide nanotubes and nanovesicles [13—19], all of which were inspired from the Z-DNA-binding zuotin discovery.

The property of the self-assembling peptide systems

The scaffolds consist of alternating amino acids that contain 50% charged residues [3—8,20—24]. These peptides are characterized by their periodic repeats of alternating ionic hydrophilic and hydrophobic amino acids with a typical β-sheet structure. Thus, these β-sheet peptides have distinct polar and non-polar surfaces (Fig. 1). The self-assembly event creating the peptide scaffold takes place under physiological conditions of neutral pH and milli-molar salt concentration. They are like gel—sponge in aqueous solution and readily transportable to different environments. Individual fibers are ~10 nm in diameter. A number of additional designer self-assembling peptides including RADA16-I (AcN-RADARADARADARADA-CNH_2) and RADA16-II (AcN-RARADADARARADADA-CNH_2), in which arginine and aspartate residues replace lysine and glutamate have been designed. The alanines form overlapping hydrophobic interactions in water, a structure that is found in silk fibroin

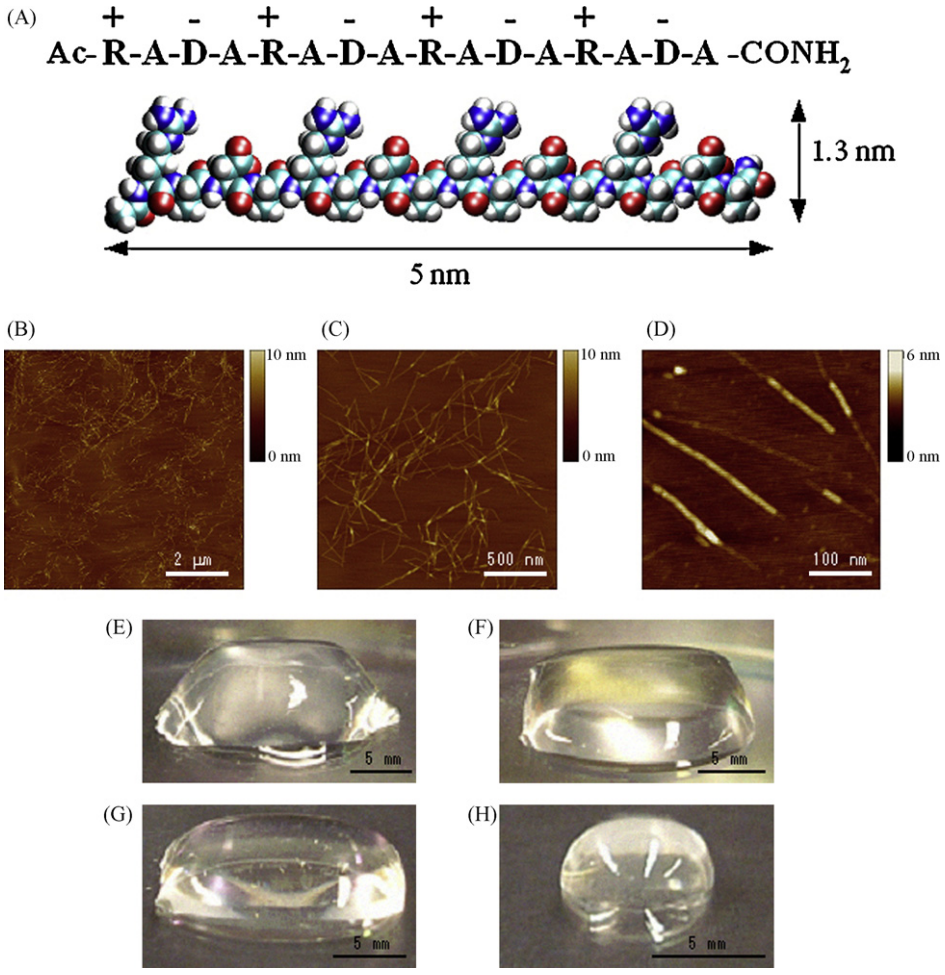

Figure 3 Peptide RADA16-I. (A) Amino acid sequence and molecular model of RADA16-I, the dimensions are ~5 nm long, 1.3 nm wide and 0.8 nm thick; (B) AFM images of RADA16-I nanofiber scaffold, 8 μm × 8 μm; (C) 2 μm × 2 μm; and (D) 0.5 μm × 0.5 μm. Note the different height of the nanofiber, ~1.3 nm, in (D) suggesting a double layer structure; photographs of RADA16-I hydrogel at various condition; (E) 0.5 wt% (pH 7.5); (F) 0.1 wt% (pH 7.5, Tris—HCl); (G) 0.1 wt% (pH 7.5, PBS) before sonication (H) re-assembled RADA16-I hydrogel after four times of sonication, respectively.

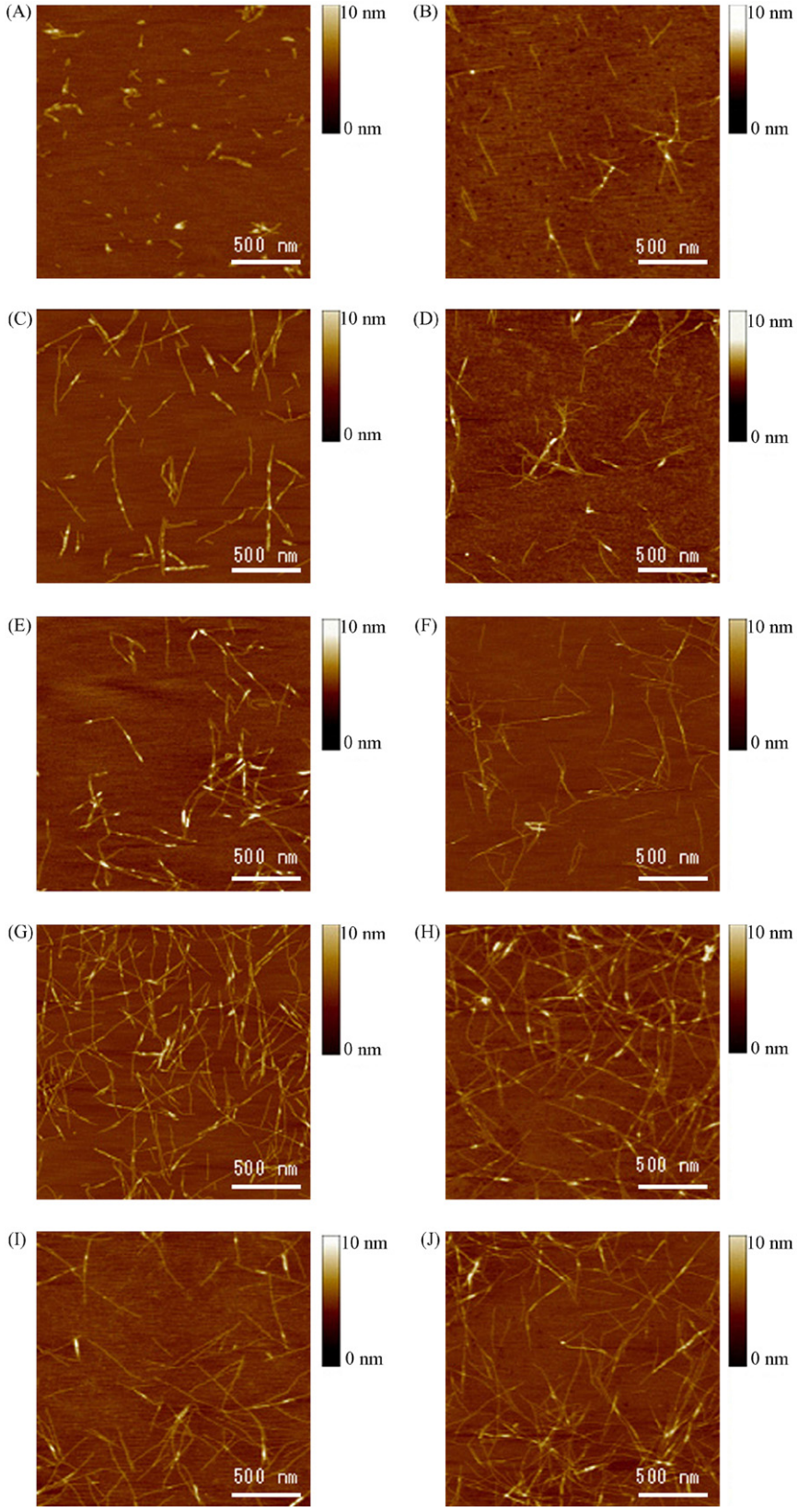

Figure 4 AFM images of RADA16-I nanofiber at various time points after sonication. The observations were made using AFM immediately after sample preparation. (A) 1 min after sonication; (B) 2 min; (C) 4 min; (D) 8 min; (E) 16 min; (F) 32 min; (G) 64 min; (H) 2 h; (I) 4 h; (J) 24 h. Note the elongation and reassembly of the peptide nanofibers over time. By ~1—2 h, these self-assembling peptide nanofibers have nearly fully re-assembled.

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. In general, these self-assembling peptides form stable β-sheet structures in water, which are stable across a broad range of temperature, wide pH ranges in high concentration of denaturing agent urea and guanidium hydrochloride. The nanofiber density correlates with the concentration of peptide solution and the nanofibers retains extremely high hydration, >99% in water (1—10 mg/ml, w/v) (Fig. 2).

The peptide synthesis method uses conventional mature solid phase or solution peptide synthesis chemistry. Depending on the length of the motifs, high purity of peptides can be produced at a reasonable cost. Since cost of the peptide synthesis has steadily decreased in recent years, it has become more and more affordable.

Many self-assembling peptides that form scaffolds have been reported and the numbers are still expanding [4,24]. The formation of the scaffold and its mechanical properties are influenced by several factors, one of which is the level of hydrophobicity [21,25]. That is, in addition to the ionic complementary interactions, the extent of the hydrophobic residues, Ala, Val, Ile, Leu, Tyr, Phe, Trp (or single letter code, A, V, I, L, Y, P, W) can significantly influence the mechanical properties of the scaffolds and the speed of their self-assembly. The higher the content of hydrophobicity, the easier it is for scaffold formation and the better for their mechanical properties [21,23,25].

Self-assembling peptide nanofiber scaffolds

A hierarchical scaffold self-organization starting from a single molecule of the ionic self-complementary peptide RADA16-I is shown in Fig. 2. Millions of peptide molecules self-assembled into individual nanofibers that further form the nanofiber scaffold (Fig. 2). The nanopores range from a few nanometers to a few hundred nanometers, the scales are similar in size as most biomolecules, so that these molecules or drugs may not only defuse slowly but also may establish a molecular gradient in the scaffolds.

The AFM image shows the individual nanofibers ranging from a few hundred nanometers to a few microns and can be aligned through microfluidic device. Peptide samples in aqueous solution using environmental AFM examination showed the similar nanofiber results suggesting the nanofiber formation is independent of drying process.

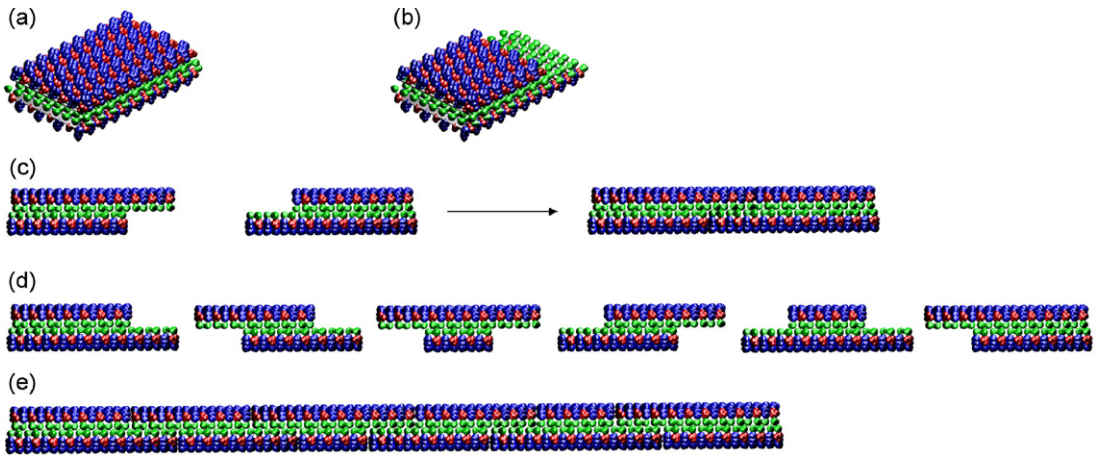

Figure 5 A proposed molecular sliding diffusion model for dynamic reassembly of self-assembling RADA16-I peptides. When the peptides form stable β-sheets in water, they form intermolecular hydrogen bonds along the peptide backbones. The β-sheets have two distinctive sides, one hydrophobic with an array 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. These nanofiber fragments can form various assemblies similar to restriction digested DNA fragments: (A) blunt ends; (B) semi-protruding ends. (C) These fragments with protruding ends could reassemble readily through hydrophobic interactions. (D) The fragments with semi-protruding and various protruding ends. (E) These fragments can reassemble readily. A proposed molecular sliding diffusion model for dynamic reassembly of self-assembling a single peptide nanofiber consisting thousands of individual 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 nanofiber first meet, the hydrophobic sides may not fit perfectly but with gaps. However, the non-specific hydrophobic interactions permit the nanofiber to slide diffusion along the fiber in either direction that minimizes the exposure of hydrophobic alanines and eventually fill the gaps. The sliding diffusion phenomenon was also proposed for nucleic acids of polyA and polyU in 1956 [27,28]. For clarity, these β-sheets are not presented as twisted strands. Color code: green, alanines; red, negatively charged aspartic acids; blue, positively charged arginines.

It is interesting to observe that at high resolution, the nanofibers appeared to have distinct layers, especially in some segments (Fig. 3). The difference in height is about 1.3—1.5 nm, the similar dimension as a single thickness of a peptide. Fig. 3E—H shows the peptide scaffold hydrogel at various concentrations, 0.6—3 mM, (1—5 mg/ml, w/v, or 99.5—99.9% water content) [9]. The scaffold hydrogel is completely transparent, which is a very important requirement for accurate image collections for uses in 3D tissue cell cultures.

Dynamic reassembly of self-assembling peptides

The self-assembling process is reversible and dynamic (Fig. 4) since these peptides are short and simple, numerous individual peptides can be readily self-organized through the weak interactions including hydrogen bonds, ionic bonds, hydrophobic and van der Waals interactions as well as water-mediated hydrogen bond formations. These nanofibers can be broken mechanically with sonication [9]. However, they can undergo dynamic re-assembly repeatedly (Fig. 4), similar as material self-healing process. Since the driving energy of the assembly in water is not only through hydrophobic van der Waals interactions, but also the arrays of ionic interactions as well as the peptide backbone hydrogen bonds, this phenomenon can be further exploited for production and fabrication of many self-assembling peptide 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 is likely to be wide spread in many unrelated fibrous biological materials where numerous weak interactions are involved. Self-assembly and reassembly are a very important property for fabricating novel materials, and it is necessary to fully understand its detailed process in order to design better biological materials.

AFM images revealed that the nanofibers range from several hundred nanometers to a few microns in length before sonication. After sonication, the fragments were broken into ~20—100 nm. The kinetics of the nanofiber reassembly is followed closely at 1, 2, 4, 8, 16, 32 and 64 min as well as 2,

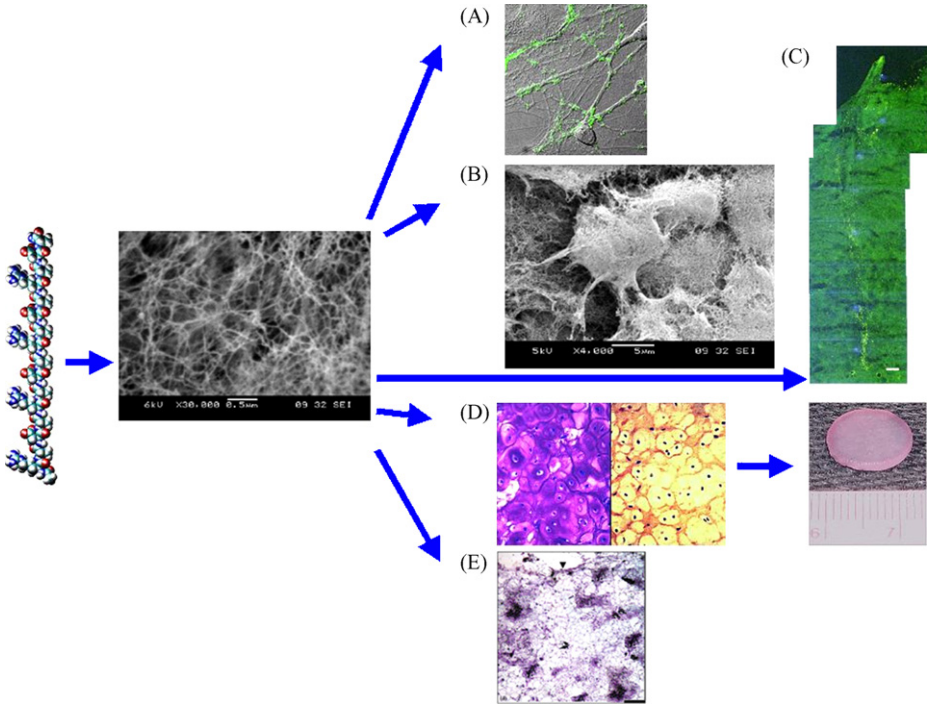

Figure 6 From designer peptide to scaffold to tissues. (A) Active synapses on the peptide surface. Primary rat hippocampal neurons form active synapses on peptide scaffolds. The confocal images shown bright discrete green dot labeling indicative of synaptically active membranes after incubation of neurons 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 3D scaffold (image courtesy of Fabrizio Gelain). (C) Brain damage repair in 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 2 days. A great number of neurons form synapses (image courtesy of Rutledge Ellis-Behnke). (D) Peptide KLD12 (KLDLKLDLKLDL), chondrocytes in the peptide scaffold and cartilage. The chondrocytes stained with TB showing abundant GAG production (left panel) and antibody to type II collagen demonstrating abundant Type II collagen production (right panel). A piece of pre-molded 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 John 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 bone nodules forming (image courtesy of Maria Bokhari).

4, and 24 h (Fig. 4). The nanofiber length reassembly is as a function of time: by 2 h, the peptide nanofibers have essentially reassembled to their original length. This remarkable and rapid reassembly is interesting because there may be a little nucleation for re-growth of the nanofiber from the addition of monomers that could only be produced during sonication. It is plausible that a large population of the sonicated nanofiber fragments contains many overlap cohesive ends due to un-disrupted alanine hydrophobic side that may quickly find each other (Fig. 5). The situation is analogous and commonly found in sonicated and enzymatic digested DNA fragments.

Kinetics of nanofiber reassembly and a plausible reassembly process

The re-assembly kinetics is as a function of time. Perhaps, similar to DNA re-assembly, the re-assembly largely depends on the concentrations of the short complementary fragments. In this case, the fragments are the sonicated peptide nanofibers with possible presence of sonicated monomers.

In order to understand the dynamic re-assembly, we proposed a plausible sliding diffusion molecular model to interpret these observations of reassembly of the self-assembling RADA16-I peptides (Fig. 5). Unlike the left-handed super-helical structures observed in KFE8 [25], a different self-assembling peptide, no super-helical structures were observed for RADA16-I using AFM [9] and TEM [6,22].

Molecular modeling of the self-assembly process

For molecular modeling clarity, these RADA16-I β-sheets are presented as non-twisted strands. It is known that these peptides form stable β-sheet structure in water, thus they not only form the intermolecular hydrogen bonding on the peptide backbones, but they also have two distinctive sides, one hydrophobic with array of overlapping alanines (Fig. 5, green color sandwiched inside), similar as found in silk fibroin or spider silk assemblies [26]. The other side of the backbones have negatively charged (−) aspartic acids represented as red, and positively charged (+) arginines represented as blue.

The alanines form packed hydrophobic interactions in water, during sonication the hydrophobic interaction could be disrupted mechanically. However, these hydrophobic cohesive ends could find each other quickly in water since the exposure of hydrophobic alanine arrays to water is energetically unfavorable. Since the hydrophobic alanines interaction is non-specific, they can slide diffuse along the nanofiber, similar as trains on the train tracks. The same sliding diffusion phenomenon was also observed in nucleic acids where polyA and polyU form complementary base pairings that can slide diffuse along the chains [27,28]. If however, the bases are heterogonous, containing G, A, T, C, the bases cannot undergo sliding diffusion. Likewise, if the hydrophobic side of the peptides does not always contain alanine, such as valine and isoleucine, it would become more difficult for sliding diffusion to occur due to structure constraint.

On the charged side, both positive and negative charges are packed together through intermolecular ionic interactions in a checkerboard manner (looking from the top). Likewise, the collectively complementary + and − ionic interactions may also facilitate the reassembly. Similar to restriction-digested DNA fragments, these nanofiber fragments could form various assemblies: blunt, semi-protruding, and protruding ends. The fragments with semi-protruding and various protruding ends as well as blunt ends can reassemble readily through hydrophobic and ionic interactions (Fig. 5).

Self-assembling peptides nanofiber scaffolds

The importance of nanoscale becomes obvious in 3D cell culture. It is clearly visible in the SEM images that the cells embedded in the self-assembling peptide nanofiber biological scaffolds in the truly 3D culture (Fig. 6) [6]. Here, the cells and cell clusters are intimately interact with the extracellular matrix where cells make on their own over time during cell growth and differentiation. Since the scaffolds are made mostly water, ∼99.0—99.5% water with 0.5—1% peptide, cells can migrate freely without hindrance, similar as fish swim freely in seaweed forest.

Likewise, another self-assembling peptide KLD12 (Ac-KLDLKLDLKLDL-NH_2) was used to culture primary bovine chondrocytes (Fig. 6) [23]. The chondrocytes not only maintained their phenotype but also produce abundant type II collagen and glycosaminoglycans. Previously, it was known that chondrocytes dedifferentiate into fibroblast cell types and no longer produce type II collagen and glycosaminoglycans in coated 2D cell cultures. This showed the critical importance of 3D culture using the simple self-assembling peptide nanofiber scaffold.

These new self-assembling peptide nanofiber biological scaffolds have become increasingly important not only in studying 3D spatial behaviors of cells, but also in developing approaches for a wide range of innovative medical technologies including regenerative medicine (Fig. 6). One example is the use of the peptide scaffolds to support neurite growth and differentiation, neural stem cell differentiation, cardiac myocytes, bone and cartilage cell cultures. The peptide scaffolds from RADA16-I and RADA16-II formed nanofiber scaffold in physiological solutions that stimulated extensive rat neurite outgrowth and active synapses formation on the peptide scaffold [22]. This observation stimulated and inspired further experiments to directly repair the brain of animals.

The peptide nanofiber scaffold has been used to inject into hamster's brain to reconnect the cut optical track to restore animal vision [29] because the peptide nanofiber scaffold encouraged abundant neural migration and high density synapses. In addition the same peptide nanofiber scaffold also instantly stops bleeding, ∼10 s, in several tissues [30]. These examples are direct applications of self-assembling peptide materials for medical applications.

Designer peptides scaffold 3D cell cultures

Although self-assembling peptides are promising scaffolds, they show no specific cell interaction because

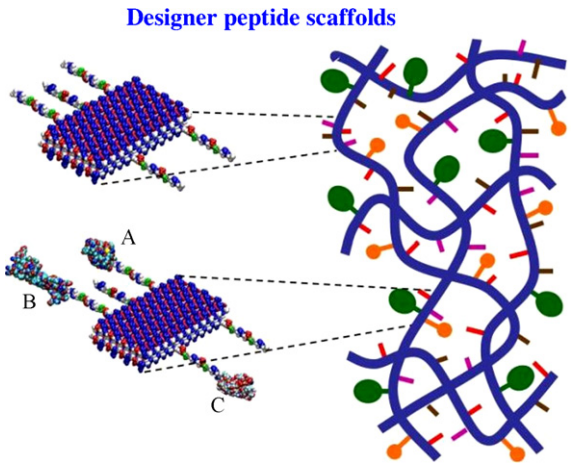

Figure 7 Molecular and schematic models of the designer peptides and of the scaffolds. RADA16 is an alternating16-residue peptide with basic arginine (blue), hydrophobic alanine (white) and aspartic acid (red). (A) Direct extension of the self-assembling peptide sequence by adding different functional motifs. Light turquoise cylinders represent the self-assembling backbone and the yellow, pink, and tan lines represent various functional peptide motifs. (B) Molecular model of a self-assembling peptide nanofiber with functional motifs flagging from both sides of the double β-sheet nanofibers. Either mono or multiple functional (or labeled) peptide motifs can be mixed at the same time. The density of these motifs can be easily adjusted by simply mixing them in various ratios, 1:1—1,000,000 or more before the assembling step. (C) They then will be part of the self-assembled scaffold.

their sequences are not naturally found in living systems. The next logical step is to directly couple biologically active and functional peptide motifs found in literature, accordingly the 2nd generation of designer scaffolds will significantly enhance their interactions with cells and tissues.

The simplest way to incorporate the functional motifs is to directly synthesize it by extending the motifs on to the self-assembling peptides themselves (Fig. 7). The functional motifs are coupled on the C-termini since solid phase peptide synthesis starts from C-termini to avoid deletion during elongation synthesis (the longer the peptide extended from the C-termini, the more likely there will be deletion errors). Usually a spacer comprising two glycine residues is added to guarantee a flexible and correct exposure of the motifs to cell surface receptors. If one combines a few designer peptides with different active motifs, these different functional motifs in various ratios can be incorporated in the same scaffold (Fig. 7). Upon exposure to solution at neutral pH, the functionalized sequences self-assemble, leaving the added motifs on both sides of each nanofiber (Fig. 7). Nanofibers take part to the overall scaffold thus giving microenvironments functionalized with specific biological stimuli (Fig. 7).

The self-assembling peptide scaffolds with functional motifs can be commercially produced at a reasonable cost. Thus, this method can be readily adopted for wide spread uses including study how cell interact with their local- and micro-environments, cell migrations in 3D, tumor and cancer cells interactions with the normal cells, cell processes and neurite extensions, cell-based drug screen assays and other diverse applications.

We have produced different designer peptides from a variety of functional motifs with different lengths [6—8]. We showed that the addition of motifs in some cases to the self-assembling peptide RADA16-I did not significantly inhibit self-assembling properties. Furthermore, one can combine the RADA16-I nanofiber with the active designer self-assembling peptides through mixing the modified peptides. Although their nanofiber structures appear to be indistinguishable from the RADA16-I scaffold, the appended functional motifs significantly influenced cell behaviors.

Using the designer self-assembling peptide nanofiber system, every ingredient of the scaffold can be defined. Furthermore, it can be combined with multiple functionalities including the soluble factors [31]. Cells reside in a 3D environment where the extracellular matrix receptors on the cell membranes can bind to the functional ligands appended to the peptide scaffolds. It is likely that higher tissue architectures with multiple cell types, rather than monolayers, could be constructed using these designer 3D self-assembling peptide nanofiber scaffolds.

Even if only a fraction of functionalized motifs on the 3D scaffold are available for cell receptor binding, cells may likely receive more external stimuli than when in contacts with coated 2D Petri dishes or RGD-coated (or other motifs) polymer micro-fibers, which is substantially larger than the cell surface receptors and in most cases, larger than the cell themselves. These cells are not in real 3D, rather, they are in 2D wrapping around the micro-polymers with a curvature depending on the diameter of the polymers. In a 2D environment, where only one side of the cell body is in direct contact with the surface, receptor clustering at the attachment site may be induced; on the other hand, the receptors for growth factors, cytokines, nutrients and signals are on the other sides that expose directly with the culture media. Thus cells may become partially polarized. In the 3D environment, the functional motifs on the nanofiber scaffold surround the whole cell body in all dimensions and the factors may form a gradient in 3D nanoporous microenvironment.

In our search for additional functional motifs, we found that a class of bone marrow homing peptides BMHP [6,32] is one of the most promising active motifs for stimulating adult mouse neural stem cells (NSC) adhesion and differentiation [7]. This observation suggests a new class of designer self-assembling peptides for 3D cell biology studies.

Designer peptide scaffolds for bone cells and 3D migration

The designer self-assembling peptide nanofiber scaffolds have been shown not only to be an excellent biological material for 3D cell cultures and are capable to stimulate cell migration into the scaffold [7,8]. We developed several peptide nanofiber scaffolds designed specifically for osteoblasts [7]. We designed one of the pure self-assembling peptide scaffolds, RADA16-I, through direct coupling to short biologically active motifs. The motifs included osteogenic growth peptide ALK (ALKRQGRTLYGF) bone-cell secreted-signal peptide, osteopontin cell adhesion

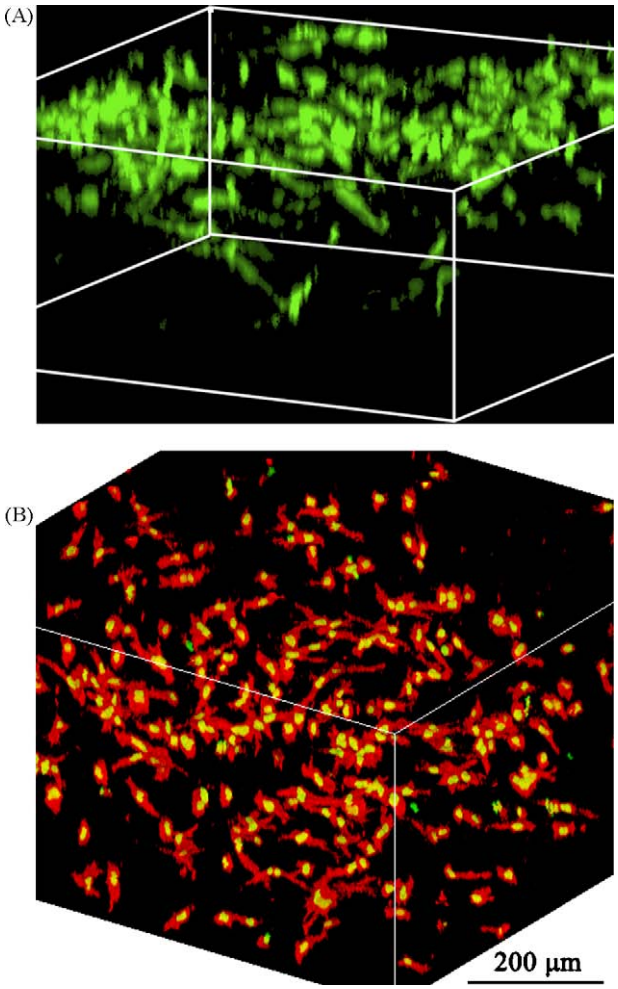

Figure 8 Laser confocal scanning microscopy images of bone cells (left panel) and HUVECs (right panel) show the cell migrated several hundred microns into the peptide scaffold. The 3D culture system is more realistic for real body 3D environment, in sharp contrast to the conventional 2D artificial culture system.

motif DGR (DGRGDSVAYG) and 2-unit RGD binding sequence PGR (PRGDSGYRGDS). The new peptide scaffolds are made by mixing the pure RADA16-I and designer peptide solutions and the molecular integration of the mixed nanofiber scaffolds was examined using AFM. Compared to pure RADA16-I scaffold, it was found that these designer peptide scaffolds significantly promoted mouse pre-osteoblast MC3T3-E1 cell proliferation. Moreover, alkaline phosphatase (ALP) activity and osteocalcin secretion, which are early and late markers for osteoblastic differentiation, were also significantly increased, thus demonstrating that the designer, self-assembling peptide scaffolds promoted the proliferation and osteogenic differentiation of MC3T3-E1.

Under the identical culture medium condition, confocal images unequivocally demonstrated that the designer PRG peptide scaffold stimulated cell migration into the 3D scaffold (Fig. 8) [7,8]. Without the modified active motif, cells stayed in the same scaffold. These observations will likely stimulate further research to study cell migration in 3D under well-defined conditions since the designer scaffolds can be fine-tuned and well controlled. This is in sharp contrast with the current cell culture conditions using collagens and Matrigel that contain unknown ingredients thus difficult to reproduce the experimental results (Fig. 9).

Designer self-assembling peptide nanofiber scaffolds for reparative, regenerative medicine and tissue engineering

Designer self-assembling peptide nanofiber scaffolds have a wide spectrum of uses in additional to 3D cell culture [33,34]. Bokhari et al. in UK produced a peptide hydrogel—polyHIPE polymer hybrid material to enhance osteoblast growth and differentiation [35]. Lee and his colleagues at Harvard Medical School used the scaffolds to promote angiogenesis [36] and when inject into mice heart muscle, self-assembling peptide nanofibers create intramyocardial microenvironments for endothelial cells [37]. Moreover, local myocardial IGF-1 delivery with biotinylated peptide nanofibers improves cell therapy for myocardial infarction suggesting the peptide scaffolds can be used for healthcare technology [38].

Designer self-assembling peptide nanofiber scaffolds for and controlled molecular releases

When one examines the scaffold under SEM and AFM, it is apparent that the self-assembling peptide form ordered 3D scaffold with pores range from 5 to 200 nm [3,6], similar to size as most small molecular drugs and therapeutic proteins. This simple observation suggests that scaffolds themselves may be useful for sustained molecular release. When some small molecules are encapsulated in the peptide nanofiber scaffolds, these small molecules released slowly depending on their charges and other characteristics [39]. These observations inspired us to directly encapsulate protein molecules since proteins are increasingly become effective therapeutics including insulin, and monoclonal antibodies [40]. The proteins showed a sustained release mostly depend on the protein molecular size and shape. The smaller proteins, such as lysozyme with a molecular weight ~14 kDa, it releases faster than antibodies which are ~10 times larger, with a molecular weight ~150 kDa [40]. Furthermore, the release profiles can be fine-tuned since the lower concentrations of peptides form lower density of scaffolds, thus large pores. On the other hand, higher concentrations of peptide form higher density scaffolds, thus having smaller pores. If amplify 10 billion times, this situation is like a forest or grassland, the sparser the trees, shrub and grass, the faster one moves, conversely, the denser the trees, shrub and grass, the slower one moves.

Chen and his colleagues also used peptide scaffold to encapsulate hydrophobic anti-cancer drug ellipticine [41]. They showed that the peptides stabilized the drug and increased its efficacy since the non-stabilized ellipticine degraded quickly thus lose its potency. One of the peptide EFK16-II stabilized neutral ellipticine molecules and ellipticine microcrystals [41]. Since many anti-cancer drugs are either not water-soluble or unstable in water, their findings could be further developed for effective anti-cancer drug delivery.

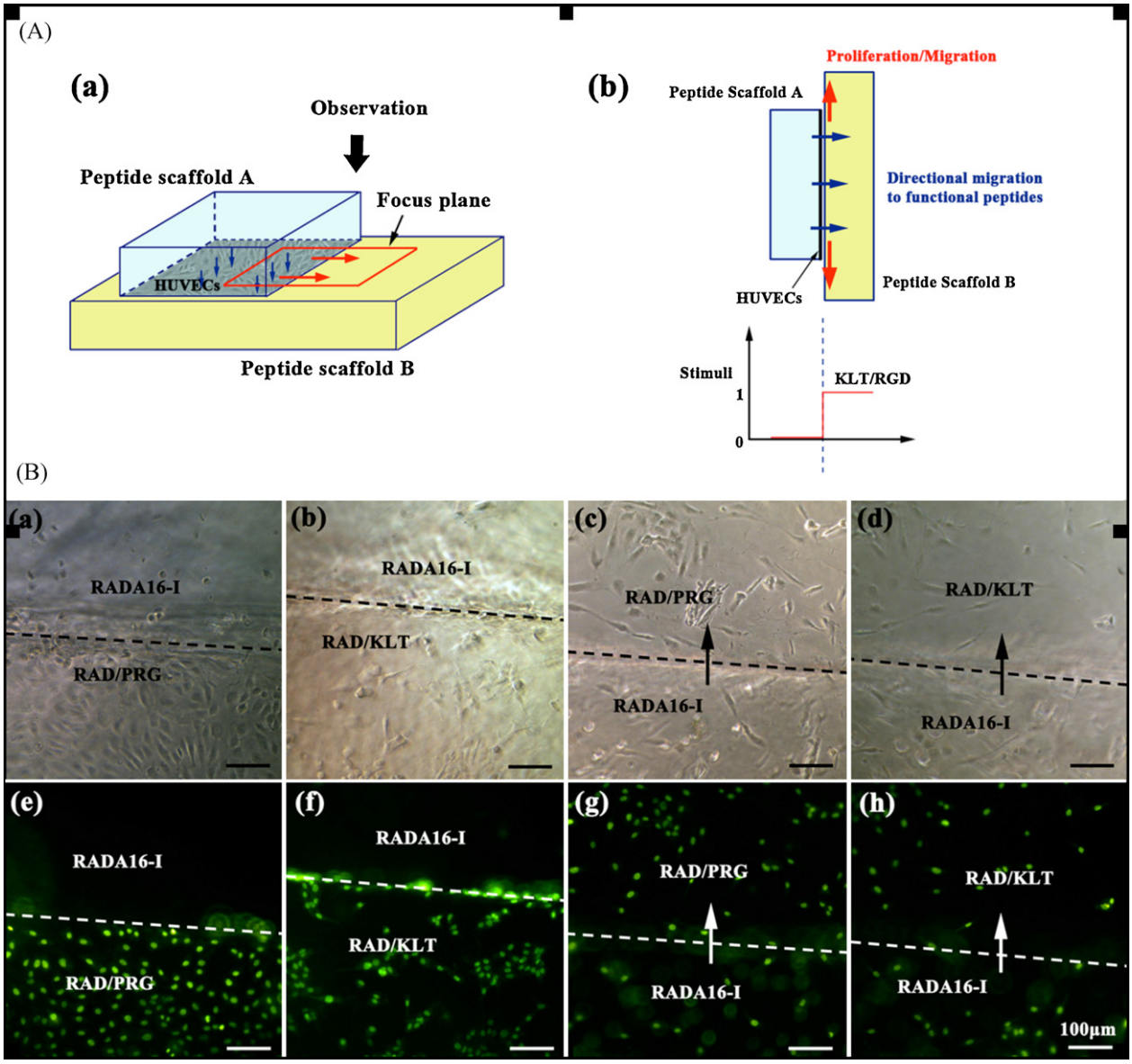

Figure 9 Endothelial cell unidirectional migration in responds to functional peptide scaffolds. (A) Schematic illustrations of cell directional migration. (a) Clear-boundary-sandwich cell migration assay. (b) Directional migration induced by functional motifs. (B) Phase contrast microscopy images of HUVECs seeded on peptide scaffolds: (a) RAD/PRG; (b) RAD/KLT; (c) and (d) RADA16-I, and fluorescent SYTOX Green nuclear staining for (e) RAD/PRG; (f) RAD/KLT; (g) and (h) RADA16-I. Cells directionally migrated from RADA16-I to RAD/PRG (c and g) and RAD/KLT (d and h). The scale bar is 100 μm for all panels.

Chiral designer self-assembling peptide nanofiber scaffolds

All amino acids, peptides and proteins are naturally chiral molecules with distinctive handedness. It is often asked if different chiral peptides can also undergo self-assembly, form stable and well-ordered nanofibers and further self-organize into scaffolds. This is an intriguing question since nature only produced L-amino acids, thus L-peptides and L-proteins. Luo Zhongli and his colleagues used the identical sequences but the chiral D-form amino acids to produce peptides with only D-amino acids. They showed that the D-peptides have inverted circular dichroism spectra, mirror images of the L-peptides of identical sequence [42]. The D-peptide is less stable than the L-peptide in high temperature and can undergo secondary structural transition from β-sheet to α-helix. This raises a very interesting question why nature selected L-amino acids for all living systems on the planet Earth. However, the nanofibers and scaffolds formed from the peptides with only D-amino acids are indistinguishable from their L-counter part [43].

Designer lipid-like peptide surfactants

We not only designed self-assembling peptides that form nanofibers, but we also designed peptides that are lipid-

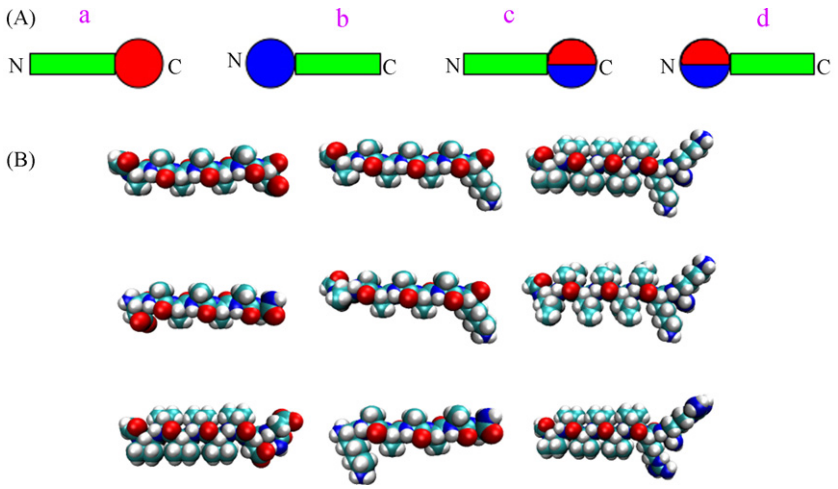

Figure 10 The designer lipid-like peptide surfactants. (A) These peptides have a hydrophilic head and a hydrophobic tail, much like lipids or detergents. They sequester their hydrophobic tail inside of micelle, vesicles or nanotube structures and their hydrophilic heads expose to water. At least three kinds molecules can be made, with −, +, −/+ heads. (B) Six examples of designer lipid-like peptide surfactants. There are (from left to right and top down): Ac-A_6D-OH, Ac-A_6K-NH_2, Ac-V_6KK-NH_2, DA_6-NH_2, Ac-A_6K-OH, Ac-I_6KK-NH_2, Ac-V_6DD-OH, KA_6-NH_2, Ac-V_6RR-NH_2. Each one has different CAC and behaves differently in water and in PBS. They have been used to stabilize membrane protein and membrane protein complexes.

like, both the structure and the chemical properties. They have hydrophobic tail and hydrophilic head (Fig. 10). They also have defined critical aggregation concentrations [16—19]. They also undergo self-assembly to form well-ordered nanostructures including nanotubes, nanovesicles and micelles (Fig. 11) [13—17].

Not only do their shape and physical structure of these lipid-like peptides resemble lipids and other organic surfactants, but their chemical properties do as well. For example, peptides have either six hydrophobic alanine or valine residues from the N-terminus, followed by a negatively charged aspartic acid residue (A_6D = Ac-AAAAAAD-OH; V_6D Ac-VVVVVVD-OH); thus, they possess two negative charges, one from the charged terminal side chain and the other from the C terminus [13]. In contrast, several simple peptides, G_4DD (Ac-GGGGDD-OH), G_6DD (Ac-GGGGGGDD-OH), G_8DD (Ac-GGGGGGGGDD-OH), have 4, 6, and 8 glycines, followed by two aspartic acids with three negative charges [14]. Similarly, A_6K (Ac-AAAAAAK-NH_2) or KA_6 (KAAAAAA-NH_2) has 6 alanines as the hydrophobic tail and a positively charged lysine as the hydrophilic head [15]. These lipid-like peptides can self-organize to form well-ordered nanostructures, including micelles, nanotubes, and nanovesicles in water (Fig. 11). Furthermore, the structure formation is concentration-dependent; namely, at low concentration, there are no defined structures; these structures spontaneously form at a critical aggregation concentration (CAC) [16—18], similar to lipids and other surfactants.

Six amino acids of varying hydrophobicity (Gly, Ala, Val, Ile, Leu, and Phe) can be used to generate the nonpolar tails. Such hydrophobic tails never exceed six residues, so that the total length of the peptide detergents will be seven, about 2.4 nm in length; interestingly, this is a similar size to that of the phospholipids abundant in membranes. The first lipid-like peptide surfactant was designed by modeling a peptide using the phosphatidylcholine as a size guide.

However, when more than six hydrophobic residues (except glycine) are used, the peptide surfactants become less soluble in water. Although we only focused to study Asp (−) and Lys (+) as the hydrophilic head groups, it must be emphasized that Glu (−), Arg (+), and His (+) can also be used the same combinatorial ways. Therefore, they can broaden the spectra of variations and increase the possible number of peptide surfactants.

Synergistic effect of the lipid-like peptides

Based on the peptide structure design, the unique property of this class of lipid-like peptide can be exquisitely fine-tuned both for their tails and heads. Similar to the common surfactants, the properties of the peptide surfactants can also be fine-tuned by the concentration and the component of the solution. Synergistic effects have been reported (Fig. 12) [18] in mixed peptides Ac-A_6D-OH and Ac-A_6K-NH_2 by measuring the critical aggregation concentrations (CACs) using DLS and the assembly nanostructures using AFM.

The lowest CAC values are obtained for Ac-A_6D-OH/Ac-A_6K-NH_2 = 1:2 in both water and PBS solutions. These decreased CAC values reflect the free energy favored synergistic effect [18] when the two negative charges in one Ac-A_6D-OH molecule can be totally neutralized by two positive charges of two Ac-A_6K-NH_2 molecules. The nanoropes and nanorods can be formed at different concentration and different molar ratio of mixed peptides (Fig. 12). When the peptide solution contents only Ac-A_6D-OH, the structure transformation of nanorods to chiral nanoropes can be observed from lower concentration to higher concentration (Fig. 12A—C). The high-resolution images in this study reveal that the nanoropes with left-handedness are self-assembled by twisting together of numerous nanorods (the inset image in Fig. 10B). This intermediate state capturing the nanorope formation process supports a nucleation-growth pathway

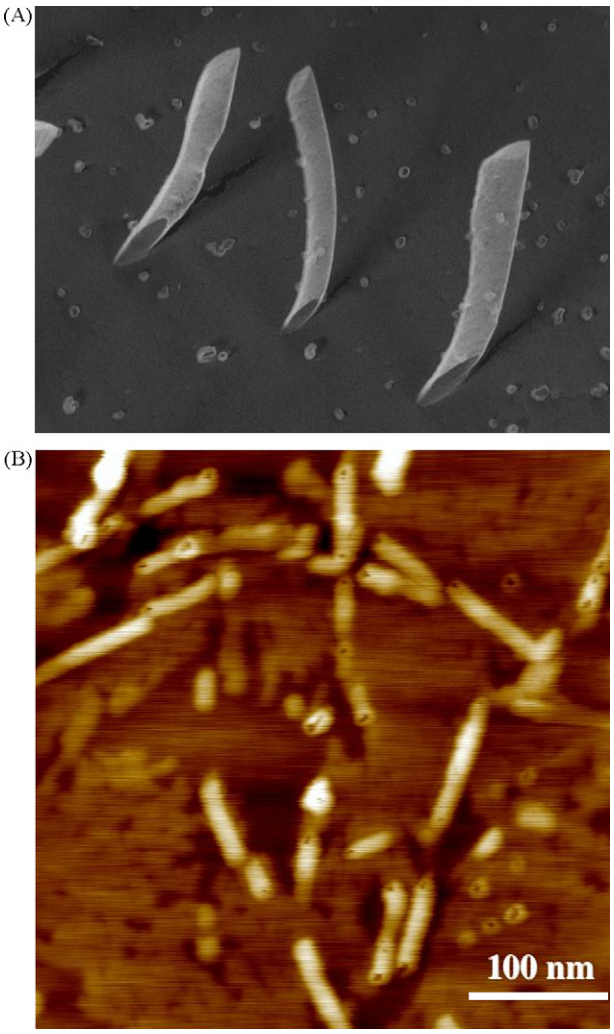

Figure 11 (A and B) TEM and AFM image of designer lipid-like peptide surfactant. These peptides undergo self-assembly to form nanotube structure. Quick-freeze/deep-etch TEM image of Ac-V_6D-OH dissolved in water (4.3 mM at pH 7). The TEM images show the dimensions, ~30—50 nm in diameters with openings of nanotube ends. Note opening ends of the peptide nanotube may be cut vertically. The strong contrast shadow of the platinum coat also suggests the hollow tubular structure.

with a high degree of cooperativity. For solution with Ac-A_6K-NH_2 peptide, structure transformation from spherical nanoparticles to rigid nanorods to stacking of nanorods was observed (Fig. 12D—F). For self-assembly of mixed peptide Ac-A_6D-OH/Ac-A_6K-NH_2, uniformed nanoropes were observed in AFM images at the molar ratio of Ac-A_6D-OH/Ac-A_6K-NH_2 = 1:1, and the thin film formation with aligned nanoropes was reported at a molar ratio of 2:1. The well-aligned nanoropes at a molar ratio of Ac-A_6D-OH/Ac-A_6K-NH_2 = 2:1 indicated the competition factor between the electrostatic repulsion according to the DLVO theory [44] and the hydrophobic interaction arising from the long side chains on lysine residues.

Based on the DLS and AFM observations, we propose a plausible assembling mechanism to explain the observed aggregation process of the nanorope structure in the mixed peptide population. At lower concentration well below the CAC value, Ac-A_6D-OH and Ac-A_6K-NH_2 are randomly oriented and distributed as monomers in solution (Fig. 13). Because of the electrostatic attraction between opposite charges and the hydrophobic interactions between alanine tails, the interdigitated short and thin nanorods, or spherical particles, could be formed in the solution (Fig. 13B). The negatively charged and positively charged peptides tend to position themselves alternatively for lowering the energy associated with the electrostatic interaction. At higher concentration, the nanoropes can be formed, which can be considered as an extended structure of the spherical particles (Fig. 12C—D). When the molar ratios of the two peptides are at an appropriate concentration, the fine-tuning of the charges and the hydrophobic interaction would produce the alignment of the nanoropes into thin film (Fig. 12E). This study will be valuable for designing new nanomaterials based on mixing different ratios of designer lipid-like peptide surfactants, and the increased surface activity by mixing lipid-like peptides is also helpful in stabilizing membrane proteins ([45—48], Mershin unpublished results).

Design new geometry of lipid-like peptide

Besides the electrostatic and hydrophobic interactions, the dimensions and shapes of the supramolecular structures also depend on other factors, such as the geometry of the polar head group and the geometrical constraints of the peptide itself [19]. Thus, it is possible to fine-tune the supramolecular structures with expanded functionalities of peptide surfactants by introducing different shapes and structures. The donut-shaped nanostructure formation was observed from self-assembly of a cone-shaped designer lipid-like peptide.

The cone-shaped amphiphilic peptide, Ac-GAVILRR-NH_2, has a hydrophilic head with two positive charges and with a relatively large size, and has a hydrophobic tail with decreasing hydrophobicity and side-chain size (Fig. 14). The CAC values were measured using dynamic light scattering in water (0.82 mM) and in phosphate-buffered saline (0.45 mM) as well. The AFM image in Fig. 8B shows the typical donut-shaped nanostructures of the cone-shaped peptide assembly at a concentration of 1 μM. We have studied numerous peptides over 16 years [3,4] and this is the first time ever we observed the nanodonut structure from the cone-shaped peptides. The dimensions of the donut-shaped assemblies were measured using the section analysis. The measured outer diameter D_2 = ~105 nm, and the inner diameter D_3 = ~22 nm. The average thickness of the donut-shaped structures is around ~41.5 nm, which is similar to the diameter of the spherical nanoparticles. The schematic illustration of the nanodonut structure is presumed to be the elongated micelle and curved to be a closed ring (right panel of Fig. 15).

Interestingly, AFM observations of some short nanopipe structures with different bending angles are captured in a single image [19]. Based on this observation, we propose a plausible self-assembling pathway of the nanodonut structure that was self-assembled through fusion or elongation of spherical micelles. Furthermore the bending of the nanostructure gives rise to the nanodonut structures due to the tension originated from the interaction of the cone-shaped

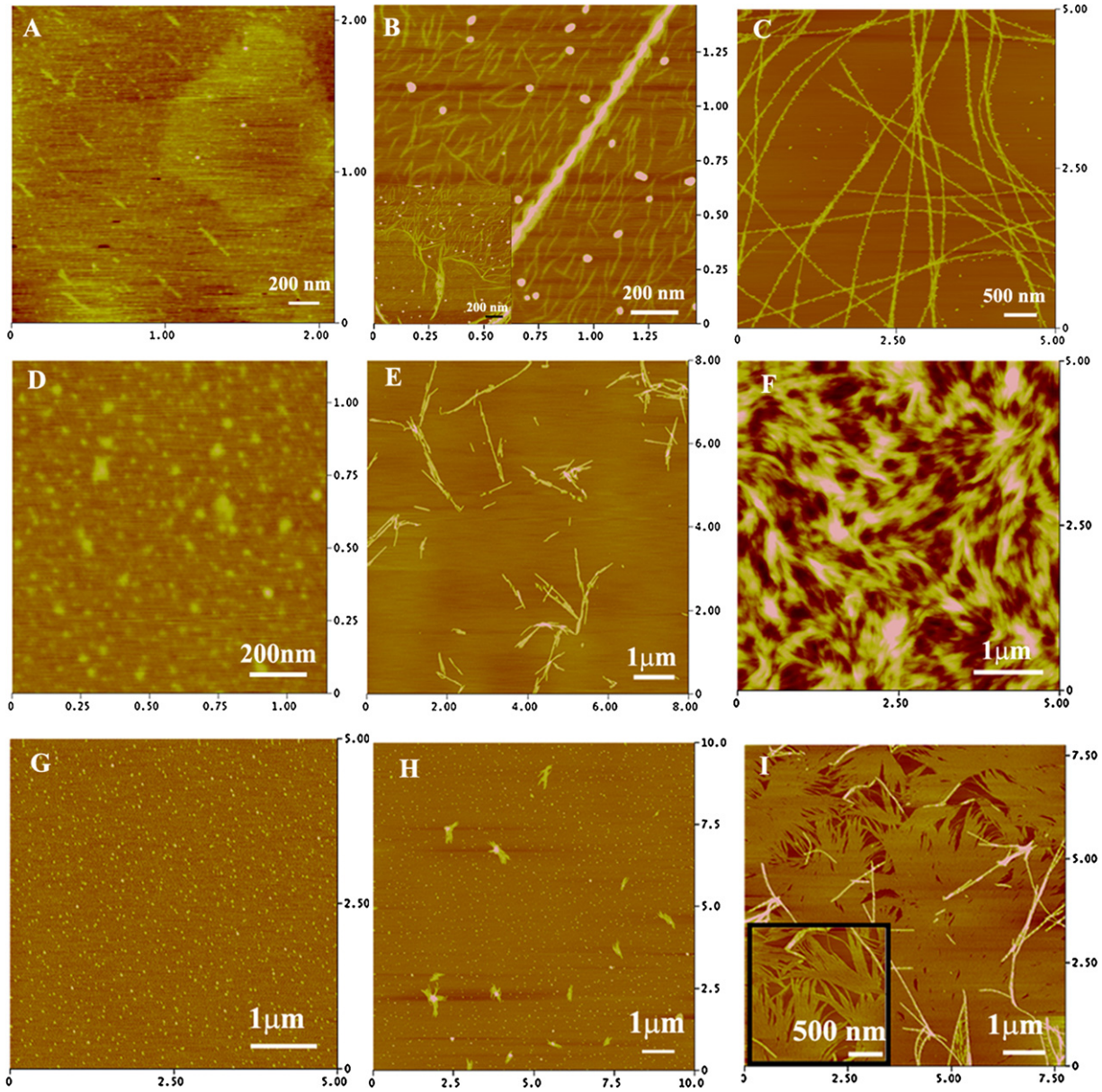

Figure 12 Synergistic effects of mixed designer lipid-like peptides illustrated by the AFM images of Ac-A_6D-OH, Ac-A_6K-NH_2, and mixed peptides Ac-A_6D-OH/Ac-A_6K-NH_2 on fleshly cleaved mica surfaces under ambient conditions. The AFM images for the nanorod and nanorope structures of Ac-A_6D-OH (A—C) with the concentrations of 1 μM (A), 70 μM (B), and 1 mM (C). The inset image in (B) illustrates the intermediate state for the twisting of nanofibrils to form the helical nanoropes. (D—F) illustrate AFM images for the nanorod structures of Ac-A_6K-NH_2 at the concentrations of 1 μM, 70 μM, and 1 mM, respectively. The AFM images for the nanofilm structures consisted of nanoropes for mixed peptides Ac-A_6D-OH/Ac-A_6K-NH_2 (G—I) with the concentrations of 1.5 μM (G), 15 μM (H), and 1.5 mM (I). The concentrations of the total peptides were assigned to stand for the concentration of the mixture solutions. The molar ratios for the solutions are Ac-A_6D-OH/Ac-A_6K-NH_2 = 2:1 and the inset in (I) is the high-resolution image showing the nanorope structure at domain boundaries.

peptide side-chains. Our observations may be useful for further fine-tuning of the geometry and shape of a new class of designer peptides and their self-assembled supramolecular materials for diverse uses. This unique cone-shaped geometry would not only add repertoire of the self-assembling nanostructures but also deepen the understanding of the mechanisms of the peptide self-assembling.

One of the questions that frequently asked is if the lipid-like peptide can indeed mix well with real lipid molecules. Experiments using monoolein bilayers to incorporate the designer lipid-like peptides showed that these peptides could indeed interact with the monoolein very well [45]. The impact of these lipid-like peptide surfactants and the ternary MO/peptide/water system has been studied using small-angle X-ray scattering (SAXS). At higher peptide concentration ($R = 0.10$), the lipid bilayers are destabilized and the structural transition from the *Pn3m* to the inverted hexagonal phase (H(2)) is induced. For the cationic pep-

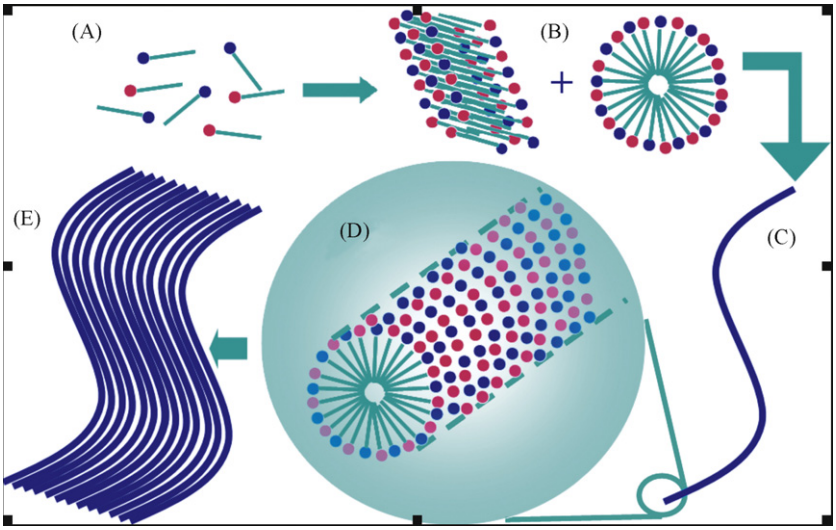

Figure 13 A proposed plausible mechanism for the assembly process of the nanorope structure. (A) The randomly oriented and distributed peptide monomers at low concentration, the color code: cyan sticks represent the hydrophobic tails consisting of six alanine residues; red balls represent for the hydrophilic head aspartic acid of Ac-A_6D-OH with 2 negative charges; the blue balls represent for the hydrophilic head lysine Ac-A_6K-NH_2 with 1 positive charge. (B) The spherical particle formation or nanorod structure. (C) The formation of the nanorope structure. (D) The schematic illustration of the section structure of the nanoropes. (E) The alignment of the numerous nanoropes leading to the formation the thin film.

tides, our study illustrates how even minor modifications, such as changing the location of the head group Ac-A_6K-NH_2 vs. KA_6NH_2 affects significantly the peptide's effectiveness. Only KA_6NH_2 displays a propensity to promote the formation of H(2), which suggests that KA_6NH_2 molecules have a higher degree of incorporation in the interface than those of Ac-A_6K-NH_2 [45].

Peptide Sequence

PEPTIDE SEQUENCE	PI	MW	soluble at pH	Appearance
Ac-GAVILRR-NH_2	12	783.97	13.21	Clear

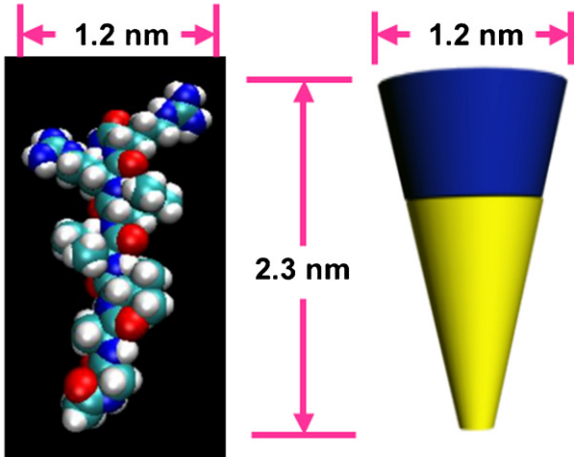

Figure 14 Molecular model of Ac-GAVILRR-NH_2. The peptide length is approximately 2.3 nm and the width is 1.2 nm. Color code: hydrogen = white, carbon = cyan, oxygen = red and nitrogen = blue. The cone-shaped model is simplified for the shape of Ac-GAVILRR-NH_2. The blue part indicates the positively charged hydrophilic region and the yellow part indicates the hydrophobic region.

Designer lipid-like peptide stabilize membrane proteins

Membrane proteins play vital roles in all living systems. They are crucial for biological energy conversions, cell—cell communications, specific ion channels and pumps involving our senses: sight, hearing, smell, taste, touch and temperature sensing. Approximately ~30% of all genes in almost all sequenced genomes code for membrane protein [46—48]. However, our understanding of their structures and function falls far behind that of soluble proteins. As of February 2009, there are only 182 unique membrane protein structures of total 428 variations known (http://www.blanco.biomol.uci.edu/Membrane_Proteins_xtal.html) among over 55,000 structures in the Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). The reason is that there are several notoriously difficult steps to obtain membrane proteins.

Membrane proteins are exquisitely fine nature-made molecular devices that will be very useful for a wide range of applications including solar energy harvesting and ultrasensitive sensing. In order to accelerate membrane protein structural studies and use them for design and fabrication of nanobiodevices, new and simple methods are crucial. We found that simple lipid-like peptides are excellent materials to solubilize and stabilize these proteins.

Detailed structural analyses of membrane proteins as well as their uses in advanced nanobiotechnology applications require extended stabilization of the functional protein conformation. We have shown that the designer surfactant-like peptides can significantly increase the activity and stabilize diverse membrane proteins including *E.coli* Glycerol-3-phosphate dehydrogenase [49], G-protein coupled receptor-bovine rhodopsin [50], the functional form of the multi-domain protein complex Photosystem-I (PS-I) on surface in dry form [51] and in aqueous solution

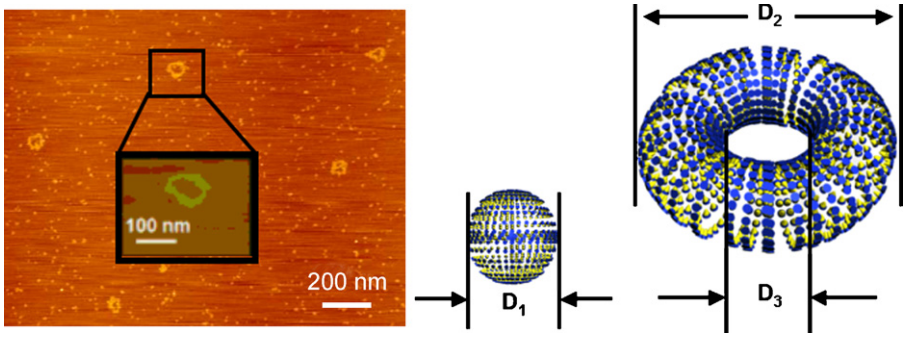

Figure 15 AFM image for the aggregation structures of Ac-GAVILRR-NH_2 in water at concentration of 1 μM. The AFM image indicates the coexistence of nano-donut structures and the spherical micelles, the scale bar is 1 μm. The inset shows a zoomed image of the donut-shape structure, the scale bar is 100 nm. Schematic illustration of the structure of the spherical micelles and the nano-donuts. The average diameter of the micelles (D_1 = ~30 nm) and the outer and inner diameters of the nano-donut structures (D_2 = ~110 nm; D_3 = ~27 nm) are obtained based on section analysis of tens of nano-donuts in different AFM images.

[52]. We carried out a systematic analysis using a series of such peptides to identify the chemical and structural features that enhance the photochemical activity of PS-I. We observed that lipid-like peptide surfactant amphiphilicity is necessary, but not sufficient to stabilize PS-I in its functional form. Furthermore, we showed that not only the polarity and number of charges on the hydrophilic head are important, but also the hydrophobicity and size of the amino acid side groups in the hydrophobic tail play an important role. For bovine rhodopsin, A6D-OH is the best [52]. For Photosystem-I, the best performing peptides for the stabilization of functional PS-I are in order of effectiveness: ac-I_6K_2-$CONH_2$, ac-A_6K-$CONH_2$, ac-V_6K_2-$CONH_2$, ac-V_6R_2-$CONH_2$ (Fig. 16) [52]. These simple and inexpensive peptide surfactants will likely make significant contributions to stabilize the functional form of diverse and currently elusive membrane proteins and their complexes with important applications (Fig. 17).

Self-assembling peptide extend prion-infected animal life

Recently, Hnasko and Bruederle reported unexpected observations from studying prion-infected animals [53]. They used the self-assembling peptide RADA16-I that shares neither sequence homology at all to any prion PrP^c nor any other amyloid proteins. When they mixed the infected animal prion brain with 1% RADA16-I and subsequently injected the mix into new animals. They found the mix with the peptide delayed the infection and extended animal's life ~40 days, a 50% increase of infected animal life span. This is significant since there is no cure for any prion diseases including ''mad cow'' disease, scrapie in sheep, chronic wasting disease in deer as well as Creutzfeldt—Jakob disease (CJD), Gerstmann—Sträussler—Scheinker syndrome (GSS), Kuru as well as other transmissible spongiform encephalopathy in human. This is another example that self-assembling peptide may have unexpected applications in alleviate prion-disease in clinical medicine.

A hierarchical α-helical self-assembling peptide

We not only designed numerous β-sheet forming peptides, lipid-like peptides, secondary structural transitional peptides, but we also designed a helical coiled-coil hierarchical self-assembling peptide that self-organized into fractal structure [54] that fulfills the fractal definition of self-similarity at multiple length scales. In this design, the hierarchical self-assembling peptide with a cross-linkable coiled-coil contains an internal cysteine. The fractal structure of the hierarchical assemblies can be seen using Atomic force microscopy (AFM), low-power optical microscope and unaided-eyes. Molecular simulations showed that the peptides cross-linked to form clusters of coiled-coils, which further assembled to form globules of tens of nanometers

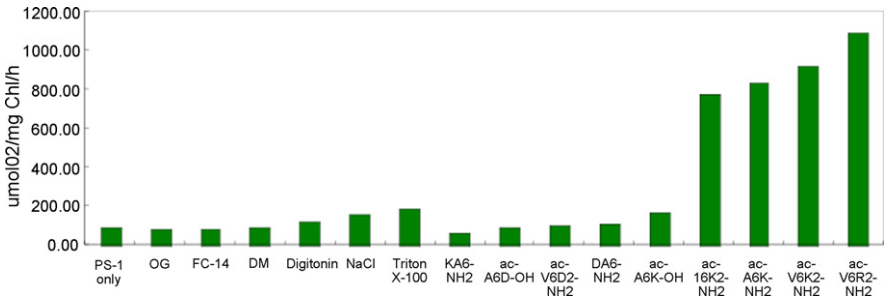

Figure 16 Comparative analysis of the photosystem I (PS-I) activities in the presence of commercial detergents that are commonly used for the stabilization of membrane proteins and of peptide surfactants. The cationic designer peptides showed markedly better capabilities to stabilize the photosystem I for up to a few months.

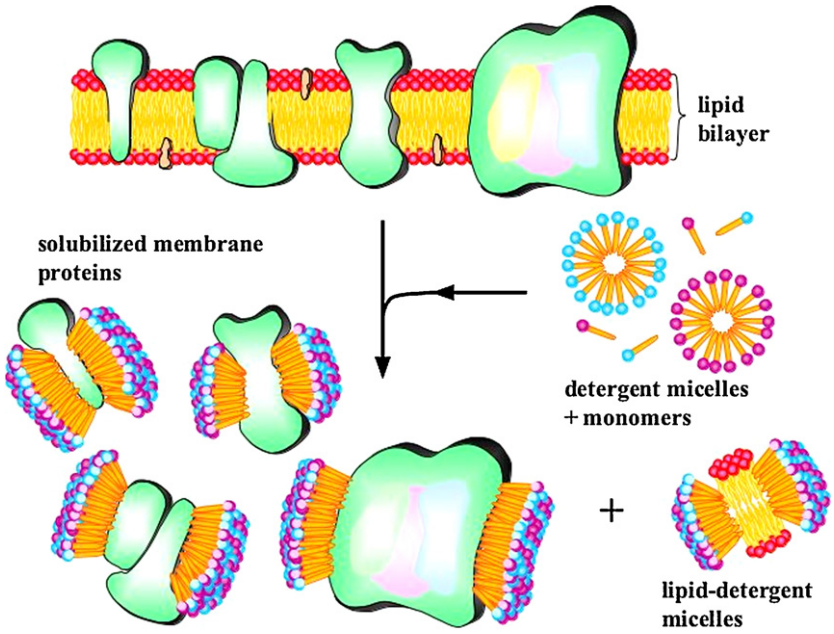

Figure 17 A proposed scheme for how the designer lipid-like peptides stabilize membrane proteins. These simple designer self-assembling lipid-like peptides have been used to solubilize, stabilize and crystallize membrane proteins. These peptides have a hydrophilic head and a hydrophobic tail, much like other biological lipids. They use their tail to sequester the hydrophobic part of membrane proteins, and the hydrophilic heads 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 overcoming the barrier of high resolutions of molecular structure for challenging membrane proteins.

in diameter. The hierarchical organization is modulated by pH or thiol-reducing agent. Exploitation of the fractal structures through chemical methods may be valuable for the fabrication of materials spanning multiple length scales with a wide range of applications.

Conclusion remarks

We believe that these simple and versatile designer self-assembling peptides will provide us many opportunities to study complex and previously intractable biological phenomena. Molecular engineering through designer self-assembling peptides is an enabling technology that will play an increasingly important role in nanoscience, nanotechnology, nanobiotechnology and nanomedicine.

A few words for students and young researchers: in conducting frontier and original research, it is not infrequent that one encounters unexpected observations and experiments that mystify the observer since no one has reported it before. If the observations and the experiments are reproducible and repeatable, then one must choose either ignore it, or continue to pursue what is the funded project, or to pursue the unexpected observation relentlessly to find the answers. Most people would choose the former since it is funded, and it is the goal of the research to satisfy the assigned project and the granting agencies. A few daring and risk-takers may chase the unexpected observations persistently. Perhaps most people would consider that this is chasing the rainbow. There are many difficulties, set backs, unaccepted by colleagues and peers. Manuscripts are ridiculed and rejected. The grant applications are turned down repeatedly. It takes a lot of courage to overcome peer pressure and eventually to persuade colleagues and peers that the unexpected observations are worthwhile and with merit. There are many such examples in scientific discoveries including Nicolaus Copernicus' scientifically based heliocentric cosmology that displaced the Earth from the center of the universe; Charles Darwin's theory of evolution; Alfred Wegener's plate tectonics; DNA as the genetic material, Alexander Rich's DNA—RNA and RNA—RNA perfect pairing without enzymes, Benoit Mandelbrot's fractals; Carleton Gajdusek's seemingly incurable Kuru disease, Thomas Cech's RNA self-splicing, Victor Ambros and Gary Ruvkun's mircoRNA, and many more. The resistance from the establishment is very strong. One must trust one's own reproducible experiments and careful observations must be completely self-confident and persistent.

Since we started our serendipitous journey of working on designer self-assembling peptide systems, we have encountered many surprises, from developing a class of pure peptide nanofiber scaffolds for 3D tissue culture and regenerative medicine, to design lipid-like peptides that solubilize, stabilize and crystallize membrane proteins, to study the model system of protein conformational diseases. As Nobel laureate D. Carleton Gajdusek best put it ''*It is important to explore, to do things others ignore but that will become important in 10—20 years*''.

Acknowledgments

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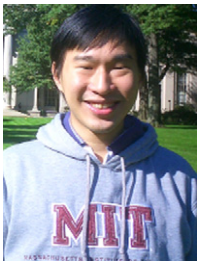

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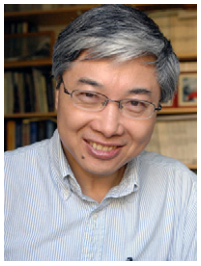

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