# Molecular designer self-assembling peptides†‡

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Chemistry has generally been associated with inorganic and organic syntheses, metal–organic composites, coordinate metal chemistry, catalyses, block copolymer, coating, thin film, industrial surfactants and small-molecule drug development. That is about to change. Chemistry will also expand to the discovery and fabrication of biological and molecular materials with diverse structures, functionalities and utilities. The advent of biotechnology, nanotechnology and nanobiotechnology has accelerated this trend. Nature has selected and evolved numerous molecular architectural motifs at nanometer scale over billions of years for particular functions. These molecular nanomotifs can now be designed for new materials and nanodevices from the bottom up. Chemistry will again harness Nature's enormous power to benefit other disciplines and society. This *tutorial review* focuses on two self-assembling peptide systems.

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† The HTML version of this article has been enhanced with additional colour images.
‡ A tease: a serendipitous discovery of a few new classes of self-assembling peptides has changed our view of peptides as emerging materials ranging from 3-D cell culture, tissue engineering to the study of membrane protein structures.

## Introduction

Through billions of years of molecular selection and evolution, Nature has selected and evolved numerous and diverse chemical and molecular structural motifs.[1,2] These motifs are the basic building blocks of a wide range of sophisticated nanomachines that work at astonishing speed and efficiency with the finest controls, such as directly and efficiently harvesting solar power by converting it into chemical energy that we depend on today, using the same building blocks to faithfully copy and evolve all life forms on Earth, and balancing the complex ecosystem.

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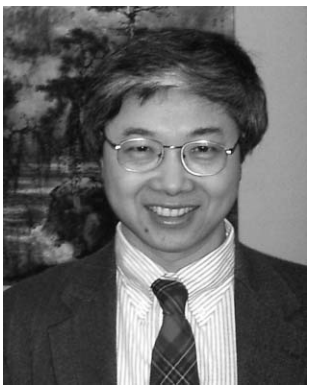

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Only now we begin to learn from Nature—in its finest molecular details and intricate interactions of numerous fine parts—to go one step further and reap Nature's power to benefit humankind while maintaining its delicate balance. We are learning the basic molecular engineering principles for nano- and micro-fabrication at the exquisitely fine scale through the understanding of molecular self-assembly phenomena.

Molecular self-assembly phenomena are ubiquitous in nature: lattice packing in crystals and minerals, shell and tooth growth, oil droplets forming in water, triple helical collagen structures, and the formation of numerous complex molecular machines (such as ribosomes and light-harvesting photosystems). Molecular self-assembly is not only well-studied chemistry as we know it, but essential in all chemical and life sciences. The key elements in molecular self-assembly are chemical complementarity and structural compatibility through numerous noncovalent weak interactions.

Molecular self-assembly is a rather broad and fast-moving field; it is impossible to fully cover the entire spectrum, so here we will only focus on the self-assembling peptide systems from our own laboratories.

Several self-assembling peptide systems have been developed, ranging from models for studying protein folding and protein conformational diseases, to molecular materials that produce peptide nanofibers, peptide scaffolds, peptide surfactants and peptide ink.[3–6] These self-assembling peptide systems are rather simple, versatile, economically affordable and easy to produce on a large-scale to spur new industries. These self-assembling peptide systems represent a significant advance in molecular design and engineering for diverse technological innovations. Those who are interested in a broad view are encouraged to consult earlier reviews.[7–10]

Molecular self-assembly is facilitated through numerous weak, noncovalent bonds—especially hydrogen bonds, ionic bonds (also called electrostatic interactions, or salt bridges as commonly referred to in biology), hydrophobic interactions, van der Waals interactions, and water-mediated hydrogen bonds. Although these weak bonds are rather insignificant in isolation, when combined they not only govern the 3-D structural conformations of all proteins, nucleic acids and other molecules, but also dictate their interaction with other molecules. The water-mediated hydrogen bond is particularly important for living systems since all biological molecules interact with water.

These weak interactions promote the assembly of molecules into units of well-defined and stable hierarchical macroscopic structures. Although each of the bonds or interactions is rather weak, the collective interactions can result in very stable structures and materials. Like hands and gloves, both the size/shape and the correct orientation, *i.e.* chirality, are important in order to have a complementary and compatible fit.

Molecular self-assembly is ubiquitous in Nature and has recently emerged as a new approach in chemical synthesis, nanotechnology, polymer science, materials and engineering. Molecular self-assembly systems lie at the interface between molecular biology, protein science, biochemistry, polymer science, materials science and engineering.[3–10] Many self-assembling systems have been developed, and they represent a significant advance in the molecular engineering of simple molecular building blocks useful for a wide range of applications. This field is growing at an accelerating pace, riding on the tide of biotechnology and nanotechnology.

## Self-assembling peptides as structural building motifs

In the construction of a building, the doors, windows, and other components can be prefabricated, programmed, and assembled according to the architectural plans. If we shrink the construction units millions or billions of times into nanoscale, we can apply similar principles to construct molecular materials and nanodevices through molecular self-assembly and programmed molecular assembly. Given the growing trend and interest but limited space, only two self-assembling systems are reviewed here (see Fig. 1). They include "Peptide Lego", which forms well-ordered nanofiber scaffolds for 3-D cell culture and for regenerative medicine as well as for drug and protein deliveries; and "lipid-like peptides", not only for solubilizing, stabilizing and crystallizing membrane proteins but also for drug formulations. These building block designer peptide motifs are structurally simple and versatile for a broad spectrum of applications.

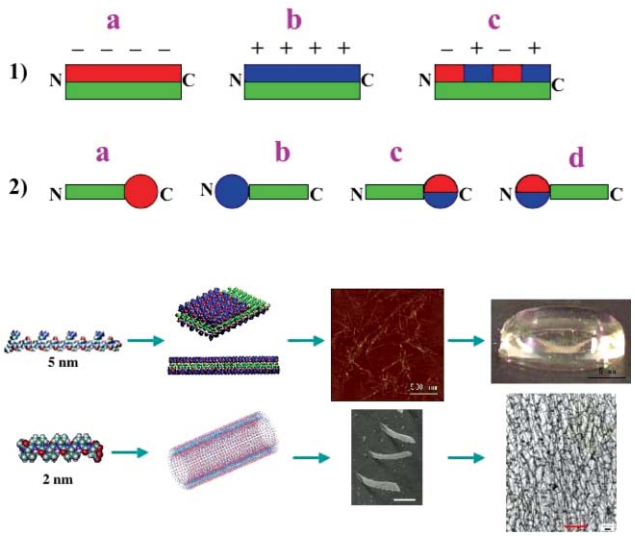


**Fig. 1** Fabrication of various peptide materials. Upper panel: Peptide Lego, also called ionic self-complementary peptide has 16 amino acids, ~5 nm in size, with an alternating polar and nonpolar pattern. They form stable β-strand and β-sheet structures, thus the side chains partition into two sides, one polar and the other nonpolar. They undergo self-assembly to form nanofibers with the nonpolar residues inside (green) and + (blue) and – (red) charged residues form complementary ionic interactions, like a checkerboard. These nanofibers form interwoven matrices that further form a scaffold hydrogel with very high water content, >99.5% water. Lower panel: Lipid-like peptides, ~2 nm in size, which have a distinct head group, either positively charged or negatively charged, and a hydrophobic tail consisting of six hydrophobic amino acids. They can self-assemble into nanotubes and nanovesicles with a diameter of ~30–50 nm. These nanotubes go on to form an inter-connected network, which has also been observed in other nanotubes.


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## Peptide Lego

At the nanometer scale, molecular "peptide Lego" resembles the Lego bricks that have both pegs and holes in a precisely determined manner. They can be designed to self-assemble into stable and fine structures. This class of "peptide Lego" spontaneously assembles into well-formed nanofibers at the molecular level.[11] The first member of the peptide Lego class was serendipitously discovered from a segment of a left-handed Z-DNA binding protein in yeast, Zuotin (Zuo means left in Chinese, tin means protein in biology).[12]

This class of designer self-assembling peptides forms β-sheet structures in water and in aqueous solution, thus forming two distinct surfaces: one hydrophilic and the other hydrophobic, like the pegs and holes in Lego bricks. In aqueous solution, the hydrophobic sides shield themselves from water, thus facilitating the peptide to undergo intermolecular self-assembly, similar to what is seen in the case of intramolecular protein folding. The unique structural feature of these "peptide Lego" systems is that they form complementary ionic bonds with regular repeats on the hydrophilic surface (Fig. 2). The complementary ionic sides have been classified into several moduli, *i.e.* modulus I, II, III, IV, *etc.*, and mixed moduli. This classification is based on the hydrophilic surface of the molecules that have alternating + and − charged amino acid residues, either alternating by 1, 2, 3, 4 and so on. For example, charge arrangements for the different moduli are as follows: modulus I, − + − + − + − +; modulus II, − − + + − − + +; modulus III, − − − + + +; and modulus IV, − − − − + + + +. The charge orientation can also be designed in reverse orientations that yield entirely different molecules with distinct molecular behaviors. These well-defined sequences allow them to undergo ordered self-assembly, resembling some situations found in well-studied polymer assemblies.[9,10]

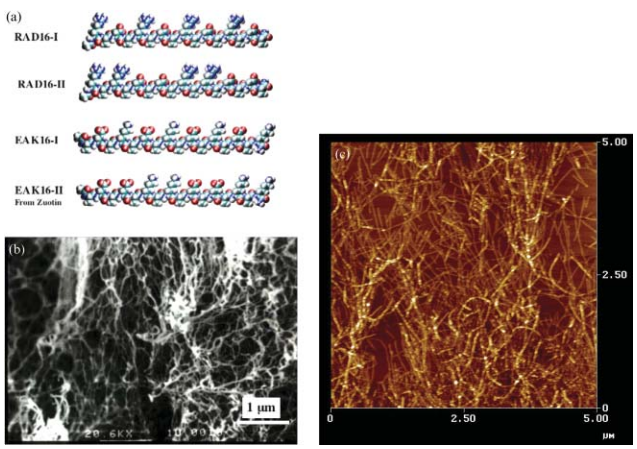


**Fig. 2** A) Molecular models of several self-assembling peptides, RAD16-I, RAD16-II, EAK16-I and EAK16-II. Each molecule is ~5 nm in length with 8 alanines on one side and 4 negatively and 4 positively charged amino acids in an alternating arrangement on the other side. B) SEM image of EAK16-II nanofiber scaffold. Note the nanopores ~5–200 nanometers in diameter, the right pore size for biomolecular diffusion. C) AFM image of RADA16-I nanofiber scaffold (also called PuraMatrix). The nanoscale is in sharp contrast to the microfibers of traditional polymer scaffolds, where the fiber diameter is ~10–50 microns and the pores range from 10–200 microns.

The peptide Lego molecules readily undergo self-assembly in aqueous solutions to form well-ordered nanofibers that further associate to form nanofiber scaffolds with well-ordered nanopores averaging 5–200 nm.[11,13–18] One of them, RADA16-I, has been widely used and commercialized; it is now called PuraMatrix because of its purity as a molecular designer biological scaffold in contrast to other biologically derived scaffolds from animal collagens and Matrigel which contain unspecified components in addition to known materials.

Since these nanofiber scaffolds contain 5–200 nm pores with extremely high water content (~99.5% or 5 mg/ml w/v), they were used for the preparation of three-dimensional (3-D) cell-culture media.[19,20] These scaffolds closely mimic the porosity and gross structure of extracellular matrices, not only allowing cells to reside and migrate in a 3-D environment, but also allowing molecules, such as growth factors and nutrients, to diffuse in and out very slowly;[21] therefore, these peptide scaffolds are ideal materials for 3-D cell culture, controlled cell differentiation, regenerative medicine and slow drug release applications.[14–26]

## Lipid-like self-assembling peptides

Inspired from building blocks of cell membranes using Nature's lipids as a guide, we also designed a class of lipid-like self-assembling peptides with hydrophobic tails and hydrophilic heads that all undergo self-assembly in water.[27–30] These peptides have tunable hydrophobic tails with various degrees of hydrophobicity, a hydrophilic head, and either negatively charged aspartic and glutamic acids or positively charged lysine, histidine or arginine (Fig. 3). The individual peptides contain 7 to 8 amino acid residues with a hydrophilic head composed of aspartic acid and a tail of hydrophobic amino acids such as alanine, valine or leucine. The length of each peptide is ~2.5 nm, similar to that of natural phospholipids.[27–30] However, the peptide length can also be

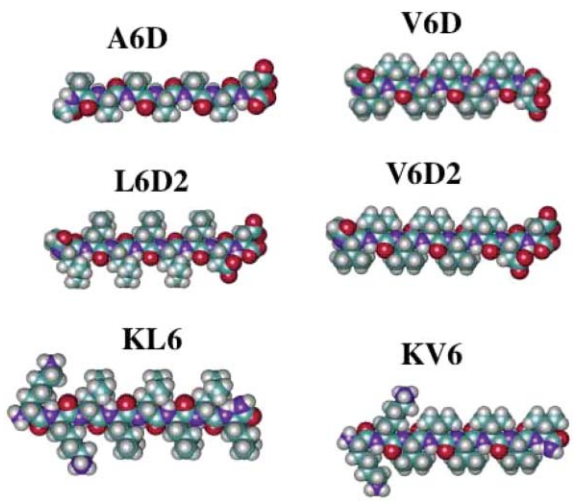


**Fig. 3** Molecular models of lipid-like peptides. $A_6D$, $V_6D$, $V_6D_2$ and $L_6D_2$. $KKL_6$, $KKV_6$. D (Aspartic acid) bears negative charges, K (lysine) bears positive charge, A (alanine), V (valine) and L (leucine) constitutes the hydrophobic tails with increasing hydrophobicity. Each peptide is about 2–3 nm in length, similar to biological phospholipids. Color code: carbon, green; hydrogen, white; red, oxygen; and blue, nitrogen.

fine-tuned by adding or removing amino acids one at a time to a desired length.

Although individually these lipid-like peptides have completely different composition and sequences, they share a common feature: the hydrophilic heads have 1–2 charged amino acids and the hydrophobic tails have four or more consecutive hydrophobic amino acids. For example, $A_6D$ (AAAAAAD), $V_6D$ (VVVVVVD) peptides have six hydrophobic alanine or valine residues from the N-terminus followed by a negatively charged aspartic acid residue, thus having two negative charges, one from the side chain and the other from the C terminus. In contrast, $K_2V_6$ (KKVVVVVV) has two positively charged lysines as the hydrophilic head, followed by six valines as the hydrophobic tail.[27–30] Both heads and tails can be finely tuned with a wide spectrum of chemical properties of the 20 natural amino acids. In addition, there are hundreds of artificial amino acids that can also be used to design the lipid-like peptides. Furthermore, we can mimic phospholipid even more closely using phosphoserine as the hydrophilic heads and alanine or valine as hydrophobic tails, pSAAAAAA ($pSA_6$), pSVVVVVV ($pSV_6$). They also exhibited similar self-assembly behaviors to phospholipids, with distinct critical aggregation concentrations, forming well-ordered nanostructures.[31] They represent another class of designer, lipid-like self-assembling peptides.

In the homogeneous population of the lipid-like peptides and absence of proteins, these peptides, like lipids, undergo self-assembly in water to form nanotubes and nanovesicles with an average diameter of 30–50 nm.[27–30] The tails consisting of alanine and valine produce more homogeneous and stable structures than those of glycine, isoleucine and leucine. It is plausible that this self-assembling behavior may be due to their hydrophobic and hydrophilic ratios. These monomer, lipid-like peptides were used for molecular modeling (Fig. 3). The negatively charged aspartic acid is modeled as red, positively charged lysine is blue, and the hydrophobic tails are green. Numerous such lipid-like peptides can readily self-assemble into dynamic tubular and vesicle structures.

Quick-freeze/deep-etch sample preparation where the sample was instantly flash-frozen at −190 °C produced a 3-D structure with minimal structural disturbance. It revealed a network of open-ended nanotubes observed under transmission electron microscopy (Fig. 4A).[27–30] Over time, there seems to be dynamic molecular behavior. Likewise, $A_6K$ cationic peptides also exhibited similar nanotube structures with the opening ends clearly visible (Fig. 4B).

It is of great interest that these simple lipid-like peptides readily produce remarkable complex and dynamic structures (Fig. 5). If we can fully understand the correlation of their chemical properties and self-assembling behaviors, we will then be able to gain freedom to build materials from the bottom up.

How could these simple lipid-like peptides form such well-structured nanotubes and nanovesicles? It seems there are striking molecular and chemical similarities between some single-tail lipids and the lipid-like peptides, since both have a hydrophilic head and a hydrophobic tail. However, the structural packing between lipids and peptides is likely to be quite different. In lipids, the hydrophobic tails pack tightly against each other to completely displace water, precluding the formation of hydrogen bonds. On the other hand, in addition to hydrophobic tail packing between the amino acid side chains, the lipid-like peptides also interact through intermolecular hydrogen bonds along their backbone.

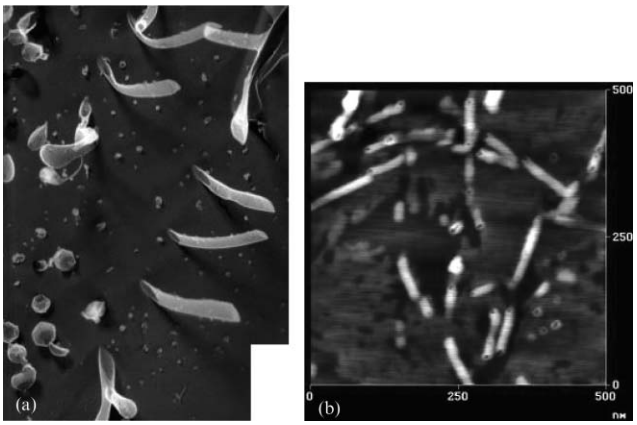


**Fig. 4** Quick-freeze/deep-etch TEM image of $V_6D$ dissolved in water (4.3 mM at pH 7) at high-resolution and AFM image of $A_6K$. A) The TEM images show the dimensions, ~30–50 nm in diameter with openings of nanotube ends. Note opening ends of the peptide nanotube may be cut vertically. The strong contrast shadow of the platinum coat also suggests a hollow tubular structure. There are openings at the ends with the other ends possibly buried. The diameter of the $V_6D$ nanotubes is ~30–50 nm. B) The nanotubes of $A_6K$ lipid-like peptide. Note the unmistakable openings at the end of the nanotubes.

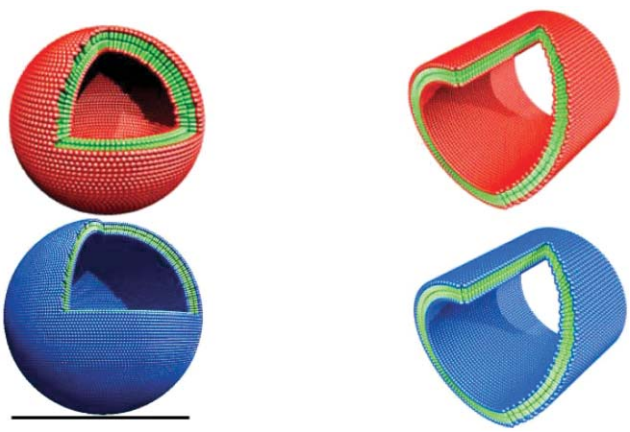

**Fig. 5** Molecular models of lipid-like peptide nanostructures. The bilayer structures were determined by neutron scattering with an estimated thickness of 5 nm.[32] Color code: hydrophobic tail, green; red, negatively charged head ($V_6D$); and blue, positively charged head ($V_6K$, or $KV_6$). The scale bar is 50 nanometers.

**Lipid-like peptides stabilize diverse membrane proteins.** To our great delight, we found that in the presence of membrane proteins, these lipid-like peptides cannot only solubilize, stabilize, and maintain the functions of several membrane proteins,[33–36] but also crystallize a membrane protein glycerol-3-phosphate dehydrogenase (GlpD).[37] These designer lipid-like peptides may now open a new avenue to overcome one of the biggest challenges in structural biology:[38,39] to obtain high-resolution structures of membrane proteins.


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Study of membrane proteins will not only enrich and deepen our knowledge of how cells communicate with their surroundings (the response of all living systems to their environments), but also these membrane proteins can be used to fabricate the most advanced molecular devices, such as energy harnessing devices, extremely sensitive sensors, medical detection devices, and other applications we can't now even imagine.

Membrane proteins 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. However, membrane proteins are extremely difficult to work with and their high-resolution structural determinations lag far behind those of soluble protein structures. We found that simple lipid-like peptides are excellent materials to solubilize and stabilize these proteins. For example, a membrane protein glycerol-3 phosphate dehydrogenase (GlpD) with 6 transmembrane spanning redox enzymes[35] was crystallized in a very short time.[40] The lipid-like peptides work similarly as other chemical surfactants that encapsulate and protect membrane proteins from undesirable self-aggregation as schematically illustrated in Fig. 6. This finding encouraged us to tackle the structures of membrane proteins and turned our attention toward one of the biggest challenges in biology for the next few decades.[38,39]

## Other peptide construction motifs as material building blocks

Aggeli and colleagues reported formation of nanofibers self-assembled from several peptides that lead to $\sim$10 nm fibrils with various extents of left-handed helical twist.[41]

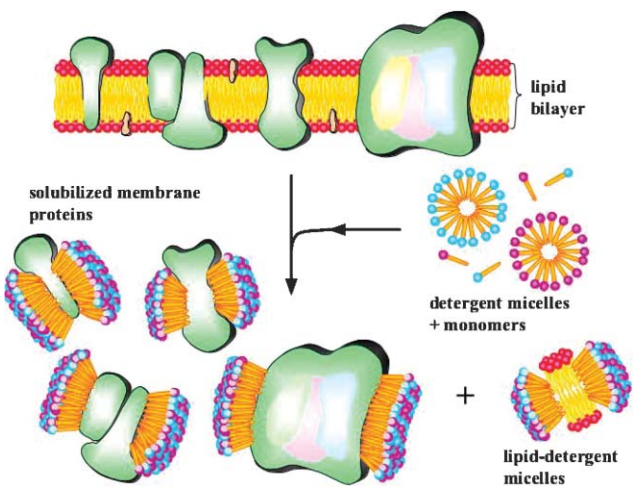


**Fig. 6** A proposed scheme for how the designer lipid-like peptides stabilize membrane proteins. These simple designer self-assembling lipid-like peptides have been used to solubilize, stabilize and crystallize membrane proteins. These peptides have a hydrophilic head and a hydrophobic tail, much like other biological lipids. They use their tail to sequester the hydrophobic part of membrane proteins, and the hydrophilic heads are exposed to water. Thus, they make membrane proteins soluble and stable outside of their native cellular lipid milieu. These lipid-like peptides are very important for overcoming the barrier to obtaining high resolution molecular structures for challenging membrane proteins.

Reches and Gazit demonstrated that the shortest peptide, a Phe–Phe dipeptide, can form stable nanotubes.[42] By diffusing silver ions into the well-formed tubes, then removing the peptide either enzymatically, chemically, or through heat burning, a silver wire was revealed.[42]

Amyloid protein nanofibers have also been used as scaffolds to align gold nanocrystals. Scheibel and colleagues reported that a bioengineered prion-determining (NM) domain of yeast prion protein Sup35 provided a scaffold for fabricating nanowires. They also tested the resulting wires' conducting capability.[43]

## Perspectives in chemistry and materials biotechnology

The future of chemistry and materials biotechnology is bright. The development of designer self-assembling peptide biological materials will in turn broaden the questions we address, thereby deepening our understanding of seemingly intractable biological phenomena. The designer self-assembling peptide systems will create many new classes of wide length scale materials from the molecular scale up and will have a broad and high impact in emerging fields.

However, big challenges still remain. For example, synthetic peptides are currently expensive as materials for widespread use. Although peptide cost has been decreasing steadily, currently \$100–\$200 per gram, it is still beyond the affordability of most industries. The cost bottleneck must be overcome. New innovative synthesis technologies including novel chemistry of peptide synthesis and cell-based, large-scale production will play an increasingly important role for wider applications of self-assembling peptide materials. It is one thing to publish a paper for a new discovery, but it is entirely another to spur a new industry.

It is believed that these simple and versatile self-assembling peptides will provide us with new opportunities to study complex and previously intractable biological phenomena. Molecular engineering through designer self-assembling peptides is an enabling technology that will likely play an increasingly important role in the future of chemical biology and will likely change our lives in the coming decades.

Since we started our serendipitous journey of working on various self-assembling peptide systems, we have encountered many surprises, from developing a class of pure peptide nanofiber scaffolds for 3-D tissue culture[19,44] and for regenerative medicine,[15–26] to finding lipid-like peptides[27–30] that solubilize, stabilize and crystallize membrane proteins,[33–36] to studying the model system of protein conformational diseases.[45–47] 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*".

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