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Lipid-like Self-Assembling Peptides

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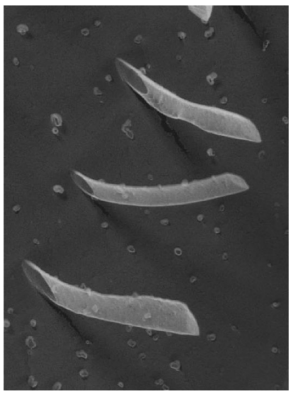
CONSPECTUS



One important question in prebiotic chemistry is the search for simple structures that might have enclosed biological molecules in a cell-like space. Phospholipids, the components of biological membranes, are highly complex. Instead, we looked for molecules that might have been available on prebiotic Earth. Simple peptides with hydrophobic tails and hydrophilic heads that are made up of merely a combination of these robust, abiotically synthesized amino acids and could self-assemble into nanotubes or nanovesicles fulfilled our initial requirements. These molecules could provide a primitive enclosure for the earliest enzymes based on either RNA or peptides and other molecular structures with a variety of functions.

We discovered and designed a class of these simple lipid-like peptides, which we describe in this Account. These peptides consist of natural amino acids (glycine, alanine, valine, isoleucine, leucine, aspartic acid, glutamic acid, lysine, and arginine) and exhibit lipid-like dynamic behaviors. These structures further undergo spontaneous assembly to form ordered arrangements including micelles, nanovesicles, and nanotubes with visible openings. Because of their simplicity and stability in water, such assemblies could provide examples of prebiotic molecular evolution that may predate the RNA world. These short and simple peptides have the potential to self-organize to form simple enclosures that stabilize other fragile molecules, to bring low concentration molecules into a local environment, and to enhance higher local concentration. As a result, these structures plausibly could not only accelerate the dehydration process for new chemical bond formation but also facilitate further self-organization and prebiotic evolution in a dynamic manner.

We also expect that this class of lipid-like peptides will likely find a wide range of uses in the real world. Because of their favorable interactions with lipids, these lipid-like peptides have been used to solubilize and stabilize membrane proteins, both for scientific studies and for the fabrication of nanobiotechological devices. They can also increase the solubility of other water-insoluble molecules and increase long-term stability of some water-soluble proteins. Likewise, because of their lipophilicity, these structures can deliver molecular cargo, such as small molecules, siRNA, and DNA, in vivo for potential therapeutic applications.

Introduction

A Simple Question: What Is the Simplest Enclosure System in Water in a Prebiotic Environment? In June 1992 at the "Origin of Life" Gordon Conference in New Hampshire, almost all active players in the origin of life field in the world gathered. The participants include scientists from diverse fields: astronomy, atmospheric science, geology, geochemistry, organic chemistry, biochemistry, and molecular biology, as well as patent law. They included the legendary Stanly Miller and Leslie Orgel, as well as leading figures Jack Szostak, Gerry Joyce, Christopher Chyba, James Ferris, André Brack, Günter von Kiedrowski, and Gunter Wächtershäuser.

There was then much excitement about RNA since RNA was found to catalyze self-splicing and self-replicating and take part in a wide range of other biological actions that were seemingly impossible to imagine even in early 1980s. There was a sense of overturning the protein dominant world, as it was called the RNA World.

I at the meeting openly asked two seemingly very simple questions that did not directly related to protein or RNA. The two questions were (1) what could be the simplest amphiphilic biomolecules that could self-organize to enclose other biological molecules in a primarily aqueous prebiotic environment and (2) could such structures be made and self-assembled from the simplest of amino acids?

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FIGURE 1. The lipid-like peptides. These peptides have a hydrophilic head and a hydrophobic tail, much like lipids or detergents. They sequester their hydrophobic tail inside of micelles, vesicles, or nanotube structures, and their hydrophilic heads are exposed to water. At least three kinds molecules can be made, with −, +, or ∓ heads and in two orientations.

I reasoned that the prebiotic enclosure biopolymers could neither be phospholipids nor nucleic acids because they are relatively complex multicomponent molecules containing several distinctive parts.[1,2] Furthermore, it takes much chemical energy to make a lipid molecule with a long chain to form reduced carbons. Similarly, nucleic acids are complex molecules themselves and may not be able to form tight enclosures, like a membrane system. Likewise, proteins as we know them today seem to be unlikely actors in the prebiotic environment because polymerization of any specific sequence from a "soup" of possible monomers encounters unavoidable problems of combinatorial mathematics. In short, abiotic syntheses of all three types of complex molecules in the prebiotic environment seem rather unlikely.

I therefore asked whether it was plausible that the simplest peptides with hydrophobic tails and hydrophilic heads (Figure 1), comprising just two or three kinds of the simple amino acids, as short as a few amino acids, could function in this simplest enclosure-forming capacity. Although several groups had studied the chemistry of various amino acids and amino acid biopolymers,[3−6] distinctive enclosure structures were then not reported in the literature.

Simple Amino Acids and Their Condensation under Prebiotic Conditions

Glycine (side chain R = H), alanine (R = CH_3), and aspartic acid (R = CH_2COOH) are among the chemically and structurally simplest amino acids. They are of particular interest to prebiotic molecular evolution because of their presence not only in the products of biochemical simulations of Earth's presumed prebiotic environment,[7−10] but also in the CI-type carbonaceous chondrites, including Orgueil, Ivuna, and Murchison meteorites.[11−15] Specifically, glycine is the simplest possible amino acid, and it is an achiral molecule without any true side chains and is presumed to be the most abundant amino acid in abiotic environments.

Beyond synthesis of the amino acids themselves, it has been experimentally demonstrated that amino acids and their derivatives can form peptides when subjected to repeated hydration−dehydration cycles under microwave heating, in aqueous ammonia, or on heated clays that mimic various hypothesized conditions of early life on the planet.[6,16,17] Indeed, oligoglycine appears to be produced abiotically, as it has been synthesized by subjecting glycine monomers to ∼40 h of supercritical water conditions at 270 °C and high-pressure at 10 MPa.[18]

These high-temperature and high-pressure conditions are similar to those found in deep-sea volcanoes and hydrothermal vents, a favorite hypothesized venue for the origin of life. It is plausible that some of the simplest biochemical building blocks could have produced complex life forms over eons of natural selection and evolution. The challenge, however, is to explain how sufficiently complex proteins or ribozymes could have been produced in the lipid membranes necessary for the metabolism of their own catalysis and reproduction.

If, instead of lipid membranes, simple peptides with hydrophobic tails and hydrophilic heads that are made up of merely a combination of these robust, abiotically synthesized amino acids could self-assemble into nanotubes or nanovesicles, they would have the potential to provide a primitive enclosure for the earliest RNA-based[19,20] or peptide enzymes and other molecular structures with a variety of functions.

In other words, if such structures could be demonstrated to exist, this would provide a plausible idea that in the prebiotic world, these lipid-like peptides of various lengths could form and self-organize into distinct vesicles and tubes, which could act as naturally formed enclosures, isolated from the environment, for prebiotic rudimentary enzymes and ribozymes to accumulate. From this starting point, it is far easier to envision how a diverse population of peptides and RNA could not only condense into complex structures but also increasingly evolve into sophisticated systems, stimulate their own synthesis, and replicate and evolve ultimately into the wondrously efficient chemical and biological catalysts ubiquitous today.[21,22]

Lipid-like Peptides

A class of simple amphiphilic lipid-like peptides that consist of plausible prebiotic amino acids was designed (Figure 2). The first peptide, Ac-AAAAAAD-OH, was designed with computer modeling, linking amino acids together one at a time to achieve a similar length and shape to lipids. This class of these molecules comprises peptides that exhibit lipid-like or surfactant properties.[23−27]

Not only do the shape and physical structure of these lipid-like peptides resemble lipids and other organic surfactants, but

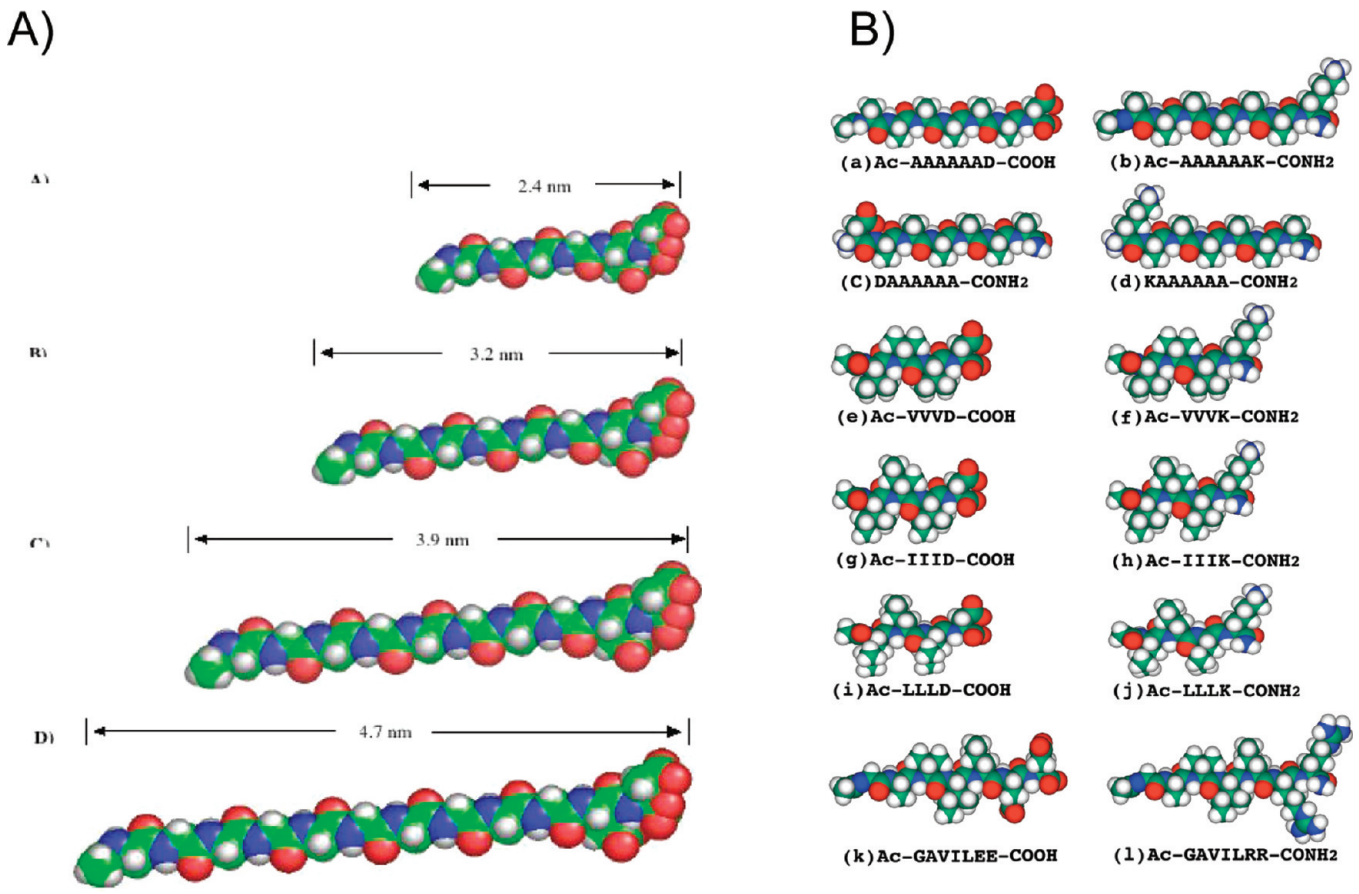

FIGURE 2. Molecular models of peptide detergents at neutral pH. (A) Molecular structures of individual glycine tail-based surfactant peptides: (a) G_4D_2, (b) G_6D_2, (c) G_8D_2, and (d) $G_{10}D_2$. The tail length of glycines varies depending on the number of glycine residues. The lengths of these molecules in the extended conformation range from 2.4 nm for G_4D_2 to 4.7 nm for $G_{10}D_2$. (B) Other amino acid-based tails: (a) Ac-AAAAAAD-COOH; (b) Ac-AAAAAAK-$CONH_2$; (c) DAAAAAA-$CONH_2$; (d) KAAAAAA-$CONH_2$; (e) Ac-VVVD-COOH; (f) Ac-VVVK-$CONH_2$; (g) Ac-IIID-COOH; (h) Ac-IIIK-$CONH_2$; (i) Ac-LLLD-COOH; (j) Ac-LLLK-$CONH_2$; (k) Ac-GAVILEE; (l) Ac-GAVILRR.[43] Aspartic acid (D) is negatively charged, and lysine (K) is positively charged. The hydrophobic tails of the peptide detergents consist of alanine (A), valine (V), isoleucine (I), and leucine (L). Each peptide is ~2−2.5 nm long, similar in size to biological phospholipids. Color code: teal, carbon; red, oxygen; blue, nitrogen; and white, hydrogen.

TABLE 1. A List of Ultrasmall Peptides[a]

head group	heptamer	hexamer	pentamer	tetramer	trimer
aspartic acid (D)	LIVAGDD	**LIVAGD***, ILVAGD*, LIVAAD, LAVAGD, **AIVAGD***	LIVAD, LIVGD	IVAD*, IIID	**IVD**, IID
glutamic acid (E)	LIVAGEE	LIVAGE *			
lysine (K)		LIVAGK		IIIK	
serine (S)		LIVAGS*, ILVAGS, AIVAGS			
threonine (T)		LIVAGT, AIVAGT			

[a]A group of 27 peptides that self-assembled to ordered supramolecular networks, resulting in hydrogel formation. All peptides were acetylated at the N-terminus, while the carboxyl group at the C-terminus was unchanged, except for LIVAGK, which was amidated to suppress the charge at the C-terminus. The D-isoform of some peptides (marked with *) were also studied. Peptides that were investigated more extensively are shown in bold, namely, LIVAGD (also named Ac-LD_6), AIVAGD (also named Ac-AD_6), and IVD (also named Ac-ID_3). From Hauser et al., 2011.[28] Reprinted with permission from PNAS.

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; V_6D Ac-VVVVVVD); they possess two negative charges, one from the charged terminal side chain and the other from the C terminus.[23] In contrast, several simple peptides, G_4DD (Ac-GGGGDD), G_6DD (Ac-GGGGGGDD), G_8DD (Ac-GGGGGGGGDD), have four, six, or eight glycines, followed by two asparatic acids with three negative charges.[24] Similarly, Ac-A_6K (Ac-AAAAAAK-NH_2) or KA_6 (KAAAAAA-NH_2) has six alanines as the hydrophobic tail and a positively charged lysine as the hydrophilic head.[25]

Charlotte A. E. Hauser designed a unique class of ultrasmall peptides as small as only three amino acids, Ac-IVD

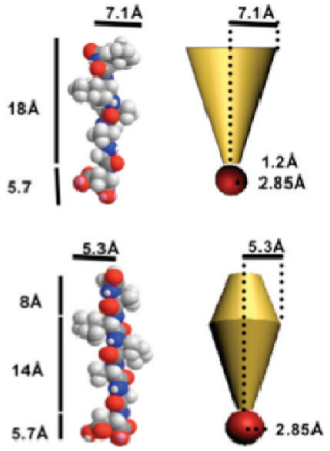

FIGURE 3. Molecular models of the ultrasmall self-assembling peptides and dimensions. Schematic representation of the peptide motif Ac-LIVAGD (Ac-LD6) and Ac-AIVAGD (Ac-AD6). From Mishra et al.[29] Reprinted with permission from Elsevier.

and Ac-IID (~1 nm), or four amino acids, Ac-IVAD, Ac-IIID and Ac-IIIK (Table 1; Figure 3). These peptides readily undergo self-assembly to form stable and well-ordered nanostructures.[28−30] Despite their small size, these peptides show a secondary conformational transition from structurally unorganized monomers into metastable α-helical intermediates that terminate in cross-β structures. The peptides have a characteristic sequence motif that consists of an aliphatic amino acid tail of decreasing hydrophobicity capped by a polar head, which makes them amphiphilic. The self-assembled nanostructures formed by this peptide class include long helices, straight fibers, and hollow nanospheres that could form the simplest enclosures in the prebiotic environment.[28−30] In some cases, these peptide form the shortest helical structure and very stable and strong hydrogels.[28−30] Although individual chemical species within this population of peptides have completely different composition and sequence, they share a common feature: a hydrophilic head comprising one or two charged amino acids and a hydrophobic tail comprising two or more consecutive hydrophobic amino acids (Figure 3; Table 1). Furthermore, noncharged hydrophilic heads using serine and threonine have also been made (Table 1), in their possession of a hydrophobic tail and a hydrophilic head without charges.[28−30]

Self-Organized Nanotubes, Nanovesicles, and Other Nanostructures

These lipid-like peptides self-organize in water to form well-ordered nanostructures, which include micelles, nanotubes, and nanovesicles (Figures 4−6). Furthermore, the structure formation is concentration-dependent, namely, at low concentration, there are no defined structures, these structures spontaneously assemble at a critical aggregation concentration (CAC),[26−30] and they behave similarly to lipids and other surfactants.

Five amino acids of varying hydrophobicity (Gly, Ala, Val, Ile, and Leu) have been used for the nonpolar tails. Such hydrophobic tails are two to six residues so that the total size of the peptide detergents is between three and seven amino acids, about 1−2.4 nm in length. Interestingly, 2.4 nm is a similar size to that of the phospholipid, which is very abundant in cell membranes. The common lipid bilayer membrane is ~5 nm, and the hydrophobic part is ~4.8 nm.

The first lipid-like peptide was designed by modeling the Ac-A_6D-OH peptide using phosphatidylcholine as a size guide. However, when peptides with more than six hydrophobic residues (except glycine) were synthesized, the peptides become less and less soluble in water. We used aspartate (−), glutamate (−), and lysine (+), arginine (+), and histidine (+) as the hydrophilic head groups; they can also be used in various combinatorial ways. Therefore, they can broaden the range of fine variations and increase the possible number of peptides.

Bucak and colleagues used small-angle X-ray scattering and cryo-SEM image to study AAAAAAK and found that above its critical concentration (CAC), A_6K self-assembles into several-micrometer-long hollow nanotubes with a monodisperse cross-sectional radius of 26 nm. Because the peptides carry a positive charge, the nanotubes are charge-stabilized. Because of the very large aspect ratio, the tubes form an ordered phase that presumably is nematic, suggesting the favorable self-assembly of antiparallel peptide dimers into β-sheet ribbons that wrap helically to form the nanotube wall.[31,32]

Moreover, similar to the dynamic behavior of phospholipid vesicles and other microstructures,[33] these simplest of peptide nanostructures appear to behave as dynamic entities in water—they fuse, divide, and change shape as a function of time and environmental influence (Figures 4−6).[23−30]

Cones and Other Shapes of Peptides

It is known that lipids have different shapes depending on their aliphatic chains, some with straight aliphatic chains and some with bent shapes with unsaturated chains. These lipids can make curvatures of a variety of cellular structures.

Inspired by natural lipid shapes, I also designed some cone-shaped lipid-like peptides. For example, Ac-GAVILRR-NH_2 has a cone shape, with the largest part made of arginine and smallest part glycine (Figure 7).[34] Thus this peptide

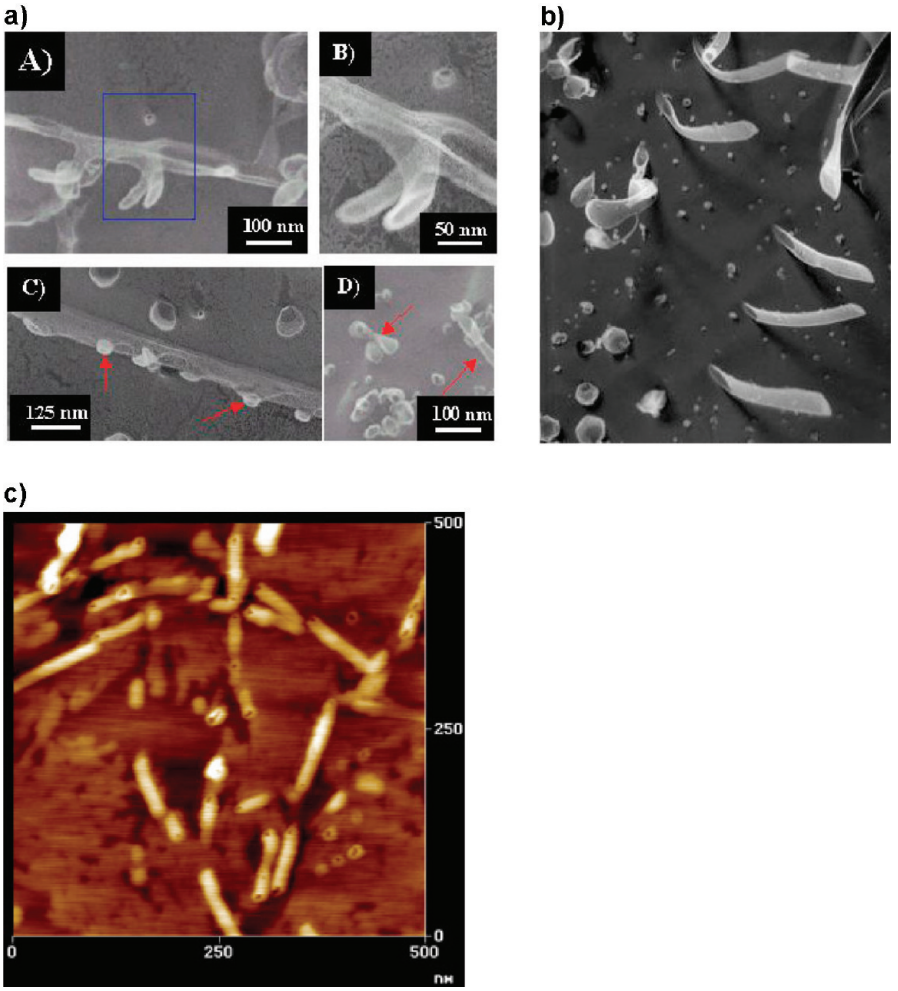

FIGURE 4. (A, B) High-resolution TEM images of G_6D_2 showing different structures and dynamic behaviors of these structures: (A) (a) A pair of finger-like structures branching off from the stem. (b) Enlargement of the box in image a, the detail opening structures are clearly visible. (c) The openings (arrows) from the nanotube which may have resulted in the growth of the finger-like structures. Some nanovesicles are also visible. (d) The nanovesicles may undergo fission (arrows). (b) Nanotubes and nanovesicles of lipid-like peptide ac-VVVVVVD-OH in water. Micelles are also present. Examples of vesicles budding off of a nanotube are included. Image courtesy of Dr. Steve Yang. (C) AFM image of nanotubes of A_6K lipid-like self-assembling peptides. When the solution pH is less than the lysine pK_a of 10, the peptide bears a positive charge. The openings of peptide nanotubes are clearly visible. These nanotube structures can also undergo structural changes depending on various conditions, particularly pH changes, ionic strength of salts, temperature, and incubation time. The other sheet-like materials are likely the unassembled peptides at the time of image collection.

formed a structure with curvature when it self-organized into nanostructures in water. A donut-shaped structure has been observed under AFM (Figure 8). Several cone-shaped peptides from Hauser's laboratory, such as Ac-LIVAGK-NH_2, Ac-LIVAGEE-OH, Ac-LIVAGD-OH, and Ac-AIVAGD-OH, self-assemble into nanospheres and other interesting nanostructures, thus further broadening the repertoire (Figure 3).[28−30]

Synergistic Self-Assembly of Lipid-like Peptides

The lipid-like peptides have two different charges of head groups, one positively charged with lysine and arginine or histidine, the other negatively charged with aspartic acid or glutamic acid.[23−30,35] When the lipid-like peptides with positively and negatively charged peptides are combined together, they further interact to form some interesting structures; however individually they do not form such structures.[36] The synergistic assembly is worth further study since in nature, there are always molecular complexes and multiple molecular interactions.

Lipid-like Peptides Interact with Lipid and SDS

Since the lipids and the lipid-like peptides share many common chemical and structural features, we asked whether the lipid-like peptides interact with lipids. Experiments with monoolein showed close interactions of these

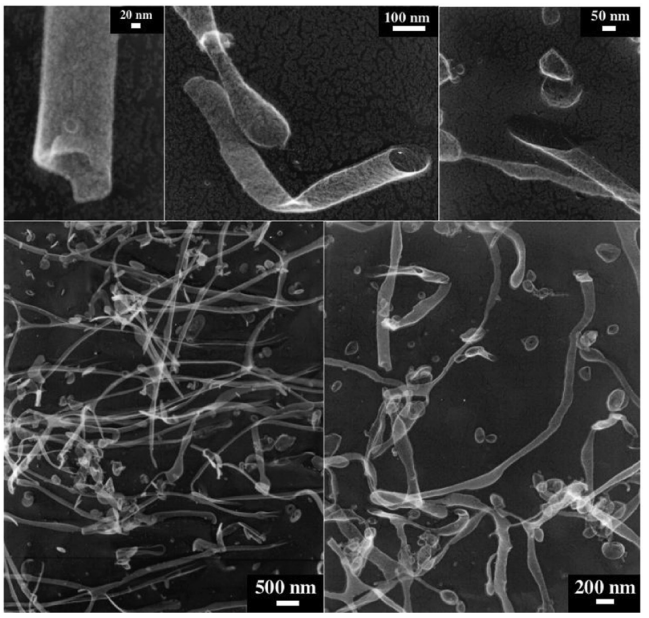

FIGURE 5. Quick-freeze/deep-etch TEM images of pSA6. Upper panels show examples of several individual nanotubes. The nanotube openings are clearly visible. A magnified image shows the budding vesicles. Lower panels give an overview of two different regions of the areas, showing the presence of nanotube and nanovesicle structures. The areas with many budding vesicles can be discerned.

peptides with monoolein.[37] The ternary MO/peptide/water system has been studied using small-angle X-ray scattering (SAXS), within a certain range of peptide concentrations and temperatures (25−70 °C). We demonstrated that the bilayer curvature and the stability are modulated by (i) the peptide/lipid molar ratio, (ii) the peptide molecular structure, namely, the degree of hydrophobicity, the type of the hydrophilic amino acid, and the headgroup location, and (iii) the temperature. The anionic peptide surfactants, Ac-A_6D and DA_6, exhibit the strongest surface activity.

At low peptide concentrations, the *Pn*3*m* structure is still preserved, but its lattice increases due to the strong electrostatic repulsion between the negatively charged peptide molecules, which are incorporated into the interface. This means that the anionic peptides have the effect of enlarging the water channels, and thus they serve to enhance the accommodation of positively charged water-soluble active molecules in the *Pn*3*m* phase.

At higher peptide concentration, the lipid bilayers are destabilized, and the structural transition from the *Pn*3*m* to the inverted hexagonal phase (*H*2) is induced. For the cationic peptides, our study illustrates how even minor modifications, such as changing the location of the headgroup (Ac-A_6K vs KA_6), significantly affects the peptide's effectiveness.[37] Only KA_6 displays a propensity to promote

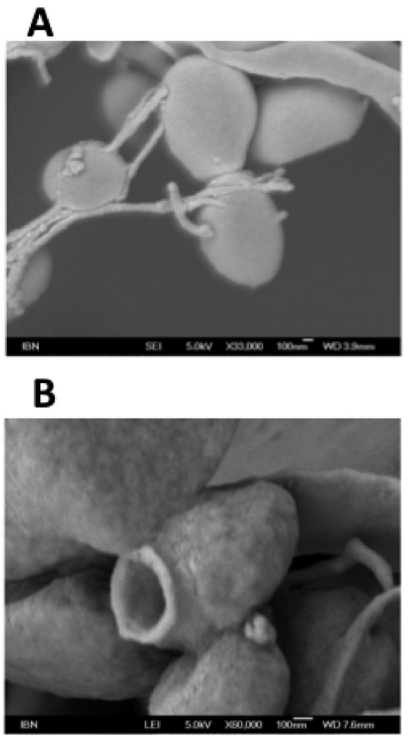

FIGURE 6. Filed emission scanning electron microscope (FESEM) images of different structures formed from two ultrasmall self-assembling peptides: (A) Ac-AIVAGD-OH (5 mg/mL) and (B) Ac-LIVAGD-OH (0.1 mg/mL). From Mishra et al.[29] Reprinted with permission from Elsevier.

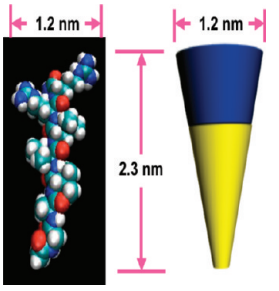

FIGURE 7. Molecular models and self-assembled donut-shaped structure of Ac-GAVILRR-NH_2. The peptide length is approximately 2.3 nm, and the width is 1.2 nm. Color code: hydrogen = white, carbon = cyan, oxygen = red, and nitrogen = blue. The cone-shaped model is simplified for the shape of Ac-GAVILRR-NH_2. The blue part indicates the positively charged hydrophilic region, and the yellow part indicates the hydrophobic region.

the formation of hexagonal phase, which suggests that KA_6 molecules have a higher degree of incorporation in the interface than Ac-A_6K.

Although as short as only seven residues, Ac-A_6K formed stable α-helical structures in sodium dodecyl sulfate (SDS). These short α-helices are able to stabilize α-helical motifs, which are commonly found in transmembrane domains of diverse membrane proteins. Experiments also showed that

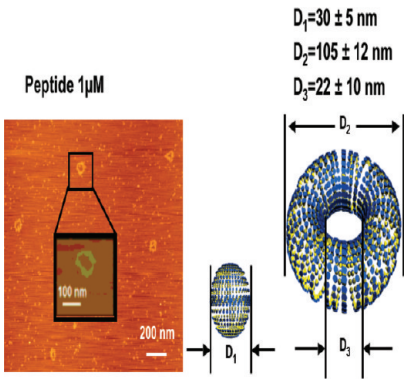

FIGURE 8. AFM image of the donut structure of the self-assembled Ac-GAVILRR-NH_2 is presented. (A) The AFM image of the aggregation structures at 2 μM in water. The square enlarges one of the nanodonut structures. (B) The dimensions of the donut structure.

Ac-A_6D and Ac-A_6K could form β-sheets and appear as hydrogels at higher concentrations. Furthermore, Ac-A_6D and Ac-A_6K together in SDS formed expected β-sheet structures via a surprising α-helical intermediate.[36]

Lipid-like Peptides Stabilize Membrane Proteins

Since they behave like lipids and interact with monoolein lipid and other surfactants, I asked that whether these peptides could stabilize membrane proteins. Our studies using these peptides showed that they indeed stabilized a wide range of membrane proteins (Figure 9) including *Escherichia coli* glycerol-3-phosphate dehydrogenase,[38] the multidomain protein complex photosystem-I (PSI) on a surface in dry form[39] and in aqueous solution,[40] G-protein coupled receptor (GPCR) bovine rhodopsin,[41] and olfactory receptors.[42,43] Furthermore, we have demonstrated that these lipid-like peptides are excellent materials for cell-free production of over 20 GPCRs with milligram yields.[42,43]

The Lipid-like Peptides and Relevance to Prebiotic Molecular Evolution

It is presumably plausible that in the prebiotic world, under the influence of water, lipid-like peptides of various lengths might self-organize into distinct vesicles and tubes regardless of sequences that could enclose prebiotic rudimentary enzymes so as to isolate them from the environment. Thus, a diverse population of peptides and RNA might condense into complex structures that evolve to different functions.[21,22]

The existence of diverse and stable nanostructures demonstrates how biochemical molecular selections could give rise to complex entities, presumably through a process

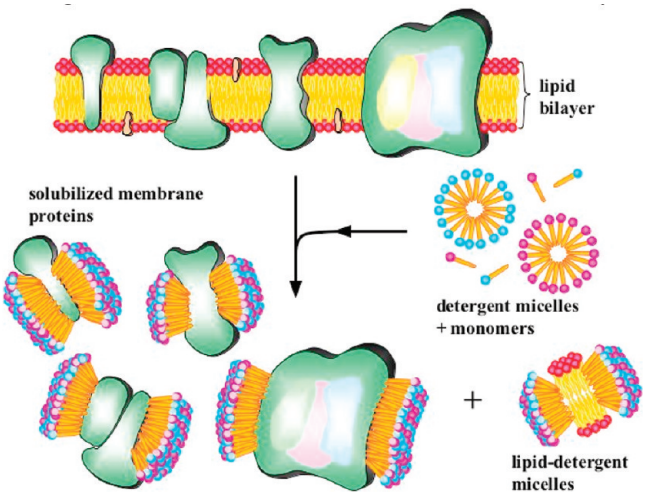

FIGURE 9. A proposed scheme for how the designer lipid-like peptides stabilize membrane proteins. These simple designer self-assembling lipid-like peptides have been used to solubilize, stabilize, and crystallize membrane proteins. These peptides have a hydrophilic head and a hydrophobic tail, much like 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.

of prebiotic molecular evolution applied to primitive, quasi-living autocatalytic networks.

When one considers prebiotic molecular selection and evolution in the context of the origin of life, the enormously powerful force of water must never be underestimated. All life forms as we know are based on water, and all molecules in living systems interact with it; thus water has likely driven molecular evolution from the very beginning, here on earth or plausibly elsewhere in the universe.

Perspective

In science, there are numerous examples of curiosity-driven research and unintentional discoveries that led to technological breakthroughs and new economic development. They include the mad pursuit of the DNA double helix structure, DNA−RNA and RNA−RNA hybridizations, RNA splicing, RNA as enzymes, telomeres, programmed cell death, and the indispensible Worldwide Web. The discovery of the lipid-like peptide is no exception. I started from asking a question about the plausibly simplest enclosure that led to pursue the lipid-like peptides. This unexpected curiosity-driven question later led to the development of surfactants for stabilizing diverse membrane proteins that will be important for design and fabrication of membrane-protein-based molecular devices. Furthermore one of the lipid-like

peptides has been shown to effectively deliver siRNA to treat naturally occurring breast cancers in dogs. Thus research based on curiosity-driven questions must be strongly encouraged and supported despite the current emphasis on application-driven research. The recent successful example of a wide range of applications provides a glimpse of what is coming for widespread uses of lipid-like peptides, from a seemingly simple and curious question about the chemical origin of life.

BIOGRAPHICAL INFORMATION

Shuguang Zhang earned his Ph.D. in Biochemistry & Molecular Biology from University of California at Santa Barbara in 1988. He published over 150 scientific papers in the areas from designer self-assembling peptides to the study of membrane proteins to emerging biosolar energy. He was an American Cancer Society Postdoctoral Fellow and a Whitaker Foundation Investigator at MIT. He is a distinguished Changjiang scholar in China and a 2003 Fellow of Japan Society for Promotion of Science. His work on designer peptide scaffolds won a 2004 R&D100 award. He was a 2006 John Simon Guggenheim Fellow and a winner of the 2006 Wilhelm Exner Medal of Austria. He was inducted as a foreign member of the Austrian Academy of Science in 2010. He is also a Fellow of the American Institute of Medical and Biological Engineering. He founded several biotech startup companies using knowledge from curiosity-driven research.

FOOTNOTES

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The authors declare no competing financial interest.

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