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Review

Emerging biological materials through molecular self-assembly

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Abstract

Understanding of new materials at the molecular level has become increasingly critical for a new generation of nanomaterials for nanotechnology, namely, the design, synthesis and fabrication of nanodevices at the molecular scale. New technology through molecular self-assembly as a fabrication tool will become tremendously important in the coming decades. Basic engineering principles for microfabrication can be learned by understanding the molecular self-assembly phenomena. Self-assembly phenomenon is ubiquitous in nature. The key elements in molecular self-assembly are chemical complementarity and structural compatibility through noncovalent interactions. We have defined the path to understand these principles. Numerous self-assembling systems have been developed ranging from models to the study of protein folding and protein conformational diseases, to molecular electronics, surface engineering, and nanotechnology. Several distinctive types of self-assembling peptide systems have been developed. Type I, ''molecular Lego'' forms a hydrogel scaffold for tissue engineering; Type II, ''molecular switch'' as a molecular actuator; Type III, ''molecular hook'' and ''molecular velcro'' for surface engineering; Type IV, peptide nanotubes and nanovesicles, or ''molecular capsule'' for protein and gene deliveries and Type V, ''molecular cavity'' for biomineralization. These self-assembling peptide systems are simple, versatile and easy to produce. These self-assembly systems represent a significant advance in the molecular engineering for diverse technological innovations.

Keywords: Chemical complementarity; Structural compatibility; Hydrophobic interactions; Molecular engineering; Nanostructures; Biological scaffold

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1. Introduction

Human civilizations are often divided into ages according to the materials that dominate in the society. For example, in the Stone Age, when the tools were largely made of stones, people used those simple stone tools to improve their lifestyle and advanced their civilization. In the Bronze Age, when most tools were made of bronze, a number of technological innovations were fostered and eventually altered the landscape of the civilization that made the Stone Age obsolete. Into the Iron Age, when the processing of iron was discovered, society advanced tremendously. Many new tools were built, so that rapid and farther transportation became possible. People then explored many lands and seas. We have entered the modern materials age, the plastic age in the last half of the 20th century and the silicon age in the last quarter of the 20th century continuing to this day. These new materials have fundamentally transformed our lifestyle forever and made a vast world into a global village. What kinds of materials lie ahead in the future? It is believed that the ''Designed Materials'' will likely play a key role in constructing and processing the future composite and integrated materials. This will be achieved both through discovering nature's designs that have evolved from eons of selection, and through knowledge-based designs. These materials will again undoubtedly transform our civilization as we know it today. These new materials will be the foundation for the future generations of ''Designed Materials Age''. Leonardo da Vinci stated eloquently hundreds of year ago ''What nature finishes to produce its own species, man begins using natural things in harmony with this very nature to create an infinity of species''.

The Designed Materials Age requires new knowledge to build advanced materials. One of the approaches is through molecular self-assembly. Molecular self-assembly is ubiquitous in nature. It has recently emerged as a new approach in chemical synthesis, nanotechnology, polymer science, materials and engineering. Molecular self-assembly systems lie at the interface of molecular and structural biology, protein science, chemistry, polymer science, materials science and engineering. Many self-assembling systems have been developed. These systems range from bi-, triblock copolymers, and complex DNA structures, simple and complex proteins and peptides. Molecular self-assembly systems represent a significant advance in the molecular engineering of simple molecular building blocks useful for a wide range of applications.

2. The basis of self-assembly and molecular self-assembly

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

Molecular self-assembly, by definition, is the spontaneous organization of molecules under thermodynamic equilibrium conditions into structurally well-defined and rather stable arrangements through a number of noncovalent interactions (Whitesides et al., 1991; Lehn,

1993; Ball, 1994). These molecules undergo self-association forming hierarchical structures. The key engineering principle for molecular self-assembly is to artfully design the molecular building blocks that are able to undergo spontaneously stepwise interactions and assemblies through the formations of numerous noncovalent weak chemical bonds. These typically include hydrogen bonds, ionic bonds and van der Waals' bonds (Pauling, 1960) to assemble these molecules into some well-defined and stable hierarchical macroscopic structures. Although each of the bonds is rather weak, the collective interactions can result in very stable structures and materials. The key elements in molecular self-assembly are chemical complementarity and structural compatibility. Like hands and gloves, both the size and the correct orientation, i.e. chirality, are important in order to have a complementary and compatible fitting.

3. Molecular self-assembly in nature

Biomimicry and designing nature-inspired materials through molecular self-assembly is an emerging field in the coming years of the 21st century. Nature is a grand master at designing chemically complementary and structurally compatible constituents for molecular self-assembly through eons of molecular selection and evolution. Chemical evolution from the first groups of primitive molecules through countless iterations of molecular self-assembly and disassembly has ultimately produced more and more complex molecular systems.

In the last decade, considerable advances have been made in the use of peptides, phospholipid and DNA as building blocks to produce potential biological materials for a wide range of applications (Schnur, 1993; Ghadiri et al., 1994; Bong et al., 2001; Zhang et al., 1993, 1995; Holmes et al., 2000; Aggeli et al., 1997, 2001; Alivisatos et al., 1996; Mirkin et al., 1996; Winfree et al., 1998). The constituents of biological origins, such as phospholipid molecules, amino acids and nucleotides have not been generally considered to be useful materials for traditional materials science and engineering. The advent of biotechnology and genetic engineering coupled with the recent advancement in chemistry of nucleic acids and peptide syntheses has resulted in a conceptual change. Molecular self-assembly is emerging as a new route to produce novel materials and to complement other materials, i.e. ceramics, metals and alloys, synthetic polymers and other composite materials. Several recent discoveries and rapid developments in biotechnology, however, have rekindled the field of biological materials engineering (Urry and Pattanaik, 1997; Huc and Lehn, 1997; Petka et al., 1998).

There are ample examples of molecular self-assembly in nature. One of the well-known examples is the silk assembly. The monomeric silk fibroin protein is approximately 1 μm but a single silkworm can spin fibroins into silk materials over 2 km in length, two billion times longer! (Feltwell, 1990; Winkler et al., 1999). Such a marvelous engineering skill can only make us envy. Human ingenuity and current advanced technology is far behind the seemingly easy task by the silkworm. Likewise, spiders are grand master materials engineers who can produce many types of spider silks through self-assembly of the building blocks in a variety of ways, thus, producing spider silk fiber with tremendous strength and flexibility. These building blocks are often at the nanometer scale. However, the resulting materials could be measured at

Table 1
Type I self-assembling peptides studied

Name	Sequence (N → C)	Ionic modulus	Structure
RADA16-I	+ − + − + − + − n-RADARADARADARADA-c	I	beta
RGDA16-I	+ − + − + − + − n-RADARGDARADARGDA-c	I	r.c.
RADA8-I	+ − + − n-RADARADA-c	I	r.c.
RAD16-II	+ + − − + + − − n-RARADADARARADADA-c	II	beta
RAD8-II	+ + − − n-RARADADA-c	II	r.c.
EAKA16-I	− + − + − + − + n-AEAKAEAKAEAKAEAK-c	I	beta
EAKA8-I	− + − + n-AEAKAEAK-c	I	r.c.
RAEA16-I	+ − + − + − + − n-RAEARAEARAEARAEA-c	I	beta
RAEA8-I	+ − + − n-RAEARAEA-c	I	r.c.
KADA16-I	+ − + − + − + − n-KADAKADAKADAKADA-c	I	beta
KADA8-I	+ − + − n-KADAKADA-c	I	r.c.
EAH16-II	− − + + − − + + n-AEAEAHAHAEAEAHAH-c	II	beta
EAH8-II	− − + + n-AEAEAHAH-c	II	r.c.
EFK16-II	− − + + − − + + n-FEFEFKFKFEFEFKFK-c	II	beta
EFK12-I	− + − + − + n-FEFKFEFKFEFK-c	I	beta
EFK8-II	− + − + n-FEFKFEFK-c	I	beta
ELK16-II	− − + + − − + + n-LELELKLKLELELKLK-c	II	beta
ELK8-II	− − + + n-LELELKLK-c	II	beta
EAK16-II	− − + + − − + + n-AEAEAKAKAEAEAKAK-c	II	beta
EAK12	− − − − + + n-AEAEAEAEAKAK-c	IV/II	beta/alpha
EAK8-II	− − + + n-AEAEAKAK-c	II	r.c.
KAE16-IV	+ + + + − − − − n-KAKAKAKAEAEAEAEA-c	IV	beta
EAK16-IV	− − − − + + + + n-AEAEAEAEAKAKAKAK-c	IV	beta
KLD12-I	+ − + − + − n-KLDLKLDLKLDL-c	I	beta

Table 1 (*continued*)

Name	Sequence (N → C)	Ionic modulus	Structure
KLE12-I	+ − + − + − n-KLELKLELKLEL-c	I	beta
RAD16-IV	+ + + + − − − − n-RARARARADADADADA-c	IV	beta
DAR16-IV	− − − − + + + + n-ADADADADARARARAR-c	IV	beta/alpha
DAR16-IV[a]	− − − − + + + + n-DADADADARARARARA-c	IV	beta/alpha
DAR32-IV	− − − − + + + + n-(ADADADADARARARAR)-c	IV	beta/alpha
EHK16	+ − + − + + + + + − + − + + + + n-HEHEHKHKHEHEHKHK-c	N/A	r.c.
EHK8-I	+ − + − + + + + n-HEHEHKHK-c	N/A	r.c.
VE20[a] (NaCl)	− − − − − − − − − − n-VEVEVEVEVEVEVEVEVE-c	N/A	beta
RF20[a] (NaCl)	+ + + + + + + + + + n-RFRFRFRFRFRFRFRFRF-c	N/A	beta

Beta, beta sheet; alpha-helix; r.c., random coil; N/A, not applicable. The numbers that follow the name denotes the length of the peptides.

[a] Both VE20 and RF20 are in beta-sheet form when they are incubated in solution containing NaCl.

meters and kilometer scales. Likewise, the size of individual phospholipid molecules is approximately 2.5 nm in length, but they can self-assemble into millimeter-size lipid tubules with defined helical twist, many million times larger. A number of applications have been developed (Schnur, 1993; Spector et al., 1996). The power of molecular self-assembly can never be underestimated. Molecular self-assembly can also build sophisticated structures and materials. For example, collagen and keratin can self-assemble into ligaments and hair, respectively. In cells, many individual chaperone proteins assembly into a well-defined ring structure to sort out, fold and refold proteins (Sigler et al., 1998). The same is true for other protein systems, such as seashell biomineralization (Morse, 1999; Weiner and Addadi, 1991). Likewise, mammals build their teeth through self-assembly of a protein scaffold that is made of many individual proteins first and recruit calcium ions to the sites for biomineralization.

4. Self-assembling peptide systems

A new class of peptide-based biological materials was serendipitously discovered in yeast (Zhang et al., 1993) from the self-assembly of ionic self-complementary peptides (Zhang et al., 1993). A number of peptide molecular self-assembly systems have been designed and developed (Table 1). This systematic analysis provided insight into the chemical and structural principles of peptide self-assembly. These peptides are short, simple to design, extremely versatile and easy to synthesize. Three types of self-assembling peptides have been

systematically studied thus far. It is believed additional different types will be discovered and developed in the coming years. This class of biological materials has considerable potential for a number of applications, including scaffolding for tissue repair and tissue engineering, drug delivery of molecular medicine, as well as biological surface engineering. Similar systems have also been described wherein these peptide systems undergo self-assembly to form gel with regular beta sheet tapes of well-defined structures (Aggeli et al., 1997, 2001). The self-assembly of peptide nanotubes that allow ions to pass through and to insert themselves into lipid bilayer membranes were also described (Bieri et al., 1999; Fernandez-Lopez et al., 2001). Furthermore, a number of fascinating biomimetic peptide and protein structures have been engineered, such as helical coil–coils, di-, tri- and tetrahelical bundles (O'Shea et al., 1989; Hecht et al., 1990; Baker and DeGrado, 1999). However, their applications for materials science and engineering remain under explored. It is likely that these stable coiled coils will be developed as nanomaterials in the future.

4.1. Type I self-assembling peptides

Type I peptides, also called "molecular Lego", form beta sheet structures in aqueous solution because they contain two distinct surfaces, one hydrophilic, the other hydrophobic.

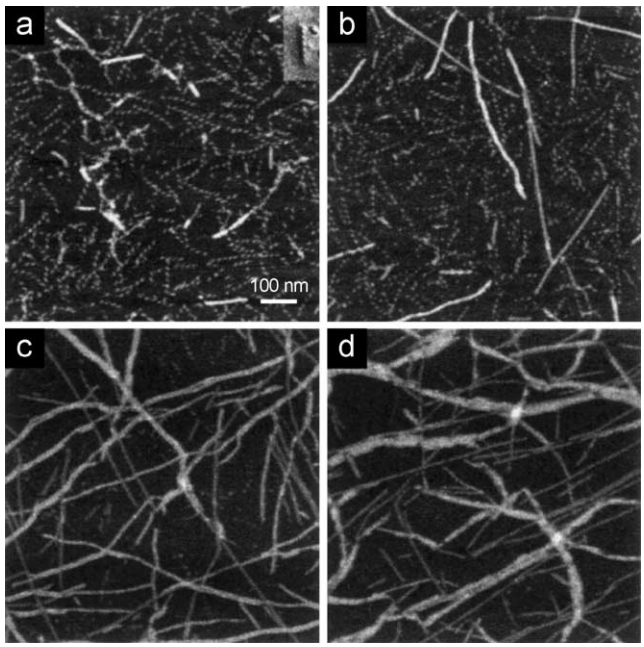

Fig. 1. Intermediate structures in the self-assembly of KFE8 in aqueous solution. Images are AFM scans (the brightness of features increases as a function of height) of a freshly cleaved mica surface over which aliquots taken from solution were deposited at different times after preparation of the solution. (a) After 8 min. Inset: electron micrograph of a sample of peptide solution obtained using the quick-freeze/deep-etch technique; (b) 35 min after preparation; (c) 2 h; (d) 30 h.

Like Lego bricks that have pegs and holes and can only be assembled into particular structures, these peptides can do so at the molecular level. The unique structural feature of these peptides is that they form complementary ionic bonds with regular repeats on the hydrophilic surface (Zhang et al., 1993, 1995). 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, molecules of modulus I have − + − + − + − +, modulus II, − − + + − − + +, modulus, IV − − − − + + + +. These well-defined sequences allow them to undergo ordered self-assembly, resemblance of some situation found in well-studied polymer assemblies.

Upon the addition of monovalent alkaline cations or the introduction of the peptide solutions into physiological media, these oligopeptides spontaneously assemble to form microscopic (Fig. 1) and macroscopic structures that can be fabricated into various geometric shapes (Fig. 2) (Zhang et al., 1995; Holmes et al., 2000). Scanning EM and AFM reveals that the matrices are made of interwoven nanofibers having 10–20 nm in diameter and pores

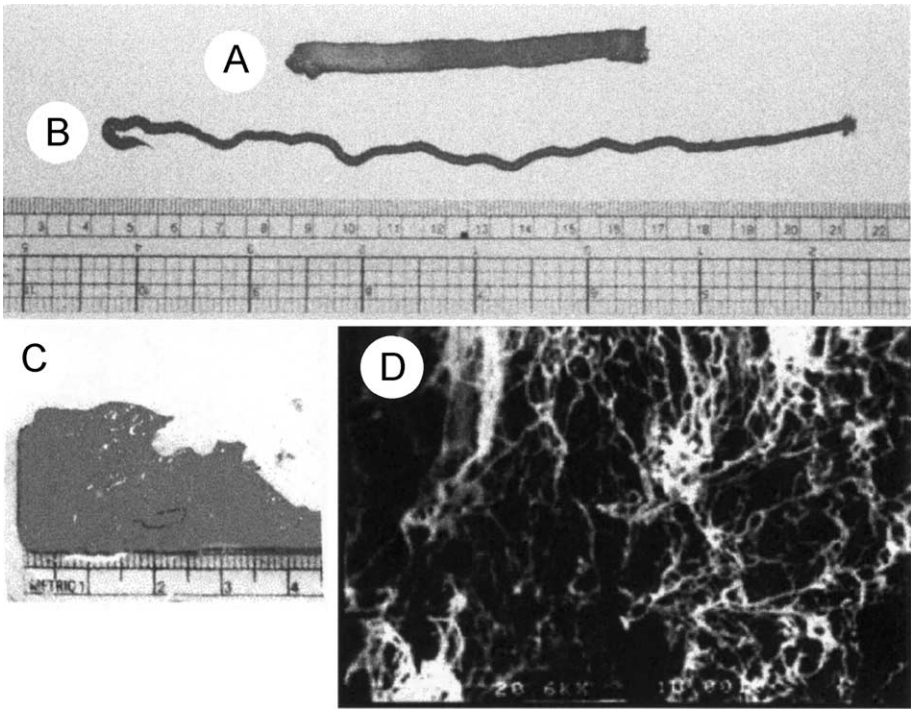

Fig. 2. Hydrogel form of biological materials from self-assembly of the Type I peptides (A–C). (D) The SEM structure of peptide filaments. The material is self-assembled from individual interwoven fibers. The diameter of the fiber is about 10–20 nm and the enclosures are about 50–100 nm. Under high resolution by atomic force microscopy, the filaments are revealed to be a twisted helix with regular helical repeats.

about 50–200 nm in diameter (Zhang et al., 1993, 1995; Leon et al., 1998; Holmes et al., 2000; Caplan et al., 2000; Marini et al., 2002).

The molecular structure and proposed complementary ionic pairings of the Type I peptides between positively charged lysines and negatively charged glutamates in an overlap arrangement are modeled in Zhang et al., 1995. This structure represents an example of this class of self-assembling beta sheet peptides that undergo spontaneous association under physiological conditions. If the charged residues are substituted, i.e. the positive charged lysines are replaced by positively charged arginines and the negatively charged glutamates are replaced by negatively charged aspartates, there are essentially no drastic effects on the self-assembly process. However, if the positively charged resides, Lys and Arg are replaced by negatively charged residues, Asp and Glu, the peptide can no longer undergo self-assembly to form macroscopic materials although they can still form beta-sheet structures in the presence of salt. If the alanines are changed to more hydrophobic residues, such as Leu, Ile, Phe or Tyr, the molecules have a greater tendency

Table 2
Cell attachment to the self-assembling peptide scaffold hydrogel

Cell type	Cell line
Mouse fibroblast	NIH-3T3
Chicken embryo fibroblast	CEF
Chinese hamster ovary	CHO
Monkey kidney cells	Cos7
Human cervical carcinoma	HeLa
Human osteosarcoma	MG63
Human hepatocellular carcinoma	HepG2
Hamster pancreas	HIT-T15
Human embryonic kidney	HEK293
Human neuroblastoma[a]	SH-SY5Y
Rat pheochromocytoma[a]	PC12
Mouse cerebellum granule cells[a,b]	
Mouse and rat hippocampal cells[a,b]	
Rat adult neural stem cells[a,b]	
Rat CNS neural tissues[a,b]	
Rat adult liver stem cells[b]	
Rabbit cardiac myocytes[b]	
Bovine aortic endothelial cells[b]	
Bovine chondrocytes[b] (calf and adult cells)	
Human endothelial cells[b]	
Human foreskin fibroblast[b]	
Human epidermal keratinocytes[b]	

Various cell types attachment or encapsulated to the peptide scaffold. Visual assessment of cell attachment was performed using phase contrast microscopy for over a period of 2 weeks.

[a] Refers to neuronal cells.

[b] Refers to cells derived from primary cultures.

to self-assemble and form peptide matrices with enhanced strength (Leon et al., 1998; Caplan et al., 2001; Marini et al., 2002).

A number of mammalian cell types have been tested and all have been found to form stable attachments with the peptide materials (Table 2) (Zhang et al., 1995). Several peptide materials have been used to test for their ability to support cell proliferation and differentiation. These results suggested that the peptide materials cannot only support various types of cells attachments, but can also allow the attached cells to proliferate and differentiate. For example, rat PC12 cells on peptide matrices were exposed to NGF, they underwent differentiation and exhibited extensive neurite outgrowth. In addition, when primary mouse neuron cells were allowed to attach the peptide materials, the neuron cells projected lengthy axons that followed the specific contours of the self-assembled peptide surface and made active and functional connections (Holmes et al., 2000).

The fundamental design principles of such self-assembling peptide systems can be readily extended to polymers and polymer composites, where copolymers can be designed and produced. Humans have learned a great deal from nature, have gone many steps further and will continue to create new materials.

4.2. Type II self-assembling peptides

Several Type II peptides have been developed as "Molecular Switches" in which the peptides could drastically change its molecular structure (Figs. 3 and 4). One of the peptides with 16 amino acids, DAR16-IV, has a beta-sheet structure at ambient temperature with 5 nm in length but can undergo an abrupt structural transition at high temperatures to form a stable alpha-helical structure with 2.5 nm length (Zhang and Rich, 1997; Altman et al., 2000). Similar structural transformations can be induced by changes of pH. This suggests that secondary structures of some sequences, especially flanked by clusters of negative charges on the N-terminus and positive charges on the C-terminus, may undergo drastic conformational transformations under the appropriate conditions. These findings cannot only provide insights into protein–protein interactions during protein folding and the pathogenesis of some protein conformational disease, including scrapie, prion, kuru, Huntington's, Parkinson's and Alzheimer's diseases, but can also be developed as molecular switches for a new generation of nanoactuators and nanoswitches.

The peptides of DAR16-IV (DADADADARARARARA) and EAK12 (AEAEAEAEAKAK) have a cluster of negatively charged Aspartate glutamate residues close to N-terminus and a cluster of positively charged lysine or arginine residues near C-terminus. It is well known that all alpha-helices have a helical dipole moment with a partial negative C-terminus toward a partial N-terminus (Hol et al., 1981; Hol, 1985). Because of the unique sequence of DAR16-IV and EAK12, their side chain charges balance the helical dipole moment, therefore, favoring helical structure formation. However, they also have alternating hydrophilic and hydrophobic residue as well as ionic self-complementarity, which have been previously characterized to form stable beta-sheets. Thus, the behavior of these Type II molecules is likely to be more complex and dynamic than other stable beta-sheet

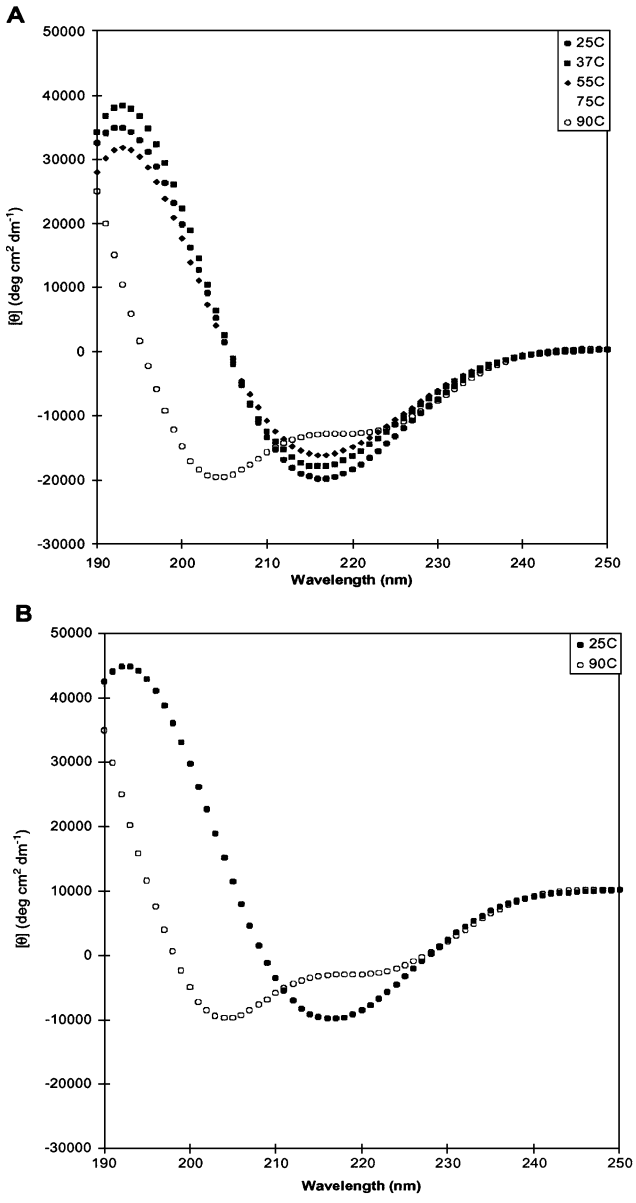

Fig. 3. Type II peptides. Temperature effect on DAR16-IV* structural transition. DAR16-IV* was incubated at various temperatures for 10 min and measured at 25 °C. (A) Structures of DAR16-IV* from 25 to 90 °C. At 25 °C, it forms a stable beta sheet. This beta sheet structure is stable until 75 °C. Here, the beta sheet structure is abruptly converted to an alpha-helical structure with no detectable intermediate. The conversion in DAR16-IV* is much more abrupt than in EAK12-c, as only two distinct structural forms are observed. (B) DAR16-IV exhibits two distinctive structures at two different temperatures, 25 and 90 °C. Note the imperfect isosbestic point at 211 nm, and the sharp contrast between the two peptide structures.

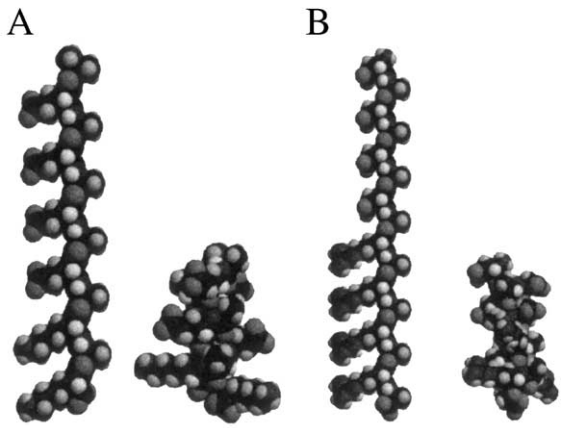

Fig. 4. (A) Molecular models of EAK12 in beta-strand and alpha-helical form. (B) Molecular models of DAR16-IV in beta-strand form and alpha-helical form. The length of the beta-strand is about 5 nm and the length of the alpha-helix is about 2.3 nm.

peptides. Additional molecules with such dipoles have been designed, studied and confirmed the initial findings.

Others have also reported similar findings that proteins and peptides can undergo self-assembly and disassembly or change their conformations depending on the environmental

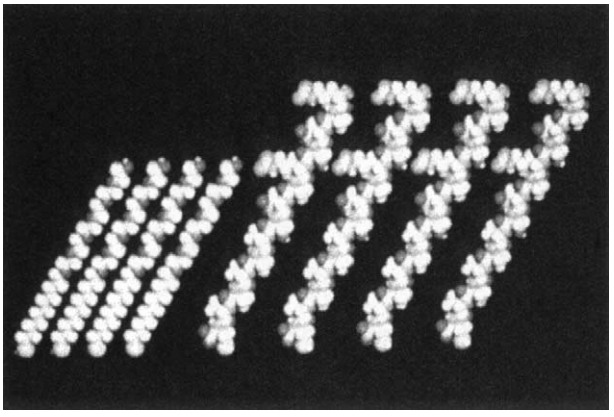

Fig. 5. Self-assembling peptides for biological surface engineering. Molecular models of the Type III oligopeptide RADSC-14 with the sequence RADSRADSAAAAAC (right) and of ethylene glycol thiolate (EG6SH) (left). The N-terminal segment $(RADS)_2$ is the ligand for cell attachment, the five-alanine segment, AAAAA, is a linker to the anchoring cysteine. The cysteine anchor is covalently bound to the gold atoms on the surface. Molecular models are shown on the surface where both molecules form self-assembled monolayers with different heights. The extended lengths of RADSC-14 and EG6SH are approximately 5 and 4 nm, respectively.

influence, such as its location, pH change and temperature or crystal lattice packing (Minor and Kim, 1996; Takahashi et al., 1999; Tan and Richmond, 1998).

4.3. Type III self-assembling peptides

Type III peptides, like "Molecular Paint" and "Molecular Velcro", undergo self-assembly onto surface rather with among themselves. They form monolayers on surfaces for specific cell pattern formations or to interact with other molecules. These peptides have three distinct features (Fig. 5). The first feature is the terminal segment of ligands that incorporate a variety of functional groups for recognition by other molecules or cells. The second feature is the central linker where a variable spacer is not only used to allow freedom of interaction at a specified distance away from the surface but also permit the flexibility or rigidity. The third feature is the surface anchor where a chemical group on the peptide can react with the surface to form a covalent bond. This simple system using Type III self-assembling peptides and other substances to engineer surfaces is an emerging technology that will be a useful tool in biomedical engineering and biology. This biological surface engineering technique will provide new methods to study cell–cell communication and cell behavior (Fig. 6) (Zhang et al., 1999).

Similar kinds of molecular self-assembly systems, through incorporating a segment of organic linker for surface anchoring, have been developed by George Whitesides and his colleagues (Whitesides et al., 1991; Chen et al., 1997; Mrksich and Whitesides, 1996).

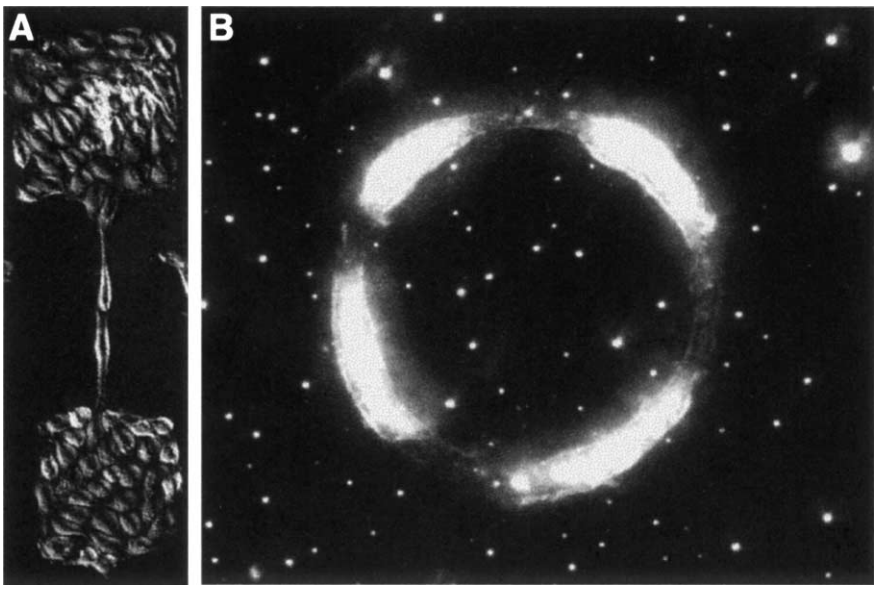

Fig. 6. Cell arrays and patterns. The images of cells were taken with a Normarski microscope. (A) Endothelial cells formed the patterns of squares connected with linear cell tracks in line and patch form. (B) Four individually separated cells formed a circle.

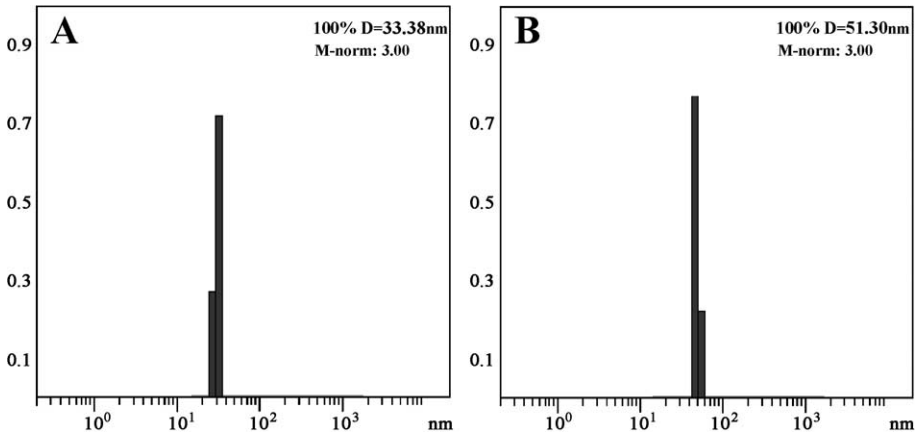

Fig. 7. Dynamic light scattering (DLS) measurement of surfactant peptide nanostructures. The peptides V_6D, V_6D_2, and A_6D gave similar results. Intensity data were collected five times, each looking nearly identical with the rest. The *X*-axis is the size in nanometers and the *Y*-axis is the fraction distribution. The average diameter is approximately 30–50 nm. (A) A_6D. (B) V_6D. The other dimension along the length of the nanotube is beyond the range of DLS measurement.

4.4. Type IV self-assembling peptides

Several surfactant-like peptides undergo self-assembly to form nanotubes and nanovesicles having an average diameter of 30–50 nm (Vauthey et al., 2002). Dynamic light scattering studies showed structures with very discrete size. The structures showed some tail sequence preference. The distribution becomes broader over time, indicating a very dynamic process of assembly and disassembly (Fig. 7). Visualization with transmission electron microscopy of quick-freeze/deep-etch sample preparation revealed a network of open-ended nanotubes with a helical twist (Figs. 8 and 9). Many three-way junctions that may act as links between the nanotubes have also been observed (Fig. 9). Studies of peptide surfactant molecules have significant implications in the design of non-lipid biological surfactants and understanding the complexity and dynamics of self-assembly processes. The self-assembly of surfactant-like peptides containing four or more glycines as the component of the hydrophobic tail and aspartic acids in the polar heads has also been studied (Santoso et al., 2002).

These surfactant peptide monomers contain seven to eight amino acid residues and have a hydrophilic head composed of aspartic acid and a tail of hydrophobic amino acids, such as alanine, valine or leucine. The length of each peptide is approximately 2 nm, similar to that of biological phospholipids. How could these simple surfactant-like peptides form such well-ordered nanotubes and nanovesicles? There are molecular and chemical similarities between lipids and the peptides since both have a hydrophilic head and a hydrophobic tail. The packing between lipids and peptides are likely to be quite different, however. 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

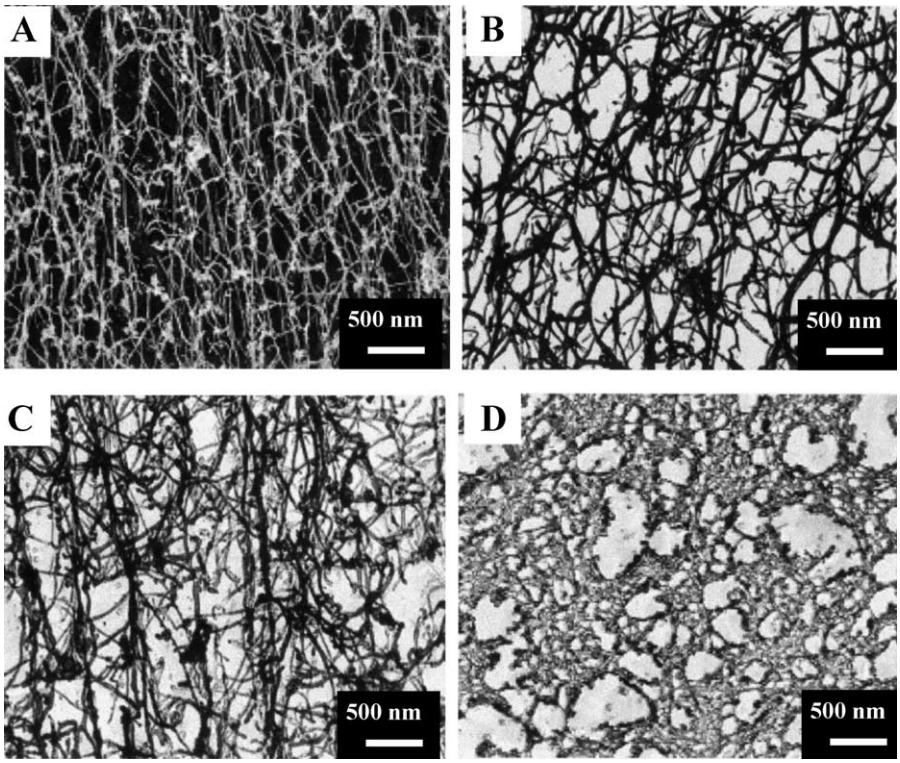

Fig. 8. Quick-freeze/deep-etch TEM image of surfactant peptides, A_6D, V_6D, V_6D_2 and L_6D_2 in water (4.3 mM). These peptides are self-assembled into a dense network extending to several microns in length. Because the droplet solution containing the peptide nanotubes is in three-dimensions, the network of a two-dimensional image appears denser than the actual structure, similar to looking at a picture of the branches on a tree without leaves. (A) A_6D, (B) V_6D, (C) V_6D_2, and (D) L_6D_2. Bar is 500 nm.

acid side chains, surfactant peptides may also interact through intermolecular hydrogen bonds along the backbone. A proposed model is illustrated in Fig. 10.

The molecular engineered non-lipid building may also open an avenue for fabricating new generations of nanomaterials and molecular scaffolds. These structures may be relevant to prebiotic enclosures that sequester the earliest enzymes from their environment.

4.5. Additional self-assembling peptide systems

Several other types of self-assembling peptide systems are currently being developed as emerging materials. In one of these systems, part of the peptide binds to and condenses nucleic acids, the other parts facilitate membrane trafficking to translocate them across the cellular lipid membranes (Schwartz and Zhang, 2000). This system will likely have applications for delivery of molecular drugs, including DNA and RNA for gene therapy,

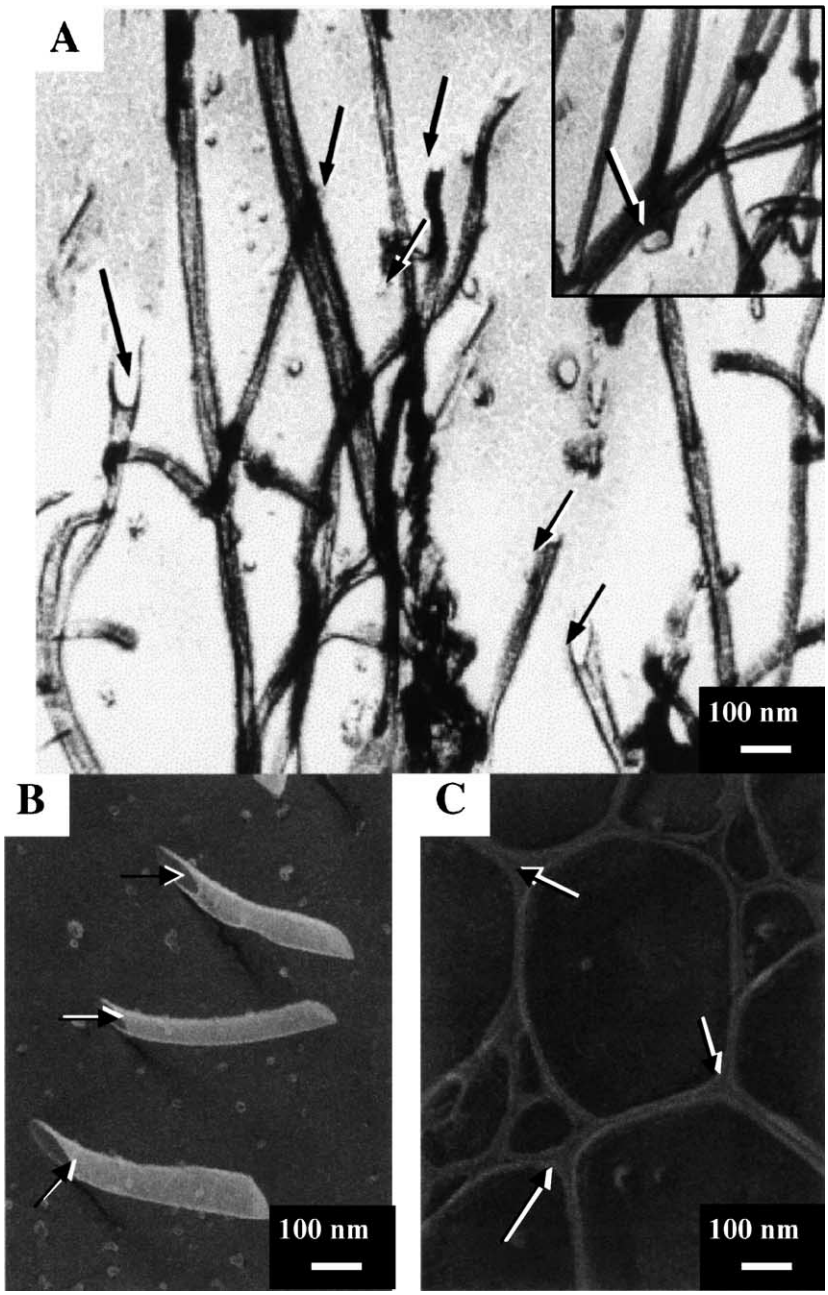

Fig. 9. Quick-freeze/deep-etch TEM image of A_6D and V_6D dissolved in water (4.3 mM at pH 7) at high resolution. The images show the dimensions, 30–50 nm in diameters with openings of nanotube ends (arrows). Insert shows an opening end in more detail. Note that some opening ends of the peptide nanotube may be cut vertically. The strong contrast shadow of the platinum coat also suggests the hollow tubular structure. Similar lipid right-handed helical tubular nano- and microstructures have been reported (Schnur, 1993; Spector et al., 1996). Images show openings at the ends (B, indicated by arrows) and many three-way junctions (C, arrows).

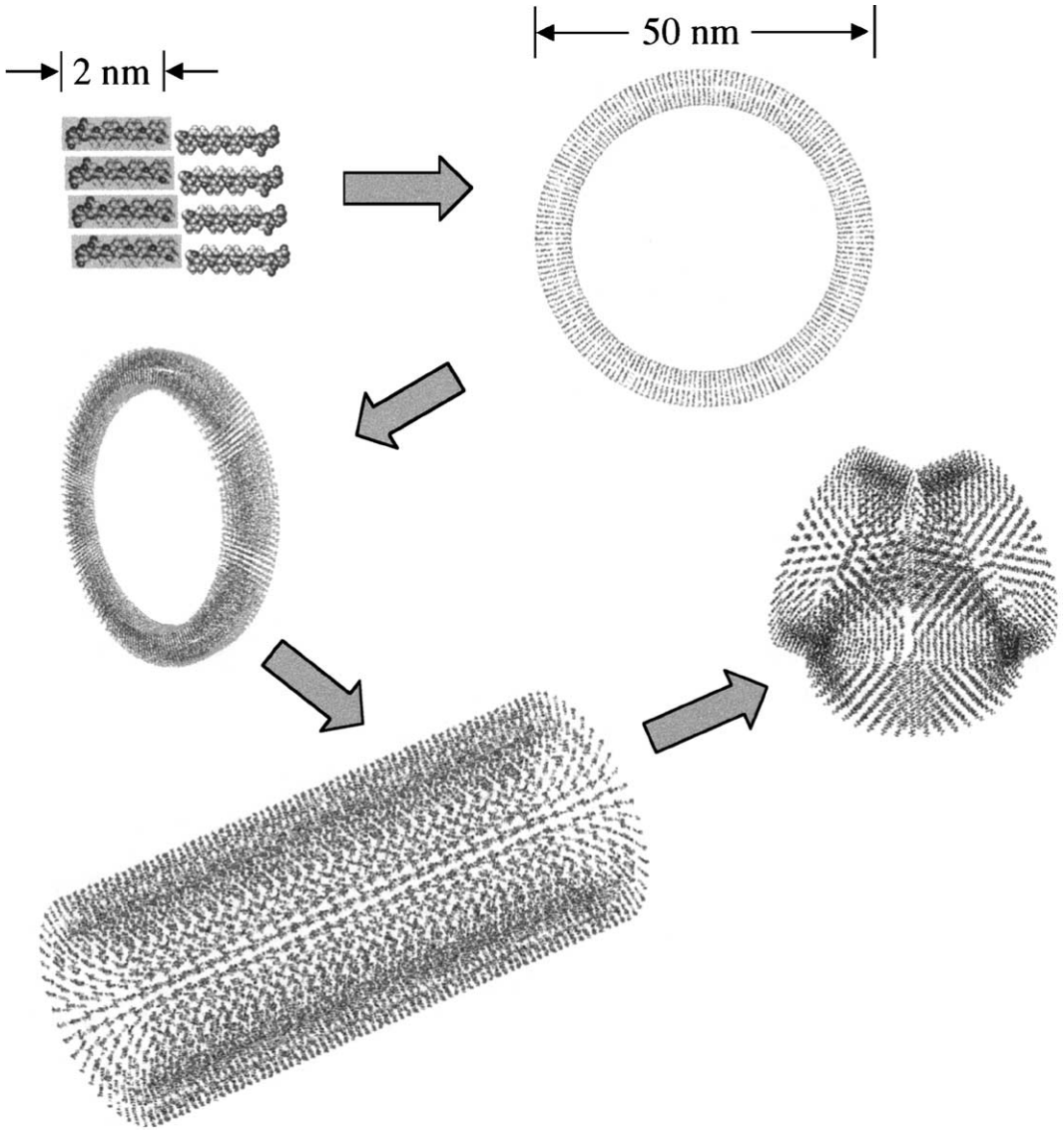

Fig. 10. Potential packing pathway of V_6D peptide nanotube formation. Each peptide monomer is 2 nm, and the diameter of the modeled bilayer nanotube is 50 nm. Each peptide may interact with one another to form a closed ring, which in turn would stack upon top of one another, ultimately yielding a nanotube. Three nanotubes are connected to each other through a three-way junction. This phenomenon mirrors the lipid microtubule structures.

as well as protein therapy and delivery of other therapeutics. The other system is aimed to produce new biological scaffolding material to facilitate biomineralization (Hartgerink et al., 2001). In such a system, the material has a peptide and a polymer segment. The peptide is negatively charged with a molecular recognition site and the polymer is a hydrophobic tail. The hydrophobic segments of the chain in aqueous solutions can self-assemble into regular

intersections of the scaffold, much like the joints in the construction of buildings; therefore forming negatively charged compartments. These negative compartments can then attract the positively charged ions, thus building the mineral phases.

5. The emerging biological materials for new technologies

Development of new materials and technologies often broadens the questions we can address, therefore, deepening our understanding of seemingly intractable phenomena. Development of nanotechnology requires nanoscale materials for nanodevices. Molecular self-assembly systems will undoubtedly create a new class of nanomaterials at the molecular level. It is believed that applications of these simple and versatile molecular self-assembly systems will provide us with new opportunities to study some complex and previously intractable biological phenomena. Molecular engineering through molecular design and self-assembly of biological building blocks is an enabling technology that will likely play an increasingly important role in future technology and will change our lives.

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