Building from the bottom up

by Shuguang Zhang

Designed materials and new tools hold the key for future science and technologies. Building materials from the bottom up is complementary to traditional top-down materials processing, but requires a deep understanding of the individual molecular structures, their assemblies, and dynamic behaviors. This approach, using molecular self-assembly as a fabrication tool, will become an integral part of materials production, especially nanomaterials, in the coming years. Two key elements in molecular self-assembly are chemical complementarity and structural compatibility through weak and noncovalent interactions. We have defined the path to understand these principles. The self-assembly systems represent a significant advance in the molecular engineering of advanced materials and nanomaterials.

Center for Biomedical Engineering, Building NE47,
Center for Bits & Atoms, The Media Lab,
Massachusetts Institute of Technology,
77 Massachusetts Avenue,
Cambridge, MA 02139-4307 USA
E-mail: Shuguang@mit.edu
URL: http://web.mit.edu/lms/www

Nature is a grand master who builds materials from the bottom up, one atom or molecule at a time. These materials include inorganic minerals, crystals, clays, inorganic/organic composite seashells, pearls, bone and teeth, wood, silk, horn, collagen, and extracellular matrices. The sophistication of nature's bottom-up fabrication and construction has inspired us to learn, in order to go beyond nature's materials.

The 'designed materials age' requires new knowledge to build advanced materials. It not only requires full understanding of the individual building blocks, but also deep knowledge of the simple construction units. It is a path from simplicity to complexity. Much like the constructions of the Great Wall of China, the Pantheon in Athens, or Florence's Duomo (Fig. 1), the 'designed materials age' needs basic building blocks and construction units. But these building blocks and units are at the atomic and molecular scale.

Alphabet of materials building blocks

There is an analogy between the construction of the English language and the basic building blocks for materials. Combinations of the 26 letters of the alphabet make countless words that can be strung into sentences and phrases, which, in turn, can be used to construct increasingly complex paragraphs, chapters, and, eventually, books or volumes of books (Fig. 2). Assimilated foreign words and phrases are also adopted into the language. Designing and building new materials follows a similar pattern.

In nature, through billions of years of prebiotic and molecular selection and evolution, there are bio-organic

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molecules, including 20 amino acids, a few nucleotides, and dozens of lipid molecules and sugars. There are also some naturally modified building blocks, or metabolic intermediates. Now, hundreds of synthetic derivatives have been added to the list. These natural and synthetic building blocks, much like the letters of the alphabet with foreign additions, are the key elements for designed materials. Metals, salt composites, and other simple elements are complementary inorganic building blocks. When these bio-organic and inorganic building blocks are coalesced together, either through molecular self-assembly or programmed assembly, to form stable structures with new functionality, a new 'designed' material is born.

"When nature finishes to produce its own species, man begins using natural things in harmony with this very nature to create an infinity of species." (Leonardo da Vinci)

One approach is to design new materials through molecular self-assembly, which is ubiquitous in nature. Molecular self-assembly involves mostly weak and noncovalent bonds, which are individually quite insignificant. Collectively, however, these weak interactions, notably (i) hydrogen bonds, (ii) ionic bonds (electrostatic interactions), (iii) van der Waals interactions, (iv) hydrophobic interactions, and (v) water-mediated hydrogen bonds, play an indispensable role in all biological structures and their interactions. The water-mediated hydrogen bond is especially important for biological systems, because all such materials interact with water. Consider, for example, the structure of collagen. Water-mediated hydrogen bonds are crucial to hold the three-stranded collagen helix together, both intra- and intermolecularly.

Self-assembly has recently emerged as a new approach in chemical synthesis, nanotechnology, polymer science, materials science, and engineering. Molecular self-assembly systems lie at the interface of these disciplines and many self-assembling systems have been developed. These systems range from bi- or tri-block copolymers, complex DNA structures, and lipids to simple or complex peptides and proteins. Self-assembly systems represent a significant advance in the engineering of simple molecular building blocks useful for a wide range of applications.

Since the field of designed nanomaterials is growing at an accelerated pace, it is impossible to summarize all the latest advances in this article. I will, therefore, mainly focus on our own efforts in facilitating this endeavor.

Designed peptide construction units

Much like the construction of a house, where doors, windows, and many other parts can be prefabricated and assembled according to architectural plans, we can apply similar principles to construct nanomaterials and devices, through molecular self-assembly and programmed molecular assembly. Given the growing trend and interest in this field,

Fig. 1 Built from the bottom up, a single brick at a time. (a) The Great Wall of China, started in 700 BC, is 5600 km in length and each brick is ~10 x 20 x 30 cm; (b) Florence's 14th century Duomo, many small bricks and tiles add up to a majestic dome ~44 m in diameter.

this review will focus on only four self-assembling construction units:

- 'Molecular Lego', which forms well-ordered nanofiber scaffolds for three-dimensional cell culture and reparative or regenerative medicine;
- Peptide nanotubes and/or 'molecular cargo' for drug, protein, and gene delivery;
- Segmented surface-assembling peptides, 'molecular carpets' for biological surface engineering;
- Dipolar molecules, which change conformation as a 'molecular switch', especially when covalently attached to an electronically responsive molecular antennae.

These designed construction peptide units are structurally simple, versatile for a spectrum of applications, and easy to produce in large scale at an affordable cost.

'Molecular Lego' peptides

'Molecular Lego' peptides are much like Lego™ bricks with pegs and holes arranged in a precisely determined manner that can be programmed (often by children of very young ages) to assemble in well-formed structures. This class of peptides can spontaneously assemble into well-formed nanostructures at the molecular level. The first of these was discovered serendipitously from a segment of a left-handed Z-DNA binding protein in yeast, Zuotin ('zuo' means 'left' in Chinese, 'tin' means 'protein' in biology)[1].

These peptides form β-sheet structures in aqueous solution with two distinct surfaces, one hydrophilic and the other hydrophobic, like the pegs and holes in Lego bricks. The presence of hydrophobic sides facilitates self-assembly in water, similar to the case of protein folding. The unique structural feature of these 'molecular Lego' peptides is that they form complementary ionic bonds with regular repeats on the hydrophilic surface (Fig. 3a). 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, alternating by one, two, three, four, and so on. For example, the charge arrangements for modulus I is '- + - + - + - +'; modulus II is '- - + + - - + +'; modulus III is '- - - + + +'; and modulus IV is '- - - - + + + +'. The charge orientation can also be designed in the reverse orientation to yield entirely different molecules. These well-defined sequences allow ordered self-assembly, resembling some well-studied polymer assemblies.

Upon the addition of alkaline cations or the introduction of peptide solutions into the physiological media, these peptides spontaneously assemble to form nanofibers with defined structure (Fig. 3b and c), which go on to form macroscopic structures (Fig. 3d). Because they have an extremely high water content, >99% water (1-10mg/ml, w/v), they can be fabricated into various geometric shapes (Fig. 3e)[2-6]. Scanning electron and atomic force microscopy reveal that the matrices are made of interwoven nanofibers 10-20 nm in diameter with pores about 10-200 nm in diameter[2-7].

An example of the molecular structure of the KFE8 peptides as a left-handed double helix is modeled and simulated in Fig. 3c[7-8]. This structure represents a class of self-assembling β-sheet peptides that spontaneously undergoes association under physiological conditions. If a residue on the hydrophobic side is changed, there is a significant effect on the self-assembly, such as the mechanical strength and the speed of self-assembly, but little on overall nanofiber formation. Likewise, if a charged residue is substituted, e.g. a positively-charged lysine (Lys) is replaced by a positively-charged arginine (Arg), or a negatively-

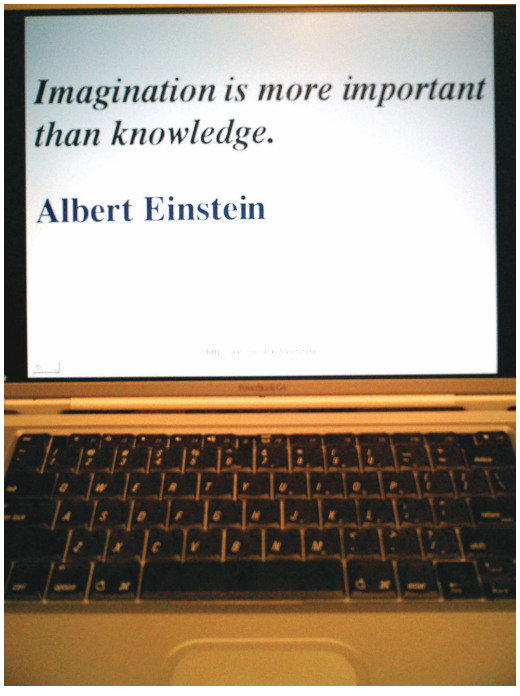
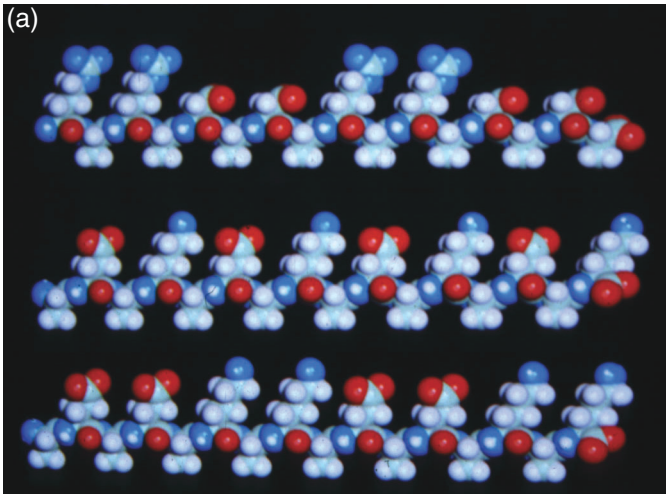
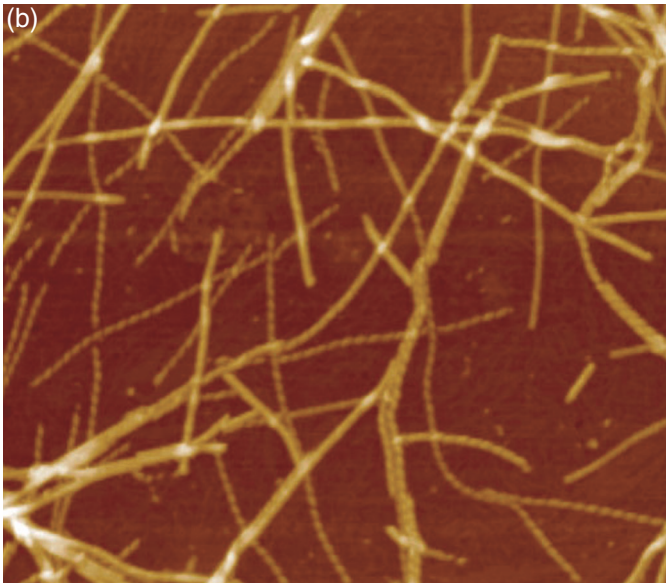
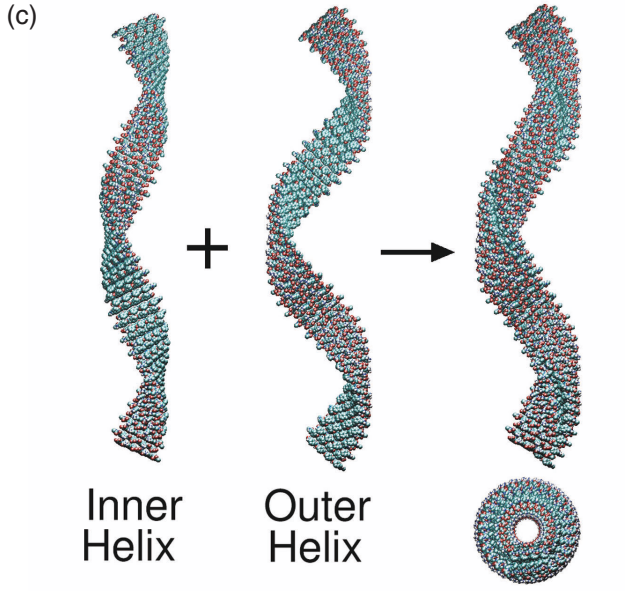
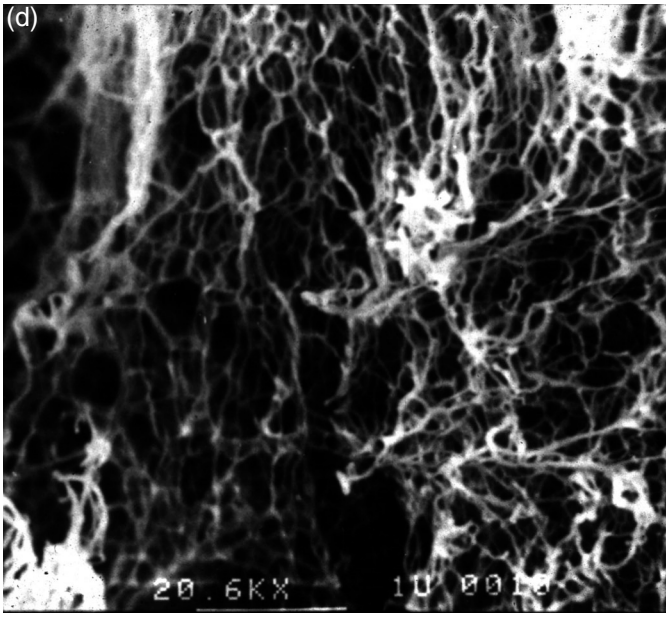


Fig. 2 The top-down versus bottom-up approach. Einstein's beautiful phrase, shown here on a computer screen, illustrates the approach of building from the bottom up, one letter and one word at a time. The same approach can be used to build materials, one atom, one molecule, and one construction unit at a time.









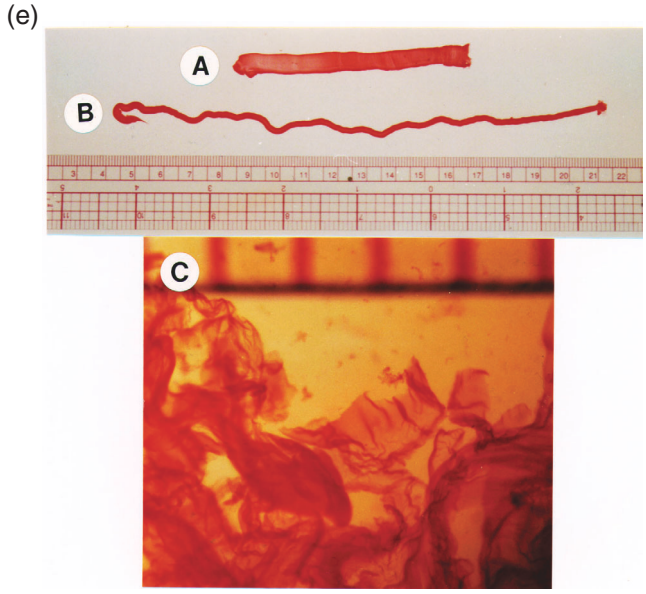


Fig. 3 'Molecular Lego' peptides. Extended β-strand structures of individual molecules are shown for (a) RAD16-I, RAD16-II, EAK16-I, EAK16-II. The distance between the charged side chains along the backbone is approximately 6.8 Å; the methyl groups of alanines are found on one side of the sheet and the charged residues on the other side, like a Lego brick's pegs and holes. Conventional β-sheet hydrogen bond formation between the oxygen and hydrogen on the nitrogen of the peptide backbone is perpendicular to the page. (Color code: carbon – light green, oxygen – red, nitrogen – blue, hydrogen – white.) (b) Atomic force microscopy (AFM) image of KFE8. The peptide in water was imaged on a mica surface. KFE8 peptides undergo self-assembly to form single, left-handed nanofibers ~7 nm in diameter and thicker bundles[7]. The left-handedness is unmistakable in AFM and quick-freeze/deep-etch transmission electron micrographs. (c) Molecular simulation, using CHARMM program[8], of KFE8 in a left-handed double helix. There are inner and outer helices with hydrophobic Phe inside away from water and hydrophilic Lys and Glu exposed to water. The diameter is 7 nm and the pitch is 19 nm[7]. (d) Scanning electron micrograph of EAK16-II nanofiber scaffold[2]. The diameter of the fibers is ~10-20 nm with pores ~10-200 nm in a self-similar fractal manner[2-4,6]. RAD16-I and several other peptides show similar nanofiber scaffolds[3-4,6]. (e) Variety of geometric forms of fabricated peptide scaffolds. Since the scaffolds are made of >99% water (1-10 mg/ml, w/v), they can be fabricated or molded into arbitrary shapes[2-6].

charged glutamate (Glu) is replaced by negatively-charged aspartate (Asp), there is little change to the nanofiber self-assembly, although the details of the nanofiber structures are different[7-8]. If, however, the positively-charged residues, Lys and Arg, are replaced by negatively-charged residues, Asp and Glu, the peptides can no longer undergo self-assembly to form macroscopic materials, although they can still form β-sheet structures in the presence of salts. If the alanines are replaced with more hydrophobic residues, such as Leu, Ile, Phe, or Tyr, there is a greater tendency to self-assemble and form peptide matrices with enhanced strength[6-11].

Surfactant peptides

We have designed other types of biphase hydrophobic-hydrophilic peptides, taking advantage of their self-assembly properties in water. Several surfactant-like peptides have been designed using natural lipids as a guide. These surfactant-like peptides, as we call them, have a hydrophobic tail with various degrees of hydrophobicity and a hydrophilic head (Fig. 4a and b). The peptide monomers contain seven to eight amino acid residues, the head is composed of negatively-charged aspartic and glutamic acids or positively-charged lysine or histidine, and the tail consists of amino acids such as alanine, valine, or leucine. The length of each peptide is ~2 nm, similar to that of biological phospholipids.

Dynamic light scattering studies show structures with very discrete sizes and some tail sequence preference. These peptides undergo self-assembly in water to form nanotubes and nanovesicles with an average diameter of 30-50 nm[12-14]. Tails consisting of alanines and valines produce more homogeneous and stable structures than those of glycines, isoleucine, or leucine. This may be because of their hydrophobic and hydrophilic ratios. These monomer surfactant peptides can be molecularly modeled (Fig. 4). The negatively-charged aspartic acid is shown in red, positively-charged lysine in blue, and green for the hydrophobic tails.

Quick-freeze/deep-etch sample preparation, whereby samples are flash-frozen at -196°C, produces three-dimensional structures with minimal structural disturbance. Transmission electron microscopy reveals a network of open-ended nanotubes with a helical twist (Fig. 5a and b). There also seems to be some dynamic molecular behavior in solution. Some vesicles may be able to 'fuse' and 'bud' out of the peptide nanotubes.

Recently, we also designed another peptide with a phosphorylated serine at the N-terminus followed by either six alanines or valines as the tail. The phosphorylated serine at the N-terminus still bears a positive charge from the free amine terminus, thus it has both positive and negative charges, somewhat like the phosphatidyl-c found in the cellular membrane. This simple surfactant peptide produces structures similar to those previously observed with other surfactant peptides. Interestingly, one of the peptides, pSV6, produces an exceptionally beautiful structure: two round heads with a slim body, much like a Q-tip (Fig. 5c).

How can these simple surfactant-like peptides form such well-ordered nanotubes and nanovesicles? There are molecular and chemical similarities between lipids and peptides – both have a hydrophilic head and a hydrophobic tail. The packing between lipids and peptides is, however,

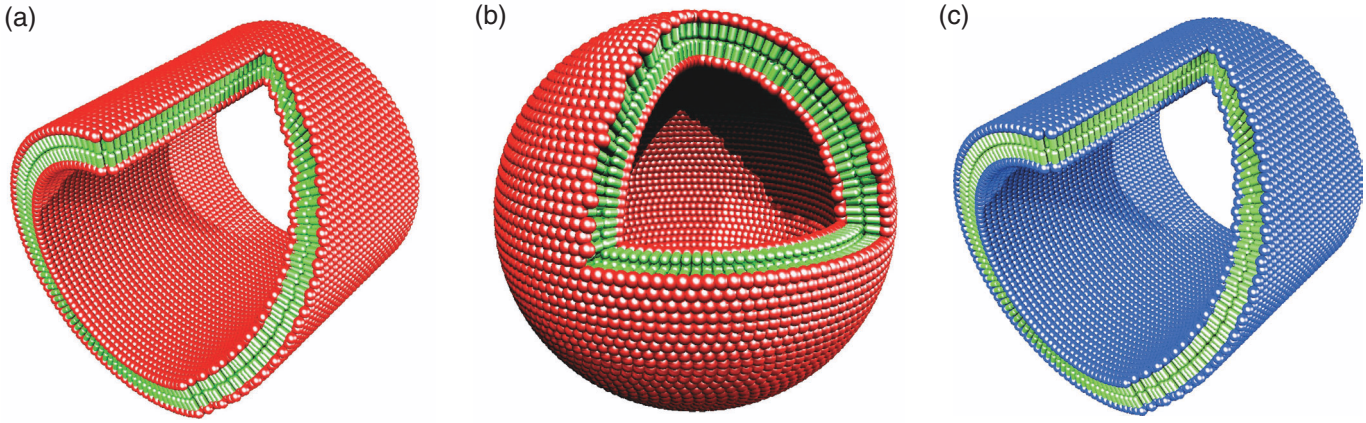


Fig. 4 Molecular models of surfactant peptides V_6D and K_2V_6. These peptides have hydrophilic heads, either negatively charged aspartic acid or positively charged lysine with hydrophobic valine tails[12-14]. (a) V6D in nanotube form. Billions of these molecules self-assemble to sequester the valine tails from water[12,13] in (b) vesicle form[12,13] or (c) nanotube form with positively charged heads[14]. These nanostructures are rather dynamic undergoing assembly and disassembly. Color code: green – hydrophobic tails, red –aspartic acid, and blue – lysine.

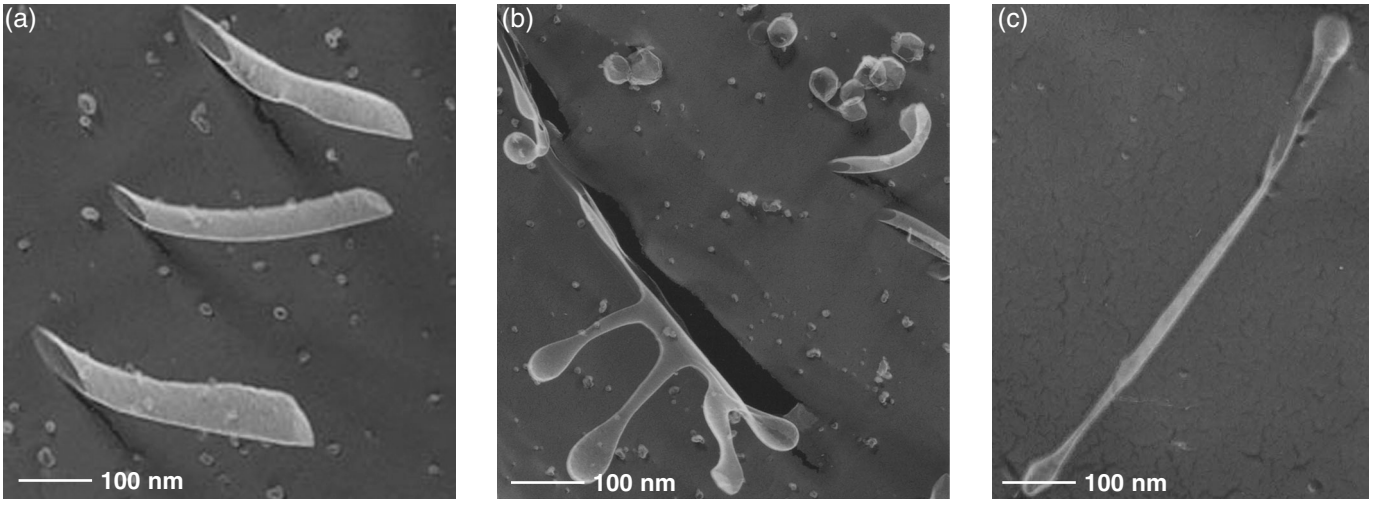


Fig. 5 Quick-freeze/deep-etch transmission electron micrographs of structures from surfactant peptides. (a) The nanotube are clearly represented, with a diameter ~30-50 nm. (b) The nanotubes and vesicles are visible in the same frame suggesting that these structures are quite dynamic. It is plausible that the vesicles may be budded out from the nanotubes and/or they may fuse to form nanotubes in a reversible manner[12-14]. The diameter of these nanostructures is ~30-50 nm. (c) Phosphor-serine surfactant peptides form nano Q-tips.

likely to be quite different. In lipids, the hydrophobic tails pack tightly together to completely displace water, precluding the formation of hydrogen bonds. On the other hand, surfactant peptides may interact through intermolecular hydrogen bonds along the backbone in addition to hydrophobic tail packing between the amino acid side chains.

Molecular 'paint' and 'carpet' peptides

In a painting or carpet, the surface coating thickness is only a few hundred microns or a few millimeters. Can we use this analogy on the nanometer scale to produce a 'molecular paint' or 'molecular carpet', in other words, design molecules that undergo self-assembly on a surface? We have designed some biologically-active peptides, one molecule at a time from the bottom-up. These 'molecular paint' and 'molecular carpet' peptides are able not only to form monolayers a few nanometers thick on a surface, but can also be used for specific cell patterns or to interact with or trap other molecules[15].

These 'molecular paint' and 'molecular carpet' peptides consist of three distinct segmental features (Fig. 6a and b). The first, and most important, feature is the ligand segment that can be designed to incorporate a variety of functional groups for specific recognition by other molecules or cell surface receptors. The second feature is the central linker, where a variable spacer is not only designed to allow freedom of interaction at a specified distance away from the surface but also determines the flexibility or rigidity of the structure. The third feature is the surface anchor, where a chemical group on the 'molecular carpet' peptide can react with a particular surface to form a covalent bond, thus immobilizing the peptides. This simple system using peptides and other substances to engineer surfaces is an emerging technology that will surely prove to be a useful tool in biomedical engineering and biology. It could provide new methods to study cell-cell communication and behavior (Fig. 6c and d)[15]. Similar kinds of molecular self-assembly systems incorporating a segment of organic linker for surface anchoring have been developed by George Whitesides and coworkers[16-18].

'Molecular switch' peptides

'Molecular switch' peptides can drastically change their molecular structure (Fig. 7a). One of these dipolar peptides with 16 amino acids, DAR16-IV, has a β-sheet structure at ambient temperatures 5 nm in length, but can undergo an abrupt structural transition at high temperatures to form a stable α-helical structure 2.5 nm in length[19,20]. Similar structural transformations can be induced by changing the pH. This suggests that secondary structures of some sequences, especially segments flanked by clusters of negative charge on the N-terminus and positive charges on the C-terminus, may undergo drastic conformational transformations under the appropriate conditions. These findings not only provide insights into protein-protein interactions during folding and the pathogenesis of some protein conformational diseases, such as scrapie, Kuru,

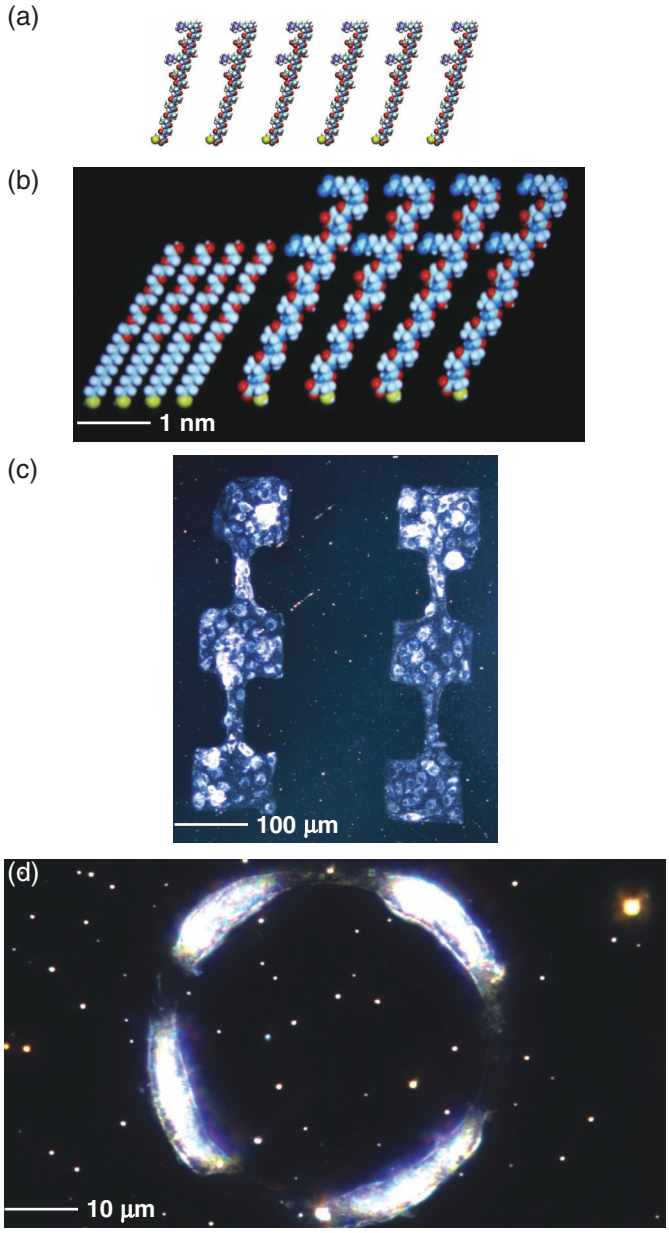


Fig. 6 'Molecular paint' and 'molecular carpet' peptides. (a) Molecular models of RADSC-14. They not only form a monolayer only 5 nm thick on surface, but are also biologically-active to trap specific molecules and cells onto the surface. RADS sequence is a modified cell adhesion motif[15]. A5 is the linker, which can be either flexible, if using glycine, or stiff, with isoleucine or leucine. Cysteine is the anchor to bind the gold atoms covalently onto the surface. (b) Monolayer of ethylene glycol thiolate (left). 'Molecular paint' and 'molecular carpet' peptides (right). Molecular models show the surface where both molecules form self-assembled monolayers with different heights. The extended lengths of RADSC-14 and EG6SH are approximately 5 nm and 4 nm, respectively. (c) Cell stations and tracks. These cells are confined in the designed pattern coated with both EG6SH and 'molecular carpet' peptides. (d) Four cells arranged in a ring with well-defined locations and shapes. Such a molecular-engineering tool ushers in new ways to study biology and to develop new bionanotechnologies.

Huntington's, Parkinson's and Alzheimer's, but can also be developed as 'molecular switches' for a new generation of nanoactuators and nanoswitches.

In order to make a true molecular switch, an electronic component is necessary. The question is how to add an electronic component to molecules, which are often only a few nanometers in size. Fortunately, a variety of metal nanocrystals may be used for such a purpose because they are similar in size to biomolecules. Joseph Jacobson, a quantum physicist at MIT's Media Lab, came up with an ingenious idea to couple these nanocrystals covalently to biological molecules through a unique chemical bond, either using a -SH on cysteine or -NH_2 on Lys (Fig. 7b). These biomolecules with nanocrystals can become electronically responsive. The first example was to electronically control DNA behavior as a switch (Fig. 7c)[21]. Additional examples of electronic control of a broad range of biomolecules or molecular machines will likely follow.

Advantage of the bottom-up approach

Materials built from the bottom-up one molecule at a time, as nature does, can incorporate specific features at will. Such a molecular engineering feat is, however, extremely difficult using conventional materials processing methodology. A few promising examples are listed here.

A number of cell tissue types have been cultured with 'molecular Lego' peptide materials[3-5]. Several peptide scaffolds have been used for cell proliferation and differentiation. These results demonstrate that peptide materials not only support various types of cell proliferation, but also differentiation into desired cell types in a controlled manner. In addition, when primary rat neurons were allowed to attach to the peptide materials, the cells projected lengthy axons that followed specific contours on the self-assembled peptide surface and made active and functional connections[4].

Surfactant peptides are now being developed as targeted delivery systems for drugs, genes, and RNAi. They are being considered for many other applications including cosmetics and cleaning materials since the peptides are nontoxic, gentle, water-soluble and bio-absorbable. 'Molecular paint' and 'carpet' peptides have found uses in a diverse range of industries where a biologically-active coating is desired. Not only can they be used in bio-related industries, but they have also been considered as nano-organizers to align other nonbiological materials, analogous to the biomineralization process. 'Molecular switch' materials are also likely to find their way into a wide range of unanticipated uses. They could transform an obsolete industry or usher in a new one.

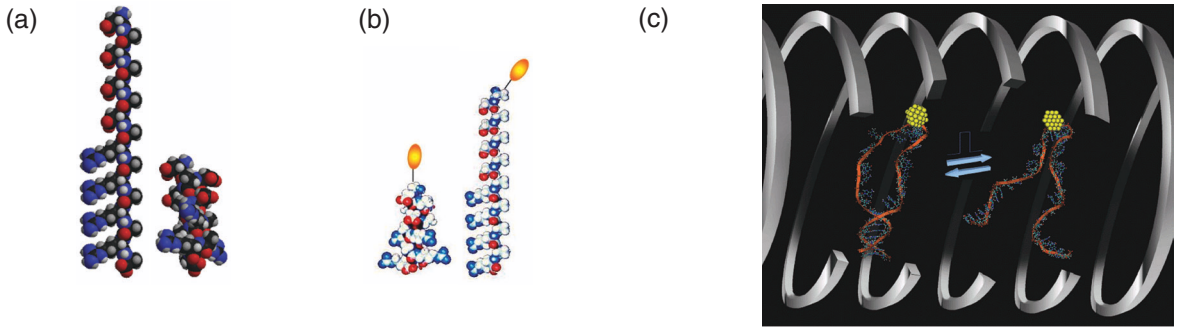


Fig. 7 'Molecular switch' peptides. (a) Structures of DAR16-IV in two distinct stable forms. The β-sheet structure is abruptly converted to an α-helical structure with no detectable intermediate. The conversion in DAR16-IV produced distinct stable structural forms[19-20]. (b) If metal nanocrystals are covalently coupled, the molecules may be responsive to electronic control. (c) The first example of reported electronic control is DNA-coupled with nanocrystals[21]. We call this new endeavor radio frequency biology, or RF Biology. It could have tremendous promise for producing new materials from the bottom-up at the nanoscale in a programmable manner.

Remarks and perspective

We are just at the beginning of a great journey that will have many unexpected findings. There is no turning back. There is much work under way actively pursuing molecular engineering and the building of composite materials, but there is still an enormous challenge ahead.

As I have briefly summarized here, peptides can be considered as the building blocks of new materials[22-26]. They can be designed combinatorially to incorporate other building blocks, such as sugars, lipids, nucleic acids, or inorganic metallic nanocrystals. Nature has inspired us and opened the door. Our imagination is now the only limit. The fundamental design principles of building from the bottom up can be readily extended to polymers and polymer composites, where copolymers can be designed and produced.

Building materials from the bottom up requires a multidisciplinary approach. This arena is unquestionably in the nano-dimension, where all fields of science and engineering meet. New ideas and collaborations will be fostered when scientists from diverse background collaborate. As Francis Crick put it, "In nature, hybrid species are usually sterile, but in science the reverse is often true. Hybrid subjects are often astonishingly fertile, whereas if a scientific discipline remains too pure it usually wilts." MT

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