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REVIEW

Fabrication of novel biomaterials through molecular self-assembly

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Two complementary strategies can be used in the fabrication of molecular biomaterials. In the 'top-down' approach, biomaterials are generated by stripping down a complex entity into its component parts (for example, paring a virus particle down to its capsid to form a viral cage). This contrasts with the 'bottom-up' approach, in which materials are assembled molecule by molecule (and in some cases even atom by atom) to produce novel supramolecular architectures. The latter approach is likely to become an integral part of nanomaterials manufacture and requires a deep understanding of individual molecular building blocks and their structures, assembly properties and dynamic behaviors. Two key elements in molecular fabrication are chemical complementarity and structural compatibility, both of which confer the weak and noncovalent interactions that bind building blocks together during self-assembly. Using natural processes as a guide, substantial advances have been achieved at the interface of nanomaterials and biology, including the fabrication of nanofiber materials for three-dimensional cell culture and tissue engineering, the assembly of peptide or protein nanotubes and helical ribbons, the creation of living microlenses, the synthesis of metal nanowires on DNA templates, the fabrication of peptide, protein and lipid scaffolds, the assembly of electronic materials by bacterial phage selection, and the use of radiofrequency to regulate molecular behaviors.

Molecular self-assembly is a powerful approach for fabricating novel supramolecular architectures. It is ubiquitous in the natural world (see **Box 1**): lipid molecules form oil drops in water; four hemoglobin polypeptides form a functional tetrameric hemoglobin protein; ribosomal proteins and RNA coalesce into functional ribosomes.

Molecular self-assembly is mediated by weak, noncovalent bonds—notably hydrogen bonds, ionic bonds (electrostatic interactions), hydrophobic interactions, van der Waals interactions, and water-mediated hydrogen bonds. Although these bonds are relatively insignificant in isolation, when combined together as a whole, they govern the structural conformation of all biological macromolecules and influence their interaction with other molecules. The water-mediated hydrogen bond is especially important for living systems, as all biological materials interact with water.

All biomolecules, including peptides and proteins, interact and self-organize to form well-defined structures that are associated with functionality[1]. By observing the processes by which supramolecular architectures are assembled in nature[1–3], we can begin to exploit self-assembly for the synthesis of entirely novel synthetic materials. Peptides and proteins are versatile building blocks for fabricating materials. Nature has already used them as scaffolds to produce a dizzying array of materials, including collagen, keratin, pearl, shell, coral and calcite microlenses, and optical waveguides.

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In this review, I focus on the fabrication of diverse molecular structures through self-assembly of peptides, proteins, and lipids. As the use of genetically engineered polypeptides for specifically binding selected inorganic compounds to assemble functional nanostructures has recently been reviewed[4], it will not be discussed in detail here. For other aspects of nanomaterials synthesis, namely chemistry-driven approaches to materials synthesis (*e.g.*, self assembly of organic ligands and metal ions into three-dimensional hollow cages or metallodendrimers) and *in vitro* systems for artificial protein synthesis, the reader is referred elsewhere[5–8].

Fabrication of nanofibers

Work in my laboratory has focused on fabricating several self-assembling peptides and proteins for a variety of studies and biomaterials (**Fig. 1**). Examples include ionic self-complementary peptides[9–11], which form β-sheet structures in aqueous solution with two distinct surfaces—one hydrophilic, the other hydrophobic (rather like the pegs and holes in Lego bricks). The hydrophobic residues shield themselves from water and self-assemble in water in a manner similar to that seen in the case of protein folding *in vivo*. The unique structural feature of these 'molecular Lego' peptides is that they form complementary ionic bonds with regular repeats on the hydrophilic surface (**Fig. 1a**). The complementary ionic sides have been classified into several moduli (modulus I, modulus II, modulus III, modulus IV, *etc.*, and mixtures thereof). This classification scheme is based on the hydrophilic surfaces of the molecules, which have alternating positively and negatively charged amino acids alternating by one residue, two residues, three residues and so on. For example, charge arrangements for modulus I, modulus II, modulus III and modulus IV are −+−+−+−+, −−++−−++, −−−+++ and −−−−++++, respectively. The charge orienta-

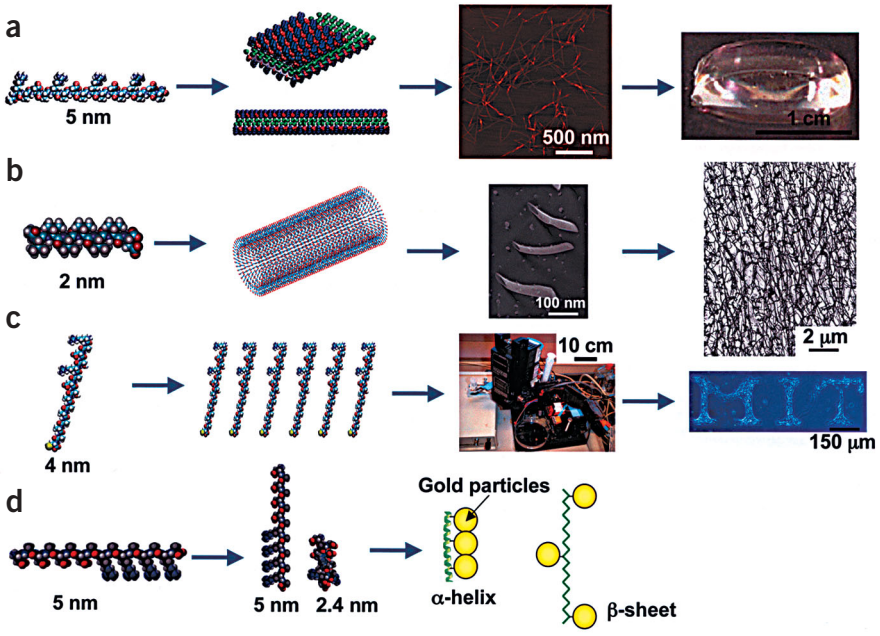

Figure 1 Fabrication of various peptide materials. (**a**) The ionic self-complementary peptide has 16 amino acids, ~5 nm in size, with an alternating polar and nonpolar pattern. The peptides form stable β-strand and β-sheet structures; thus, the side chains partition into two sides, one polar and the other nonpolar[48–50]. 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%. This is similar to agarose gel and other hydrogels. (Images courtesy of H. Yokoi.) (**b**) A type of surfactant-like peptide, ~2 nm in size, that has a distinct head charged group, either positively charged or negatively charged, and a nonpolar tail consisting of six hydrophobic amino acids. The peptides can self-assemble into nanotubes and nanovesicles with a diameter of ~30–50 nm. These nanotubes go on to form an inter-connected network[33–35] similar to what has been observed in carbon nanotubes. (Image courtesy of S. Santoso.) (**c**) Surface nanocoating peptide. This type of peptide has three distinct segments: a functional segment, which interacts with other proteins and cells; a linker segment that not only can be either flexible or stiff, but also sets the distance from the surface; and an anchor for covalent attachment to the surface[47]. These peptides can be used as ink for an inkjet printer to print directly on a surface, instantly creating any arbitrary pattern, as shown here. Neural cells from rat hippocampal tissue form defined patterns. (Images courtesy of S. Fuller and N. Sanjana.) (**d**) Molecular switch peptide, a type of peptide with strong dipoles that can undergo drastic conformation changes, between α-helix and β-strand or β-sheet, under external stimuli[87]. It is conceivable that metal nanocrystals could be attached to these dipolar peptides to fabricate them into tiny switches.

tion can also be designed in the reverse orientation, which can yield entirely different molecules. These well-defined sequences allow the peptides to undergo ordered self-assembly, in a process resembling some situations found in well-studied polymer assemblies.

A broad range of peptides and proteins have been shown to produce very stable nanofiber structures, also called amyloid fibers[12–22]. (In physiological settings, the formation of such amyloid fibrils is known to have a role in several diseases, including bovine spongiform encephalopathy, Alzheimer disease, type II diabetes and Creutzfeld–Jakob disease; *e.g.*, see ref. 23.) These nanofibers are very well ordered and possess remarkable regularity and, in some cases, helical periodicity. The mechanism whereby they undergo self-assembly is now being elucidated[24–27]. These nanofibers are similar in scale to extracellular matrices that are crucial in allowing a variety of cells to adhere together to form functional tissues. Furthermore, these nanofibers, if their formations can be precisely controlled, could serve as scaffold to organize nanocrystals for use in the electronic industry (see below).

Fabricating bionanotubes

Nature has selected, evolved and produced a host of amphiphilic molecules that contain distinct hydrophobic and hydrophilic segments. These amphiphilic molecules readily partition in water to form various semienclosed environments. One of the best examples is phospholipids—the predominant constituents of the plasma membrane that encapsulate and protect the cellular contents from the environment, an absolute prerequisite for almost all living systems.

Phospholipids readily undergo self-assembly in aqueous solution to form distinct structures that include micelles, vesicles and tubules. This is largely a result of the hydrophobic forces that drive the nonpolar region of each molecule away from water and toward one another. The dimensions and shape of the supramolecular lipid structures depend upon various factors, such as the geometry and curvature of the polar head and the shape and length of the nonpolar tails[28].

Schnur and colleagues[29,30] pioneered lipid tubule self-assembly to build materials and ushered in a new era of fabricating novel materials using simple building blocks[31,32]. They have not only extensively studied all aspects of the materials from single molecular chemistry and chirality to all size scales, but have also developed unexpected applications, such as an anti-fouling coating for ships comprising self-assembled lipid tubules[31,32].

These experiments stimulated my research group to ask what might be the simplest amphiphilic biopolymers in the prebiotic environment. Accordingly, we designed many simple amphiphilic peptides that consist exclusively of natural amino acids. One such class of molecules is surfactant-like peptides (**Fig. 1b**)[33–35]. Although individually they have completely different compositions and sequences, these surfactant-like peptides share a common feature: the hydrophilic heads have one or two charged amino acids and the hydrophobic tails have four or more consecutive hydrophobic amino acids (see figures in refs. 33–35). For example, the peptide V_6D (VVVVVVD) has six hydrophobic valine residues beginning 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[33]. In contrast, G_8DD (GGGGGGGGDD) has eight glycines followed by two aspartic acids, with three negative charges[34]. Similarly, A_6K (AAAAAAK) or KA_6 (KAAAAAA) has six alanines as the hydrophobic tail and a positively charged lysine as the hydrophilic head[35].

Perutz, in his last set of papers[20,36,37], unequivocally demonstrated the formation of nanotubes from polyglutamines. He showed that the length of the polyglutamines—the number of consecutive glutamines on the polypeptide chain—plays a key role in the formation of β-helix structure. These experimental results were confirmed using computer modeling and simulations (ref. 38; A. Windle, personal

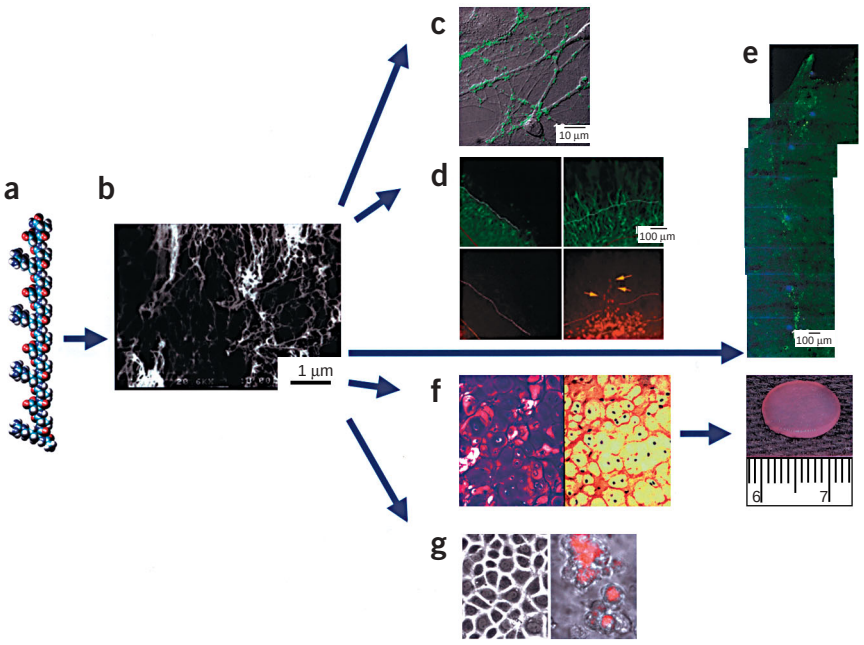

Figure 2 Self-assembling peptides form a three-dimensional scaffold woven from nanofibers ~10 nm in diameter. The scaffolds have been applied in several three-dimensional cell culture studies and in tissue engineering applications[49,50,53,62,63]. (**a**) Representation of self-assembling peptide. (**b**) Electron micrograph of three-dimensional scaffold formed *in vitro*. (**c**) Rat hippocampal neurons form active nerve connections; each green dot represents a single synapsis[50]. (**d**) Neural cells from a rat hippocampal tissue slide migrate on the three-dimensional peptide scaffold. Cells on the polymer membrane (left) and on the peptide scaffold (right) are shown. Both glial cells (green) and neural progenitors (red) migrate into the three-dimensional peptide scaffold[61]. (Image courtesy of C. Semino.) (**e**) Brain damage repair in hamster. The peptide scaffold was injected into the optic nerve, which was first severed with a knife. The cut was sealed by the migrating cells after 2 days. A great number of neurons form synapses (R. Ellis-Behnke, personal communication). (Image courtesy of R. Ellis-Behnke.) (**f**) Chondrocytes from young and adult bovine encapsulated in the peptide scaffold. These cells not only produce a large amount of glycosaminoglycans (purple) and type II collagen (yellow), characteristic materials found in cartilage, but also a cartilage-like tissue *in vitro*[53]. (Images courtesy of J. Kisiday and A. Grodzinsky.) (**g**) Adult rat liver progenitor cells encapsulated in the peptide scaffold. The cells on the two-dimensional dish did not produce cytochrome P450–type enzymes (left). However, cells in three-dimensional scaffolds showed cytochrome P450 activity (right)[62]. (Image courtesy of C. Semino.)

communication). The polyglutamine nanotubes are likely to provide a very interesting scaffold for the design of new materials.

Nanometer-thick coatings on surfaces

Molecular assembly can be targeted to alter the chemical and physical properties of a material's surface. Surface coatings instantly alter a material's texture, color and compatibility with, and responsiveness to, the environment. Conventional coatings are typically applied by painting or electroplating. The coatings are usually in the ten- and hundred-micron size ranges and the interface is often not complementary at the molecular level. Thus, erosion is common.

Whitesides and collaborators[38–41] have developed a microcontact printing technology that combines semi-conducting industry fabrication, chemistry, and polymer science to produce defined features on a surface down to the micrometer or nanometer scale. Following microcontact printing, a surface can be functionalized with different molecules using a variety of methods (*e.g.*, covalent coupling, surface adhesion, coordination chemistry, and dip-pen nanolithography)

Box 1 Following nature's lead

Nature is the grandmaster when it comes to building extraordinary materials and molecular machines—one atom, and one molecule, at a time. Masterworks include such materials as minerals, well-ordered clays and photonic crystals, and, in the biological world, composites of inorganic and organic shells, pearls, corals, bones, teeth, wood, silk, horn, collagen, muscle fibers and extracellular matrices. Multifunctional macromolecular assemblies in biology, such as hemoglobin, polymerases, ATP synthase, membrane channels, the spliceosome, the proteosome and ribosomes, are all essentially exquisitely designed molecular machines (**Table 1**).

Through billions of years of prebiotic molecular selection and evolution, nature has produced a basic set of molecules that includes 20 amino acids, a few nucleotides, a dozen or so lipid molecules and two dozen sugars as well as naturally modified building blocks or metabolic intermediates. From these seemingly simple molecules, natural processes are capable of fashioning an enormously diverse range of fabrication units, which can further self-organize into refined structures, materials and molecular machines that not only have high precision, flexibility and error-correction capacity, but also are self-sustaining and evolving.

Indeed, nature shows a strong preference for bottom-up design, building up molecular assemblies, bit by bit, more or less simultaneously on well-defined scaffolds. Take, for example, egg formation in oviparous animals. The fabrication of an egg involves not only the creation of the ovum, its various protective membranes and accompanying nutritive materials (*i.e.*, yolk) but also simultaneous synthesis of the eggshell from an extremely low concentration of calcium and other minerals, all in a very limited space. Oviparous animals synthesize eggshell against an enormous ionic and molecular concentration gradient as a result of the high levels of minerals at the site of eggshell assembly. Similar challenges are overcome in dental tissues when they form teeth during early childhood.

Nature accomplishes these feats effortlessly, yet recreating them in the laboratory represents an enormous challenge to the human engineer. The sophistication and success of natural bottom-up fabrication processes inspire our attempts to mimic these phenomena with the aim of creating new and varied structures with novel utilities well beyond the gifts of nature.

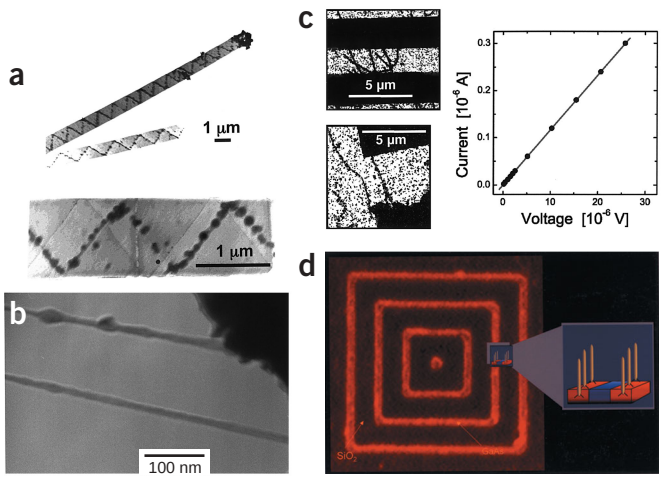

Figure 3 Lipid, peptide and protein scaffold nanowires. (**a**) Lipid tubule–coated wire. Nanoparticles are coated on the left-handed helical lipid tubules. The nanoparticles are aligned inside the tubule along the regular helical pattern[65]. (Image courtesy of J. Schnur.) (**b**) Silver ions fill the nanotubes formed from a dipeptide, Phe-Phe, the shortest peptide possible. The silver alone formed a wire after removal of the Phe-Phe peptide scaffold[66]. (Image courtesy of M. Reches and E. Gazit.) (**c**) The NM segment of the yeast prion protein Sup35 can produce stable and long nanofibers. Through genetic modification, a cysteine was incorporated that can covalently bind to gold nanocrystals and the nanofibers enhanced with metallic silver and gold. The NM–gold nanowire complex carries electric signals[67]. (Images courtesy of S. Lindquist.) (**d**) Discovery and selection of electronic materials using bacteriophage display system. A combinatorial phage library was used to selectively bind to electronic materials. Selected recombinant phage peptide has a high affinity for GaAs. Fluorescently labeled phage displaying a peptide specifically selected for affinity to GaAs bind to the patterned GaAs nested square pattern on a wafer. The redline (1 μm in diameter) corresponds to GaAs and the black spaces (4 μm in diameter) are SiO_2. This peptide-specific binding could also potentially be used to deliver nanocrystals to specific locations[68]. (Image courtesy of A. Belcher.)

according to the application. Surfaces have now been modified with a vast family of chemical compounds, and peptides and proteins have also been printed onto surfaces. Mirkin and colleagues[42–44] have also developed a dip-pen nanolithography to directly print micro- and nano-features onto surfaces. They use a modified AFM tip to dip into protein ink or other solutions, and then transfer these substrates directly onto a previously modified surface, enabling the inscription of diverse patterns. This is much like writing with a feather pen in the Middle Ages. These developments have spurred new research into the control of molecular and cellular patterning, cell morphology, and cellular interactions[40,41,45–47] and fueled new technology development.

Work in my laboratory has focused on designing a variety of peptides to self-assemble into a monolayer on surfaces and to allow adhesion molecules to interact with cells and adhere to the surface (**Fig. 1c**; for reviews, see refs. 40,41,47). These peptides have three general regions along their lengths: a ligand for specific cell recognition and attachment, a linker for physical separation from the surface, and an anchor for covalent attachment to the surface[47]. The ligands may be of the RGD (arginine–glycine–aspartic acid) motif that is known to promote cell adhesion, or other sequences for specific molecular recognition or specific cell interactions. The linker is usually a string of hydrophobic amino acids such as alanine or valine. The anchor can be a cysteine residue for gold surfaces, aspartic acid linking on amine surfaces or lysine linking on carboxylic acid surfaces.

We have used this method, in conjunction with self-assembled monolayers prepared through microcontact printing, to place cells into complex patterns[47]. This approach may facilitate research into cell-cell communication. Recently, we have moved one step further: using peptides and proteins as ink, we have directly microprinted specific features onto the nonadhesive surface of polyethylene glycol to fabricate arbitrary patterns rapidly without preparing a mask or stamps (**Fig. 1c**). The process is similar to using an ink pen for writing—here, the microcontact printing device is the pen and the biological or chemical substances are the inks.

Nanofiber peptide and protein scaffolds

Other work in my group has focused on fabricating three-dimensional peptide scaffolds by exposing the self-assembling peptide to a salt solution or to physiological media that accelerate the formation of macroscopic structures[48–50]. Scanning electron microscopy (SEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM) reveal that the matrices formed are made of interwoven nanofibers with diameter of ~10 nm and pores of ~5–200 nm in size[48–52]. If the alanines are replaced by more hydrophobic residues, such as valine, leucine, isoleucine, phenylalanine or tyrosine, the molecules have a greater tendency to self-assemble and form peptide matrices[51–53]. These simple, defined and tailor-made self-assembling peptides have provided the first *de novo*–designed scaffolds for three-dimensional cell culture, with potential implications for basic studies of cell growth, applied studies in tissue engineering and, ultimately, regenerative medicine.

Several polymer scientists, chemical engineers and chemists, notably Tirrell and his group[54,55], have also fabricated artificial amphiphilic protein scaffolds[54–58]. To accomplish this feat, investigators used a genetically designed polypeptide consisting of over 200 amino acids arranged as a di-block copolymer similar to copolymers found in structural proteins that support internal cell shapes. The resulting designed proteins have considerable mechanical strength and fast-recovering scaffolds that are responsive to changes in pH and can resist temperatures of up to 90 °C[54–58]. Ultimately, they may prove useful for engineering functionality and biodegradability into materials destined for biomedical applications[54–58].

Table 1 What do they have in common? Machines and molecular machines

Machines	Molecular machines
Vehicles	Hemoglobin
Assembly lines	Ribosomes
Motors, generators	ATP synthases
Train tracks	Actin filament network
Train controlling center	Centrosome
Digital databases	Nucleosomes
Copy machines	Polymerases
Chain couplers	Ligases
Bulldozer, destroyer	Proteases, proteosomes
Mail-sorting machines	Protein sorting mechanisms
Electric fences	Membranes
Gates, keys, passes	Ion channels
Internet nodes	Neuron synapses

Box 2 Reflections on self-assembly

The chirality of molecular building blocks plays an important role in self-assembly processes. An example of how chirality at the molecular level influences the supramolecular structures is evident in the self-assembly of an eight-residue peptide, KFE8 (FKFEFKFE)[52]. The innate right-handed twist of the β-stranded peptide backbone in β-sheet conformation leads to the formation of left-handed double-helical ribbon of regular pitch at the nanometer scale. Chirality in fibrous molecular structures is common. Interestingly, many nanofibers self-assembled from a wide range of peptides and proteins form ~10-nm fibrils with various degrees of left-handed helical twist, regardless of the size of the biomolecules[20,84–86]. However, the detailed molecular structure of these nanofibers is still difficult to determine because they are not amenable to either single-crystal X-ray diffraction or solution nuclear magnetic resonance at present. New tools will be needed to obtain high resolution of fibrous structures.

Building blocks other than naturally occurring amino acids can be used to create amphiphilic peptides. For example, Stupp and colleagues[59,60] have designed a 'peptide-amphiphile' derivatized with nonpolar hydrocarbon tails, illustrating how a composite of hydrophobic polymer tails and hydrophilic peptide heads can be used to form nanocylinders. The molecule has a long alkyl chain at the N terminus of a peptide that acts as the hydrophobic tail. By incorporating a phosphorylated serine within the peptide end of the molecule, calcium or other ions can be attracted, organized and regulated to facilitate the mineralization of hydroxyapatite[59,60]. The C terminus of the peptide can be further functionalized with the RGD motif, promoting cell attachment to the nanofibers.

Because these nanofiber scaffolds contain 5–200 nm pores and have an extremely high water content (>99.5%, or 1–5 mg/ml), they are of potential utility in three-dimensional cell-culture media. The scaffolds closely mimic the porosity and gross structure of extracellular matrices, allowing cells to reside in a three-dimensional environment and molecules, such as growth factors and nutrients, to diffuse in and out very slowly.

Work in our group[49,50,53,61,62] has demonstrated that a variety of cells encapsulated and grown in three-dimensional peptide scaffolds show interesting functional cellular behaviors, including proliferation, functional differentiation, active migration, and extensive production of their own extracellular matrices (**Fig. 2**). When primary rat neuronal cells are allowed to attach to the peptide scaffolds, the neuronal cells not only project lengthy axons that follow the contours of the scaffold surface, but also form active and functional synaptic connections (**Fig. 2c**)[50]. Furthermore, some neuronal progenitor cells migrate long distances into the three-dimensional peptide scaffold (**Fig. 2d**)[62]. Recently, the same peptide scaffold has been used for repair of animal brain lesions where the severed brain section could be re-patched (**Fig. 2e**; R. Ellis-Behnke, personal communication).

Similarly, when young and adult bovine chondrocytes are encapsulated in the peptide scaffold, they not only maintain their differentiated state but also produce abundant type II and type XI collagen as well as glycosaminoglycans (**Fig. 2f**)[53]. Moreover, adult liver progenitor cells proliferate and differentiate into cells that express enzyme activities which metabolize toxic substances (**Fig. 2g**)[61].

Fabricating nanowires using bioscaffolds

In the computing industry, the fabrication of nanowires and nanofeatures using the 'top-down' approach faces tremendous challenges. Thus, the possibility of fabricating conducting nanowires by molecular means using peptide scaffolds is of particular interest to the electronics industry. One can readily envision that nanotubes made from self-assembling peptides might serve as templates for metallization. Once the organic scaffold has been removed, a pure conducting wire is left behind and immobilized on a surface. There is great interest in developing various methods for attaching conducting metal nanocrystals to a peptide for such a purpose.

Matsui and colleagues[63,64] have reported success in functionalizing peptide nanotubes into nanowires. They not only coated the peptide nanotube with copper and nickel but also showed that their nanotubes can be coated with avidin, making them able to bind specifically to gold surfaces that have previously been treated with biotinylated self-assembled monolayers[63,64].

Lvov *et al.*[65] have fabricated nano- and microwires by coating the lipid tubules with silica and gold nanocrystals. They found that these nanocrystals linked to the tubules according to the tubules' helical

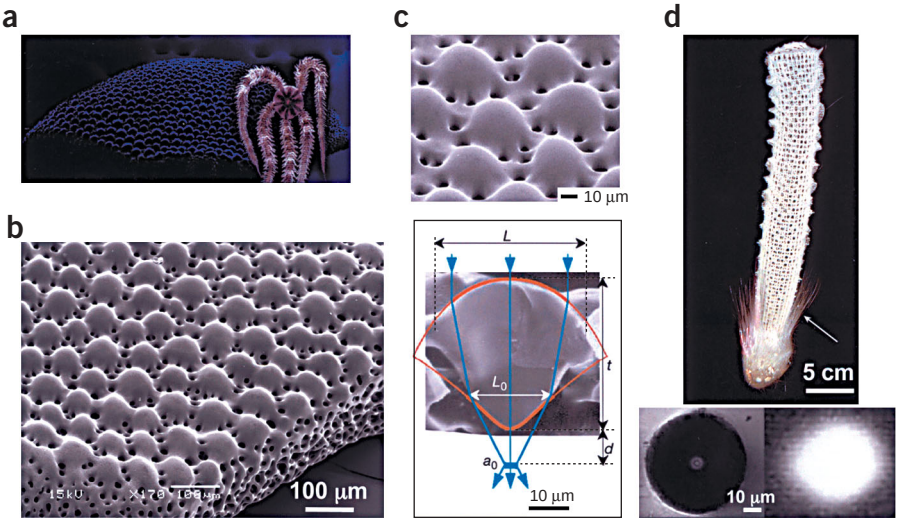

Figure 4 Microlenses and fiber-optics fabricated from protein scaffolds. Nature builds scaffolds for many unexpected uses. (**a**) A marine brittlestar and its lens. (**b**) The calcite microlenses of *Ophiocoma wendtii* after chemical cleaning to remove proteins and other organic substances. (**c**) Top, high-magnification image of *O. wendtii* microlenses. Bottom, the pathway of light is focused by the microlens[71]. The functional region of this lens (L_0) closely matches the profile of a lens that compensates for spherical aberration (red lines). The light paths are shown as blue lines. (**d**) Structure and fiber-optical properties of spicules in the glass sponge *Euplectella*. Top, photomicrograph showing the basket-like cage structure and basalia spicules (arrow). Bottom, wave guiding for individual spicules upon coupling with white light. Spicules embedded in epoxide act as single-mode or few-mode waveguide (left); free-standing spicules act as multimode waveguide (right panel)[72]. (All images courtesy of J. Aizenberg.)

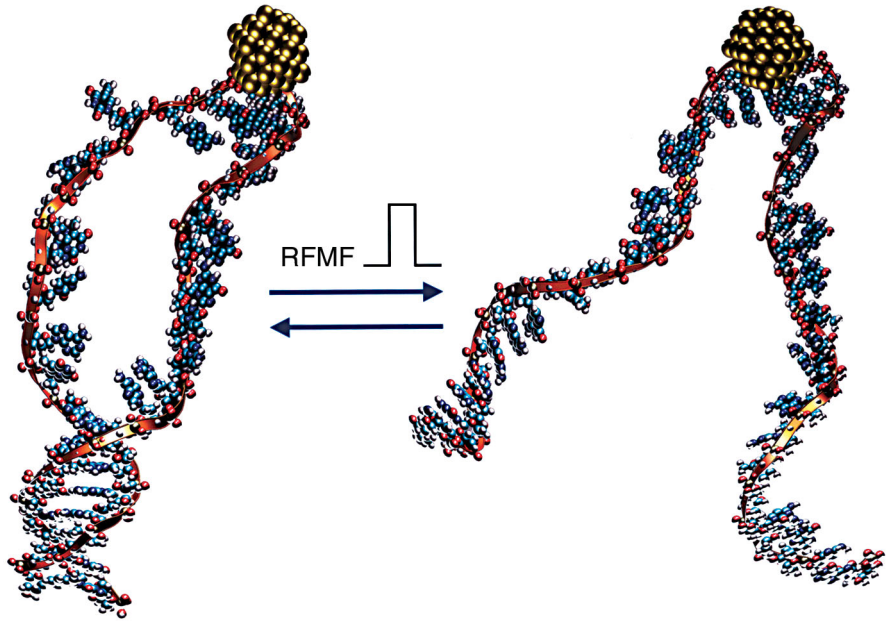

Figure 5 Metal nanocrystal–coupled biomolecule DNA. Gold or other metal nanocrystals can be covalently couple to biomolecules through specific amino acid side chains or DNA, as in example shown here[86]. When the radiofrequency magnetic field (RFMF) is off, a segment of the DNA forms a stable double helix; when the RFMF is turned on, the DNA deforms and changes its conformation[82]. When an RFMF is applied, the metal nanocrystals can respond to external signals so that the biomolecules attached with it also respond accordingly. Electric signals can be controlled finely, rapidly, reversibly and with extremely high precision. Furthermore, electric signals can penetrate many barriers and turbid solutions, including cells and tissue. Thus, the system could potentially be used to follow complex cellular events, such signal transduction, *in vivo* protein folding and molecular interactions. (Images courtesy of W. Hwang, K. Hamad-Schifferli and J. Jacobson.)

periodicity (**Fig. 3a**). These wires have been used for coating in a number of industrial applications.

Recently, Reches and Gazit[66] have demonstrated that a Phe-Phe dipeptide—the shortest peptide length possible, consisting of only two amino acids with a single amide bond—can form stable nanotubes. They then diffused silver ions into the defined tubes and were able to remove the peptide either enzymatically, chemically or through heat burning to reveal the silver wire (**Fig. 3b**)[66].

In other recent work, amyloid protein nanofibers have been used as scaffold to align gold nanocrystals. Lindquist and colleagues[67] have reported how a bioengineered version of the prion-determining (NM) domain of the yeast prion protein Sup35 can provide a scaffold for fabricating nanowires, and tested the conducting capability of the resulting wires (**Fig. 3c**). These efforts collectively open a new direction in the fabrication of electronic nanomaterials.

Belcher and colleagues[68–70] take a very different approach toward not only discovering, but also fabricating, electronic and magnetic materials, departing sharply from traditional materials process technology. Their strategy is to genetically engineer self-assembling bacteriophage so that they can be used to select conducting, semiconducting and magnetic materials (**Fig. 3d**). The researchers have also evolved the phages (and other microbial organisms) for additional material fabrications. This strategy may lead to the discovery of new electronic and magnetic materials[4].

Such approaches for producing finer and finer features at the nanoscale, with increasing density and in finite areas, may prove complementary to microcontact printing process. Although the latter approach has become widely used and is rapidly being perfected, fabrication of the finest feature using microcontact printing is limited by the capabilities of lithography technologies currently used in the semiconductor industry.

Bio-optical structures and optical waveguides

The most advanced top-down technology for fabricating a complex optical systems falls far short when compared with the accomplishments of living organisms at ambient temperature and low pressure (and without clean rooms)[71,72]. Several groups have studied biomineralization in diverse marine organisms, notably the brittlestar *Ophiocoma wendtii* and the sponge *Euplectella*. Some remarkable living optical systems have been uncovered, such as the fiber-optical spicules from *Euplectella* that have the dimensions of a single human hair and can act as multimode waveguides (**Fig. 4**). Compared with contemporary synthetic optical fibers, the microfibers are much more fracture resistant (as a result of the presence of organic ligands connecting the nanofibers), have high refractive indices and considerable flexibility, with the capacity to act as single-mode or few-mode (where light waves are effectively confined to the core when embedded in epoxide) waveguides. The microfibers can act as multimode waveguides when light is coupled to free-standing spicules. The enhanced refractive-index contrast between the spicule and air allows most of the light to fill the entire cladding area, providing a highly effective fiber-optical network[72]. These discoveries have inspired Aizenberg *et al.*[73] to fabricate micropatterned single crystals and photonics with potential applications in communication technology (**Fig. 4**).

Future perspectives

The burgeoning interest in nanotechnology has stimulated the discovery and development of new biomaterials that can undergo self-assembly into well-ordered structures at the nanometer scale[1–8,74–76]. Although the fabrication of ordered structures is reproducible and ubiquitous in nature, it remains an enormous challenge for scientists and engineers. Undoubtedly, future efforts will focus on the discovery, selection and development of peptides from combinatorial peptide and protein libraries[77-80] for use in nanofabrication.

The semiconductor and telecommunication industries currently face an enormous challenge in attempting to apply the top-down approach to the manufacture of smaller and smaller features in a limited space in a cost-effective manner. In this respect, biology fabricates elegant and seemingly impossibly tiny features at the nanoscale with consummate ease[1–3,71–75]. The electronics, materials and polymer industries will benefit tremendously from close study of living systems and biomimicry[1–3,71–75]. At the same time, the dramatic loss of biodiversity resulting from human activities threatens to extinguish exotic creatures that could provide both valuable lessons and resources for nanomaterials researchers[81].

The refinement of nanomaterials strategies also offers potential benefits in biological research. At present, the study of membrane proteins and their use as devices is especially challenging. Current

estimates from genomic information suggest that membrane proteins comprise ~20–30% of all cellular proteins, yet only a handful of high-resolution structures have been elucidated thus far. It is not unlikely that new materials may allow membrane proteins to be organized in a three-dimensional membrane lattice, opening new avenues to their biochemical study, crystallization and integration into nanodevices.

At present, tracking of the activities of proteins inside a cell is extremely difficult. The high concentration of proteins (protein represents around 25% of dry weight in *Escherichia coli*) means that cellular density is probably thicker than a bowl of oatmeal. However, in this incredibly dense environment, intracellular signaling and molecular transportation and movement, DNA replication, transcription and translation of the genetic material are all carried out very rapidly, often in microseconds or seconds. How can we follow and understand these subtle and precise molecular interactions without significantly disturbing the cellular environment?

In this regard, Joseph Jacobson, a quantum physicist at the MIT Media Lab, has conceived the ingenious idea of covalently attaching metal nanocrystals directly to biomolecules. Our laboratories have just begun to collaborate on developing an approach termed radiofrequency (RF) biology, wherein a biomolecule is covalently attached to a metal nanocrystal antenna similar in size (~1–3 nm) to most biomolecules. The tagged molecule is responsive to external electric signals and can be controlled precisely. Such tagged biomolecules can be subjected to radiofrequency electronic control (**Fig. 5**)[82].

Though the field is still in its infancy, the self-assembly of biological molecules ultimately promises materials, devices and technologies beyond our current imaginations. As Susan Lindquist has put it[83]: "About 10,000 years ago, [humans] began to domesticate plants and animals. Now it's time to domesticate molecules."

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