

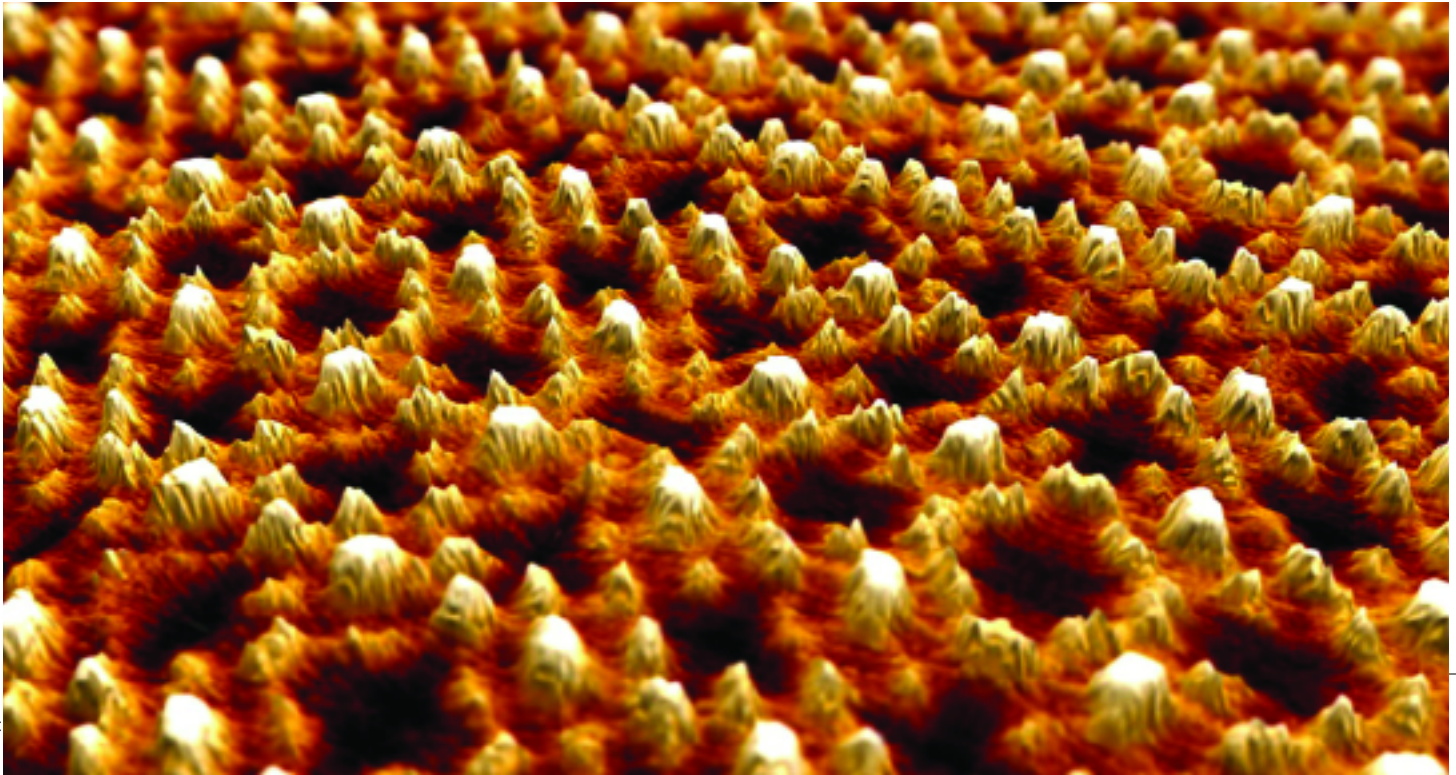
# Biomimetic Nanotechnology

FEATURE

## Researchers mimic biology to form nanoscale devices

by Eric J. Lerner

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**Figure 1. Scanning-force-microscope surface-relief reconstruction of a crystalline protein S-layer from *Bacillus sphaericus* CCM2177 with a center-to-center spacing of 13.1 nm.**

**N**anotechnology involves the creation and manipulation of complex structures on the scale of nanometers—something organisms have done for about 3.8 billion years. Using DNA, RNA, and a huge variety of proteins, living cells build complex molecules and nanoscale organelles, and create nonliving materials, such as tooth enamel, with nanoscale structures. So it is logical for nanotechnologists to seek to duplicate organisms' own techniques to try to create new nanomachines from the bottom up.

Although biomimetic nanotechnology is in its infancy, with no applications yet reaching commercialization, the barriers in some cases lie mainly in scaling up production processes to industrial levels. In others, researchers must make significant basic breakthroughs to bridge the gap between laboratory experiments and usefulness.

### Imitating nature

Researchers are exploring several ways to imitate biology at the submicrometer level. One approach tries to inorganically duplicate biological materials that have extraordinary properties. A recent successful example of this comes from a study of geckos at the University of Manchester's Centre for Mesoscience and Nanotechnol-

ogy in England. The little lizards have a remarkable ability to cling to almost any surface, no matter how smooth, even when they are upside down (Figure 2).

To imitate nature, one must first understand it. Not until 2000 did researchers determine that the gecko's sticking abilities stemmed from the 200-nm-wide keratin hairs that coated the soles of their feet. Capillary forces cause hairs with that diameter to stick to films of water or wet surfaces. Equally strong van der Waals forces enable them to attach to dry surfaces as well. Each hair exerts only  $10^{-7}$  N of force, but they are densely packed enough to collectively have an adhesive force of  $10$  N/cm<sup>2</sup>—enough to suspend a 100-kg mass from a patch 10 cm on each side.

Inspired by these findings, the Manchester team attempted to reproduce the gecko hairs as an array of plastic fibers. These rigid fibers, however, did not work because only a few of them would make contact with an uneven surface, and the fibers lacked sufficient strength to resist breaking when the adhesive was pulled away from the surface. So the team tried a polyimide plastic film and patterned the fibers using electron beam lithography.

They found that if the fibers were too close together, they stuck to each other, which reduced their stickiness

to the surface. The optimum geometry proved to be a spacing of  $1.6\ \mu\text{m}$ , a diameter of  $500\ \text{nm}$ , and a length of  $2\ \mu\text{m}$ . With a flexible backing applied to the fibers—so that they could more easily accommodate irregular surfaces—the team achieved an adhesion of  $3\ \text{N}/\text{cm}^2$ , almost 30% that of the real gecko. This adhesion strength would be sufficient to suspend a man with just adhesive gloves covering his palms.

Although the experimental version of gecko tape lasted through several cycles of attachment and detachment, the team contemplates making future versions based on hydrophobic materials, such as the gecko's keratin. In concept, these materials would not stick to each other and would last longer. Of course, researchers must develop less expensive techniques than electron lithography to mass-produce such tape.

## Building with proteins

Dropping in scale from hundreds of nanometers to  $10\ \text{nm}$  brings researchers to the realm of large molecules. Organisms build structures with proteins, so a second major biomimetic approach uses natural or newly designed proteins to create nanostructures. For one thing, natural proteins can form repetitive, crystalline structures to serve as substrates for arrays of nanomachines or for nanoelectronics.

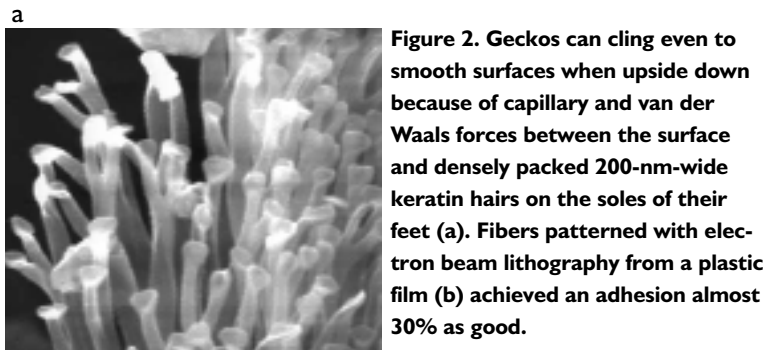
Bacteria form a one-molecule-thick layer of crystalline proteins on their exteriors, called S-layers, which repeat on a  $10\text{-nm}$  crystalline grid. A number of groups, including Uwe Sleytr and colleagues at the Center for Nanobiotechnology, University of Natural Resources and Applied Life Sciences, in Vienna, Austria, seek to use bacterial S-layers as superstructures for artificial arrays. This effort involves first chemically removing the S-layer from the bacteria and breaking it up into individual molecular subunits. The subunits, when placed in solution, reassemble into ordered arrays on solid supports, such as silicon wafers, metal electrodes, or synthetic polymers (Figure 1).

Once an S-layer attaches to a substrate, specific sensor molecules can be attached to the molecular array to form a bioanalytical sensor. For example, Sleytr's group made a glucose sensor by binding glucose oxidase molecules to the S-layer and measuring the current passing through the electrodes as the oxidase reacted with the glucose.

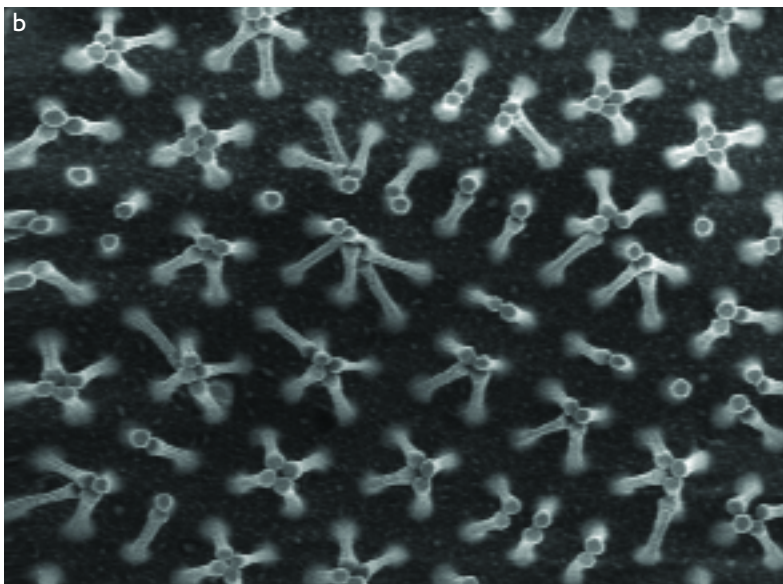
Another application under development uses S-layers as photoresists in conventional lithography. Exposure to UV light destroys the S-layer proteins in the same way that exposure to radiation changes a conventional photoresist. However, S-layers are only  $5\text{--}10\ \text{nm}$  thick, much thinner than conventional photoresists, which makes possible the replication of narrower features.

## Binding proteins

Other researchers are experimenting with proteins in a far more complex way—using their ability to specifically bind with each other and with inorganic materials as a way to build new materials. One of the characteristics of



**Figure 2. Geckos can cling even to smooth surfaces when upside down because of capillary and van der Waals forces between the surface and densely packed 200-nm-wide keratin hairs on the soles of their feet (a). Fibers patterned with electron beam lithography from a plastic film (b) achieved an adhesion almost 30% as good.**

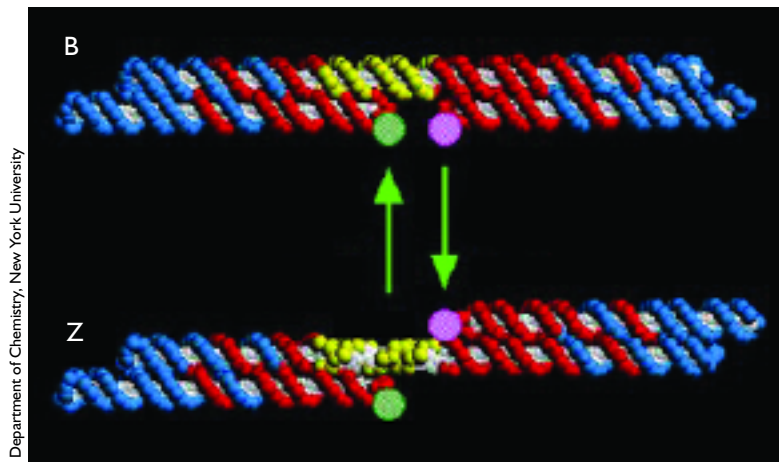


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biologically produced nonliving materials, such as abalone shell and spider silk, is a hierarchical structure. That is, structures exist not just at the macroscopic level and the crystalline level, but at many scales in between. This structuring often imparts remarkable characteristics to a material, such as silk's great strength. If researchers can design appropriate new proteins, they could be used to produce similarly complex artificial materials in an industrial process.

However, scientists as yet cannot predict the shape of proteins or their binding properties just from the sequences of their constituent amino acids, because protein-folding simulations have not advanced that far. An alternative approach selects proteins with the desired binding properties from a large number of randomly generated molecules. This can be done by the genetic engineering of bacteriophage viruses—viruses that infect bacteria—an approach pioneered by Angela M. Belcher, associate professor of materials science at the Massachusetts Institute of Technology (MIT), and other researchers.

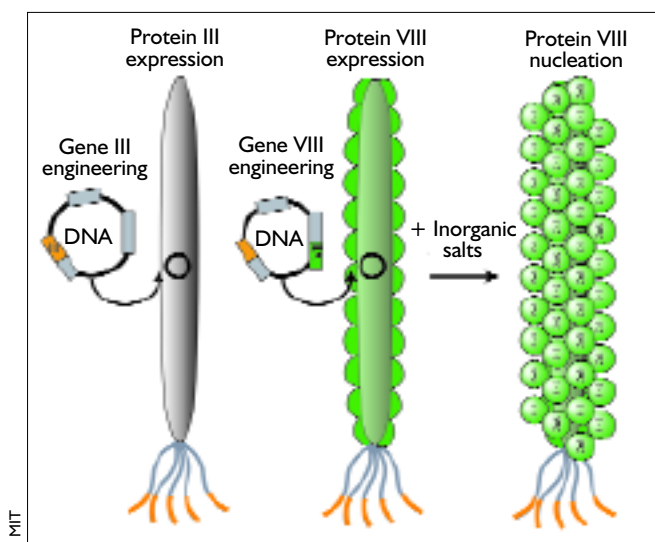
In the first step, DNA fragments with random sequences coding for many different proteins are incorporated into the DNA of bacteriophages in such a way that one of the proteins forms on the exterior of a virus. The viruses replicate and are placed in a solution in contact with the material to which they are supposed to bind. After washing away the viruses that do not bind, the few that do attach are chemically freed from the target and allowed to repli-



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**Figure 3.** Two double-crossover molecules (red and blue) connected by a bridge element (yellow) that can be converted from B-DNA (top) to Z-DNA (bottom) by the addition of hexaaminocobalt(III) chloride (and converted back again by its removal) form the basis of a DNA nanomechanical device. The change is monitored by attached fluorescent dyes represented by the stippled circles.

cate again. The sequence is repeated until only the protein with the strongest binding remains. That protein can then be sequenced for future use. In this manner, researchers at various laboratories are creating a library of proteins that bind to specific elements and inorganic compounds, including gold, platinum, silver, zinc oxide, gallium arsenide, and iron oxide.



**Figure 4.** DNA fragments coding for proteins are incorporated into bacteriophage viruses such that one of the proteins forms on the exterior. The viruses replicate, bind to a target substance such as zinc sulfide, and form wires from which the viruses may be removed by heating.

One possible application of such inorganic-binding proteins (also referred to as genetically engineered polypeptides for inorganics, or GEPIs) is in the assembly of nanoparticles into specific nanoscale devices, such as quantum dots. Because protein-binding reactions occur at or near room temperature in solutions, they could be con-

siderably less expensive than conventional vacuum techniques, such as molecular-beam epitaxy. In addition, such proteins could prove useful in creating smaller devices.

A separate approach to creating nanostructures uses viruses as part of the structure itself, not just to produce the right proteins. In joint work by Belcher's MIT group and researchers at the University of Texas at Austin, genetically engineered bacteriophages align themselves into long filaments. Their outer proteins bind with inorganic materials, such as zinc sulfide and cadmium sulfide, to form long nanowires up to 600 nm long and only 20 nm across. Heating the resulting wires to 350 °C removes the virus, leaving only the metallic wire behind.

The viruses used consist of only six proteins, two of which bind with the selected inorganic material. The researchers hope to modify some or all of the remaining proteins to produce more-complex self-assembled structures than wires (Figure 4).

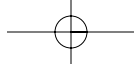
## Structuring DNA

Producing the proteins needed for nanostructures involves DNA, of course, because it is the DNA in the virus that codes for the amino acid sequence in the proteins. But another biomimetic approach uses the DNA itself as the structural element, not proteins. The idea—developed by Nadrian C. Seeman, professor of chemistry at New York University, among others—is to unravel the two intertwined helices at the end of DNA molecules and then stick them together with the matched ends of two other DNA molecules. Because the specific sequence of nucleic acids in a given DNA strand will only match with the corresponding sequence in another DNA strand, specific molecules can be fit together like a jigsaw puzzle, with only one possible structure at each point where one DNA strand attaches to two others.

The process of joining one DNA molecule to two others occurs in organisms during meiosis, the cell-division process that produces germ cells (egg and sperm) and temporarily forms X-shaped structures called Holliday junctions. However, with appropriately designed DNA sequences, a molecule can have Holliday junctions at both ends, thus allowing them to form two- and even three-dimensional arrays.

Normal DNA molecules are too flexible to form rigid scaffolding. In chromosomes, DNA is twisted into densely packed hierarchies of helices, not a rigid array. But if two DNA molecules attach to each other twice at crossover points, the resulting double-crossover DNA (DX DNA) is stiff. By 2000, Seeman was able to use these molecules to produce two-dimensional arrays of DNA molecules.

However, progress toward the practical application of these DNA arrays has been modest. Three possible applications have been discussed. One would use the arrays,



eventually elaborated into three-dimensional arrays, as scaffolding for nanoelectronic components. Specific components, attached to DNA sequences, would bind to the matching sequence in the right place in the array.

A second application, perhaps closer to realization, would use the arrays to bind large biological molecules into an artificial crystal for X-ray crystallography studies. Conventionally, such studies rely on the molecules forming crystals on their own, but in many cases, natural crystallization does not occur. Putting large numbers of molecules into identical spaces in a regular array would form an artificial crystal, making crystallography studies possible.

DNA arrays also could form the basis for nanomachines. In one effort in this direction, Seeman and colleagues developed a DNA structure that could be rotated back and forth between two positions. To do this, they connected two DX molecules with a DNA “shaft” that can be converted from right-handed B-DNA to left-handed Z-DNA by the addition of  $\text{Co}(\text{NH}_3)_6\text{Cl}_3$  (Figure 3).

Significant obstacles stand in the way of practical applications of DNA structures, which remain in the early research phase. For one thing, DNA nanomachines would appear slow, taking a relatively long time—seconds or at least milliseconds—for chemical messengers to reach a machine and change its state. Perhaps more fundamentally, researchers have not figured out how to replicate DNA structures on a large scale. In organisms,

DNA molecules replicate with the aid of enzymes that unzip and rezip them. But such replication appears difficult in the complexly branched structures of DNA constructs. Clever topological tricks could possibly overcome this problem, but they have not been worked out yet in practice.

## Prospects

Although DNA structures are not a near-term technology, other approaches seem closer to realization. At the mesoscale—the gecko tape, for example, where existing fabrication technology could produce structures imitating biological ones—obstacles to commercialization involve the usual challenges of scaling up a laboratory-created item to an industrial product and improving durability.

Protein-based techniques are at an intermediate stage—neither entirely a pure research subject, nor one verging on commercial application. For the most part, these techniques aim at using biologically based processes to produce artificial structures that could, in principle, be built by entirely inorganic means. For example, many groups working with nanotubes are also looking at ways to form regular arrays that incorporate appropriate metals and other materials. One key question that remains unresolved is whether protein-based methods can come on-line faster or less expensively than nanotube-based methods. ■

