Self-Assembly Comes Together

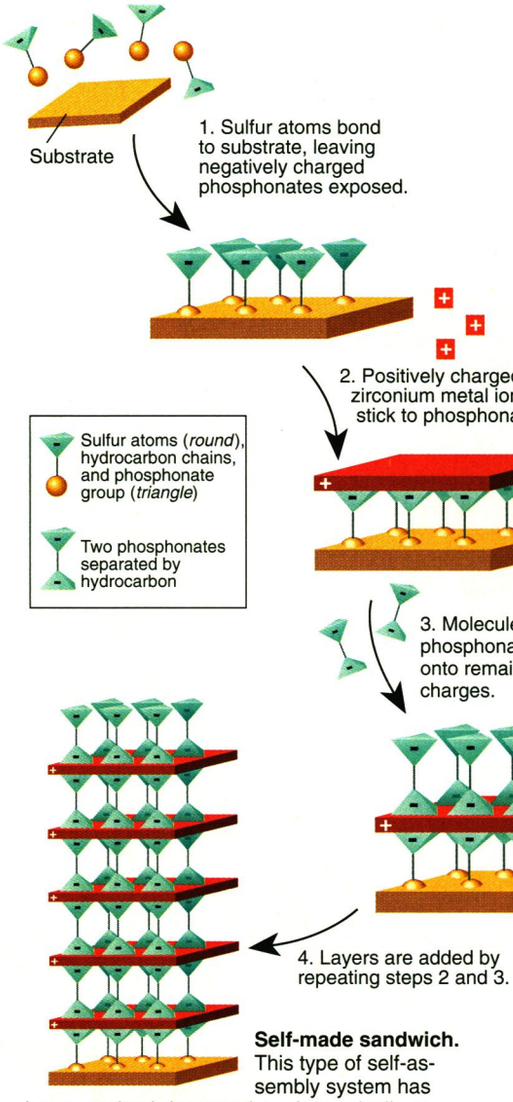
Materials that make themselves are beginning to make their presence felt as novel drug-delivery vehicles and electronic components

When it comes to assembling molecules into complex structures, materials scientists have been outclassed by nature. Compare, for example, chemists and clams. Both make high-strength ceramic composites. But while chemists must rely on crude methods—including extreme temperatures and the use of molds—to fuse neighboring molecules into specific shapes, certain clams take a more elegant approach. These mollusks engineer a shiny, tough mother-of-pearl shell by using a series of proteins that assemble themselves into a scaffolding. The scaffolding guides tiny ceramic plates, created by the mollusk, into precise shell layers.

Materials scientists would love to be able to emulate the clam and duplicate this type of "self-assembly," because it offers tremendous advantages in control and economy over conventional manufacturing. To control the makeup of microchips, for example, manufacturers need billion-dollar plants with clean rooms and vacuum chambers. Self-assembled structures, on the other hand, simply put themselves together based on attractive and repulsive forces between molecules. And the apparatus for doing this type of self-assembly—essentially a beaker on a tabletop—"costs about a dollar," says Tom Mallouk, a chemist at Pennsylvania State University.

So, hoping to borrow from biology, materials scientists are increasingly attempting to use materials that make themselves. Indeed, the growing use of self-assembly is part of what chemist Stephen Mann of the University of Bath in England calls a "quiet revolution" in materials science. The revolution, says Davis, is "the most important thing in materials synthesis right now. Biological organisms already know how to do this with unbelievable sophistication. They can organize structures at the angstrom, micron, and centimeter level. We're still at the beginning stages. But we're learning fast."

Although scientists are nowhere near duplicating nature's more elegant self-assemblies, they are already beginning to register their first practical successes. Several drug companies are in late-stage clinical trials with self-assembled microscopic vesicles that ferry potentially lifesaving drugs to cancer patients. And by getting organic, metal, and phosphonate molecules—complexes of phosphorus and oxygen atoms—to assemble themselves into conducting materials, researchers are turning electronic fabrication into a benchtop affair.



Self-made sandwich. This type of self-assembly system has been used to bring together electronically active materials.

SOURCE: TOM MALLOUK ILLUSTRATION: K. SUTLIFF

For now, most of this work remains in the early stages, particularly for electronics. Though most self-assembly setups are cheap by the standards of the semiconductor industry, they face stiff competition from existing technology, which has decades of research behind it and elaborate manufacturing facilities that are already scaled up to keep the cost of individual components low. Although self-assembly is a seductive prospect, "the ultimate deciding factor for all these applications will be cost," says Joel Schnur, a physical chemist at the Center for Bio/Molecular Science and Engineering at the Naval Research Laboratory (NRL) in Washington, D.C.

Delivering the goods

Today, self-assembly appears to be coming together most quickly in drug delivery. But it's been a long—and not particularly smooth—path. In order to succeed, medical shuttles must ward off the destructive attacks set in motion by metabolic processes. When they are injected directly into the bloodstream, many drugs, including a number of cancer chemotherapy agents, are broken down within hours by—among other things—enzymes in the blood and liver. Self-assembly gives researchers a tool to quickly and easily manufacture microscopic structures to protect these drugs so they can remain active in the body for longer periods, from days to weeks, it is hoped.

As early as the 1960s, researchers realized that one possible way to sneak drugs into the body would be to hide them in a material imitating the membranes that make up the body's own cells and vesicles. Those membranes are largely made of phospholipid molecules, which have balloon-like head groups that are attracted to water, and tails made up of long hydrocarbon chains that flee from water's presence. This "split personality" gives these molecules the ability to self-assemble. When phospholipids, like the biologically common phosphatidylcholine (PC), are placed in water, the tails arrange themselves end-to-end in an attempt to flee from the water. The result is a double layer, or bilayer, of phospholipids arranged tail-to-tail with the heads pointing out into the water. This bilayer then grows into a sphere, called a liposome, with water both inside and outside, but not in contact with the tails.

By dissolving therapeutic drugs in the water at the start of this process, researchers such as Alex Bangham of Cambridge University were able to use these "lipid bilayers" as drug carriers, which can vary in size from

25 nanometers to more than a micron. The drugs were released either by slowly leaking out through the porous membranes or all at once when the bilayers ruptured.

Trial, error, success

Although the idea was appealing, and spheres containing anti-cancer drugs were heavily hyped as a way to beat the disease, early versions of these self-assembling delivery trucks had problems. The spheres were attacked by immune cells called macrophages. How the macrophages recognized these spheres isn't known, though it is most likely due to proteins in blood plasma that attached themselves to the spheres, tagging them for removal by macrophages. Natural membranes and vesicles escape this fate because they incorporate other molecules, such as glycoproteins and carbohydrates, that stick out of the membrane and apparently help shield them from attachment by proteins.

At the time, researchers couldn't figure out a way around this problem, and the effort stalled. It started up again in the late 1980s, when two independent groups, led by Demetrios Papahadjopoulos, a biophysicist at the University of California at San Francisco, and Terry Allen at the University of Alberta in Edmonton, began mimicking the protection that natural membranes use by incorporating glycolipids into the liposomes. It worked: In animal tests the liposomes escaped assault by macrophages. In the last few years academic and biotechnology company researchers have pulled off the same trick using synthetic glycolipids such as polyethyleneglycol (PEG).

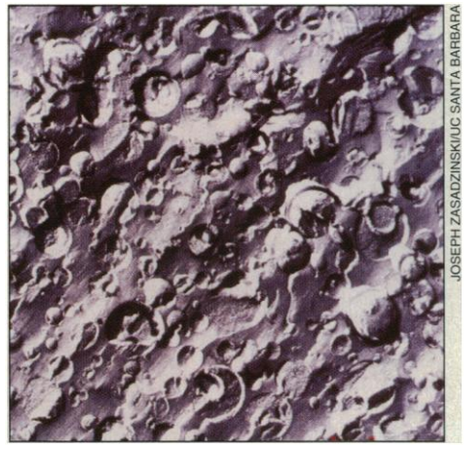
Based on that and other successes, liposomes are now being employed as transport vehicles for several different types of drugs. In early trials on animals, two companies—Liposome Technologies of Menlo Park, California, and The Liposome Company of Princeton, New Jersey—have shown that encasing the anti-cancer drug doxirubicin in liposomes and injecting it into cancer-stricken mice can increase survival rates of the mice over others that receive the drug all by itself. Both companies are currently in late-stage clinical trials with humans.

Liposomes now look promising for drug delivery, but they would be even more attractive if they could be specifically targeted to, say, home in on a tumor and deliver their cargo just to the tumor cells. And that approach—"targeted spheres"—is also being tried. Last year, Allen successfully attached an antibody for mammalian squamous-cell carcinoma to a sphere that successfully attacked lung tumor cells in mice. Also at an early developmental stage is an effort by several researchers, including Robert Langer at the Massachusetts Institute of Technology, to self-assemble drug-carrying spheres out of polymers, some of which may break down more slowly, thereby releasing their contents over a longer period of time.

Although this work holds promise, given liposomes' uneven track record to date, cancer researchers are watching these tests with some skepticism. High expectations for the vehicles have been quashed before, says Sydney Salmon, director of the Arizona Cancer Center at the University of Arizona. "[Liposomes] do appear to improve the half-life of drugs [in the bloodstream] and reduce toxicity. But they will need to be demonstrated in clinical trials," he says.

The tubular route

Spheres aren't the only structures that phospholipids can form. Lipids other than PC have features that cause them to assemble into tubes that resemble microscopic soda straws. And the ability of these "microtubules" to form spontaneously allows researchers to explore material structures that would be time consuming and expensive to create through chemical synthesis.



JOSEPH ZASADZINSKI/UC SANTA BARBARA

Molecular shuttles. Self-assembling spheres such as these encapsulate anti-cancer chemotherapy agents and are currently in clinical trials.

Tubule-forming phospholipids such as diacetylenic lipids have a slightly different shape than sphere-forming phospholipids like PC, says NRL's Schnur, who worked out a model of microtubule formation with NRL colleague Jonathan Selinger. According to this model, when these phospholipids begin stacking in bilayers, the molecules' asymmetrical shape keeps them from forming a sphere. Instead, the bilayer becomes a long, narrow strip. The disjointed packing within the bilayer then causes each strip to curl, rather like a spiral staircase. Attraction between intermolecular forces at the edges winds the staircase tighter until the edges fuse to form a tubule.

Once formed, these tubules can then be stuffed with a Jellolike polymer matrix containing drugs or other chemicals that will slowly drain out, as the polymer is porous. Earlier this year NRL biologists Alan Rudolph and Barry Spargo used this strategy to turn microtubules into timed-release drug capsules. In animal studies, they showed that tubules stuffed with the polymer matrix and a cell growth factor called transforming growth factor–β, when placed at the site of a wound, would slowly release the growth factor for up to 5 days, speeding wound healing.

The microtubules also make dandy microscopic templates. Coated with metals, they are stronger and have useful electronic properties. Physicists Ranganathan Shashidhar of NRL and Eric Cross of Pennsylvania State University are currently exploring the use of nickel and permalloy tubules to reduce the electromagnetic interference between adjacent microchips in ultradense circuit boards. Such microchips constantly give off and absorb electromagnetic waves, like radiowaves and microwaves. If strong enough, the electromagnetic waves given off from one chip can disrupt the operation of its neighbors. Up to now this hasn't been a problem because chip designers have been able to space the chips far enough apart so that interference isn't a concern. But as computer manufacturers strive to further miniaturize components, they are packing the chips closer together, boosting the interference between chips.

"The trick is to have very small pieces of material between each chip that will block electromagnetic fields," says Schnur. And microscopic rods of metal are ideal for this, as their long, thin shape enhances their ability to block electromagnetic waves. So Shashidhar and Cross have turned to self-assembled metal-clad tubules as a simple and cheap way to get that structure.

Sheets and sandwiches

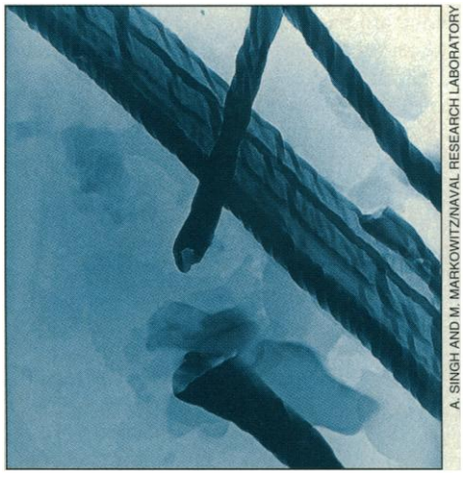
Electronics applications are also driving researchers to experiment with self-assembly as a way of layering sheets of materials, an arrangement that has myriad applications. Laying down successive single-atom-thick layers of semiconductor materials for devices such as laser diodes currently requires a $1-million molecular beam epitaxy machine to "spray paint" molecular layers. Self-assembly doesn't. The molecular building blocks are chosen so that they can only combine in a single-layered arrangement. "Self-assembly means you don't have to do very much work to get something that is well ordered and controlled," says Penn State's Mallouk. "It's like a poor man's molecular beam epitaxy."

Mallouk and others have been using this poor man's technique to layer electronically active materials for devices such as solar cells and light-emitting diodes (LEDs). To make such self-assembled sandwiches, says Mallouk, he has to control the chemistry so that "each layer serves as the bed for the next layer." In 1988, Mallouk first did this by layering a series of molecules on a gold-plated

silicon bed. He started by dipping this base into a solution containing molecules with a sulfur atom at one end, a hydrocarbon chain in the middle, and a phosphonate group at the end (see diagram on p. 316). The sulfur atoms tightly bonded to the gold surface, leaving phosphonate groups sticking out at the surface.

These phosphonates carry a net negative charge. So Mallouk placed the structures in a bath containing zirconium ions, which have a positive charge. The zirconium bonded to the phosphonates, forming a single-atom-thick layer of sheet metal. Then Mallouk dipped the structures in a bath containing molecules with phosphonate groups at both end, which readily bound to the zirconium, leaving another group of unbound phosphonates sticking out at the top. From there he could simply repeat the process, building layer after layer.

In general, layered structures are essential for semiconductor devices, which work by separating opposite electric charges between different layers, or at least different levels of the same layer. In conventional solar cells,



A. SINGH AND M. MARKOWITZ/NAVAL RESEARCH LABORATORY

Drawing straws. Used for applications ranging from drug delivery to microwave electronics, strawlike "microtubules" can be as small as half a micron in diameter.

for example, silicon atoms capture photons, emitting negatively charged electrons and positively charged "holes"—effectively the absence of an electron—in the process. These opposite charges migrate to the top and bottom of the silicon layer, where they are collected and shuttled to a battery for storage. If the charges recombine before reaching the battery, the result is heat instead of electricity.

In 1992, Howard Katz and his colleagues at AT&T used Mallouk's so-called "metal-phosphonate" self-assembling system to emulate part of a solar cell. They began by depositing several layers of organic molecules, known as porphyrins and vilogens. Like silicon, porphyrin molecules capture sunlight, giving off electrons and holes. In Katz' layered device, the porphyrins then donate the electrons to the vilogen molecules, allowing the charges to remain separate.

Of course, this is still far from a working solar cell, admits Katz, as he and his colleagues need to figure out how to collect the electrons and pass them to a battery. But if such problems can be solved, self-assembled layers hold the promise of drastically reducing the manufacturing cost of solar cells, since self-assembly makes forming multilayered electronic structures nearly as simple as dipping plates into successive buckets of dishwater and rinse water. With that promise in mind, researchers such as Princeton's Mark Thompson are tinkering with self-constructing layers as possible routes not only to solar cells but to LEDs, which run the same reaction in reverse, combining opposite electric charges and emitting light.

Constructing the field

Whether or not such applications will one day find their way to the marketplace remains to be seen. Applications like liposomes for delivering anti-cancer drugs face not only questions of efficacy but competition from more conventional therapies such as radiation and traditional chemotherapy. For applications such as electronics even proving cost-effectiveness may not be enough, since manufacturers have already spent billions to tool up for making silicon-based microchips. Like many materials science innovations, self-assembled structures are likely to be used for niche applications first, says Leslie Smith, a physical chemist at the National Institute of Standards and Technology in Gaithersburg, Maryland.

Whatever strategies these newcomers adopt for the moment, to insiders "it's clear that self-assembly is here to stay," says Davis. While some scientists refine current techniques in the hopes of proving cost-effectiveness, new applications keep appearing on the horizon. Researchers are trying to get self-assembling molecules to aid in orienting liquid crystal polymers for optical displays, as well in making nonlinear optical materials, which are essential for routing optically transmitted data. And though this quiet revolution is just beginning, one day researchers may be as proficient, if not as happy, as clams.

—**Robert F. Service**

Additional Readings

G. Cao *et al.*, "Layered Metal Phosphates and Phosphonates: From Crystals to Monolayers," *Accounts of Chemical Research* **115**, 11855 (1993).

S. Mann, "Molecular Tectonics in Biomineralization and Biomimetic Materials Chemistry," *Nature* **365**, 499 (1993).

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EVOLUTIONARY BIOLOGY

A Boost for "Adaptive" Mutation

British geneticist John Cairns touched off a firestorm in genetic and evolutionary circles 6 years ago by proposing that bacteria could, in his words, "choose" the mutations they acquire. Assigning such freedom of choice to bacteria violates a basic tenet of evolutionary theory, which holds that mutations arise at random. But Cairns and his colleagues claimed to have evidence for their view: They put bacteria unable to digest lactose into a petri dish with only lactose for nourishment—and found that the bacteria preferentially acquired the crucial mutations they needed to become lactose-eaters.

Then came the firestorm. Other researchers also saw evidence of these apparently nonrandom mutations in their counts of various bacterial and yeast colonies. But a legion of neo-Darwinists and population geneticists, who considered Cairns' idea heresy of the first order, pounced on this work, charging that the results were due to experimental artifacts. Now, according to two papers in this issue of *Science*, a distinct type of mutation is indeed at work under certain selective conditions—but neither paper proves that useful mutations arise faster than non-useful ones.

In essence, some critics had claimed that Cairns and others had miscounted garden-variety mutations. Patricia Foster and Jeffrey Trimarchi of Boston University, on page 407, and Susan Rosenberg of the University of Alberta and her colleagues, on page 405, go beyond colony counts to present gene sequence data from *Escherichia coli*, showing that under selection pressure, a novel mutation mechanism is creating specific types of mutations. Says geneticist Franklin Stahl of the University of Oregon, "With essentially one [stroke], this qualitative observation rules out the possibility that the whole thing was an unintentional fake."

Not only do these papers show that something exceptional was happening in Cairns' experiments, they offer clues to the molecular nuts and bolts of the mutational mechanism, implicating the same kind of genetic mistake that has recently been linked to colon cancer and some inherited diseases. With these results in hand, even longtime skeptics like Richard Lenski of Michigan State University say they are now persuaded that an unusual mutational process is at work—at least in this experimental system.

But Lenski is quick to point out that