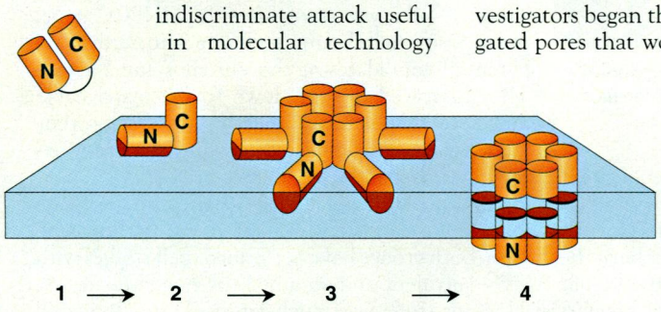
Materials Science Comes to Life at Boston Gathering

Last week, more than 3500 scientists met in Boston for the fall meeting of the Materials Research Society, which is celebrating its 20th anniversary. As the topics below indicate, materials modeled on biology, or borrowed from it, have become one of the field's hottest areas; this year they were the focus of two symposia.

Switching a Bacterial Toxin to Good Use

For more than a decade, molecular biologist Hagan Bayley of the Worcester Foundation for Experimental Biology in Shrewsbury, Massachusetts, has been trying to refine a bacterial weapon: a protein called alpha-hemolysin, which ruptures cells by shooting their membranes full of holes. To make that indiscriminate attack useful in molecular technology and medicine, Bayley must tame the toxin so that it will open—or close—pores on command.



Poring it on. Alpha-hemolysin molecules adhere to a cell surface, partially open, aggregate, and snap fully open, piercing the membrane and creating a minute pore.

SOURCE: BAYLEY, *JOURNAL OF BIOLOGICAL CHEMISTRY* ILLUSTRATION: L. CARROLL

In two talks at this year's meeting, Bayley and one of his collaborators, John Kasianowicz, a biophysicist at the National Institute of Standards and Technology, reported taking a large step toward fulfilling that vision. (Some of the results will appear in *Protein Engineering*.) By manipulating the gene for alpha-hemolysin, they have been able to equip the protein with "switches" that respond to proteases—protein-cutting enzymes—or metal ions. Thus modified, Bayley and Kasianowicz think, such proteins could serve as molecular biosensors and possibly join the arsenal of cancer treatments.

The switches are based on earlier research that had dissected the workings of alpha-hemolysin (*Science*, 7 February 1992, p. 684). After its gene was cloned in the mid-1980s, Bayley and other researchers learned that the 293 amino acid long protein has two primary domains, C and N, connected by a 44 amino acid loop. Normally, the C and N domains jackknife together with the loop as a hinge. But when alpha-hemolysins bind to the surface of a cell membrane, researchers believe, each jackknife partially unfolds, allowing the C domains of six proteins to associate with each other in a circular pre-pore complex while the N domains remain splayed on the cell surface. At that point, the hinges snap open and the N domains of the six proteins pierce the membrane to open a nanometer-sized pore.

Once this mechanism was revealed, investigators began thinking up ways to create gated pores that would open or close in response to external stimuli, such as chemicals. At the meeting, Kasianowicz described experiments with one altered pore-forming protein that may fit the bill. Bayley's lab has created a mutant alpha-hemolysin in which the central loop contains the amino acid histidine in five consecutive positions. The mutant protein forms pores perfectly, but when exposed to micromolar concentrations of metal ions like copper and zinc, which bind to histidine, the pores snap closed, apparently because the proteins return to their pre-pore position. A chelating agent, which mops up metal ions, can reverse the process, reopening the holes. Kasianowicz showed that such pores might serve as metal-ion biosensors, since their opening and closing causes measurable changes in the conductance of cell-like membranes.

In another effort to gain control over pore formation, Bayley's lab has created alpha-hemolysin "overlap mutants," in which two overlapping fragments of the alpha-hemolysin protein are synthesized by expressing two gene fragments in a plasmid. The first fragment codes for alpha-hemolysin's first 172 amino acids, the second for amino acids 132-293. Together, these two sub-units form an abnormally long molecule, but when the altered molecules encounter a cell membrane, they can still gather into pre-pore complexes.

That's as far as overlap mutants go, however, because the extra amino acids disrupt the connecting loop, jamming the hinge. But if the first fragment is mutated so that it incorporates a protease-recognition site, the hinges can be unjammed on command by a protease enzyme, which cleaves out the extraneous amino acids, leaving alpha-hemolysins that can snap completely open and form pores. "We've built a biochemical trigger into the protein that's triggered selectively by an enzyme," says Bayley.

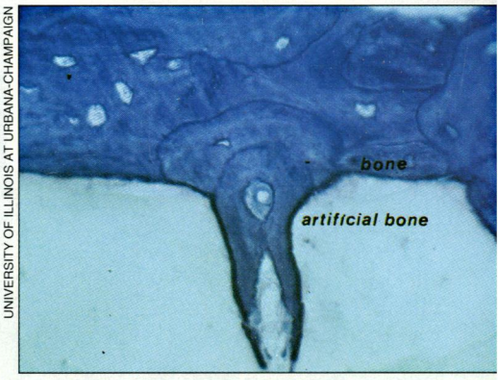
Bayley's lab is already modifying this approach for the war on cancer. He and his colleagues are linking alpha-hemolysin molecules to antibodies, creating a class of proteins they call "immunolysins." Guided to tumor cells by the antibodies, these proteins would pierce cells, killing them or making them more vulnerable to toxic agents. To further ensure that nearby healthy cells are not ripped open, the researchers plan to engineer the alpha-hemolysin so it is activated only by specific tumor-associated proteases. In this way, says Bayley, "we may be able to get tumor cells to commit suicide."

That notion, while speculative, delights Bayley's colleagues who work with alpha-hemolysin. Says Vanderbilt University microbiologist Sidney Harshman, "I think he's devised a cute, ingenious way to have an immediate application. It's a really neat idea, even if it doesn't work."

–John Travis

Boning Up With Organoapatites

In the past few years, biomaterials researchers have been assembling an impressive kit of human replacement parts: Artificial skin, bone, cartilage, even complicated organs like the liver are in the works. At this year's materials' meeting, Samuel Stupp, a materials scientist at the University of Illinois at Urbana-Champaign, described his own lab's efforts to add to the repair kit with a novel class of materials that mimic the structure of natural bone. His eventual goal is to develop a bone substitute that could coat and



UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

A healthy apatite? Organoapatite—a polymer-mineral composite that is a potential bone substitute—gives way to natural bone growth.

bond metal implants, fill up defects or cracks in bone, or even serve as an implant material itself.

To create these new materials, which Stupp calls organoapatites, his lab grows crystals of hydroxyapatite—the same calcium phosphate mineral that adds hardness to natural bone and teeth—in a solution of polymeric long-chain organic molecules. As the crystals form, they capture the polymers, creating a material in which the polymers are threaded throughout the ordered crystal lattice of the apatite, much as organic molecules and proteins are threaded through the mineral lattice of natural bone. The polymer network toughens the replacement material and helps control the size of the crystals. Laboratory-grown hydroxyapatite crystals are usually far larger than the nanometer-sized ones found in bone, but, says Stupp, "the crystals of our artificial bone look like those of natural bone."

That could be a major advantage. Stupp's research has shown that, unlike normal hydroxyapatite, which is sometimes sprayed on metal implants to encourage tissue adhesion, organoapatites are naturally degraded by osteoblasts, the bone-forming cells of normal bone. "This material is recognized by the cells as dead bone," he says. "I think the crystal size is important to the ability of the cells to recognize and break down the apatite." In its place, the osteoblasts deposit natural bone—a better material than any substitute.

Stupp foresees the possibility of endowing the artificial bone with other properties by manipulating the incorporated polymers. For example, says Stupp, "You can use growth factors or drugs as the organic factors you thread through the lattice," turning the organoapatite into a slow-release drug-delivery system. His lab has already made an organoapatite that combines a biocompatible polymer found in soft contact lenses—for strength and flexibility—with an anti-inflammatory drug and a series of amino acids that promotes tissue regeneration. The idea, says Stupp, would be to ward off any immune attack on the bone substitute—while hastening its natural demise by encouraging new bone formation.

With only very preliminary animal testing done on a few types of organoapatites, Stupp admits it's too early to say whether this novel class of materials will fulfill the promise he envisions. Moreover, Stupp faces stiff competition from other investigators and biotech firms who have their own candidate bone substitutes. Comments Myron Spector, director of orthopedic research at Brigham and Women's Hospital in Boston, "I believe organoapatite is a particularly innovative approach [to a bone substitute]. But it's difficult to say how valuable it will prove to be in a clinical setting."

–J.T.

BIOTECHNOLOGY

Can DNA Mimics Improve On the Real Thing?

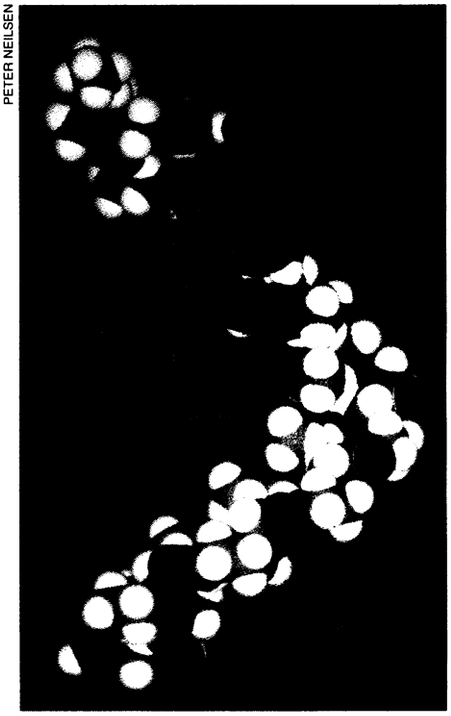
You wouldn't think DNA and RNA needed to be replaced or updated. After all, those two nucleic acids have done a pretty good job for billions of years in their traditional roles of coding and passing on genetic information. But with the coming of modern biomedicine and its vision of using bits of DNA and RNA to tag or combat harmful genes, the natural compounds don't always fit the bill anymore. They break down quickly in the body, and they don't bind to their targets as eagerly as some researchers would like. Those faults make the possibility of improving on nature irresistibly attractive to some biotech companies.

At the moment the highest hopes of these biotechnicians lie in a class of manmade compounds, invented a mere 2 years ago, that look like and act like DNA—but are completely different under the skin. The new molecules, brainchildren of chemists Michael Egholm, Peter Nielsen, Ole Buchardt, and Rolf Berg at the University of Copenhagen in Denmark, string the four chemical bases that serve as coding characters in DNA and RNA along a chemical backbone borrowed from a completely different class of molecules: proteins. These new compounds, known as peptide nucleic acids (PNA), are not only more stable in cells than their natural counterparts, but also bind natural DNA and RNA 50 to 100 times more tightly than the natural nucleic acids cling to each other (*Science*, 6 December 1991, p. 1497).

This discovery has attracted interest from basic scientists, who are intrigued by the notion that, as Jack Cohen of Georgetown University puts it, "man is beginning to come up with analogs of things nature made with evolution." And it has spurred a flurry of activity at biotech companies, among them Denmark's PNA Diagnostics, which is on the verge of marketing the first PNA-based test kits, and Isis Pharmaceuticals of Carlsbad, California, which is starting animal tests of PNA therapies. And researchers have been studying PNA at several other companies, including Glaxo in Research Triangle Park, North Carolina.

As the development of PNAs moves forward, however, Cohen and many of his academic colleagues are feeling qualms. They worry that these doppelgängers could reveal nasty traits that might preclude their use as drugs. Indeed, biochemists working with PNAs admit that the properties that make them so promising—tight binding and longevity—could also worsen side effects. "These [compounds] might be of immense therapeutic value," says biologist Malcolm Pluskal of Millipore in Bedford, Massachusetts. "But from an ethical standpoint I think it's important to look at these concerns." Isis research scientist John Keily also tempers enthusiasm with caution. "There will be some nonspecific effects, so you have a legitimate concern," he says. "It's just too new to know how serious the concerns are."

PETER NEILSEN



Risky embrace? A peptide nucleic acid *(orange)* binds to a DNA strand far more tightly than can the complementary DNA sequence.

The biotech executives who have jumped into PNA development are willing to run that risk on the chance they will be able to find ways to avoid the dangers and exploit PNA's attractive qualities. The most important of them is that, like natural DNA and RNA, it can seek and bind another strand with the complementary sequence of nucleotide bases. That's the idea behind the DNA-based diagnostic probes that are often used to seek out mutated genes or genes of pathogens such as the AIDS virus. It's also the basis of an emerging form of genetic therapy known as antisense. The idea behind antisense is to block the action of unwanted genes—either mutations or viral genes—with nucleic acid strands carrying the opposite