Protein shells out guidance to crystals

The glimmering, opalescent beauty of mother-of-pearl arises from the intricate layers of proteins and calcium carbonate crystals laid down as a mollusk generates its shell. The proteins do more than just coexist with the crystals: They influence their shape and guide their direction of growth.

Researchers at Boston University have taken a step toward understanding how proteins issue calcium carbonate crystals their marching orders. Richard A. Laursen and Daniel B. DeOliveira designed a protein that binds to a form of calcium carbonate called calcite. Although calcite usually grows in a rhombohedral, or diamond, shape, the crystals metamorphosed into tall pillars or boxy nuggets in the presence of the protein.

This experiment, says David Kaplan of Tufts University in Medford, Mass., "is the first to demonstrate that you can design a protein from first principles and define it to interact with the crystal faces. I think [Laursen and DeOliveira's] work is just terrific because it gives people a new direction to think about."

Several researchers have previously studied how the mix of natural proteins produced by mollusks influences the growth of crystals. Daniel E. Morse and his colleagues at the University of California, Santa Barbara, for example, found that they could abruptly switch growing calcite crystals to another form called aragonite by applying protein mixtures (SN: 4/12/97, p. 228). Aragonite is a major component of shells.

No one, however, has isolated and purified any of the natural proteins that control calcium carbonate growth, says Kaplan.

Rather than working with the poorly known mollusk proteins, Laursen and DeOliveira started from a well-characterized protein that prevents a fish called grubby sculpin from turning into an ice sculpture in the winter (SN: 4/19/97, p. 237). This antifreeze protein and others like it circulate in the blood of many Arctic and Antarctic fish, binding to the surface of ice crystals to prevent them from growing too large.

The grubby sculpin protein has a tight spiral structure known as an alpha helix. The researchers substituted a few negatively charged amino acids for neutral ones in the antifreeze protein so that it would bind to the positively charged ions on the surface of calcite. Calcite crystals are formed from charged ions, whereas ice builds up from neutral water molecules connected through hydrogen bonds.

The researchers allowed rhombohedral calcite seed crystals to grow in a solution of calcium carbonate and the protein. At 3°C, most of the protein is in its alpha helical shape, and the crystals

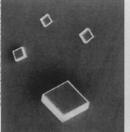
grow in only one direction, forming long columns. "That's what we'd hoped they'd do," says Laursen. He and DeOliveira had designed the protein to coat specific faces of the crystal and block the addition of ions there.

At 25° C, however, the proteins lose their shape and the crystals build up on all sides, resulting in a "totally unexpected" studded

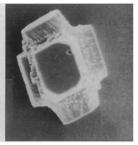
shape, Laursen says. Unraveled, the proteins may act like a collection of negatively charged ions, influencing the crystal shape in a gross way instead of interacting directly with the crystal surface, he suggests.

DeOliveira and Laursen report their findings in the Nov. 5 JOURNAL OF THE AMERICAN CHEMICAL SOCIETY.

Although the team has shown it can make a protein that controls crystal growth, Laursen cautions that the results don't explain how biomineralization occurs. So far, there's no evidence that the proteins that guide shell growth in mollusks have an alpha helical structure like the fish antifreeze proteins. The mollusk proteins probably contain accordion-pleated structures known as beta sheets, but those are much more difficult to synthesize and work with, he says. —C. Wu







Calcite crystals shaped like rhombohedrons (left) grow into long columns (middle) in the presence of a specially synthesized protein. At a higher temperature, the protein loses its well-defined three-dimensional structure and interacts differently with the calcite. The rhombohedral crystals then build up on all sides, acquiring a studded appearance (right).

Compromise reached on census sampling

After months of wrangling, the Clinton administration and Republican congressional leaders agreed last week on a compromise plan to prepare for the year 2000 census.

The agreement requires the Bureau of the Census to pursue a dual track. The bureau may experiment with the use of statistical sampling to supplement a traditional head count (SN: 10/11/97, p. 238), but at the same time it must test enumeration without sampling as part of its dress rehearsal, scheduled for early 1998.

Republicans, meanwhile, will pursue an expedited review by the Supreme Court of the legality and constitutionality of using statistical sampling to adjust census numbers.

The measure also establishes an eight-person, bipartisan Census Monitoring Board to oversee preparation and implementation of the 2000 census.

In effect, the compromise delays until 1999 a decision on whether nationwide sampling can be used to supplement and adjust a traditional enumeration. "We've come to an operational truce," says Martha Farnsworth Riche, Census Bureau director. The compromise lets the bureau move forward while judicial review occurs, she adds.

The bureau will test its new procedures, which include sampling, in Sacramento, Calif., and on the Menominee Reservation in Wisconsin. As a contingency plan, it will conduct a more traditional enumeration in a number of rural counties in South Carolina.

Members of Congress have expressed a variety of concerns about making statistical adjustments to census numbers, including the possibility of undermining public confidence in the decennial census and reducing participation. Several have noted the complexity of the statistical approach and the inherent uncertainties and assumptions in the chosen statistical methods.

"I'm concerned about how the sampling is actually implemented," says David W. Murray of the Statistical Assessment Service in Washington, D.C. Enumeration does not catch everyone, he concedes, but with a statistical approach, there is a danger of building in other sorts of errors because of the choice of sampling methodology.

Moreover, such errors are much more difficult to pinpoint and understand than those associated with enumeration, Murray contends. "Is one type of error being replaced by another?"

"The fact is that traditional census operations give us an undercount," says Charles L. Schultze of the Brookings Institution in Washington, D.C. Those most often missed include rural renters, migrant workers, elderly women living alone, minorities, mobile young men, and particularly children.

Sampling offers a substantial improvement in accuracy, Schultze argues. Indeed, there may no longer be a realistic alternative to sampling because of the escalating costs and diminishing returns of conducting a conventional enumeration.

"It's an issue of trust," Riche says. "We want to make the process as transparent as possible."

—I. Peterson

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