X-RAY CRYSTALLOGRAPHY

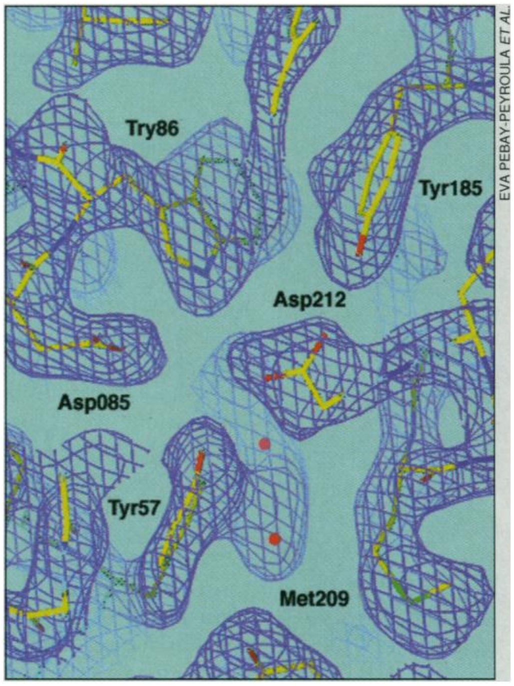
Opening the Door to More Membrane Protein Structures

If one picture is worth a thousand words, recent advances in x-ray crystallography methods are providing the equivalent of the *Encyclopedia Britannica*. Crystallographers are now churning out the three-dimensional (3D) structures of proteins at the rate of one or more per day. But so far one key group of proteins—those normally located in cell membranes—has been badly underrepresented in this ever-expanding book of structural knowledge. The problem is that researchers have to crystallize proteins in order to probe their structure with x-rays, and integral membrane proteins often can't withstand being removed from their normal environment. A solution to this problem may be at hand, however.

On page 1676 of this issue, Eva Pebay-Peyroula of the Institute of Biological Structure in Grenoble, France, and Gabriele Rummel, Jurg Rosenbusch, and Ehud Landau of the University of Basel, Switzerland, offer a newly detailed 3D structure of bacteriorhodopsin, a key protein enabling the salt-loving bacterium *Halobacterium salinarium* to convert energy from sunlight to chemical energy that the bacterial cells can use. The structure, with a resolution of 2.5 angstroms, offers the most detailed look yet inside this solar power plant.

Bacteriorhodopsin absorbs photons of light and uses their energy to pump protons out of the bacterial cell, generating a chemical and electrical gradient across the membrane that can serve as an energy source. Water molecules located in the coils of the protein are thought to act as a kind of bucket brigade that helps the protein's amino acids achieve this proton transport. By showing the precise positions of eight water molecules, as well as of the amino acid side chains, the new structure is helping investigators understand how the bacteriorhodopsin choreographs this process. The structure may also provide a model for understanding the operation of other membrane proteins with similar overall architecture, which include several receptors for hormones and neurotransmitters.

But even more important, the novel technique the Swiss-French team developed for crystallizing bacteriorhodopsin could make it much easier to grow crystals of membrane proteins generally, opening the way to direct analyses of their structures. The researchers grew their crystals within lattices of membranelike materials, created by mixing lipids and water under appropriate conditions. The technique, says Columbia University protein crystallographer Eric Gouaux, "offers a very creative approach to growing crystals of membrane-bound proteins that, in the past, have proved difficult to prepare."



Clarified vision. The electron density map shows the locations of some of the amino acids in or near the bacteriorhodopsin proton pathway. The red dots indicate two water molecules located in a water pocket behind tyrosine 57 and aspartic acid 212.

The problem is that proteins embedded in membranes are surrounded mainly by fat molecules. When researchers then try to coax the proteins out of the membrane so that they can be crystallized, they tend to unfold and become disorganized in their new watery surroundings. In the past, this limited investigators to making two-dimensional bacteriorhodopsin crystals, consisting of just a single layer of well-ordered protein molecules. These could be analyzed by a technique called electron crystallography, which enabled pioneering researchers such as Richard Henderson and his colleagues at the Medical Research Council Laboratory in Cambridge, United Kingdom, to solve the bacteriorhodopsin structure to a resolution of 3.5 angstroms. But that resolution was not quite good enough to see all the atoms of the protein and its associated water molecules. Currently, only x-ray crystallography can deliver a view that is sharp enough. And x-ray crystallography requires 3D crystals.

Biophysical chemist Ehud Landau reasoned that the best way to grow 3D crystals of bacteriorhodopsin would be to keep the protein in a congenial environment. He custom-built a lattice of lipids, fats similar to those in membranes, to house the bacteriorhodopsin during crystallization. "We have faked the natural environment," says Rosenbusch. As a result, extracted membrane proteins that previously fell apart in a matter of hours were "happy and functioning for months," yielding very small, 3D crystals.

But growing 3D crystals of bacteriorhodopsin for the first time was not enough to get the job done. Because the crystals were tiny and fragile hexagonal plates, only 20 to 40 micrometers (millionths of a meter) in diameter and 5 micrometers in thickness, they tended to crack easily, like potato chips. Because this distorts the spatial arrangement of bacteriorhodopsin in the crystal lattice, such crystals diffract x-rays poorly. To get the desired high resolution, the team needed an x-ray beam narrow and bright enough to produce good diffraction patterns from the very small crystal fragments remaining. The team turned for help to physicist Christian Riekel at the European Synchrotron Radiation Facility (ESRF) in Grenoble, a powerful x-ray source. ESRF has recently opened a new microfocus beamline that can pack hundreds of billions of photons a second into a beam just 10 micrometers in diameter (*Science*, 29 August, p. 1217).

The result was a high-resolution picture of bacteriorhodopsin that is broadly similar to the existing picture, based on the electron crystallographic analysis and on studies in which H. Ghobind Khorana of the Massachusetts Institute of Technology and his colleagues mutated amino acids to identify the ones associated with proton transport. But some critical differences may require researchers to modify their view of how that transport occurs.

For example, mutational studies had identified aspartic acid 85 as a key component of proton transfer. But the new structure shows that this amino acid is too far from the schiff base attached to retinal, the pigment bound to bacteriorhodopsin that puts the proton pump in operation by absorbing photons, for direct proton transfer to occur. However, because the new structure locates water molecules in the bacteriorhodopsin proton pathway, researchers do have their first clues to how some water molecules may help shuttle protons through the bacterial membrane. "The issue is, are these the interesting water molecules?" says Henderson. Rosenbusch says they soon hope to answer that question, by introducing mutations into the bacteriorhodopsin molecule that alter proton transfer and may allow the effects of the water molecules to be seen.

Crystallographers may soon be getting such intimate views of other membrane proteins, thanks to Landau's technique for mimicking the natural environment. Eventually, they may be able to design and build artificial membranes with different lattice sizes to snare and crystallize membrane proteins of varying sizes and shapes for structural studies. Riekel also predicts that further improvements in ESRF's microfocus beamline will allow analysis of crystals as small as 5 micrometers across. "Microdiffraction has a large future for difficult-to-crystallize substances," he says. If so, protein chemists will soon see a large number of membrane proteins in their structural encyclopedia.

–Anne Simon Moffat

EARTH SCIENCE

More Signs of a Far-Traveled West

For 2 decades, earth scientists have argued over the proposal that chunks of North America's western edge migrated thousands of kilometers northward to their present positions. Now, a few exquisitely preserved fossils have given the theory an extra push. A team of geologists and geophysicists reports in this issue of *Science* that the superb condition of marine fossils from near Vancouver Island provides a key test of the evidence, which consists of traces of ancient magnetism in the fossil-laden rock. Seventy million years ago, they conclude, Vancouver Island was adjacent to Baja California, thousands of kilometers to the south.

After so many years, the dispute (*Science*, 5 May 1995, p. 635) is not likely to be settled by a single finding. Indeed, critics are already identifying loopholes. Still, the study, reported on page 1642 by paleontologist Peter Ward of the University of Washington in Seattle, paleomagnetician Joseph Kirschvink of the California Institute of Technology in Pasadena, and their colleagues, "could influence the fence sitters," says geologist Darrel Cowan of the University of Washington, who has written on possible geologic tests of the so-called Baja–British Columbia hypothesis.

The hypothesis originated 20 years ago in studies of the magnetism locked in rocks from the so-called exotic terranes of the Pacific Northwest—large chunks of crust that seem to have formed elsewhere and migrated to their present positions. Because Earth's magnetic field is horizontal at the equator but vertical at the poles, the inclination of a rock's magnetism shows how far north it was when it formed and locked in Earth's field. Along the west coast of North America, researchers measured paleomagnetic inclinations smaller than they should be if the rock had formed in place, as part of North America. Many paleomagneticians took that to mean that the terranes had slid up the coast from far to the south, much as California west of the San Andreas fault is sliding now.

Most geologists and some paleomagneticians disagreed. For one thing, they couldn't see the large faults that would have guided the rocks northward. Instead, they proposed that the terranes originated offshore at roughly their present latitudes and later docked with North America. The shallow magnetic inclinations were misleading, they argued, because most of these measurements came from great masses of frozen magma, which the tectonic jostling of the coast could easily have tilted from their original orientations over tens of millions of years.

P. WARD

Telling luster. The fine preservation of mollusk fossils like this ammonite, several centimeters across, suggested that the rock holds an accurate record of its travels.

Sedimentary rock would solve that problem, because it is laid down in recognizable horizontal layers. But sedimentary rocks from the largest terrane—the Insular superterrane, which makes up much of the coastal crust from northern Washington state into Alaska—seemed to have been heated long after they formed, wiping them clean of their original magnetic signature.

Kirschvink, however, realized from unrelated work he and Ward were doing in central California that temperature-sensitive fossils could be a marker for rock that hadn't been heated and magnetically altered. Ward, in turn, knew of fossils from the Texada and Hornby islands—part of the Insular superterrane off the east coast of Vancouver Island—that fit the bill. These fossils of extinct mollusks, called ammonites and inoceramids, retained the pearly luster of the living animals, implying that the paleomagnetic inclinations in the surrounding rock could be relied on. The magnetism was about 25 degrees shallower than expected at Vancouver Island's current latitude, implying that 70 million to 80 million years ago, when the rock formed, the terrane was 3500 kilometers to the south, off Baja California.

Ward and his colleagues say the condition of the fossils also bears witness against another process that could have skewed the data: compaction shallowing. Sediment can compress by 50% or more as the weight of new sediment above it squeezes out the water between its grains. Compaction can reduce any existing paleomagnetic inclination as the magnetic grains tilt toward the horizontal under the compression.

Ward and Kirschvink note that even if there had been compaction, the 10° of compaction shallowing typically found couldn't explain the 25° in their rocks. But the fossils argue against even that much compaction, says Kirschvink: "One of the indicators [of sediment compaction] is to look for compacted fossils, and they're not present in these sediments." Further reassurance comes from the carbonate globules that tend to encase these fossils, he says. These concretions began to form soon after the fossils were buried, he says, and would have welded grains of rock in place, preventing compaction shallowing. That's "a good way to stop inclination error," he says. "We can rule it out."

"Their argument is pretty good," says paleomagnetician Kenneth Kodama of Lehigh University in Bethlehem, Pennsylvania, who has studied compaction shallowing in the lab. But, says Kodama, "they haven't thought enough about whether or not the concretions could have formed after a certain amount of compaction. As they say, it may not explain all of their shallowing of inclination, but it could explain part of it." Sedimentologist Peter Mozley of the New Mexico Institute of Mining and Technology in Socorro agrees. "Typically, concretions are thought to form early, but late-stage concretions do exist," he says.

Kodama and Mozley recommend further analysis of the concretions to pin down just how much compaction actually occurred. Until then, says longtime Baja–British Columbia critic Robert Butler of the University of Arizona, Tucson, "the whole thing goes on without a definitive closure."

–Richard A. Kerr