Membrane Protein Holds Photosynthetic Secrets

This year's Nobel Prize for chemistry recognizes the direct and indirect benefits that flowed from the crystallization of the photosynthetic reaction center from a bacterial membrane

"THEY CALLED ME CRAZY," remembers Hartmut Michel, who shared this year's Nobel Prize in chemistry with fellow Germans Johann Deisenhofer and Robert Huber. "People said it was impossible to crystallize membrane proteins, but I persisted and eventually was successful."

The protein that Michel managed to crystallize, and whose atomic structure he then analyzed in collaboration with Deisenhofer, was the photosynthetic reaction center of the purple bacterium *Rhodopseudomonas viridis*. Their joint work therefore has implications not only for preparing membrane proteins for analysis in general, a class of molecules that has great medical importance, but also for gaining a deeper understanding of the chemistry of photosynthesis. In addition, it gives researchers unprecedented insights into new areas of chemistry.

In the past 20 years more than 100 water-soluble proteins have been purified and crystallized, and, using x-ray crystallography, their structures analyzed atom by atom. Water molecules play an important role in allowing the hydrophilic protein molecules to line up in a crystal lattice, and they often insert themselves in the spaces between the proteins.

Proteins that are integral parts of membranes are, however, not soluble in water, because their normal function requires that they interact both with lipids (within the membrane itself) and water (at the membrane surface). In other words, they are partly hydrophilic and partly hydrophobic, and will not line up in neat crystalline arrays when exposed to water.

The challenge to crystallize membrane proteins had seemed insurmountable, decades of attempts having been rewarded only with failure. "People thought it couldn't be done," says William Parson of the University of Washington. "It was a psychological barrier, like the 4-minute mile." Repeated failure had given rise to rationalizations about why it would *never* be done . . . until, in 1981, Michel did it. Like most before him, Michel used detergents to free the protein from the membrane, but the key next step was to employ small "amphiphilic" molecules—having both hydrophilic and hydrophobic elements—that effectively kick the detergent molecules away from the proteins, and take the place of water in the crystal lattice.

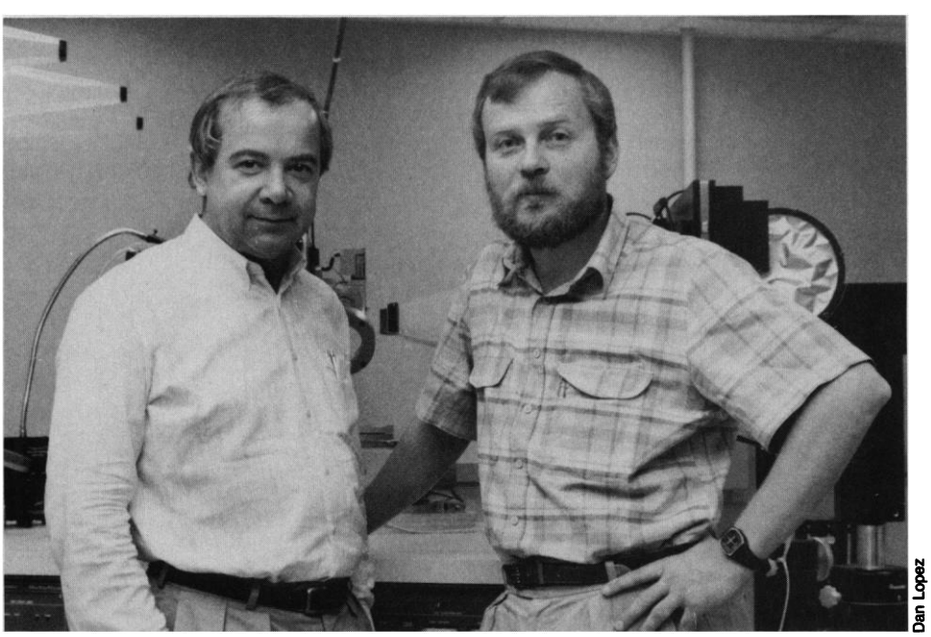
Before trying to break the biochemist's 4-minute mile with the photosynthetic complex, Michel had been attempting to crystallize another bacterial membrane protein, bacteriorhodopsin from *Halobacterium halobium*. "I did a postdoc with Dieter Oesterhelt at the University of Wurzburg, trying to fuse the bacteriorhodopsin into vesicles," explains Michel. The idea was that bacteriorhodopsin, a purple, light-sensitive pigment like the one in the vertebrate retina, might provide an energy source for driving amino acid transport in the vesicle.

During this project Michel and Oesterhelt made two-dimensional crystals of the purple pigment, but failed to make three-dimensional crystals. "The techniques and approaches we developed with bacteriorhodopsin enabled me later to succeed with the photosynthetic complex," explains Michel. For his inspiration and drive in promoting this work, Oesterhelt fully deserved recognition by the Nobel committee, Michel told *Science*. "Without his help, I would have given up."

In 1979 Oesterhelt and Michel moved from Wurzburg to the Max Planck Institute in Martinsried. Soon afterward Michel turned his attention from the bacteriorhodopsin problem, which was still proving intractable, to the *Rhodopseudomonas viridis* photosynthetic reaction center. This protein complex was one of several membrane proteins that seemed promising candidates for crystallization, because they appeared more or less as two-dimensional crystals in the living membrane. "In September 1981 I had the first *viridis* crystals," Michel recalls. The next step was structural analysis with x-ray crystallography.

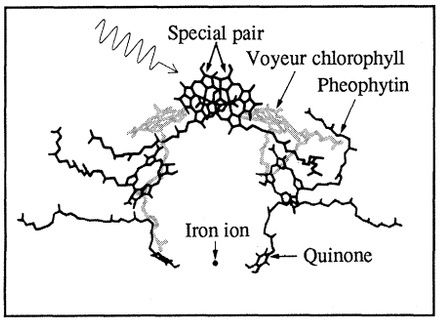
Although Michel had some experience with the technique, he says "the project was too big for one person." The following summer, therefore, he teamed up with Deisenhofer, after having given a seminar in Huber's laboratory in Martinsried, where Deisenhofer worked. Within 2 years the structural analysis was virtually complete, and the results were to have a tremendous impact on researchers' understanding of photosynthetic chemistry. "There had been a great deal of work on the chemistry of photosynthesis, particularly using spectroscopy," says Deisenhofer, "but there were still a lot of puzzles. The structural work helped solve a lot of those puzzles."

Photosynthesis occurs in a wide variety of plants, algae, and bacteria, and, although the fundamental effect is the same in all cases—converting light energy into chemical ener-



Dan Lopez

Nobel duo: *Hartmut Michel (right) visits Johann Deisenhofer at the University of Texas, Dallas, where he moved earlier this year.*



Wide World

Reaction center: *Shown here are the pigments and accessory molecules at the heart of the photosynthetic reaction center.*

gy—there are some important differences among them. For instance, plants, algae, and cyanobacteria (blue-green algae) use water as a source of electrons in the photochemical process, whereas other bacteria, including purple bacteria, employ a variety of other electron donors. In addition, in plants, algae, and blue-green algae there are two routes for trapping light energy (photosystems I and II), whereas in other bacteria there is only one. In spite of these differences, the structural information gleaned by Michel and Deisenhofer from the *Rhodopseudomonas viridis* photosynthetic reaction center has general implications for understanding the energy-converting mechanisms of photosynthesis as a whole.

By the early 1980s, chemists, biochemists, and spectroscopists had teased apart much of what was involved in photosynthesis as a whole, including the purple bacteria with which Michel and Deisenhofer worked. It was known, for instance, that in the reaction center complex in *Rhodopseudomonas* there are four proteins subunits, denoted L, M, H, and C, the last being a c-type cytochrome, associated with four heme residues. It was also known that the heart of the reaction center consists of four molecules of chlorophyll, two of pheophytin (related to chlorophyll), and two quinones. This whole package is embedded in the membrane of photosynthetic vesicles within the bacteria.

The application of optical absorption and electron spin resonance spectroscopy had also revealed in detail the events set in train by the absorption of light. A photon excites an electron in a chlorophyll molecule in the reaction center nearest the inner surface of the membrane. The electron then begins a journey through the reaction center to a point near the outer surface of the membrane, visiting first a neighboring pheophytin molecule, then a quinone (Qa), and finally a second quinone (Qb). When this happens twice the effect is to establish a charge separation across the membrane, which represents stored energy that ultimately drives chemical synthesis.

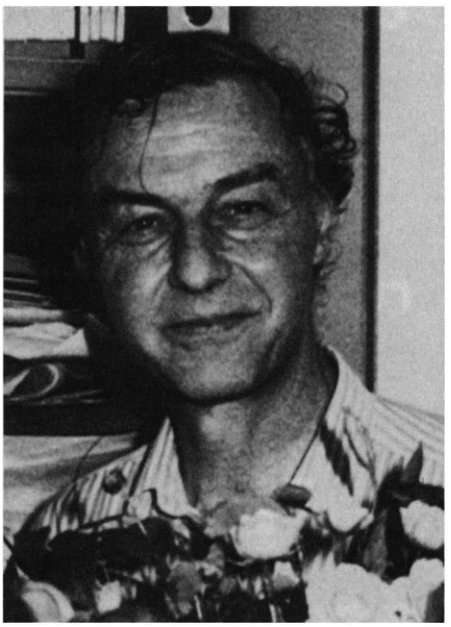
Not only was the sequence of events known, but also the time taken for each event, a fairly complete picture. However, ideas about the overall architecture were at best hazy, and the nature of the initial photon absorption was in dispute. In the early 1970s, James Norris and his colleagues at the Argonne National Laboratory, and independently George Feher and his colleagues at the University of California, San Diego, argued from electron spin resonance spectroscopy that two chlorophyll molecules—denoted the special pair—were involved in the photon absorption.

"The idea of the special pair was not well received," Norris told *Science*, "but after about 5 years it became accepted. But then it went out of favor again . . . until the structure came along." Deisenhofer describes as one of his "moments of highest excitement" when he saw "what looked like a pair of closely associated chlorophyll molecules in the electron density pattern." The notion of the special pair had been confirmed.

"What was beautiful about the structure," says Douglas Youvan of the Massachusetts Institute of Technology, "was that immediately you could see how the time series of events would fit into the spatial framework." But there were surprises, most notable of which was how very symmetrical the reaction complex was. The special pair forms the head of the complex, while dangling in a spiral each side is one molecule each of chlorophyll, pheophytin, and quinone, in that order: rotate the complex through 180° about its long axis, and you finish up with a virtually identical configuration. "And yet," says Youvan, "the electron transfer is known to go along just one of the chains. That remains a puzzle."

Also a puzzle is what role, if any, the chlorophyll molecule at the beginning of the hanging spiral plays in electron transfer from the special pair to the pheophytin. "Spectroscopy fails to detect the electron visiting this accessory chlorophyll," says Youvan, "and it is possible that it is there for structural reasons only."

Knowledge of the structure of the reaction center yields more than an ability to hang the temporal sequence of events on a concrete framework. It allows precise calculations of the kinetics of electron transfer, given that the distance traveled by the electron at each step, and its immediate atomic environment, is known. "An exact, discrete geometry for the chemistry," is how Norris describes it. This feeds into the growing field of molecular dynamics, a computer-simulation technique for studying intramolecular reactions. "With the photosynthetic reaction center, we can feed in very detailed structural and kinetic information upon which the simulations can be more accurately built," notes Parson.



Robert Huber: *The Max Planck Institute laboratory run by Huber was a powerhouse of structural analysis.*

Meanwhile, molecular genetics is also able to contribute, because mutations can be made to insert specific structural modifications in the reaction center. Such modifications—more than 50 of which have been made so far—can be used as probes, to determine which structures within the system are key to the electron transfer process.

This light-driven electron transfer of photosynthesis is a remarkable process, because it can operate under a wide range of physical conditions, including near absolute-zero temperatures. And, as Norris notes, chemists are used to thinking of electron transfer as operating in aqueous environments, and yet the photosynthetic reaction center is completely nonpolar. "That's interesting—and challenging—to a chemist," he says. "That's an impetus for us to try to make other reactions work in nonaqueous environments, and down to 2 K."

Although the photosynthetic reaction center with which Michel and Deisenhofer worked was from a purple bacterium, there are several similarities with the major system in green plants (photosystem II). Important among them is a strong sequence similarity between the major proteins L and M in the bacterial system and the equivalent proteins in the plant chloroplast. And again, a special pair seems to be at the heart of the reaction center. "There is no doubt that this work is important for solving green plant photosynthesis," says Norris, for which solution, he guesses, another trip to Stockholm surely beckons. ■ ROGER LEWIN