curved stripe would be longer than on a straight stripe, and its characteristic momentum would be smaller: This keeps the maximal Fermi momentum sharply defined. At the same time, coherent sideways motions of the stripes will give the appearance of coherent two-dimensional motions to the electrons propagating along the stripes.

If the interpretation of the Hall measurements by Noda *et al.* (*3*) is correct, this "self-organized one dimensionality" restores itself in the ground state of the stripe phase. Noda *et al.* studied samples characterized by strong stripe order. Superconductivity is largely suppressed in these samples, and a metallic-like state is realized instead, characterized by an increase of the dc resistivity. Noda *et al.* find that in a certain doping regime, the Hall voltage decreases rapidly as soon as the stripe charge order sets in. The Hall conductance (σ_{xy}) tracks precisely the inverse of the magnitude of the stripe charge-order parameter as measured by x-ray scattering. Such a behavior is difficult to understand in terms of conventional transport theory, and the authors have come up with a very simple and appealing alternative interpretation. A prerequisite for the Hall effect is that the electrical currents flow in at least two dimensions. Noda *et al.* suggest that σ_{xy} reflects the degree of one dimensionality of the current flows in the striped system, with the one dimensionality imposed by the stripe order.

If this interpretation is correct, it would mean that the system of electrons collectivizes in a state that is insulating in one direction and metallic in the other (see the bottom panel in the figure). Such a state of electron matter was predicted by Kivelson *et al.* (*7*), who introduced the concept of quantum liquid crystals, quantum analogs of the classical liquid crystals familiar from liquid crystal displays. The existence of such a state can be demonstrated on the basis of general arguments. Kivelson *et al.* also came up with a specific example: Assuming the stripes to be internally metallic, they showed that stripe fluctuations of the type illustrated in the figure could prevent the system of stripes from becoming an overall insulator. However, Noda *et al.* present evidence suggesting that the quantum liquid crystal is associated with a stripe state that is insulating on the microscopic scale, like the stripes of mean-field theory (*6*). From the doping dependence of the stripe distance as measured by neutron scattering, Yamada *et al.* (*8*) deduced that at low doping levels, every hole stabilizes a piece of stripe exactly two unit cells long, indicating that the stripes are internally Mott insulating. In this doping regime, the Hall effect collapses. In contrast, above the critical doping level for which the neutron work suggests metallic stripes, the Hall effect is unremarkable. It remains to be understood why the insulating nature of the stripes on very small scales is apparently a condition for the quantum liquid crystal behavior on large scales.

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PERSPECTIVES: STRUCTURAL BIOCHEMISTRY

Proton Pump Caught in the Act

Robert B. Gennis and Thomas G. Ebrey

On page 255 of this issue, Luecke *et al.* (*1*) reveal the structure of bacteriorhodopsin, an active transport protein, frozen in midstroke. Bacteriorhodopsin is a membrane protein that uses a photon of visible light as an energy source to transport a proton across the membrane against an electrochemical gradient, resulting in proton release on one side of the membrane and proton uptake on the opposite side. In order to take up a proton on one side and release it on the other, the affinity for the transported substance (a proton in this instance) must be altered along its path during the transport. Furthermore, a switch mechanism must assure that the pump is unidirectional. Some of the underlying molecular mechanisms are now revealed by the 2.0 Å resolution x-ray crystallographic structure of bacteriorhodopsin trapped in an intermediate state (*1*).

Bacteriorhodopsin is isolated from the purple membranes of an archaea, *Halobacterium salinarum*. The protein, although small (26 kD), contains seven transmembrane helices and a buried lysine residue that is covalently linked to a retinal molecule through a Schiff base; these motifs are also found in the eukaryotic visual pigments. Absorption of a photon by the protein-bound retinal in the initial state (BR) results in isomerization of the retinal, initiating a sequence of events as the protein adjusts and relaxes through a series of intermediates denoted K, L, M, N, and O. About 50 μs after a photon of light is absorbed, when the M state is forming, a proton is ejected on the extracellular side of the membrane. Several milliseconds later, a proton is taken up from the cytoplasmic side of the protein and, after a few more milliseconds, the protein relaxes back to the BR state, completing the photocycle (*2*, *3*). Light energy is hereby converted into a proton-motive force—a transmembrane proton electrochemical gradient that can be used to do work such as adenosine triphosphate biosynthesis. Bacteriorhodopsin is thus essentially a photosynthetic system—a light-harvesting protein that sets up a proton gradient—but without chlorophyll.

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Over the past two decades, methods have been devised for trapping the wild-type bacteriorhodopsin and its mutants in specific photo-intermediate states (*4*). The current work applies this technology to the $Asp^{96} \rightarrow Asn$ (D96N) mutant of bacteriorhodopsin in crystals formed by cubic lipidic phase crystallization, a crystallization procedure specifically designed for integral membrane proteins (*5*) that has resulted in several structures of bacteriorhodopsin at 2.9 to 1.55 Å resolution (*5*–*9*). The high quality of the crystals allows side chains and internal water molecules to be located with unusual accuracy.

Continuous illumination of the mutant D96N converts virtually 100% of the protein to one particular state, called M_N. In this state, the proton has already been ejected from the extracellular side of the protein because of marked shifts in the proton affinities of several residues, including the retinal chromophore, but the reprotonation of the retinal from the opposite side of the membrane is hindered by the point mutation.

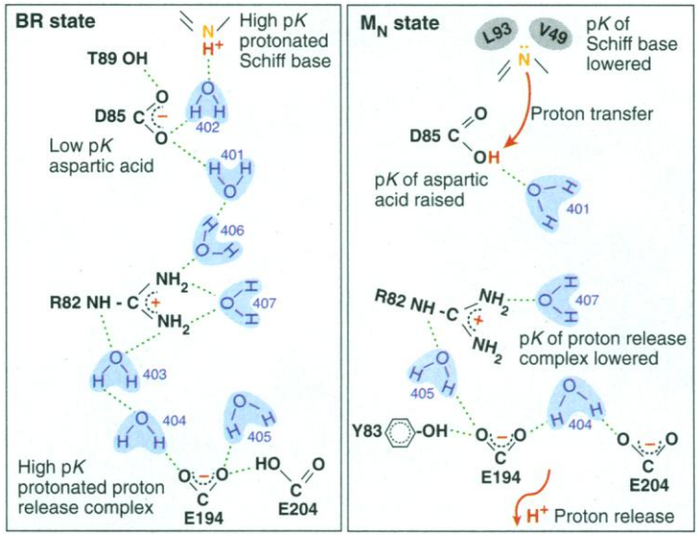
The current study reveals that the movements performed by bacteriorhodopsin as it functions are small. A key role is played by internal water molecules that first help define the initial structure of the protein and then, after photoexcitation, redistribute themselves within the protein. These internal water molecules provide hydrogen

bonds that facilitate proton diffusion and stabilize charges within the protein at different times and locations during the transport process. The observations are consistent with the large amount of information already available from biophysical studies on bacteriorhodopsin but add fascinating structural details showing conformational changes in the protein that are relevant to all studies relating protein structure and function.

In the initial BR state, a network of hydrogen bonds formed by internal, ordered water molecules stabilizes the positively charged, protonated Schiff base along with the deprotonated Asp^{85}, which serves as the counterion to the cationic Schiff base within the protein interior (see the figure) (*8*, *9*). The hydrogen bond network continues to the Glu^{204} and Glu^{194} pair near the extracellular surface of the protein. The pathway for proton release to the extracellular surface is thus already in place in the initial BR state, but release is not favored because of the pK_a's (where K_a is the acid constant) of the relevant groups within the protein. On the cytoplasmic side of the protein, there is no equivalent chain of water molecules or hydrogen bond network leading to the protein surface.

As expected from spectroscopic studies (*3*), absorption of a photon by retinal results in isomerization from the all-*trans* to the 13-*cis*, 15-*anti* configuration. The largest movements are of the retinal C14, which moves by 1.7 Å, and the 13-methyl group, which moves by 1.3 Å, both toward the cytoplasmic side. The protonated retinylidene nitrogen, stabilized by a hydrogen bond to an internal water molecule in the initial state, is no longer stabilized in the M_N state. Instead, isomerization has rotated and displaced the Schiff base to point toward the cytoplasmic side of the protein, facing the hydrophobic side chains of Leu^{93} and Val^{49}, thus decreasing their pK_a. Three internal water molecules are replaced by a single water molecule, with the result that the hydrogen bonding pattern around Asp^{85} changes to favor its protonated form, effectively raising its pK_a. Some of these internal water changes have previously been detected by the elegant Fourier transform infrared spectroscopy measurements of Maeda and co-workers (*10*).

The hydrogen bonding and associated pK_a changes result in proton transfer from the Schiff base to Asp^{85}. Arg^{82} is displaced by 1.6 Å, taking it closer to the complex of residues including Glu^{204}-Glu^{194}, lowering the pK_a of this complex (*11*), and resulting in the release of a proton to the extracellular aqueous phase (*12*–*14*). The hydrogen bond network involving internal water molecules on the extracelluar side of the protein thus predisposes the system for the shift of a proton from the Schiff base to Asp^{85} and, simultaneously, the loss of a proton from the extracellular surface.



Water, water everywhere! Arrangement of key residues and internal water molecules on the extracellular side of bacteriorhodopsin in the initial BR state and the photo-intermediate M_N state. Changes in the hydrogen bond network and the number and location of internal water molecules result in substantial changes in the pK_a of key groups in the protein. The net result is proton ejection to the extracytoplasmic surface and charge movement through the protein. As depicted, the released proton is shared between the two glutamates, but Fourier transform infrared evidence (*14*, *15*) suggests that this may not be the case.

The lack of such a network of hydrogen bonds and of a proton acceptor on the cytoplasmic side of the membrane explains why the proton lost from the Schiff base nitrogen does not move in this direction. In the M_N state, the movement of the retinal side chain has already resulted in the displacement of Leu^{93} and Phe^{219} on the cytoplasmic side of the membrane, opening up a potential pathway for proton access to the Schiff base from the cytoplasmic side. In the wild-type protein, the source of this proton is Asp^{96}, and the pathway is likely to be formed by water molecules that move into this space and provide the connection. However, the simple rotation of the Schiff base nitrogen to face different sides of the protein is not sufficient to explain the unidirectional nature of the proton transfer, and conformational changes in the protein must be essential to the switching mechanism (*4*). The ends of the F and G helices near the cytoplasmic surface are disordered in the M_N structure, perhaps indicating their role in providing access to this nascent channel. The pump does not run backward, probably because the pK_a of Asp^{85} remains high throughout; Asp^{85} holds on to its proton even when the pK_a of the Schiff base is increased to its original value. The Schiff base is thus reprotonated from the cytoplasmic side of the membrane. The detailed changes that result in the resetting of the protein back to its initial state remain to be observed.

Luecke *et al.*'s study reveals the small changes in protein conformation, and the number and position of internal water molecules, that result in large changes in the pK_a's of several groups in bacteriorhodopsin, assuring unidirectional proton release. There is no doubt that this work will be followed by future structural studies of the protein trapped in other states. Earlier states may clarify the relation between the rotation of the Schiff base nitrogen and the pathway and timing of the proton transfer to Asp^{85}. Studies on later states should define the structural changes resulting in reprotonation of the Schiff base from the cytoplasmic side of the protein. These data will provide an accurate basis to understand the dynamics of the system, with important applications not only to other membrane transporters but also to enzymes in general, where internal proton movements are often at the heart of catalysis.

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[15] **In situ Determination of Transient pK _a Changes of Internal Amino Acids of Bacteriorhodopsin by Using Time-Resolved Attenuated Total Reflection Fourier-Transform Infrared Spectroscopy**

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