BIOCHEMISTRY

# Channelrhodopsin reveals its dark secrets

## A high-resolution structure of channelrhodopsin 2 provides key insights for optogenetics

*By* **Klaus Gerwert**

A key element of information processing in the brain is the flow of ions across membranes through ion channels. For optogenetic applications, scientists replace these ion channels with the light-gated channels such as the cation channels channelrhodopsin 1 and 2 (ChR1 and ChR2) (*1*, *2*) or more recently discovered anion channels (*3*). These channels allow remote light-controlled activation and deactivation of excitable cells with high spatiotemporal resolution; desired biological signals such as action potentials can thereby be switched on and off with light (*4*). On page 1018 of this issue, Volkov *et al.* (*5*) report the high-resolution structures of ChR2 wild type in its dark-adapted state and of a ChR mutant. The structures provide a detailed understanding of site-specific mutations used in optogenetics and will enable the rational design of optimized optogenetic tools.

ChRs belong to the large family of microbial rhodopsins. They span the membrane with seven-transmembrane α-helices and contain a deeply embedded retinal chromophore, which is covalently attached to a lysine residue via a protonated Schiff base. The first-discovered member of this family, the light-driven proton pump bacteriorhodopsin (*6*), inspired researchers worldwide to develop new biophysical tools that resolve structure and function, especially of membrane proteins, with high spatiotemporal resolution. A prominent example is cryo–electron microscopy, which Henderson *et al.* developed and used to determine the first high-resolution structural model of bacteriorhodopsin in 1990 (*7*). Around the same time, we used time-resolved Fourier transform infrared spectroscopy to resolve the detailed proton transfer steps through bacteriorhodopsin (*8*). The precise timing of the proton transfer steps in bacteriorhodopsin is reminiscent of a mechanical clock. The steps are controlled by the de- and reprotonation of the Schiff base after light-induced retinal isomerization (see the figure). Proton transfer via precisely arranged protein-bound water molecules is a crucial aspect of this mechanism (*9*).

Fundamental research on bacteriorhodopsins paved the way to the discovery of microbial ChRs, which rapidly led to biological applications. However, the lack of a ChR crystal structure has limited further advances. In 2012, Kato *et al.* provided a first insight into the ChR structure with the C1C2 chimera structural model, consisting of five helices of ChR1 and two helices of ChR2 (*10*). However, the light-induced responses of the C1C2 chimera are different from those of the ChR2 wild type. Volkov *et al.* now report the wild-type ChR2 structure at a resolution of 2.4 Å and compare the structural models for C1C2 and wild-type ChR in detail. They also discuss the potential molecular mechanism of the light-gated ion channel opening, taking into account the wealth of information provided by previous time-resolved biophysical studies. Their proposed mechanism resembles that of bacteriorhodopsin but also has important differences (see the figure).

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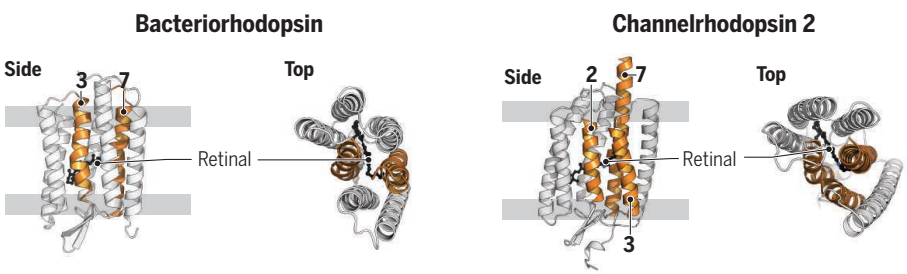
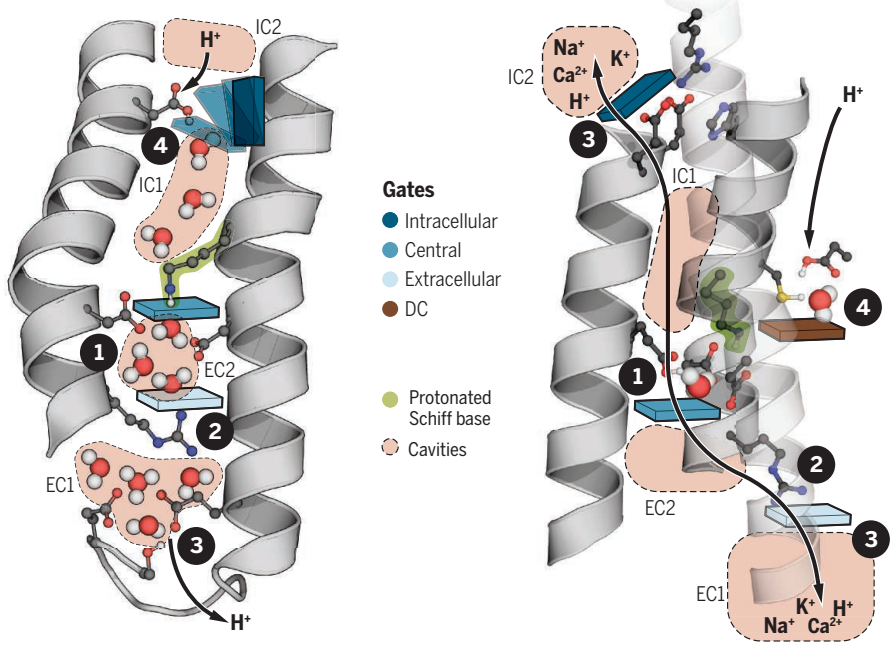
### Channel gating in microbial rhodopsins

Several gates and water cavities are known to play key roles in ion transfer through the light-driven proton pump bacteriorhodopsin. Volkov *et al.*'s structure of the light-gated ion channel channelrhodopsin 2 (ChR2) highlights similarities and differences between the two proteins.




**Coordinated transfer**
Bacteriorhodopsin is activated by light-induced retinal isomerization. Proton transfer is controlled by deprotonation and reprotonation of the Schiff base, the central proton-binding site. Gates open and close in a coordinated fashion (steps **1** to **4**).

**Proposed pathway**
ChR2 has an arrangement of cavities and gates similar to that of bacteriorhodopsin, but all gates must be open at the same time to allow ion flux (step **3**). The release pathway seems similar, but pore formation by helix 2 and reprotonation of the Schiff base by the DC gate (step **4**) are different.

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The ChR2 wild-type structure has more and larger water-filled cavities than C1C2: two on the extracellular site (EC1 and EC2) and two on the intracellular site (IC1 and IC2). The authors propose three gates for the ion flux: a central, an extracellular, and an intracellular gate. The ion-conducting water-filled cavities are separated by the three gates and are arranged in a way similar to the cavities in bacteriorhodopsin (see the figure), pointing to a common precursor protein. But in contrast to consecutive gate switching, which is needed for pumping in bacteriorhodopsin, all gates must be opened simultaneously in ChR to allow ion flux through the protein. As in bacteriorhodopsin, ChR opening and closing seems to be controlled by the de- and reprotonation of the Schiff base.

The most striking difference between bacteriorhodopsin and wild-type ChR involves helix 2, which allows water influx by its opening movement. E90 (glutamic acid 90) on helix 2 is part of the central gate and determines the ion selectivity (*11*). E90 is differently oriented in wild-type ChR than in C1C2. The closing of the channel seems to be controlled by the reprotonation of the Schiff base. An additional, newly identified water molecule between C128 (cysteine 128) and D156 (aspartic acid 156), not observed in C1C2, may be involved in reprotonation of the Schiff base by the DC gate; this gate is not located in the ion pore but seems to play an important role in the lifetime of the open channel (*5*).

Volkov *et al.*'s wild-type structure of ChR2 allows for more precise biomolecular simulations of the ChR opening mechanism. Further insights would come from the structure of the light-adapted state of the protein. A detailed mechanistic understanding is a prerequisite for rationally designed, optimized tools for advanced optogenetic applications, far exceeding most applications in the brain today. Such optogenetic tools may not only allow for the restoration of vision, but may be applied to improve other physiological processes such as hearing (*12, 13*). ■

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QUANTUM OPTICS

# Quantum interference beyond the fringe

## The discovery 30 years ago of the interference of pairs of photons signaled the onset of an era for quantum optics

*By* **Ian Walmsley**

Thirty years ago, Len Mandel, together with his students Jeff Ou and C. K. Hong, published a description of a remarkably simple experiment (*1*), the consequences of which have had dramatic implications for quantum science and technology. The experiment consisted of sending two elementary particles of light, photons, onto opposite sides of a piece of glass that had been coated with a thin film to give it a reflectivity of 50% (see the figure). They observed that the two photons always left by the same side at the output, though it was not possible to determine beforehand which side that would be. This is completely surprising if one considers the behavior of particles as indivisible, identical entities obeying the laws of classical physics. It is easy to see that in such a circumstance, one might expect there to be four equally likely output configurations, only two of which consist of the particles occupying the same outputs. The phenomenon is a beautiful manifestation of the interference of a quantum field; in this case, the bosonic field associated with photons. The effect would be entirely the opposite with fermionic fields associated with electrons—both particles would always leave by different sides.

***"The phenomenon [HOM dip] is a beautiful manifestation of the interference of a quantum field..."***

In their paper, Hong, Ou, and Mandel (HOM) emphasized the role of distinguishing information in determining the extent to which interference occurred, arguing that the mere presence of such information, whether measured or not, would abrogate the "bunching" effect. Hence, they argued that this would be a good way to measure the time delay between two single photons using a slow-responding detector. If the photons were delayed from each other so that they did not arrive together at the beamsplitter, then they would not exit together, and thus would not result in the two detectors at the output ports both registering a signal simultaneously. The change in the rate at which the detectors register in coincidence as the delay is varied gives the signature HOM "dip" (see the figure), which provides a test of the nonclassical nature of the input light.

Quantum interference between two particles has many subtle effects, including that for entangled states of the photons (states in which the photons are in quantum superpositions of two possible distinguishable configurations; for example, the photon at the upper port is red and that at the lower port blue, or vice versa), it is not even necessary that they arrive at the beamsplitter at the same time. The probability $P$ that two photons input at different ports exit at different ports is proportional to the square of the difference between the two-photon wave functions at the input and at the output of the beamsplitter. Whether or not this leads to complete suppression of the probability (that is, $P = 0$) in a 50:50 beamsplitter depends on the symmetry of the two-photon wave function under exchange of their quantum numbers (except for the port number) (*2*). If the two photons are identical in all respects (for example, the same polarization, color, arrival time, spatial shape, and direction), then exchanging any of the properties of the photons leaves the two-photon wave function unchanged. If some of the characteristics are different, then exchanging those can change the wave function. If it does change, then sometimes the two photons will exit at opposite ports, in contrast to the case first demonstrated by Hong, Ou, and Mandel. Entangled states where the photons arrive separately can still satisfy the symmetry constraints.

This property of interference can be generalized to more complicated arrangements of beamsplitters. Imagine three beamsplit-

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