

## Crystal Structure of Channelrhodopsin, a Light-Gated Cation Channel: All Cations Lead through the Monomer

Channelrhodopsin (ChR) is a light-gated cation channel derived from algae that conducts cations, including sodium ions, in a light-dependent manner. Because the inward flow of sodium ions triggers the neuron firing, neurons expressing ChRs can be controlled by light even within freely moving mammals. Although ChR has been broadly applied to neuroscience research, little is known about its molecular mechanisms. We determined the crystal structure of ChR at 2.3 Å resolution and revealed its molecular architecture, especially the cation-conducting pathway. The integration of structural and electrophysiological analyses provided insight into the molecular basis for the remarkable function of ChR, and paved the way for the design of ChR variants with novel properties.

Organisms from bacteria to humans perceive light and use the information for visual and non-visual functions, including ATP synthesis and circadian rhythm. In most cases, the perception of light is mediated by rhodopsin family proteins, which consist of seven-transmembrane (7-TM) domains and covalently linked retinal chromophores. Based on their functions, rhodopsin family proteins can be divided into four distinct classes: photoisomerase, signal transducer, ion pump, and the most recently discovered class, ion channel (Fig. 1).

Channelrhodopsin (ChR) was originally isolated from tiny green algae, *Chlamydomonas Reinhardtii*, and identified as a light-gated cation channel in 2002 [1]. In early 2005, it was found that ChRs could be expressed in mammalian neurons to mediate the precise control of action potential firing in response to light pulses. ChRs have now been used to control neuronal activity in a wide range of animals, but virtually nothing is known about how a 7-TM protein can form a light-switchable

channel for cations. Although a rough helical arrangement was visible in the recently published electron microscopic (EM) structure of ChR at 6 Å resolution [2], the details of amino acid positioning and channel function remained completely lacking. A high-resolution structure would be of enormous value, not only to enhance understanding of the mechanism of this new class of rhodopsin family proteins, but also to guide the way to designing ChR variants with novel functions related to spectrum, selectivity, and kinetics.

To solve the crystal structure of ChR, we expressed a chimeric ChR between ChR1 and ChR2 in Sf9 insect cells. The crystals were obtained in a lipidic mesophase, and the structure was solved by the multiple anomalous dispersion (MAD) method, using mercury-derivatized crystals. As far as we know, this is the first example of phase determination by MAD for a crystal obtained in lipidic mesophase. We finally determined the crystal structure of ChR in the closed state at 2.3 Å resolution.

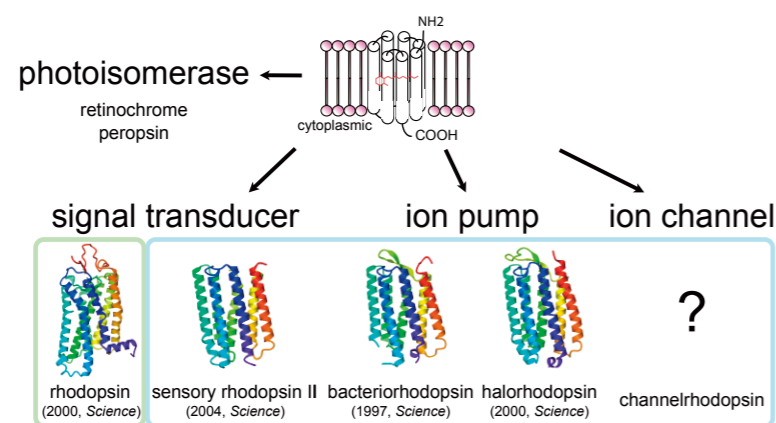


Figure 1  
Classification of rhodopsin family proteins.

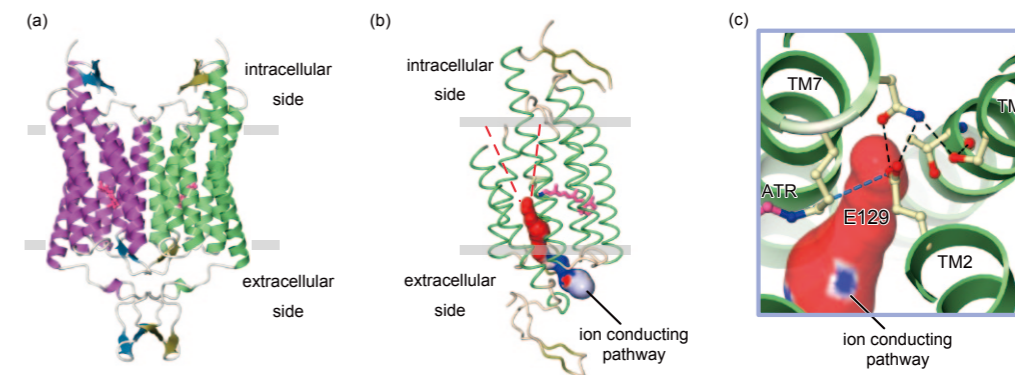


Figure 2  
The structures of ChR. (a) Overall structure of ChR dimer. Each protomer is shown in a ribbon representation (green and magenta) and retinal chromophores are depicted by a stick model (pink). (b) The ion-conducting pathway. The putative intracellular vestibule is shown by red dashed lines. (c) The possible channel gate. Hydrogen bonds are shown by black dashed lines, and the blue dashed line represents a possible proton transfer pathway.

ChR is composed of an N-terminal extracellular domain, the 7-TM domains connected by three cytoplasmic and extracellular loops, and the C-terminal intracellular domain [Fig. 2(a)]. Of particular note is that, as previously predicted from EM [2], ChR is tightly associated into a dimer *via* interfacial interactions between the N-domain, ECL1, TM3 and TM4 of each molecule. This result is surprising because all other known microbial rhodopsins form trimer or tetramer. This is the first example of microbial rhodopsin to adopt a dimer conformation.

To further understand the ChR structure, we compared our ChR with the most well studied microbial rhodopsin, bacteriorhodopsin (BR). Although the primary sequence identity between ChR and BR is as low as 15%, the overall structure of ChR is well superimposed on that of BR. TM3 to TM6 are very similar, and the position of the retinal chromophore is well conserved, whereas there are two distinct features between ChR and BR. First, ChR has additional N-terminal and C-terminal domains, and more importantly, the extracellular ends of TM1 and TM2 are tilted compared to those of BR. Because of this tilt, ChR has a larger pore formed by TM1, 2, 3, and 7. The calculated electrostatic surface potential reveals that this pore is strongly electronegative, so we assumed that this pore acts as the cation-conducting pathway in ChR [Fig. 2(b)]. To verify this hypothesis, we expressed the mutants of the pore-lining residues in HEK293 cells and recorded photocurrents in response to blue light pulses. Most mutants showed altered properties, including photo-conductance, kinetics, and ion preference. Therefore, we suggest that this pore actually acts as the cation-conducting pathway.

While this cation-conducting pathway is open toward the extracellular side, the cytoplasmic side of the pathway is occluded by Glu129 [Fig. 2(c)]. Although the calculated *pKa* of Glu129 suggests that this residue is protonated in the closed state, E129Q mutant shows a

strongly decreased photocurrent. Thus, we assume that Glu129 acts as the putative channel gate-keeper, and that the gating is regulated by the protonation change of Glu129 during the photocycle. Recent FT-IR studies also support our idea [3].

In this study [4], we determined the first crystal structure of a light-gated cation channel, in the closed/dark state at 2.3 Å resolution, and provided insights into ChR dimerization, retinal binding, and cation conductance. In the field of ChR, there are two hypotheses about the cation-conducting pathway. One is that the pathway comprises helices from a single ChR molecule, and the other is that the ChR dimer assembles to form the conducting pathway using elements from each of the two ChRs. This study strongly supports the former hypothesis and will accelerate the basic mechanistic understanding of this remarkable photoreceptor protein. This high-resolution information, along with electrophysiological analyses, will also guide the way to designing ChR variants with ideal properties, such as enhanced potassium ion selectivity and red-shifted absorption spectrum.

### REFERENCES

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