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## New Images Show Ion Channel in Its Natural Habitat

Howard Hughes Medical Institute researchers are unveiling the most detailed views yet of the structure of a voltage-dependent potassium ion channel. The new images, which show the channel in a more natural environment than previous studies, reveal that the channel's function is likely to be profoundly influenced by lipid molecules within the cell membrane in which the channel is embedded.

The research team, led by HHMI investigator Roderick MacKinnon, hopes that a technique they used to prepare the ion channel for analysis — called lipid-detergent-mediated crystallization - will make it possible to capture membrane proteins in a more native, membrane-like environment.

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— Roderick MacKinnon

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MacKinnon and his colleagues at The Rockefeller University published their findings on the structure of the ion channel in the November 15, 2007, issue of the journal *Nature*.

Voltage-dependent potassium ion channels are central to the function of nerves and muscles. Without them the brain would immediately suffer neural gridlock, and the heart would seize up. The channels are precise molecular machines that propagate electrical impulses in the brain, heart and other cell types. The potassium channels are large proteins with a central pore that pierces the cell membrane and allows only potassium ions to pass through.

When an electrical impulse travels along a nerve, it changes the charge separation across the cell membrane—with the inside becoming more positive. This electrical polarity change triggers voltage-dependent potassium ion channels to open, allowing positively-charged potassium ions to flow out of the cell. This outflow of potassium allows the membrane to return to its

resting state and prepares it for the next electrical impulse.

In earlier studies, MacKinnon and his colleagues deduced the structure of the voltage sensor, which is the component of the voltage-dependent potassium ion channels that senses changes in voltage. The voltage sensor reacts to a change in the membrane electrical polarity to open or close the pore.

MacKinnon and his colleagues used x-ray crystallography to determine the structure of the voltage sensor. In x-ray crystallography, protein crystals are bombarded with x-ray beams. As the x-rays pass through and bounce off of atoms in the crystal, they produce a diffraction pattern, which can then be analyzed to determine the three-dimensional shape of the protein.

The pictures that emerged from those structural studies showed that the voltage sensors contained a helix-turn-helix structure, which MacKinnon's group has called the voltage sensor paddle. The voltage sensor paddle contains positively charged amino acids that enable the voltage sensor to respond to the membrane's electrical polarity.

MacKinnon and his colleagues theorized that the positively charged paddle moves within the membrane at the protein-lipid interface. When the membrane becomes positively charged on the inside, the paddles is attracted to move toward the outside and open the channel, allowing potassium to flow out and restoring the membrane charge to its resting state. When the inside of the membrane becomes negatively charged, the paddles move inward snapping the channel shut.

Those earlier studies, however, left some questions about ion channel function unanswered because significant details of the structure remained unresolved. We could not see many of the individual side chains of this protein that are important to its function, MacKinnon noted. Answering remaining questions meant developing new experimental approaches. These are very difficult structures to determine, and our progress has been like taking one step at a time up a very big mountain, he explained.

The researchers' latest steps entailed engineering a new form of the channel that they could then use to obtain improved protein crystals. The higher quality crystals would enable more detailed structural and functional insights from the x-ray crystallography studies.

The researchers produced a paddle-chimera channel by swapping the normal paddles of a channel with those from a different channel. This gave us a new crystal packing that helped us get better definition of the atoms in the protein that we couldn't see in the original structure, said MacKinnon.

The scientists also attempted to mimic the oily cell membrane in which the channel exists naturally. By immersing the channel protein in a mixture of detergent and lipid -instead of the more traditional method of using detergent alone —MacKinnon's team was able to see the channel in a more natural environment.

This new approach gave us dramatic new insight, because we could actually see the lipid molecules gathered around the protein, and see them form the characteristic leaflets of the bilayer biological membrane, said MacKinnon. With an earlier structure that we published in 2005 we could only speculate why the use of lipids was important, but now we can see it very clearly, he said.

MacKinnon said that knowing the atomic structures have changed his perspective on the role of the membrane in ion channel function. I used to think that the voltage sensor didn't have much to do with the lipid membrane, he said. But these structures have informed us that the voltage sensor has a great deal to do with the lipid membrane.

When you examine the structure of usual alpha-helical membrane proteins, they look like a big disk of protein that snakes back and forth through the membrane. But when you look at the voltage-dependent potassium channel, you see the pore embedded in the membrane, but you also see the voltage sensors that stick out like Mickey Mouse's ears. They are mostly surrounded by lipid membrane, and what that means is that the voltage sensor can't help but be influenced by the lipid. This influence is so profound, that you can't simply say what the properties of a given voltage-dependent channel are without specifying the composition of the surrounding lipid. And what makes this influence of lipid biologically significant is that we know that different cells in the body do not have the same lipid composition, MacKinnon explained.

As a result of these studies, MacKinnon's group hypothesizes that the function of voltage-dependent channels in different kinds of lipid membrane may be very different. To me, this has been the most interesting aspect of our structural studies—that the lipid membrane would influence the channel's function.

MacKinnon said that his group is now exploring the influence of membrane structure on ion channel properties, in order to understand the biological context in which the ion channels function.