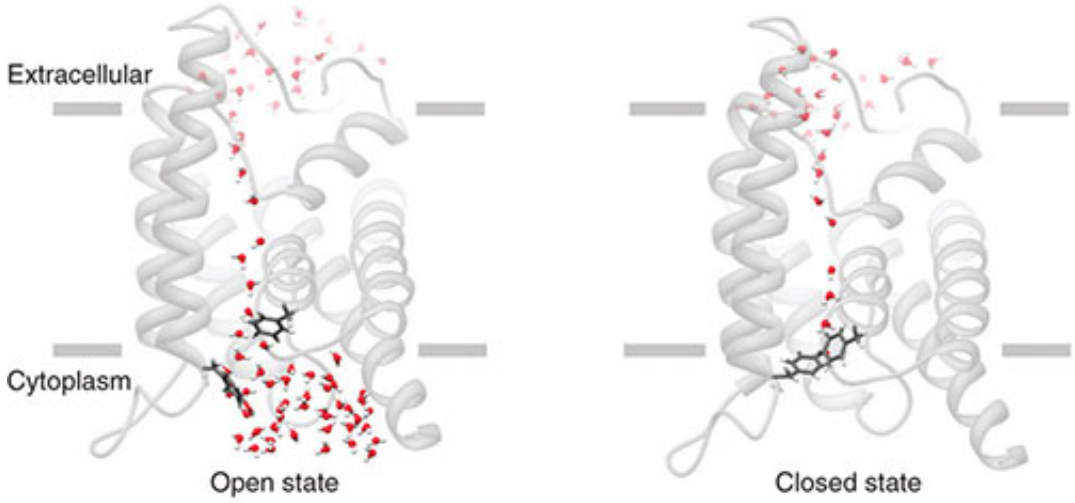
Researchers get close-up view of water pores needed in the eye's lens

August 5 2013



This illustration shows two views of a single unit of the aquaporin-0 channel. The left channel is open, allowing the passage of water molecules (red). The right channel is closed because amino acids in its core have flipped inward. Credit: Reichow et al. Nature Structural and Molecular Biology.

Researchers have achieved dynamic, atomic-scale views of a protein needed to maintain the transparency of the lens in the human eye. The work, funded in part by the National Institutes of Health, could lead to new insights and drugs for treating cataract and a variety of other health conditions.

Aquaporin proteins form water channels between cells and are found in

many tissues, but aquaporin zero (AQP0) is found only in the mammalian lens, which focuses light onto the retina, at the back of the eye. The lens is primarily made up of unique cells called lens fibers that contain little else besides water and proteins called crystallins. Tight packing of these fibers and of the crystallin proteins within them helps create a uniform medium that allows light to pass through the lens, almost as if it were glass.

Abnormal development or age-related changes in the lens can lead to cataract—a clouding of the lens that causes vision loss. Besides age, other risk factors for cataract include smoking, diabetes, and genetic factors. Mutations in the AQP0 gene can cause congenital cataract and may increase the risk of age-related cataract.

"The AQP0 channel is believed to play a vital role in maintaining the transparency of the lens and in regulating water volume in the lens fibers, so understanding the molecular details of how water flows through the channel could lead to a better understanding of cataract," said Dr. Houmam Araj, who oversees programs on lens, cataract and oculomotor systems at NIH's National Eye Institute (NEI), which helped fund the research.

Closing of AQP0 channels is regulated by a calcium-sensitive protein called calmodulin, but the precise mechanism has been unclear. Some models have suggested that calmodulin simply acts as a plug to fill the open channel. The new study, published in *Nature Structural and Molecular Biology*, reveals a more nuanced process in which calmodulin essentially grasps the open channel and forces it to close.

The research was a collaboration between investigators at the University of California, Irvine, and the Janelia Farm Research Campus in Ashburn, Va., a part of the Howard Hughes Medical Institute (HHMI). Drs. James Hall and Douglas Tobias led the effort at UC Irvine. Dr.

Tamir Gonen led the effort at Janelia Farm.

In prior studies, Dr. Gonen had examined the atomic structure of the AQP0 protein by X-ray crystallography, which involves crystallizing a protein and bombarding it with X-rays. But X-ray crystallography does not work well for large groups of proteins or for proteins in motion. So in the new study, the researchers first used electron microscopy to view AQP0 and calmodulin bound together. Then they combined their microscopy and crystallography data to generate computerized models of how the two proteins interact and to identify the most critical amino acids (the building blocks for proteins) within AQP0. To test their models, they neutralized those amino acids one by one in the actual AQP0 channel.

The AQP0 channel is made up of four identical barrel-shaped units, bundled together side by side. The researchers found that in the presence of calcium, calmodulin binds to one unit and then another, as if grabbing a pair of reins. This makes the channel twist slightly, which causes just a few amino acids within each unit to slide into the channel's core and block the flow of water.

"Calmodulin essentially throws a molecular switch that moves in and out of the water pore, like the gate valve of a plumbing fixture," Dr. Hall said.

This new view of AQP0 could help lead to new approaches for treating cataract, Dr. Hall said. Cataracts are the most common cause of blindness worldwide. In the United States, they affect about 1 in 6 people over age 40 and half over age 80. Congenital cataracts (present from birth) affect about 1 in 5,000 American children.

Cataracts can be successfully treated with surgery, in which the cloudy lens is removed and replaced with an artificial plastic lens. But the new

findings "may be a step toward learning how to prevent or delay cataracts," said Dr. Hall.

The new findings also provide inroads to understanding how calmodulin interacts with a variety of protein channels, and thus could open doors to new drugs for other common health conditions. In addition to aquaporins, our bodies rely on a vast menagerie of channels, many of which are regulated by calmodulin. For example, calmodulin helps control the gating of ion channels, which allow the passage of ions (charged particles) in and out of our cells and are essential for nerve cell firing, muscle contraction, and the rhythmic beating of the heart. This study provides the first structural model of calmodulin bound to any complete protein channel.

More information: *Nature Structural and Molecular Biology*, July 2013. DOI: 10.1038/nsmb.2630

Provided by National Eye Institute