ELUCIDATING THE MOLECULAR BASIS OF CHARGE SELECTIVITY IN PENTAMERIC LIGAND-GATED ION CHANNELS

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EXECUTIVE SUMMARY:

When activated, ion channels open an aqueous pore in the cell's membrane that allows passive movement of ions from one side of the membrane to the other. Because ions are charged particles, the movement of ions across the cell membrane creates a current that results in a change in the neuron's membrane potential. This change in membrane potential may cause a neuron to undergo an action potential, which propagates the electrical signal through the nervous system. However, ion channels can either promote or inhibit action potentials depending on what type of ion crosses a particular channel.

Pentameric ligand gate ion channels are unique in that they contain both anion- and cation-selective channels and therefore provide a great model to understand how particular proteins selectively allow the passage of unique ionic species. Using computer simulations, it is possible to understand the precise molecular basis for charge selectivity.

INTRODUCTION

Ion channels passively allow ions to diffuse in and out of a cell. However, many ion channels only allow specific ions to cross the membrane. One way an ion channel can discriminate among ions is based on the formal charge of an ionic species, known as charge selectivity. Cation-selective ion channels only allow positively charged cations to move across the membrane, whereas anion-selective ion channels only allow negatively charged ions to cross the membrane. Charge selectivity is highly important for ion channel biophysics because in adult neurons, when cations are allowed to pass through the cell, the electrical signal is said to be excitatory because it enhances the likelihood of an action potential by depolarizing the cell and therefore the propagation of the neuronal signal. However, when anions are allowed to pass, the electrical signal is observed to be inhibitory because it hyperpolarizes the cell, making it more difficult for an action potential to occur.

One particularly well-characterized superfamily of ion channels is known as the pentameric ligand-gated ion channels (pLGICs). These channels open in response to neurotransmitters such as acetylcholine, serotonin, and GABA and are important for regulating neuronal signals in various regions of the body. These channels are also targets for important classes of drugs such as anesthetics and benzodiazepines. Furthermore, they are highly unique in that they are the only superfamily of neurotransmitter-gated ion channels that contain members that are highly cation-selective, such as serotonin and acetylcholine receptors, and other members that are highly anion-selective, such as glycine and GABA receptors. This feature of pLGICs makes the family of proteins a model for understanding the molecular basis for how ion channels are able to selectively catalyze the conduction of ions based on the formal charge of the ion. Because charge selectivity is highly important for understanding how neuronal signaling can be either excitatory or inhibitory, this work has broad implications for understanding the molecular basis of neuronal processes.

METHODS & RESULTS

Ion selectivity has been studied experimentally for years using patch-clamp electrophysiology. While these experiments are able to observe patterns in ion selectivity and elucidate mutations needed to change selectivity of a particular channel, electrophysiology does not have the resolution necessary to explain the molecular underpinnings of charge selectivity. To understand charge selectivity at atomic and molecular scales, computer simulations are necessary.

To accomplish this task, we performed free-energy calculations on a variety of members of the pLGIC superfamily. Using NAMD, we completed umbrella sampling simulations on anion- and cation-selective ion channels in order to compute the free-energy profiles for both cations and anions. In doing this, one can ascertain differences in ion conduction between the cation- and anion-selective channels. Furthermore, mutations to these channels can be done *in silico* to observe how the energy landscape for both types of ion changes, and these observations can be directly compared to experimental results. The comparison with experimental results is powerful because the two techniques are highly complementary. Experimental evidence provides verification for the observations seen in simulations, while simulations provide a highly detailed atomistic picture of the ion permeation process. These simulations led us to conclude that major barriers of the free-energy landscape appear in fundamentally different locations in anion- versus cation-selective channels.

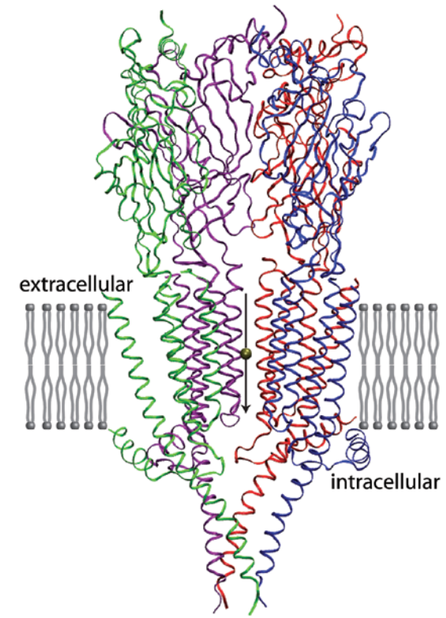

FIGURE 1: An ion, shown in gold, moves along the permeation pathway of the serotonin receptor (pdb id 4PIR), a member of the pentameric ligand-gated ion channel superfamily. One of the five subunits is removed to clearly visualize the ion, and each of the four remaining subunits is colored green, purple, red, or blue.

WHY BLUE WATERS?

Ion permeation, especially in the absence of a membrane potential, can be a rather slow process. Therefore, computational methods such as umbrella sampling have been devised in order to make the process of sampling the energy landscape of permeation much more efficient. However, while these techniques effectively use resources, the computational cost of free-energy landscapes is tremendous. In order to effectively sample the free-energy landscape for multiple ions in different channels with and without mutations, supercomputing time on Blue Waters is essential. Without Blue Waters, the project would need to be much less ambitious and smaller in scope, but with Blue Waters we are able to have a more profound understanding of the molecular basis of charge selectivity in pLGICs.