FUNCTIONAL MECHANICS OF VOLTAGE GATED LIKE ION CHANNELS

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PI: Michael L. Klein[1]
Collaborators: Christina Bergonzo[1], Rodrigo Galindo-Murillo[1], Daniel R. Roe[1]

[1]College of Science and Technology, Temple University

EXECUTIVE SUMMARY:

Ion channels are ubiquitous transducers of chemical and electrical stimuli in cells. In voltage-gated-like ion channels, conformational changes in a domain responsible for sensing a stimulus (transducer) affect the state of a gate domain (effector) that open and close, controlling the flux of permeant ions. The details of this allosteric communication are of great relevance: Pharmacological modulators of ion channels, including neurotoxins and anesthetics, are often allosteric drugs, i.e. they interfere with this coupling between the transducer and effector domains. Our calculations start to clarify the molecular underpinnings of allosteric signal propagation in voltage-gated-like ion channels. We performed molecular dynamics simulations on two classes of channels: transient receptor potential (TRP) and voltage gated cation channels. We found that, despite having similar architectures, the two classes of channels are characterized by distinct mechanical properties; accordingly each class shows unique conformational dynamics, which makes each channel responsive to a specific stimulus, i.e. ligand binding or external field.

INTRODUCTION

Ion channel proteins serve as fast sensors and transducers of chemical and electrical stimuli in cells and underpin electrical excitability. One particular ion channel family, the voltage-gated-like ion channels (VGLCs), occupy a particularly wide set of roles in the physiology of multicellular organisms. Differentiation of a single homologous transmembrane protein template has allowed an explosion of functional variability, from reporting noxious environmental conditions, to shaping the neuronal action potential, to syncing the beating of the heart. Allostery describes how certain nonlocal energetic perturbations couple to and alter the equilibrium distribution of conformations that a macromolecule visits. In ion channels, allostery provides a framework for understanding how conformational changes in domains responsible for sensing stimuli couple to channel gate domains that open and close, controlling the flux of permeant ions. Physiologically-relevant ion channel modulators, including neurotoxins and anesthetics, are often allosteric. Determining the origins of these effects in the VGLC family is crucial for controlling and correcting complex impairments in various electrically-excitable tissues. We focused on allosteric regulation of two subfamilies of the VGLC superfamily in which pore gating is regulated by different stimulus: binding of agonists and antagonists, pH and heat variation for TRP channels and membrane depolarization for voltage gated ion channels.

METHODS & RESULTS

First, we investigated whether the allosteric mechanism underlying gating is common to all TRP channels, and how this mechanism differs from that underpinning Kv channel voltage sensitivity. Thus, we performed comparative sequence analysis on large, comprehensive ensembles of TRP and Kv channel sequences. We detected sequence features that were specific to TRP channels and, based on insight from recent TRPV1 structures, we hypothesized a model of TRP channel gating that differs substantially from the one mediating voltage sensitivity in Kv channels. The hypothesized mechanism involves the displacement of a defect in the H-bond network of S6 that changes the orientation of the pore-lining residues at the hydrophobic gate (Figure 1A). To test this hypothesis and to glean additional insight into this molecular process, we performed molecular dynamics simulations of the TRPV1 channel embedded in a lipid membrane and collected trajectories spanning μs time-scales. We observed a close-to-open transition in one of the subunits and, thanks to a statistical analysis of the pattern of H-bonds, we were able to trace back the conformational transition to a collective motion compatible with the one inferred from the sequence analysis (Figure 1B-C). These results were reported in the Journal of General Physiology.

We then turned our attention to the activation mechanism of voltage gated ion channels. To investigate the activation mechanism of this class of channels, we characterized the mechanical properties of a member of this family, NavAb [1] in the context of a novel approach to identify the set of dynamical domains in proteins. We performed molecular dynamics simulations of NavAb in different conformational states and embedded in a lipid bilayer over time-scales of few μs and analyzed each trajectory. NavAb is constituted by a pore domain, assembled from the last two transmembrane helices of each monomer (conventionally referred to as S5 and S6), and four separate structural domains, each comprising the first four helices of a monomer (S1–S4), which act as voltage sensors (Figure 2A). The results from the subdivision in dynamical domains suggest an activation mechanism by which the displacement of S4 results in a motion of the linker that releases the steric hindrance exerted on the pore domain in the resting/closed state (Figure 2B-C). This observation disfavors the alternative scenario in which the linker exerts an active pulling on the pore domain. These quantitative results, reported in the journal Structure, will be valuable for designing future studies aimed at elucidating by more direct means the mechanical workings of the pore complex.

WHY BLUE WATERS

The project relied crucially on a quantitative description of complex processes occurring on time-scales of several microseconds in large membrane-protein assemblies with a typical size of approximately 300,000 atoms. Each system is constituted by an ion channel, a model lipid bilayer and an electrolyte solution and was simulated under different conditions, i.e. in presence or in absence of an applied electrostatic field or of a ligand. The capabilities of Blue Waters were key to the success of this computationally intensive project.

PUBLICATIONS

Palovcak, E., L. Delemotte, M. L. Klein, and V. Carnevale Comparative sequence analysis suggests a conserved gating mechanism for TRP channels *Journal of General Physiology*, 146:1 (2015), pp. 37-50

Ponzoni, L., G. Polles, V. Carnevale, and C. Micheletti SPECTRUS: A Dimensionality Reduction Approach for Identifying Dynamical Domains in Protein Complexes from Limited Structural Datasets *Structure*, 23:8 (2015), pp. 1516-1525

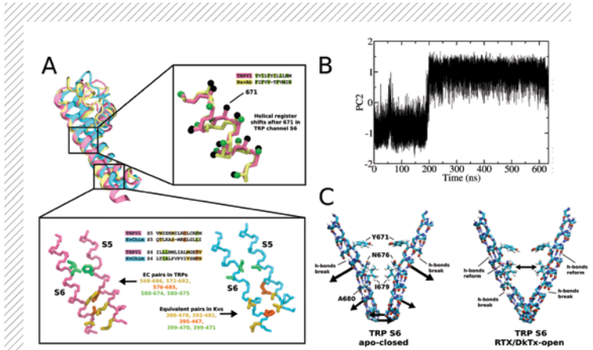

FIGURE 1: A helical distortion in the TRP channel pore.

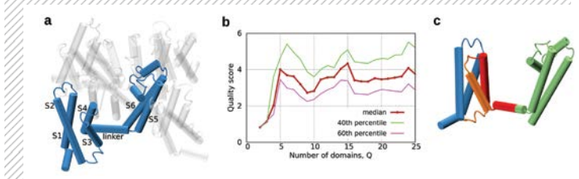

FIGURE 2: Domain Decomposition of the NavAb tetrameric ion channel.