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Insights into the function of ion channels by computational electrophysiology simulations

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ABSTRACT

Ion channels are of universal importance for all cell types and play key roles in cellular physiology and pathology. Increased insight into their functional mechanisms is crucial to enable drug design on this important class of membrane proteins, and to enhance our understanding of some of the fundamental features of cells. This review presents the concepts behind the recently developed simulation protocol Computational Electrophysiology (CompEL), which facilitates the atomistic simulation of ion channels in action. In addition, the review provides guidelines for its application in conjunction with the molecular dynamics software package GROMACS. We first lay out the rationale for designing CompEL as a method that models the driving force for ion permeation through channels the way it is established in cells, i.e., by electrochemical ion gradients across the membrane. This is followed by an outline of its implementation and a description of key settings and parameters helpful to users wishing to set up and conduct such simulations. In recent years, key mechanistic and biophysical insights have been obtained by employing the CompEL protocol to address a wide range of questions on ion channels and permeation. We summarize these recent findings on membrane proteins, which span a spectrum from highly ion-selective, narrow channels to wide diffusion pores. Finally we discuss the future potential of CompEL in light of its limitations and strengths. This article is part of a Special Issue entitled: Membrane Proteins edited by J.C. Gumbart and Sergei Noskov.

1. Introduction

Ion channels are integral membrane proteins abundant across a vast range of cell types [1]. They facilitate the passive, selective permeation of ions such as sodium, potassium and chloride through the phospholipid bilayer, which is otherwise impermeable for ions [2]. Ion channels fulfill essential functions as diverse as electrical signaling underlying neuronal function and muscular contraction, cell volume regulation, and cellular ionic homeostasis [2,3]. Whole-cell and single-channel electrophysiological experiments provide a wealth of information on channel features such as ion conductance and selectivity. Electrophysiology recordings have established that many ion channels exhibit multiple conducting states and that the gating between these states is a major hallmark of ion channel function [1,4]. As they are omnipresent and fulfill vital physiological roles, the malfunction of ion channels gives rise to several critical diseases also termed channelopathies [5]. It is therefore essential to understand the mechanisms underlying physiological as well as pathological ion permeation through ion channels and its regulation.

A major breakthrough in this field was achieved by the resolution of atomic structures of ion channels, pioneered by seminal work on potassium channels, more recently followed by chloride channels, sodium channels and calcium channels [6–10]. Prior to these advances, some wider pore non-specific channels had already been structurally characterized by X-ray crystallography [11,12]. Taken together, these structures have highlighted not only the variety of architectures employed to facilitate ion permeation, but also provided detailed insight into the interactions of ions within the pore as well as snapshots of open, closed, activated and inactivated channels, yielding a direct structural link to electrophysiological observations.

Despite the wealth of information gained from structural studies, the core of ion channel function, the actual permeation mechanism of ions across the pore, is an inherently dynamic process and therefore challenging to track with static structural studies such as X-ray crystallography. Molecular dynamics (MD) computer simulations have therefore

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been utilized extensively to study mechanisms of ion conductance, selectivity, as well as channel gating [13–16], for a review see Ref. [17]. In such simulations, the channel protein, usually embedded in a model membrane patch, is often modeled atomistically in a physiological buffer environment, which enables following the detailed motions of individual atoms and ions with high spatial and temporal resolutions.

However, there are three main challenges associated with simulating ion currents by MD techniques. First, empirical interaction parameters (force fields) in classical MD simulations are approximations and historically lack the explicit description of electronic polarization effects [18]. Therefore, it is critically important to directly evaluate the accuracy of simulation results, for example by comparison to experimental data. Second, due to the small integration time step and the number of interactions to be evaluated per step, the overall attainable simulation time is limited, currently usually to the microsecond timescale for typical simulation systems on state-of-the-art computer hardware [19]. This restricts the application of atomistic simulations to the investigation of ion currents that are minimally in the pA regime and often precludes the unbiased simulation of gating and permeation events. A third, and partially related, issue is associated with enforced ion translocation. The natural driving forces for permeating ions are a transmembrane voltage and concentration gradients across the membrane. In a normal MD simulation, however, any concentration or charge gradient will quickly dissipate unless external forces are applied or a protocol restoring the gradients.

External electric fields have long been in use for MD simulations of ion transfer [20–24]. With periodic boundary conditions (PBC) and a single-bilayer setup, both sides of the membrane effectively form the same compartment, except that their electrostatic potential differs [25,26]. Simulations of sustained ionic flow are thus possible at equal ion concentrations on both sides of the membrane. A separating vacuum or air slab has been introduced [27–29], enabling the simulation of single-membrane setups with different concentrations on both sides of the membrane, however in turn preventing sustained ionic flow as ions cannot pass the vacuum slab. Recently, an energy step was introduced in the aqueous compartment that permits different ion concentrations on both sides of a single membrane [30]. Alongside the use of external electric fields, umbrella potentials have widely been utilized to enforce ion displacements and thereby to record the free energy profile of permeation events [31,32]. This powerful method is challenged primarily by the required choice of a reaction coordinate, which is a non-trivial task for multi-ion permeation events or large pores, and therefore often necessitates the acquisition of multidimensional potentials of mean force. For small voltages, conductances can even be predicted from equilibrium simulations, if statistically sufficient spontaneous permeation events can be recorded on the timescales accessible to the simulation [33].

In this contribution, we review the results of an alternative simulation strategy termed Computational Electrophysiology (CompEL) [34], which permits the user to control both concentration gradients and voltage across the membrane. In this method, the driving force for ionic movements is a transmembrane voltage or an ion concentration gradient. As opposed to applying an external electric field, the voltage in CompEL simulations is "internal" in the sense that it results from an ion concentration difference between two aqueous compartments [27,35,36], similar to the situation in the cell, where Nernst concentration gradients occur across the cell membrane. The separation of two compartments in a periodic simulation system is obtained by duplicating the membrane patch in the direction of the membrane normal. Such an arrangement of two membranes in a periodic system results in an inner and outer compartment, of which the latter is connected across the periodic boundaries (Fig. 1).

By choosing a small ion imbalance Δq between the two compartments, a voltage across both membranes is generated, analogous to the creation of transmembrane voltages in biological membranes. As described in Ref. [28], this transmembrane voltage is due to the capacitance C of the membrane, which behaves as a capacitor separating the compartments, and the voltage ΔU is related to the charge imbalance through the equation $\Delta U = \Delta q / C$. Because the capacitance of the bilayer is relatively small (on the order of ~1 μF cm^{-2} [27,28,35,37]), an imbalance of a few ions is often sufficient to evoke a considerable voltage across the membrane in a small atomistic simulation system. However, the desired voltage can be further adjusted by slightly adapting the area of the lipid patch used in the simulations, so that physiological voltage levels are usually easily obtained.

Under normal conditions, this voltage would soon be depleted by ion translocation events through a membrane channel. However, by continuously monitoring the ion counts in the two aqueous compartments during the simulation, CompEL can maintain any desired imbalance by performing ion/water position exchanges between the compartments as needed (Fig. 3). In a similar way, independent bulk ion concentrations can be imposed on either side of the membrane. This, for example, allows the simulation of channel reversal potentials. CompEL thus enables simulations at a sustained transmembrane voltage or under a constant transmembrane ion concentration gradient. Observables that can be derived from a CompEL simulation include ionic conductances, selectivities, and pathways, as well as current–voltage (I–V) curves and the possible rectification behavior of the channel under consideration.

Our review is structured as follows. We begin with a detailed description of the simulation protocol, including a practical guide for the setup and execution of CompEL simulations. We then present five recent examples in which CompEL simulations have yielded new insights into the functional mechanisms of ion channels and pores. These include the wide beta-barrel channels PorB and VDAC, the channel-forming assembly of dermcidin, the potassium channel KcsA, and the anion channel that is intrinsic to secondary active glutamate transporters (EAATs). We conclude this review with a discussion of the limitations and strengths of CompEL and final remarks.

2. The CompEL setup: principles and application

When aiming to replicate the characteristic behavior of a protein in a molecular simulation, it is ideally placed in an environment that closely mimics its natural habitat. Therefore, membrane channels are embedded in lipid bilayers and solvated by water and ions, while PBC are used to eliminate surface artifacts. For a CompEL setup [34], two copies of such a channel/membrane system are stacked vertically, and thus PBC form two separated aqueous compartments (blue and gray in Fig. 1A, B). Under physiological conditions the lipid bilayer is virtually impermeable to water molecules and ions, which therefore need to pass through a channel to travel from one compartment to the other.

The most commonly used, 'deterministic', protocol [34] of CompEL sets reference counts for the number of positive and negative ions in each compartment. If at any time the actual number of ions differs from the reference count, ions from one compartment are swapped with water molecules from the other compartment until the reference counts are restored. This way, a gradient in ion concentration and/or electric charge between the compartments can be set and maintained over time against dissipation by ionic channel currents. The resulting steady state permits recording the flux of ions through each channel over time similar to single-channel electrophysiology measurements. The electric potential difference ΔU (Fig. 1D) is a direct result of the imposed charge imbalance Δq. In CompEL convention, each ion with charge $\pm e$ removed from one and added to the other compartment changes Δq by $\pm 2e$; however the total charge of the system remains unchanged. The total charge of a periodic simulation system should be zero when long-range electrostatics are treated with the Particle Mesh Ewald (PME) method, as a net total charge can lead to artifacts, especially for membrane systems [39]. To minimize the impact of the ion/water positional exchanges (see Fig. 5 in Ref. [34]), only particles that fulfill a maximum distance criterion to the compartment boundaries (and

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