Exploration of Na\textsuperscript{+}, K\textsuperscript{+}-ATPase ion permeation pathways via molecular dynamic simulation and electrostatic analysis

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Abstract Biologically-inspired nanodevices can serve as “natural” alternatives to conventional semiconductor devices in many applications from information storage to mechanical rotors. In this work we consider an ATP-powered transmembrane protein, the Na\textsuperscript{+}, K\textsuperscript{+}-ATPase, which has appealing functionality but still lacks an “atomistic” picture capable of elucidating its operation. The vast collection of experimental literature on the Na\textsuperscript{+}, K\textsuperscript{+}-ATPase gives a unique advantage to this protein in developing and validating computational tools. We have performed extensive molecular dynamic simulations of the Na\textsuperscript{+}, K\textsuperscript{+}-ATPase in an accurate biological environment, followed by time-averaged electrostatic analysis, to investigate the ion-binding loci and access/egress pathways that cations may take through the protein as they are transported across the membrane.

Keywords Na\textsuperscript{+}, K\textsuperscript{+}-ATPase · Bionano · Homology modeling · Electrostatics · Molecular dynamics

1 Introduction

Today’s top-down device engineering cannot continue ad infinitum due to concerns such as excessive leakage and power consumption as well as stochastic process fluctuations [1]. As a result, researchers are exploring an array of alternative methods for inspiration and construction of a new generation of devices [2]. One of the most fertile grounds for exploration is biomimetic devices born out of the convergence of biology and nanotechnology. In particular, the use of proteins has many attractive features such as a wide range of functions, higher thermal stability compared to molecular devices, and suitability for bottom-up fabrication approaches [2]. The realm of protein devices is vast and many device examples such as the optical memory based on a light-activated proton pump bacteriorhodopsin, multi-state switches using chlorophyll, mechanical rotors, and energy conversion and storage platforms have already been illustrated [3].

The transmembrane proteins, especially ion channels, have attracted the interest of the device modeling community recently [4]. In this work we focus on a ATP-driven transmembrane protein that has a crucial role on the maintenance of electrochemical gradients and the membrane potentials in all animal cells. ATPase proteins constitute a prime example of how specialized energy conversion and ion transport functions are possible via proteins. In particular, we utilize molecular dynamics (MD) simulations in connection with an electrostatic solver to explore the details of ion access/egress pathways.

2 Physiological and technological relevance

The Na\textsuperscript{+}, K\textsuperscript{+}-ATPase is a protein of \(\sim 10,000\) atoms, that plays a role in maintaining a transmembrane voltage between a cell and its environment while helping to produce a Na\textsuperscript{+} concentration gradient that powers cotransport of other molecules across the membrane. Its cyclic operation...
uses the Gibbs free energy derived from ATP dephosphorylation to exchange three intracellular Na\(^+\) ions for two extracellular two K\(^+\) ions against an electrochemical gradient. To accomplish this feat, the protein undergoes large structural changes between two primary conformations (and several minor) so that the ion permeation pathway through the transmembrane region is never fully open. Ions are exchanged in “ping-pong” style. Hydrolysis of ATP by the intracellular domains causes a rearrangement of the transmembrane helices, as shown in Fig. 1. The E1 (E2) conformational state is representative of the protein just after Na\(^+\) (K\(^+\)) ions have moved into the transmembrane region from either the intracellular (extracellular) side. Finally, an E2P state is similar to the E2 state but in a conformation that more closely resembles the open extracellular pathway [5].

Besides its relevance to biophysical and medical fields, the Na\(^+\),K\(^+\)-ATPase has several appealing advantages for use as a bionanotechnology device. The Na\(^+\),K\(^+\)-ATPase’s voltage sensitivity yields a voltage-dependent charge translocation device. Ion movements against their electrochemical gradients could be harnessed for the production or storage of energy. The Na\(^+\),K\(^+\)-ATPase’s function has been cited in biophysical and biochemical research dating back several decades [6]. However, the structural picture is cloudier. Significant questions remain unanswered, such as the loci of large movements during the pumping cycle and the specific interactions between the protein and ions [7]. The mechanism of ion translocation will only be fully understood with the integration of functional and structural results. Our goal is to explore and elucidate the Na\(^+\),K\(^+\)-ATPase’s ionic pathways via computational modeling and simulation.

3 Computational methodology

During preparation of this article, an X-ray crystallographic structure of the Na\(^+\),K\(^+\)-ATPase in the E2P conformation became available [10]. This new structural data can provide a basis for comparison of the homology models introduced in this work. Although a full analysis between the crystal structure and the homology models produced here is beyond the scope of this article, the high resemblance of the E2P model with the crystallized structure justifies our methodology. The premise behind the homology modeling approach is that proteins with similar genetic sequences share the same overall fold, implying similar function [11]. The breakthrough that facilitated Na\(^+\),K\(^+\)-ATPase modeling was the high resolution determination of a closely related Ca\(^2+\)-ATPase, SERCA [12], which has since been determined in a variety of conformations. In addition to the aforementioned Na\(^+\),K\(^+\)-ATPase structural data, various functional results (e.g. [13, 14]) were included to create a homology model via the homology modeling software, Modeller [15]. Thus three models were created based on the E1, E2, and E2P states to study the ion permeation in Na\(^+\),K\(^+\)-ATPase.

The homologues were first oriented based on structural and hydrophobic data and then included in a lipid bilayer membrane that initially consisted of 512 POPC lipid molecules. A hole suitable to accommodate the protein was created by lipid removal and repeated relaxation [16]. Each system was solvated with SPC water to create a ~200,000 atom system. NPT systems were energy minimized and then subjected to 500 ps of position restrained simulation with GROMACS 3.3.1 on a 16 node Itanium cluster. We have used a standard united-atom lipid topology and with GROMOS87 force field combination was employed [16]. Impact of different force field options is an open question that will be addressed in a future comparative study. The protein’s backbone was restrained and the lipid molecules were restrained to the bilayer plane. The lipid bilayer quickly relaxed to empirical area per lipid values. Root mean square deviation (RMSD) of subsequent fully unrestrained simulations indicated good protein stability (Fig. 2). Trajectory data analysis was performed only on data taken after the RMSD of the protein alpha carbon atoms stabilized. Further details regarding system setup and MD simulations are described elsewhere for previous simulations performed on SERCA [17].