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THE FREE ENERGY LANDSCAPES GOVERNING THE FUNCTION OF COMPLEX BIOMOLECULAR MACHINES

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EXECUTIVE SUMMARY

Proteins such as ion channels, transporters, and pumps play an essential role in controlling the bidirectional flow of material and information across the cell membrane, enabling the cell to accomplish complex tasks. In the present research, we investigated the selective ion-binding processes at the heart of the molecular mechanism of an ATP-driven ionic pump: the Na^+/K^+–ATPase. (ATP—adenosine triphosphate—is the immediate source of energy for most cellular work, in this case the transport of sodium and potassium ions across a membrane.) We also studied the key factors controlling the stability of the C-type inactivated state of the KcsA K^+ channel, a nonconducting state of K^+ channels with great physiological implications. Our project aims to gain a deep mechanistic perspective of protein function, linking structure to dynamics, by characterizing the multidimensional free energy "landscapes" that govern key functional aspects of strong electrostatic interactions associated with either ion binding or charged moieties. A free energy landscape presents a powerful and rich concept to help understand key functional processes in these systems.

RESEARCH CHALLENGE

We study complex biomolecular assemblies that consume energy in order to perform specific biological functions. The concerted action of these "molecular machines" underlies all activities of the living cell. The proteins associated with biological membranes are particularly remarkable in their control of the two-way flow of material and information across the membrane. An important question is whether one can truly design an effective and scalable computational strategy that is well adapted for a leadership-class computer such as Blue Waters to tackle processes that occur over long timescales. Unbiased molecular dynamics (MD) trajectories, while very valuable, can be limited. Advanced free energy methodologies can help overcome these limitations [1–4].

The solution that we proposed to this problem is to break down the task by calculating the free energy landscape that governs the key functional motions within a subspace of predetermined order parameters. A free energy landscape (or potential of mean force) presents a powerful and unifying concept to aid in understanding these systems. By studying a small set of biologically significant but experimentally well-characterized systems of increasing size and complexity within a unified computational perspective provided by free energy landscapes, our overarching goal is to push the envelope and advance the theory–modeling–simulation technology and open this virtual route to address fundamental biological questions. It is our hope that the present study will serve as a road map for elucidating key thermodynamic features affecting the function of important molecular nanomachines by using an extremely scalable computational strategy.

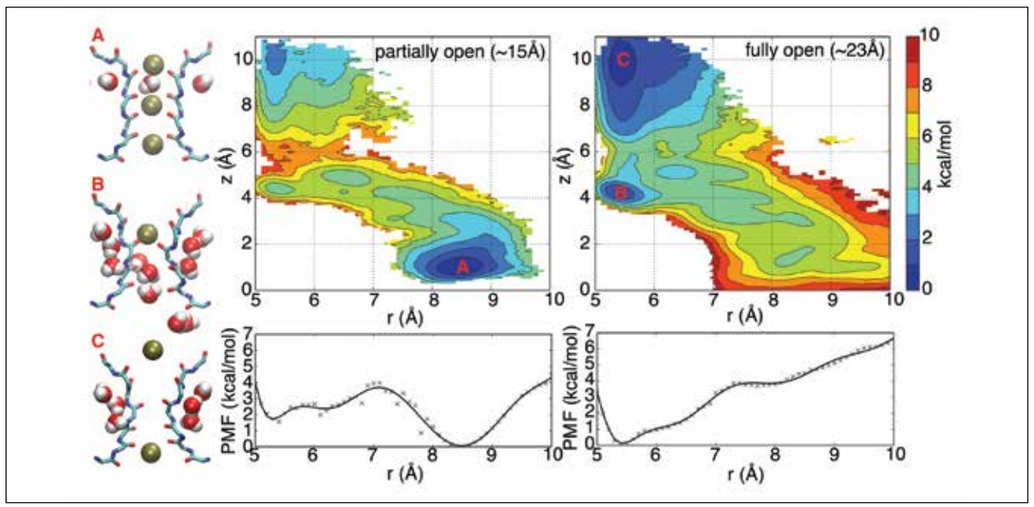

Figure 1: Two-dimensional PMF (potential of mean force) to assess the conformational preferences of the selectivity filter with partially and fully open intracellular gate in K^+ channel KcsA. The horizontal and vertical reaction coordinates, respectively, describe the width of the selectivity filter (r) and the position of the external K^+ ion along Z axis (z).

METHODS & CODES

The solution that we proposed to overcome the timescale limitation is to break down the task by calculating the free energy landscape that governs the key functional motions within a subspace of predetermined order parameters using umbrella sampling (US) Hamiltonian-tempering replica exchange MD (US/H–REMD) simulations [3,4]. Based on the knowledge of a specific system, it is possible to manage efficient US/H–REMD simulation in any multidimensional subspace of collective variables [3,5,6]. US/H–REMD simulations are extremely scalable to thousands of CPUs on high-performance computers. These computations employ NAMD extended to treat multiple-copy algorithms. (NAMD is a parallel molecular dynamics code designed for high-performance simulation of large biomolecular systems.)

RESULTS & IMPACT

C-type inactivation in K^+ channels is thought to be due to constriction of the selectivity filter. Our recent study on KcsA showed that rapid constriction occurs within 1–2 microseconds when the intracellular activation gate is fully open, but not when the gate is closed or partially open. These results imply that the observed kinetics underlying activation/inactivation gating reflect a rapid conductive-to-constricted transition of the selectivity filter that is allosterically controlled by the slow opening of the intracellular gate.

Our simulations of the SERCA (sarcoplasmic reticulum Ca^{2+}–ATPase) pump using different protonation configurations at the binding sites reveal how deprotonation events affect the opening of the cytoplasmic gate. The results show that there is a strong coupling among the chronological order of deprotonation, the entry of water molecules into the transmembrane (TM) region, and the opening of the cytoplasmic gate. From a functional point of view, the chronology of the deprotonation process described here helps explain how the transporter maintains strict coupling at physiological pH by controlling solvent accessibility to the binding sites.

The computational framework we developed is likely to impact a vast array of problems in structural biology. Our goal is to advance and expand the fundamental knowledge in the theoretical and computational methodologies that are used to characterize complex macromolecular biological systems. Technological advances in computational biophysics are expected to have a significant long-term fundamental impact on human health by furthering the development of reliable technologies for screening large biomolecular systems to databases of compounds and identifying potential lead drug molecules. All the research is done in an academic environment where education and training of students and postdocs is an intrinsic component of our activities.

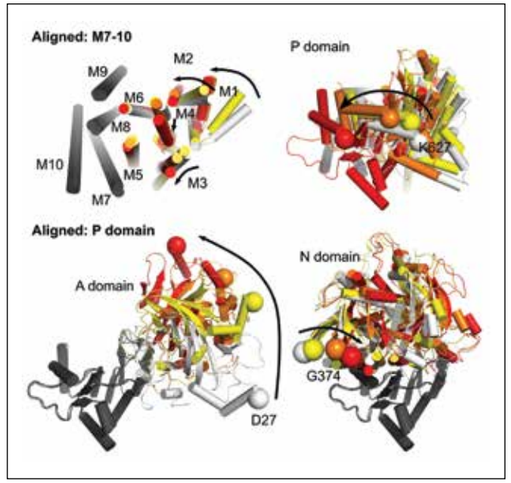

Figure 2: Main conformational changes occurring in the transmembrane (TM) region and cytoplasmic domains during the transition between major functional states of the SERCA pump. The figure shows the dominant motions in the TM (top left), the P domain (top right), the A domain (bottom left), and the N domain (bottom right).

WHY BLUE WATERS

Umbrella sampling Hamiltonian replica-exchange molecular dynamics simulations are extremely scalable to thousands of CPUs on high-performance computers; multidimensional calculations on systems that range in size from 66,104 atoms to 291,148 atoms require thousands of nodes. Thus, these computations are at the forefront of what is possible now thanks to petascale leadership computers such as Blue Waters.

PUBLICATIONS & DATA SETS

Li, J., et al., Chemical substitutions in the selectivity filter of potassium channels do not rule out constricted-like conformations for C-type inactivation. *Proceedings of the National Academy of Sciences U.S.A*, 114:42 (2017), pp. 11145–11150.

Li, J., et al., Rapid constriction of the selectivity filter underlies C-type inactivation in the KcsA potassium channel. *Journal of General Physiology*, in press (2018).

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