A computational swiss army knife approach to unravelling the secrets of proton movement through SERCA

Syma Khalid[1] and Simon Newstead[2]
[1]School of Chemistry, University of Southampton, Southampton SO17 1BJ, UK.
[2] Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK.

Correspondence:
S.khalid@soton.ac.uk
Simon.newstead@bioch.ox.ac.uk

The sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) is a member of the P-type ATPase family of cation and lipid pumps. It is responsible for the active transport of Ca^{2+} from the cytosol into the sarcoplasmic reticulum (SR) lumen of eukaryotic cells and pays an essential role in Ca^{2+} homeostasis, signalling, cell differentiation and terminating muscle contraction. Given its key role in regulating intracellular calcium stores it is unsurprising that SERCA and related P-type pumps have been the focus of much structural and functional (experimental and computational) work over the years[1]. There are over 70 crystal structures of SERCA available, capturing the protein in various conformational states of its Ca^{2+} pumping cycle making it perhaps the most structurally well characterised component of the cellular calcium transport apparatus. It is known that SERCA uses the energy produced by ATP hydrolysis to transport two Ca^{2+} ions from the cytosol into the SR lumen in exchange for two protons, which are released into the cytosol from the lumen[2]. Experimental and simulation studies have identified specific residues responsible for sequestering the protons that are exported into the cytosol. During this process of Ca^{2+}/H^{+} exchange SERCA undergoes transitions between two major conformational states; this process has been studied extensively and is discussed in detail in a number of reviews. [3-4]

What had been less well understood until recently is the movement of protons from the cytosol into the lumen, in other words movement of protons in the same direction as the Ca^{2+} ions. While biophysical studies have shown that protons indeed move into the lumen, a protein that facilitates this to date not been identified. However, structural and computational analyses of SERCA revealed the presence of hydrated pores that span the membrane, prompting speculation that SERCA itself facilitates the transport of H^{+} from the cytosol into the SR lumen[5].

In a recent article, Voth and co-workers use multi-scale molecular dynamics (MD) simulations to test this hypothesis. The pioneering study represents a computational powerhouse in terms of the combination and rigour of the methods applied to study a single process. To overcome some of the limitations of traditional MD for studying proton transfer processes the multiscale reactive molecular dynamics (MS-RMD) method is employed alongside potential of mean force calculations via umbrella sampling; in which the MS-RMD models are parameterising from QM/MM data using a force-matching protocol[6-7]. This approach enables the authors to explicitly account for Grotthuss proton shuttling[8]. The authors use the molecular system already simulated for microseconds by Espinoza-Fonseca *et al.* as input to initiate a series of further simulations[9]. The results make a compelling case for microsecond timescale passive proton transfer through SERCA.

The ‘resolution revolution’ in structural biology is yielding increasing numbers of high-resolution structures of proteins captured in various conformational states providing insights into mechanistic pathways[10]. Molecular dynamics simulations have stepped up to the challenge of complementing the structures with increasingly sophisticated and nuanced insights, such as those provided in this elegant study by Voth and colleagues. Such insights are possible due to the methodological advancements that enable for example enhanced sampling, coupling of different resolutions and explicit inclusion of reactivity. Continued method development and of equal importance, validation of force-fields and attention to water models as longer simulations of larger systems become feasible, will ensure structural biology and molecular simulations move forward together.

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