Free-energy calculations. A mathematical perspective

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Ion channels are pore-forming assemblies of transmembrane proteins that mediate and regulate ion transport through cell walls. They are ubiquitous to all life forms. In humans and other higher organisms they play the central role in conducting nerve impulses. They are also essential to cardiac processes, muscle contraction and epithelial transport. Ion channels from lower organisms can act as toxins or antimicrobial agents, and in a number of cases are involved in infectious diseases. Because of their important and diverse biological functions they are frequent targets of drug action. Also, simple natural or synthetic channels find numerous applications in biotechnology. For these reasons, studies of ion channels are at the forefront of biophysics, structural biology and cellular biology.

In the last decade, the increased availability of X-ray structures has greatly advanced our understanding of ion channels. However, their mechanism of action remains elusive. This is because, in order to assist controlled ion transport, ion channels are dynamic by nature, but X-ray crystallography captures the channel in a single, sometimes non-native state. To explain how ion channels work, X-ray structures have to be supplemented with dynamic information. In principle, molecular dynamics (MD) simulations can aid in providing this information, as this is precisely what MD has been designed to do. However, MD simulations suffer from their own problems, such as inability to access sufficiently long time scales or limited accuracy of force fields. To assess the reliability of MD simulations it is only natural to turn to the main function of channels - conducting ions - and compare calculated ionic conductance with electrophysiological data, mainly single channel recordings, obtained under similar conditions. If this comparison is satisfactory it would greatly increase our confidence that both the structures and our computational methodologies are sufficiently accurate.

Channel conductance, defined as the ratio of ionic current through the channel to applied voltage, can be calculated in MD simulations by way of applying an external electric field to the system and counting the number of ions that traverse the channel per unit time. If the current is small, a voltage significantly higher than the experimental one needs to be applied to collect sufficient statistics of ion crossing events. Then, the calculated conductance has to be extrapolated to the experimental voltage using procedures of unknown accuracy. Instead, we propose an alternative approach that applies if ion transport through channels can be described with sufficient accuracy by the one-dimensional diffusion equation in the potential given by the free energy profile and applied voltage. Then, it is possible to test the assumptions of the equation, recover the full voltage/current dependence, determine the reliability of the calculated conductance and reconstruct the underlying (equilibrium) free energy profile, all from MD simulations at a single voltage. We will present the underlying theory, model calculations that test this theory and simulations on ion conductance through a channel that has been extensively studied experimentally. To our knowledge this is the first case in which the

complete, experimentally measured dependence of the current on applied voltage has been reconstructed from MD simulations.