Abstract

Membrane transport is a crucial phenomenon that controls several biological processes such as cellular communication, signaling, ATP synthesis and muscle contraction. To ensure survival, membrane transporters are employed to carry out the transmembrane transport of ions and small molecules. Membrane transporters operate on the nanosecond to the millisecond timescale, and are miniscule in size (10^{-8}-10^{-7} meter). Therefore, membrane transporters pose significant challenges for experimental investigation, in particular with respect to the inspection of crucial dynamic atomistic details and interactions. All-atom Molecular Dynamics (MD) simulations have emerged as a useful powerful technique to investigate the structural changes on the atomic level and are often regarded as a 'computational microscope.' In this thesis, MD simulations and free energy methods are extensively used to investigate mammalian and bacterial transporters. Active ion-transport carried out by the sodium-potassium pump, gastric proton pump, and the bacterial kdpFABC complex; and lysosomal cholesterol export by the Niemann Pick Cl (NPC1) protein were studied. The investigations performed on ion-pumps sheds light on the underlying mechanisms of K^+ binding and selectivity in specific conformational states of the ion-transport cycle. A novel pKa correlation analysis was used to calculate the probability of proton transfer between acidic amino acids. For the K^+-transporting kdpFABC complex, the mechanism of regulation by a conserved phosphorylated serine was highlighted. The regulatory mechanism is essential to prevent the wasteful usage of ATP in K^+-surplus conditions. Furthermore, cholesterol transporter NPCI was examined to obtain insights into the lysosomal cholesterol export. In NPC1, we proposed for the first time, a cholesterol-dependent allosteric conformational change, which potentially regulates cholesterol export. MD simulation studies are limited by availability of three-dimensional protein structures, and the accessible time and length scales. Therefore, the studies in this thesis were performed together with experimental collaborations for validation and to obtain a holistic picture of the studied systems. The investigations in this