of substrate. The most common membrane mimetic systems for structural studies of these proteins are detergent micelles and, more recently, nanodiscs. However, these approaches have their own advantages and disadvantages: detergent micelles could allow too much flexibility, while nanodiscs could restrict the dynamics of these proteins. Therefore, we decided to explore whether these membrane mimetics affect the behavior of the biliverdin (BV) transporter ABCB10. We are measuring the basal and substrate-activated ATPase activity in detergent micelles and nanodiscs of different diameters (membrane scaffold proteins MSP1D1 and MSP2N2). We have found that ABCB10 has a very low response to BV in detergent micelles, whereas BV induces a significant activation of ABCB10 in nanodiscs. However, our data shows that the activity of ABCB10 is highly dependent on the size of the MSP used to form the nanodiscs. We found that ABCB10 in the smallest nanodiscs (MSP1D1) presents a higher basal activity and a lower activation by BV than the protein reconstituted in larger nanodiscs (MSP2N2). In addition, we have found that the response to other molecules is also affected by the size of these nanodiscs. Our data demonstrates that the nanodiscs' size can influence ABC transporters' behavior. A deeper understanding of the effect and the functional significance that artificial membranes impose on these membrane proteins' dynamics, substrate interaction, and mechanisms is essential to properly study them and find physiologically relevant results. NIH-NIGMS R01GM145938.

2230-Pos

Atomic-resolved description of anion binding and the alternating access mechanism of anion exchanger 1 (band 3)

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The red blood cell's most abundant membrane protein, band 3, is pivotal for regulating the acid-base balance and respiration through its bidirectional exchange of Cl^- and bicarbonate ions. During the transport cycle, band 3 employs the alternating access mechanism, transitioning between outward facing (OF) and inward facing (IF) states. Leveraging our recent high-resolution cryo-EM structure resolved in both states, we employed equilibrium and non-equilibrium molecular dynamic simulations to characterize spontaneous anion binding to the protein and the conformational change from the OF to the IF state during transport. An extended set of multiple μs-long equilibrium simulations revealed recurrent anion binding events, both for Cl^- and bicarbonate, at specific sites allowing for identification of the anion sites of band 3, in both IF and OF states. Next, we applied enhanced sampling methods to induce the transition from the OF to the IF state. In an elevator-like mechanism, the transport domain undergoes a combined translational/rotational motion relative to the stationary scaffold domain, as induced by specific collective variables in the simulation. The obtained transition pathway was subsequently relaxed and refined using the string method with swarms of trajectories. Then, 1D-bias-exchange, umbrella sampling was used to calculate the free energy along the refined pathway. The free energy analysis was performed on both *apo* and substrate-bound states, as well as on a system in which bound PIP2s identified in the experimental structure had been removed. The free energy profiles clearly substantiate the experimentally reported inhibitory effect of the lipid on band 3. This comprehensive computational approach studying the mechanism of band 3 sheds light on the intricate relationship between structural dynamics and free energy in a vital anion transport process.

2231-Pos

Functional evaluation of a CMT2DD-causing ATP1A1 variant

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We identified the variant c.1645G>A in *ATP1A1*, resulting in mutation G549R of the Na^+/K^+-ATPase's (NKA) α1 subunit in two unrelated patients with Charcot-Marie-Tooth disease, a peripheral neuropathy. Both patients presented with symptoms in their teens, with subsequent slow progression of distal muscle weakness, sensory loss, and areflexia. Clinical electrophysiology confirmed distal denervation, sensory axonal loss, and intermediate slowing of distal motor response. This variant was previously identified in a three-generation kindred, but its pathophysiological mechanisms remain unknown. NKA is a heterodimeric $\alpha\beta$ protein that exports $3Na^+$ and imports $2K^+$ at the cost of ATP hydrolysis, thereby establishing the electrochemical gradients required for neuronal excitability. We evaluated the function of G549R-α1β1 NKA in Na_i^+-loaded oocytes expressing these subunits using two-electrode voltage clamp. Compared to wildtype, G549R showed ~30% reduction in K^+_o-induced NKA current (n = 24, 5 oocyte batches), a result resembling previous reports of other CMT-causing variants. The $K_{0.5}$ for K^+_o was minimally increased (~30%, n = 6). We measured the ouabain-sensitive transient-charge movement elicited by voltage pulses, to evaluate NKA's partial reactions without K^+_o. The total charge moved (dependent on the number of functional NKAs at the membrane) was reduced by ~30% in G549R compared to wild type (n = 15), a result consistent with preliminary western blot analysis of plasmalemma-enriched oocyte membrane preparations. The charge-voltage curve shows a small shift, consistent with a ~20% (n = 15) reduction of Na^+_o affinity. Patch clamp experiments to evaluate interaction with intracellular ligands are underway. Preliminary results of NKA localization in HEK293 cells using YFP-tagged α1 clones show intracellular localization of G549R-NKA relative to WT-NKA. Taken together these results indicate that one of the pathophysiological mechanisms of G549R is its impaired trafficking to, and/or its half-life at, the plasmalemma, reducing the pumping capacity there. Experiments evaluating plausible dominant-negative effects are ongoing. NSF-MCB 2003251.

2232-Pos

Molecular dynamics simulations for atomic-level characterization of lipid interactions with the bovine multidrug resistance-associated protein 1 (bMRP1)

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bMRP1 is a membrane protein, and a member of the ATP-binding cassette (ABC) transporter superfamily which plays a pivotal role in cellular detoxification by pumping out a wide range of xenobiotics, including chemotherapeutic agents, from cells. This transporter uses alternating access mechanism between inward facing (IF) and outward facing (OF) conformations, during the substrate transport cycle. The transition from the protein's IF state to the OF state is driven by ATP molecules binding at the nucleotide binding sites, and subsequent dimerization of the two nucleotide binding domains. The reverse transition from OF to IF is facilitated by hydrolysis of these ATP molecules. Understanding the atomic-level interactions between membrane's lipids and bMRP1 is of paramount importance to decipher how these interactions influence the substrate and ATP driven conformational changes. In this study, we employ combination of equilibrium and nonequilibrium molecular dynamics (MD) simulations to explore the dynamic behavior of bMRP1 in the presence of various lipid species, including phospholipids and cholesterol. While nonequilibrium MD simulations is used to elucidate the transition pathway between the IF and OF states, microsecond-level equilibrium MD simulations enabled us to comprehensively analyze the distinct dynamic behaviors exhibited in these states. By simulating lipid-bMRP1 interactions under physiological conditions, we aimed to unravel the structural and dynamic features governing the binding and transport of substrate by bMRP1. This information can contribute to a better understanding of bMRP1 biological functions and inform drug development efforts aimed at overcoming multidrug resistance in veterinary and human medicine.

2233-Pos

Electron transfer directionality in type II photosynthetic reaction centers

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Photosynthetic reaction center absorbs light, converting solar energy to chemical energy through a series of electron and proton transfer reactions. The cofactors that form the pathway for electron transfer across the membrane embedded reactions centers are always in a c_2 symmetric arrangement. In type I reactions centers such at PSI both branches are used. In the type II reaction centers, PSII and purple bacterial reaction center (bRC), electron transfer uses only one branch. Thus, the electron travels from P860 in bRC goes through $BChl_A$, BPh_A, to Q_A, and then Q_B, ignoring $BChl_B$ and BPh_B. While the cofactors are arranged symmetrically, the polypeptides on the active A side (bRC L peptide or D1 in PSII) are not identical to the inactive B branch (M polypeptide bRC and D2 in PSII). MCCE calculations, use Monte Carlo (MC) sampling of protonation and redox states and side chain conformation to determine the E_m of redox cofactors. In the wild-type bRC the E_m of the active branch cofactors are more positive than those on the inactive branch, showing their reduction is thermodynamically favored. Several mutations had been made previously to