Somatic gain-of-function mutations of the Na^+, K^+-ATPase ion pump α1-subunit have been found in aldosterone-producing adenomas that are amongst the causes of hypertension. We use all-atom Molecular Dynamics (MD) simulations to investigate structural consequences of these mutations, namely Leu97 substitution by Arg (L97R), Val325 substitution by Gly (V325G), deletion of Phe93, Ser94, Met95, Leu96, Leu97 (Del93-97) and deletion-substitution of Glu953, Glu954, Thr955, Ala956 by Ser (EETA956S) that show inward leak currents under physiological conditions. First three mutations affect the structural context of the key ion binding residue Glu327 at binding site II, which leads to the loss of the ability to correctly bind ions and to occlude the pump. The mutated residue in L97R is more hydrated, which ultimately leads to the observed proton leak. V325G mutant mimics the structural behavior of L97R, however it does not promote the hydration of surrounding residues. In Del93-97, a broader opening is observed due to the rearrangement of the kinked transmembrane helix 1, M1, which may explain the sodium leak measured with this mutant. The last mutant, EETA956S, opens an additional water pathway near the C-terminus, affecting the III sodium-specific binding site. Moreover, we report for the first time the spontaneous binding of monovalent ions to the E2P outside open state of the Na^+, K^+-ATPase. The results support electrophysiology measurements and suggest how three mutations prevent the occlusion of the Na^+, K^+-ATPase, with a possibility of transforming the pump into a passive ion channel, while the fourth mutation provides a new insight into the sodium binding in the E1 state.

2945-Pos Board B637

Glutathionylation of the Na K Pump

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Reversible oxidative modifications of proteins are of importance for the normal cell functioning, e.g. for the receptor-coupled signaling. The formation of a disulfide bond between a protein and a glutathione tripeptide (Glu-Cys-Gly,) is an oxidative, posttranslational modification that might be involved in the cellular signaling. Recently, the Cys46 residue of the sarcolemmal Na K ATPAse β1 subunit has been proposed as a target for glutathionylation. Here, we use all-atom Molecular Dynamics (MD) simulations to investigate structural consequences of the Cys46 glutathionylation in the E2P state of the protein. Being negatively charged at physiological pH, the glutathione modification can induce alterations similar to the effect of phosphorylation. In contrast with previous studies, we find that Cys46, buried deeply in the membrane, can be exposed to the cytosolic glutathione due to the defect and local rearrangement of the protein-membrane interface in the E2P state, rather than the β1 helix sliding outside the membrane, previously anticipated for the E1 state. These findings are in accord with the recent crystal structure of the E1P state of Na^+, K^+-ATPase, where the position of the β1 helix is essentially the same as it is in the E2P state. We will comment on the accessibility of glutathione to Cys46, which lies at the center of the membrane.

2946-Pos Board B638

Superinhibitory Phospholemman Mutants as Potential Therapeutics for Heart Failure

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The sodium-potassium ATPase (NKA) is a target for inotropic cardiac glycosides that inhibit NKA activity and cause a downstream increase in intracellular calcium. These agents have a narrow therapeutic window and can cause arrhythmias. In contrast, the endogenous inhibitor of NKA, phospholemman (PLM), is dynamically regulated. Inhibition of NKA is relieved by elevated intracellular sodium, or when PLM is phosphorylated. In the present study, we performed scanning alanine mutagenesis of PLM to identify superinhibitory mutants of PLM that could serve as an alternative to cardiac glycosides. We hypothesized that mutations that destabilize PLM oligomers will also cause increased binding of PLM to NKA. We employed a FRET assay that simultaneously reports PLM-PLM and PLM-NKA binding for each mutant. Several mutants conformed to this prediction, showing decreased oligomerization and higher affinity for NKA compared to WT. Interestingly, other mutants demonstrated greater affinity for NKA despite no detectable decrease in oligomerization. Ongoing studies will determine whether high affinity PLM mutants function as superinhibitors of NKA activity.

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Molecular Dynamics Simulations Helps to Rationalize CopB Mutations and their Relationships to Wilson Disease

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The regulation of copper levels is central to physiology. Mutations in the ATP7B copper transporter are known to lead to Wilson's disease in humans. How these mutations lead to the disease is not fully characterized at a molecular level. An excellent model system for exploring the changes in structure and dynamics for Wilson disease mutations for the ATP binding domain is provided by CopB from A. fulgidus. This protein has high sequence similarity with the P, N and hinge regions of ATP7B. Mutations to each region have previously been characterized by experimental measurements. In this presentation we highlight implicit and explicit solvent simulations of the wild-type and mutations found within each of these three regions. The results shed new light on how the mutations impact on conformational change, on ATP-binding, and on phosphorylation within these domains.

2948-Pos Board B640

Peptide-Based Approach to Study Cytosolic Domain Interactions in a Bacterial Copper-Transporting ATPase

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Copper-transporting P_{IB}-type ATPases (CopA-family) fulfill key roles in copper homeostasis by pumping copper across bacterial or intracellular and cytoplasmic eukaryotic membranes. Mutations of the copper-transporting ATPase gene cause Wilson and Menkes diseases. CopA consists of 8 transmembrane helices and three cytosolic domains; Nucleotide binding (N), Phosphorylation (P) and Actuator (A) domains. We have used synthetic peptides to identify minimal structural motifs and physical mechanisms of cytosolic domain interactions.

Based on the crystal structure[1], we have designed decameric-peptides whose sequences are derived from the putative interaction site of the A-domain with the PN domain of CopA from *Legionella pneumophila.* The interaction of the synthetic A-domain peptide with the individually expressed PN domain was measured by Isothermal Titration Calorimetry(ITC), Circular Dichroism (CD), and stopped-flow fluorescence techniques. Preliminary ITC results show a favorable TΔS, indicating that binding of the peptide carrying the native sequence is driven by hydrophobic interactions with stoichiometry(N=1). Time-resolved fluorescence measurements with a tryptophan-carrying actuator peptide show association and dissociation rate constants with the PN domain of $22000M^{-2}s^{-1}$ and $0.7M^{-1}s^{-1}$, respectively. This corresponds to a K_d of the peptide for the PN-domain of 32μM. In the presence of the non-hydrolyzable nucleotide AMPPNP the best kinetic fit was obtained with rates of $20000M^{-2}s^{-1}$ and $1.0M^{-1}s^{-1}$, indicating an almost unaffected affinity of the peptide for the nucleotide-loaded PN-domain. In agreement with the kinetic and isothermal data, the decapeptide also shifted the melting temperature of the PN domain from 330 to 332K as monitored by CD spectroscopy.

In summary, we have good evidence that the actuator peptide binds in a predominantly hydrophobic mechanism to the PN domain in the 30-50μM K_d range with little dependence on the occupancy of the nucleotide binding site of the PN domain.

1)Gourdon,P. et.al.2011 Nature475, 59.

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Conformational Transitions in ATP-Driven Calcium Pump SERCA

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Sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) is an integral membrane protein that uses ATP hydrolysis as a source of free energy to pump two calcium ions per ATP molecule from calcium poor cytoplasm of the muscle cell to the calcium rich lumen of the sarcoplasmic reticulum, thereby maintaining a ten thousand fold concentration gradient. Detailed structural studies of the pump under different conditions provided analogues of various intermediates in the reaction cycle and revealed important changes in the tertiary structure of the protein both in the cytoplasmic and in the