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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
Wojciech Kopec, Bastien Loubet, **Himanshu Khandelia**.
University of Southern Denmark, Odense, Denmark.
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
Ryan Himes[1], Julie Bossuyt[2], Donald M. Bers[2], Seth L. Robia[1].
[1]Loyola University Chicago, Chicago, IL, USA, [2]University of California, Davis, CA, USA.
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.

2947-Pos Board B639
Molecular Dynamics Simulations Helps to Rationalize CopB Mutations and their Relationships to Wilson Disease
Samuel Jayakanthan[1], Megan M. McEvoy[2], **Thomas B. Woolf**[3].
[1]Physiology, Johns Hopkins University, Baltimore, MD, USA, [2]Chemistry and Biochemistry, University of Arizona, Tucson, AZ, USA, [3]Physiology and Biophysics and Biophysical Chemistry, Johns Hopkins University, Baltimore, MD, USA.
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
Ahmed Sayed[1], Yixin Zhang[2], Karim Fahmy[1].
[1]Resource Ecology Institute, Helmholtz-Zentrum Dresden-Rossendorf, Dresden, Germany, [2]B CUBE - Center for Molecular Bioengineering, Technische Universitaet Dresden, Dresden, Germany.
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 $22000 M^{-2}s^{-1}$ and $0.7 M^{-1}s^{-1}$, 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 $20000 M^{-2}s^{-1}$ and $1.0 M^{-1}s^{-1}$, 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.

2949-Pos Board B641
Conformational Transitions in ATP-Driven Calcium Pump SERCA
Avisek Das, Benoit Roux.
Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL, USA.
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

transmembrane parts. Two major outstanding issues are the pathways of the ions to and from the transmembrane binding sites and a detailed understanding of the large scale conformational changes among various functionally relevant states. We have applied all-atom molecular dynamics (MD) and string method with swarms-of-trajectories to study transition pathways among various experimental structures. The size of the system (291,000) and the time-scale involved in the large scale motions prohibit the use of brute-force unbiased simulations to obtain statistically meaningful information. To circumvent this challenge, the string method with swarms-of-trajectories is used to discover the optimal minimum free energy path between the two end-states. The path, which is represented as a chain of states or images in the space of relevant collective variables, is optimized by iterating two steps: moving each image along the drift calculated from an ensemble (swarm) of short unbiased MD trajectories initiated from the image and a reparametrization procedure that keeps all the images equidistant. We hope to understand the molecular details of the complex motions involved in the conformational transitions by studying the pathways produced by the string method and correlating these observations to available structural and biochemical experiments.

2950-Pos Board B642

Protonation-Dependent Structural Transitions of the Calcium Pump Studied by Microsecond Molecular Dynamics Simulations

L. Michel Espinoza-Fonseca, David D. Thomas.
Biochemistry, University of Minnesota, Minneapolis, MN, USA.

We have performed microsecond molecular dynamics (MD) simulations to investigate the protonation-dependent structural transitions in the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA). Changes in protonation states of acidic residues in the Ca^{2+}-binding sites of SERCA associated with proton countertransport play an essential role in the structural transitions between high (E1 state) and low (E2 state) Ca^{2+}-affinity conformations. In particular, deprotonation of Ca^{2+}-binding sites plays an essential role in activation as it facilitates the conversion from E2 to E1 states of SERCA. However, the mechanism by which protonation-dependent structural transitions facilitate activation of the pump is still unclear. Here, we performed 3.6-μs all-atom MD simulations of protonated and deprotonated SERCA starting from the crystal structure of the inactive E2 state. We observed that protonation of residues Glu309, Glu771 and Glu908 lock SERCA in a conformation similar to the crystal structures of the E2 state. Upon deprotonation, metal ion binding from the solution containing KCl assists partial formation of Ca^{2+}-binding site I, which results in kinking of transmembrane helix 5 at positions Ser766-Ser767. We found that kinking of helix 5 induces structural changes that propagate rapidly to the transmembrane and cytoplasmic domains, destabilizing the E2-like structure of the pump and populating an intermediate state stabilized by binding of lipid molecules to a non-annular site located between transmembrane helices 3 and 5. We propose that nonselective metal ion binding following deprotonation of SERCA initiates protonation-dependent destabilization of the E2 state and eventual formation of the partially active E1 state of the pump. This work was supported by the AHA (12SDG12060656 to L.M.E.-F.), the NIH (GM27906 to D.D.T.) and the Minnesota Supercomputing Institute.

2951-Pos Board B643

Environmental Influences on States: Molecular Dynamics Simulations of SERCA

Anu Nagarajan[1], Thomas B. Woolf[2].
[1]NINDS, National Institute of Health, Bethesda, MD, USA, [2]Department of Physiology, Johns Hopkins University, Baltimore, MD, USA.

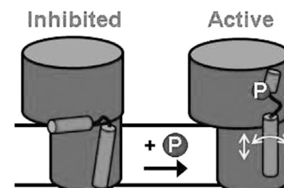
The membrane protein pump SERCA, is a Ca-ATPse that is well-characterized from a structural perspective, with x-ray conformations known for the major states of the pump cycle. Yet, understanding the role of the environment in supporting a state and in enabling transitions between states continues to be a major research challenge. To address these environmental influences we have concentrated our efforts on two of the pump cycle transitions. We now present analysis of a set of four all-atom explicit bilayer simulations of SERCA states (1WPG, 2AGV, 3AR2, and 2ZBD) that we performed using Anton (PSC) and Lonestar (XSEDE) resources. These are to be compared with our previous computations of SERCA using implicit solvent and coarse-grained models. The prior results (J. Mol. Biol.422:575-593 (2012) ; Proteins 80(8):1929-1947 (2012)) suggested an important role of compensation between different environment and protein domains as central to the transitions. We now extend those results with analysis of the explicit bilayer, water, salt and co-factor simulations. An intriguing initial result is that the presence of calcium changed the fluctuations seen in the 3AR2 state.

2952-Pos Board B644

Multifrequency EPR Detects Orientation of Calcium Transport Proteins in Lipid Bicelles

Jesse E. McCaffrey, Zachary M. James, Christine B. Karim, David D. Thomas.
Biology, Molecular Biology, and Biophysics, University of Minnesota: Twin Cities, Minneapolis, MN, USA.

We have used electron paramagnetic resonance (EPR) at X and Q band to probe the structural dynamics of the integral membrane protein phospholamban (PLB), as a function of phosphorylation and addition of its regulatory target, the sarcoplasmic reticulum calcium ATPase (SERCA). We found previously that PLB remains bound to SERCA after phosphorylation, suggesting that a structural transition within the SERCA-PLB complex is responsible for relief of inhibition. Our current goal is to elucidate this mechanism through orientation and accessibility EPR, supporting rational design of therapies to improve calcium transport in muscle cells. We used the monomeric mutant AFA-PLB with the rigid spin label TOAC incorporated within the transmembrane domain. This protein was reconstituted with purified SERCA into lipid bicelles and magnetically aligned at both X and Q band field strength, allowing for a robust global fitting approach. We measured changes in PLB tilt upon phosphorylation, in the absence and presence of SERCA. Experiments were performed in the Biophysical Spectroscopy Center at the University of Minnesota. This work was funded by grants from NIH (R01 GM27906, P30 AR0507220, and T32 AR007612).



PLB binds to SERCA forming an inhibited complex. This inhibition is relieved upon PLB phosphorylation by a structural change which includes vertical and tilting motions.

2953-Pos Board B645

The Mechanism of Uncoupling ATP Hydrolysis and Calcium Transport in Serca by Sarcolipin

Alysha A. Dicke[1], Kaustubh R. Mote[2], Gianluigi Veglia[1,2].
[1]Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, Minneapolis, MN, USA, [2]Department of Chemistry, University of Minnesota, Minneapolis, MN, USA.

The interactions of sarcolipin (SLN) with the sarco(endo)plasmic reticulum Ca^{2+} ATPase (SERCA) causes uncoupling of SERCA's ATP hydrolysis and Ca^{2+} -transport activities. SERCA is an integral component of proper muscle function. It is one of the key proteins responsible for re-establishing the low cytosolic Ca^{2+} concentrations needed for normal muscle contraction. When SLN is bound to SERCA, fewer moles of Ca^{2+} are transported per mole of ATP hydrolyzed, which leads to an increase in the heat released. By using solid-state nuclear magnetic resonance spectroscopy and functional assays, we demonstrate that it is the topological coupling between these proteins that is important for the regulation of SERCA. It is through the interactions of their transmembrane regions as well as the interactions of SLN's lumenal tail that lead to uncoupling. These results also provide insight into the mechanism for non-shivering, muscle-based thermogenesis.

2954-Pos Board B646

Sarcolipin Regulation of SERCA is Distinct from Phospholamban

Sanjaya K. Sahoo[1], Sana A. Shaikh[1], Danesh H. Sopariwala[1], Naresh C. Bal[1], Muthu Periasamy[1,2].
[1]Ohio State University, Columbus, OH, USA, [2]Davis Heart and Lung Research Institute, Columbus, OH, USA.

Sarco(endo)plasmic reticulum Ca(2+)ATPase (SERCA) pump activity is modulated by phospholamban (PLB) and sarcolipin (SLN) in cardiac and skeletal muscle. Our recent data suggest that SLN plays a role in muscle thermogenesis by promoting uncoupling of the SERCA pump (Bal, N. C., Maurya, S. K., Sopariwala, D. H., Sahoo, S. K., Gupta, S. C., Shaikh, S. A., Pant, M., Rowland, L. A., Bombardier, E., Goonasekera, S. A., Tupling, A. R., Molkentin, J. D., and Periasamy, M. (2012) Nat. Med. 18, 1575-1579), but the mechanistic details are unknown. To better define how binding of SLN not PLB to SERCA promotes uncoupling of SERCA, we compared SLN and SERCA1 interaction with that of PLB in detail. We used chemical cross-linking to study the dynamic protein-protein interaction. Our studies reveal that SLN differs significantly from PLB: 1) SLN primarily affects the Vmax of SERCA-mediated Ca(2+) uptake but not the pump affinity for Ca(2+); 2) SLN can bind to SERCA in the presence of high Ca(2+), but PLB can only interact to the ATP-bound Ca(2+)-free E2 state; and 3) unlike PLB, SLN interacts with SERCA throughout the kinetic cycle and promotes uncoupling of the SERCA pump. Using SERCA transmembrane mutants, we additionally show that PLB