725-Pos Board B505
Multiscale QM/MM Simulations of ATP Hydrolysis Mechanism in ABC-Transporters
Jingzhi Pu.
Dept. of Chem. & Chem. Biol., Indiana Univ.-Purdue Univ. Indianapolis, Indianapolis, IN, USA.
Adenosine triphosphate (ATP) binding cassette (ABC) transporters are ubiquitous molecular motor proteins that translocate various substrates across cell membranes at the expense of ATP hydrolysis. Although it is known that nucleotide binding domains in ABC-transporters contain a collection of characteristic sequence motifs, the precise mechanism under which ATP is hydrolyzed in these systems remains unknown. By using multiscale combined quantum mechanical and molecular mechanical (QM/MM) free energy simulations, we examined several possible ATP hydrolysis mechanisms in members of ABC-transporters. Our results reveal how the active site residues may participate in the catalytic mechanism and how protein dynamics may contribute to catalysis.

726-Pos Board B506
Reconstitution of Multidrug Resistance Efflux Pumps in Giant Liposomes
SooHyun Park, You Jung Kang, Sheereen Majd.
Biomedical Engineering, Pennsylvania State University, University Park, PA, USA.
Multidrug resistance (MDR), a phenomenon in which cells become resistant to a number of drugs, is a growing obstacle for treatment of cancer and bacterial infections. A group of membrane transporters, known as MDR efflux pumps that bind to a wide range of foreign molecules and pump them out of the cell, are suspected to be the main contributors to MDR. P-glycoprotein (Pgp), which is the most well studied MDR efflux pump, has a critical role in the distribution of drugs in the body. As a result, screening the interactions between this protein and candidate drugs has recently become a required step in the FDA approval process. Biophysical characterization of Pgp and its transport activity may provide key information for the design and development of effective therapeutics. Giant unilamellar vesicles (GUV) are excellent models for natural cell membranes and present appealing platforms for biophysical studies of Pgp under well-defined conditions. Here, we reconstitute Pgp from Chinese hamster ovary B30 cells in giant unilamellar vesicles and investigate its ATPase activity as well as its transport activity.
In this study, we applied small proteoliposomes with reconstituted Pgp for electroformation of Pgp-containing giant vesicles via our recently reported technique of hydrogel-assisted electroformation. These electroformed giant vesicles were characterized using fluorescence microscopy, immunohistochemistry, and ATPase assays. Fluorescence immunostaining confirmed the presence of Pgp in the membrane of GUVs. ATPase assay demonstrated that Pgp in GUVs retained its ATPase activity upon electroformtion. The specific ATPase activity of Pgp in GUVs was about 640 nmol/mg•min, comparable to that of this protein in small proteoliposomes, ~660 nmol/mg•min. Moreover, we are investigating the transport activity of Pgp in these giant liposomes using fluorescence microscopy. The present study may provide valuable insight to the function of this MDR efflux pump.

727-Pos Board B507
Exploring P-Glycoprotein Substrate Access
Laura Domicevica, Philip C. Biggin.
Department of Biochemistry, University of Oxford, Oxford, United Kingdom.
P-glycoprotein (P-gp), an ATP Binding Cassette transporter family membrane protein, extrudes mainly hydrophobic substrates in an ATP hydrolysis-dependant manner. Structurally, P-gp consists of two transmembrane domains (TMD) each comprised of 6 α-helices that together form the drug binding site, and two nucleotide binding domains (NBD) that via ATP binding and hydrolysis provide the energy for the conformational changes necessary to drive drug translocation.
Due to the wide variety of substrates it extrudes, P-gp is one of the main causes of multidrug resistance in cancer and other diseases. As multidrug resistance becomes an ever increasingly important issue, drug development is dependant on knowing what constitutes the difference between a substrate and a non-substrate of P-gp. Although the chemical structure is likely to play a role in recognition within the binding site, it is equally important to understand how access to the binding site is controlled. Therefore more information about way in which substrates access the binding site is of key interest.
In the absence of a human P-gp crystal structure, several human homology models were made from both eukaryotic and bacterial homologue crystal structures. The models were assessed for structural stability and conformational dynamics using molecular dynamics (MD) simulations. Following analysis, the homology model from the C. elegans template was chosen as the model for steered MD calculations to investigate possible substrate access pathways.
The results, when combined with bilayer localisation NMR experiments and MD simulations, give novel insights into the factors that govern P-gp substrate specificity.

728-Pos Board B508
Methionine Importers in Soil Bacteria: Potential for Transporter-Component Crosstalk
Eliza Zielazinski, Sarah Zerbs, Peter Larsen, Frank Collart, Philip D. Laible.
Biosciences Division, Argonne National Laboratory, Lemont, IL, USA.
ABC transporters are widely distributed amongst kingdoms and are responsible for nutrient transport across cellular membranes. Certain soil bacteria contain a higher than average percentage of ABC transporters, representing 40-70% of encoded transporters. While studies of SBPs have indicated that permeases can interact with multiple SBPs with varying affinity and specificity, limited information exists regarding permease specificity for ATPases. The potential for permeases to interact with multiple ATPases was suggested given the high genetic redundancy of homologous types of ABC transporters in plant growth promoting bacteria (PGPB). Genomic analyses of four *Pseudomas fluorescens* strains identified a tractable set of ABC-type amino acid importers with high sequence similarity to the structurally characterized *E. coli* methionine importer MetNI, sharing the presence of a C-terminal C2 regulatory domain on the ATPase. This domain inhibits methionine uptake by preventing ATP hydrolysis when intracellular methionine is bound.
Using a dual-expression-vector strategy, both native complexes and 'hybrid' complexes were stably isolated. Native complexes utilize permease and ATPase components from an individual operon whereas hybrid complexes partner a permease domain from one operon with an ATPase domain from another. Interestingly, three target ATPases from strain PF-5 were able to form stable complexes with a single PF-5 permease target, suggesting this strain has high potential for hybrid interactions. These PF-5 hybrid complexes are shown to be dimers by gel filtration and functional by ATPase assays.
The transinhibition mechanism of the C2 domain was also investigated, showing that D- and L-methionine differentially inhibit ATP hydrolysis of PF-5 hybrid complexes. Initial studies of SBPs associated with these complexes show a correlation between SBP ligands and compounds allosterically inhibiting ATPases. The ability for crosstalk could be especially significant for PGPB, allowing them to provide rapid and specific nutrient exchange with symbiotic species.

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EPR Accessibility Measurements of the SERCA-PLB Complex using Site-Directed Spin Labeling
Peter D. Martin, Zachary M. James, Jesse E. McCaffrey, Dave D. Thomas.
University of Minnesota, Minneapolis, MN, USA.
We investigate the structure formed byof phospholamban (PLB) bound to the sarcoplasmic reticulum (SR) Ca^{2+}-ATPase (SERCA) using site-directed spin labeling. SERCA is a 994 residue transmembrane protein that helps control Ca^{2+} concentration in the SR relative to the cytoplasm in order to facilitate muscle relaxation. PLB is a 52 residue regulatory muscle protein found in cardiac muscle which, upon binding, has been shown to inhibit SERCA activity. However, phosphorylation of PLB at Ser-16 partially relieves this inhibition while still maintaining a combined structureconserving the complex. The structural mechanism behind this relief of inhibition is largely unknown. Using EPR accessibility measurements, we have shown that phosphorylation of PLB at Ser-16 alters the binding interface between its transmembrane helix and the PLB binding groove on SERCA, likely breaking the interactions that lead to inhibition.

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Mapping of Sarcolipin Conformational States along the Enzymatic Pathway of SERCA
Alysha A. Dicke[1], Kaustubh R. Mote[2], Gianluigi Veglia[1].
[1]BMBB, University of Minnesota, Minneapolis, MN, USA, [2]TIFR Center for Interdisciplinary Sciences, Narsinghi, India.
Sarcolipin (SLN), a 31 amino acid transmembrane peptide, binds to the Sarco(endo)plasmic Reticulum Ca^{2+} ATPase (SERCA) decreasing its apparent Ca^{2+} affinity. SERCA pumps Ca^{2+} from the cytoplasm of muscle cells into the sarcoplasmic reticulum (SR) using energy derived from ATP hydrolysis. This re-establishes the Ca^{2+} gradients needed for normal muscle function. Phosphorylation of SLN relieves the inhibition of SERCA and may be involved in the beta-adrenergic response. Although a significant amount is known about SERCA, and many of the conformations throughout its transport cycle have been crystallized, the mechanism of how SLN decreases SERCA's Ca^{2+} affinity and alters the coupling between ATP