Here we are employing a new approach called the String Method with swarms-of-trajectories to study transition pathways for various zwitterionic and anionic molecules across both WT and mutant OmpF porins. Results of this work will therefore assist in the design of new antibiotics that are more effective in the treatment of bacterial infections. [Supported by NIH grant GM062342].

3564-Pos
Towards a Cell-Free Assay to Investigate Lipid Bilayer Permeation and Efflux Transport of Therapeutic Agents
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Lipid bilayers and efflux transporters like P-glycoprotein (P-gp) represent the most important *in vivo* barriers for therapeutic agents. Driven by ATP hydrolysis, P-gp exports structurally diverse hydrophobic compounds from the cell reducing intestinal absorption and blood-brain barrier passage. P-gp expression has also been linked to the efflux of chemotherapeutic drugs in human cancer cells, contributing to multidrug resistance.
The aim of this project is to adapt a liposomal permeation assay to study lipid bilayer permeation and P-gp transport in parallel. This is achieved by integrating P-gp into liposomes.
Fully functional His-tagged P-gp was overexpressed in HEK293 cells and purified by immobilized metal affinity chromatography. P-gp was reconstituted into liposomes, incorporation was verified by density gradient centrifugation. 90% of the basal ATPase activity originated from P-gp as determined with an anti-Pgp antibody. Cryo-TEM images showed unilamellar vesicles with a homogenous size distribution.
In parallel, we established liposomal assays to investigate membrane partitioning and permeation with the pH-sensitive probe fluorescein, linked to the phospholipid DHPE. A minute pH-shift at the membrane surface due to weak acids or bases entering or crossing the lipid bilayer results in a characteristic change in fluorescence. Well-known P-gp substrates and non-substrates were studied. The resulting fluorescence time-curves followed mono- or biexponential functions and the rate constants of the faster terms were used to calculate the apparent permeation coefficients ($Perm_{app}$). The $Perm_{app}$ values of the compounds investigated do not correlate with their lipophilicity or other physico-chemical parameters, which are generally used to estimate membrane permeation. The data obtained strongly emphasizes the importance of developing a simplified permeation assay.
We thank the OPO Foundation Switzerland and the Electron Microscopy Center Zurich.

3565-Pos
Classification of Human Solute Carrier Superfamily Members Reveals Functional Similarities across Families
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Solute Carrier (SLC) superfamily members are membrane transporter proteins that control the uptake and efflux of solutes, including essential cellular compounds, environmental toxins and therapeutic drugs, across biological membranes. Members of the SLC superfamily can share surprisingly similar structural features despite weak sequence similarities. Identification of sequence relationships among SLC members is needed to enhance our ability to model individual transporters and to elucidate the molecular mechanisms of their substrate specificity and transport.
Here, we describe a comprehensive sequence-based classification of SLC members into families. We classify the proteins using sensitive profile-profile alignments and two classification approaches, including similarity networks. The clusters are analyzed in view of substrate specificity, transport mode, organism conservation, and tissue specificity. SLC families with similar substrates generally cluster together, despite exhibiting relatively weak sequence similarities. In contrast, some families cluster together with no apparent reason, revealing unexplored relationships. We demonstrate computationally and experimentally the functional overlap between representative members of these families. Finally, we identify 4 putative SLC transporters in the human genome.
The SLC superfamily constitutes a biomedically important family of membrane proteins that is highly diverse in sequence. The proposed classification of the superfamily, combined with experiment, reveals new relationships among the individual families and identifies new superfamily members. The classification scheme will inform future attempts directed at modeling the structures of the SLC transporters, a prerequisite for describing their substrate specificity.

3566-Pos
Model-Structure, Mutagenesis and Functional Characteristics of the Human Transporter, NHA2
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Human NHA2 is a novel member of the Cation/Proton Antiporters-2 (CPA2) family, linked to essential hypertension. Using the crystal structure of distant bacterial transporter NhaA as template, producing a model-structure of NHA2 necessitated a composite modeling approach. Through extensive mutagenesis guided by our model, we show that while NHA2 retained some functional and structural core elements of other Na+/H+ exchangers, it exhibited other significant exclusive features. A cluster of highly conserved titratable residues was located in the so-called assembly region, made of two discontinuous helices TM4 and TM11. Whereas in NhaA, oppositely charged residues have been proposed to compensate for partial dipoles generated within this assembly, we demonstrate that in NHA2 uncharged but polar residues suffice. Instead, NHA2 possesses unique, conserved charges predicted to interact with key essential residues. Combining structural data with evolutionary conservation analysis and mutagenesis, we propose a transport mechanism for NHA2, and compare it with mechanisms proposed for NhaA and NHE1. This study illustrates an attractive approach for studying new transporters, starting from structural data to guide initial experimental efforts.

3567-Pos
Functional Reconstitution of a Bicomponent ABC Transporter
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The ATP-binding-cassette (ABC) transporters are transmembrane protein nanomachineries present in all living systems. ABC transporters utilize the energy of ATP hydrolysis to transport a variety of solutes across the membrane. *Pseudomonas aeruginosa*, a Gram-negative pathogenic bacterium, employs a bi-component ABC transporter as an active efflux of polysaccharides during the biogenesis of endotoxic lipopolysaccharides. We reconstituted the ABC transporter in various systems, including microsomes, planar lipid bilayers, and transfected mammalian N2a cell lines to obtain a mechanistic understanding of the functional properties of this nanomachinery. We employed single-channel electrical recordings to show that the transmembrane domain (TMD) of the ABC transporter features pore-forming activity. Further, our biochemical characterization of purified components sheds light on the structural assembly and stoichiometry of this bi-component ABC transporter. Our long-term goal is to detect, explore and characterize the translocation of polysaccharides at single-protein complex resolution. The use of a broad range of reconstitution systems enables a comprehensive examination of the ATP-dependent transport kinetics and thermodynamics of the large-size substrates from one side of the membrane to the other. These studies might also contribute to drug design against *Pseudomonas aeruginosa.*
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3568-Pos
New Immobilized Proteoliposome-Based Biosensor System for Investigating Human ATP-Binding Cassette Transporters
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ATP-binding cassette (ABC) transporters comprise a large family of membrane proteins that transport a variety of organic substrates across cellular membranes. Human ABC transporters are mostly efflux pumps for physiological and xenobiotic substrates related to immune response and cellular detoxification. Nine human ABC transporters play a major role in cellular multi-drug resistance (MDR), thus being called MDR-related proteins (MRPs). So far, the structure of MRPs and human ABC transporters in general is unclear. Sequence analysis and experimental data indicate that functional ABC transporters are composed of two subunits and imply strong positive cooperativity between those entities. To elucidate the transport mechanism and the molecular origin of the cooperativity a solid-supported

biosensor system with defined physicochemical properties is needed. One promising approach is the immobilization of MRP-containing proteoliposomes on functionalized surfaces. We report a new system for immobilizing biotin-doped proteoliposomes via the well-known biotin-streptavidin interaction on gold surfaces functionalized with a self assembled monolayer (SAM) of a binary thiol mixture. The SAM composed of a hydroxy-terminated 16-carbon alkanethiol and its biotinylated derivative protects the gold surfaces from unspecific adsorption and allows the immobilization of defined quantities of streptavidin. Proteoliposomes made from natural lipid compositions and doped with a biotinylated anchor lipid can readily be tethered to these surfaces. By thorough biophysical characterization using quartz crystal microbalance (QCM), atomic force microscopy (AFM) and fluorescence techniques all experimental parameters were optimized for application in biosensor systems. We successfully immobilized intact proteoliposomes containing the reconstituted human ABC transporter MRP3 on the described surfaces. Our system allows the investigation of ABC transporters by a variety of surface-enhanced techniques ranging from AFM and QCM to impedance spectroscopy and surface plasmon resonance based methods under well-defined conditions closely mimicking the protein's natural environment.

3569-Pos

Functionally Rotating Mechanism of a Multidrug Transporter Studied by Coarse-Grained Simulation

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The existence of multidrug transporters accounts for the multidrug resistance of bacteria encountered in the treatment of many infectious diseases. The recently solved crystal structure of AcrB, a major multidrug exporter in Escherichia coli, suggests a functionally rotating mechanism for such an efflux system [1]. According to this mechanism, each protomer of the trimer stays in one of the three states in an asymmetric way, and exclusively binds and extrudes drugs by accessing ''Binding'' state sequentially. To testify such a hypothesis, we have performed molecular dynamics simulation of the ''porter'' domain of AcrB around native state. The system was coarse-grained by using one bead positioned on Cα atom to represent each residue, and the energy function was described by the multiple-basin model [2]. In this work we realized, for the first time, triple-basin energy landscape for each protomer, by which frequent conformational change was simulated. Using this model, we calculated the configuration distribution of the trimer in equilibrium based on various energy landscapes, and the obtained phase diagram could be used to elucidate the mechanism of protein function. Further investigation includes the consideration of an explicit ligand bound in the ''binding'' protomer by hydrophobic interaction, and the direct simulation of functional rotation and exportation of ligand, details of which will be presented on the meeting.

Key words: AcrB, multi-basin model, re-weighting

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[2] Okazaki, K. et al., (2006) Proc Natl Acad Sci U S A 103, 11844-9.

3570-Pos

All-Atom Molecular Dynamics Simulation of Bacterial Multidrug Efflux Transporters AcrB

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Multidrug efflux transporters cause for antimicrobial resistance in the chemotherapy of cancer and antibiotic treatment of numerous different bacterial infections. In E. coli, it is known that the tripartite multidrug efflux system (AcrB/ AcrA/ TolC) exists, and AcrB resides in the inner membrane region and take part in substrate recognition and energy transduction for drug export through proton transfer. Recently, x-ray structures provided that AcrB forms trimeric protein where each subunit is different conformation, ''binding state'', ''extrusion state'' and ''access state''. These results suggest that drugs are exported by a three-step structural change. In the present study, we performed a series of all-atom molecular dynamics (MD) simulations of AcrB-membrane-water system and analyzed the structural change mechanism among three subunits.

3571-Pos

Structure-Function Analysis of ABCB1 Pharmacogenomics and Alterations in P-Glycoprotein Transport

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P-glycoprotein (P-gp) is an efflux drug transporter and member of the ATP-binding cassette (ABC) superfamily, encoded by the ABCB1 (MDR1) gene. P-gp is located in tissues important in drug disposition, including intestine, liver, kidney, and blood-brain barrier. Due to its wide tissue distribution and broad substrate specificity, P-gp is important in drug disposition. P-gp is known to transport a wide variety of structurally and functionally different drugs, but the mechanism for binding and transport is poorly understood. The ABCB1 gene is polymorphic and single nucleotide polymorphisms (SNPs) are known to alter transport via mechanisms that are unclear. Our goal is to utilize biophysical and computational methods to understand structure-function relationships in ABCB1 wild-type and its variants. We have built a wild-type human P-gp homology model based on the recently published mouse crystal structure (Aller et al., Nature, 2009), and homology models for ABCB1 SNPs 1199G>A (S400N), 1199G>T (S400I), 2677G>T (A893S), 2677G>A (A893T), and 2677G>C (A893P) using SYBYL8.0 software. Our model predicts that human P-gp has 12 transmembrane helices and an overall prolate shape, ~150x60Å, with a depth of ~60Å perpendicular to, ~40Åwithin, ~30Å above, and ~80Å below the membrane. The two intracellular nucleotide-binding domains are separated by ~20Å in the nucleotide-free state and move into contact in the nucleotide-bound state. We demonstrated that polymorphisms alter the secondary structure of P-gp. We have also modeled the hypothesized ATP-switch mechanism for P-gp transport and developed a visualization of this movement. We plan to incorporate wild-type and variant P-gp into lipid-based nanodiscs to study differential substrate binding and changes in conformation using single-molecule fluorescence. We will correlate these results, and those from in vitro transport studies, with pharmacophore modeling and QSAR studies to further understand the functional significance of genetic variation in ABCB1.

3572-Pos

Functional Rotation of the Transporter AcrB: The Essentials of Peristaltic Motion and Subsequent Substrate Extrusion

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Bacteria, such as E. coli, use multidrug efflux pumps to export toxic substrates through their cell membranes, including antibiotics. The RND transporter of the AcrAB-TolC efflux pump is able to export structurally and chemically different substrates via a functional rotation. The three major states of this rotation cycle were found in several asymmetric crystal structures. After initially analyzing the basic mechanisms of opening of the TolC channel [1] and of substrate extrusion by AcrB [2] separately, we have continued the analysis of the latter one. Thereby, we have focused both on the local interactions between substrate and protein, the properties of the extrusion pathway, as well as the principal subdomain movements which lead to the peristaltic motion. Furthermore, we have investigated the possibility to pull the substrate from the final state of the previous simulations out of the exit gate to estimate whether the substrate is already free to leave the protein via diffusion, which is usually beyond the time scale of computer simulations.

[1] R. Schulz, U. Kleinekathöfer, Biophys. J. 96, 3116 (2009)
[2] R. Schulz, A. Vargiu, F. Collu, U. Kleinekathöfer, P. Ruggerone, submitted

3573-Pos

Transport Inhibitors Cause Conformational Changes in the Yeast Mitochondrial Citrate Transport Protein Reconstituted in Liposomes as Demonstrated by EPR Spectroscopy

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In order to directly observe conformational change in the mitochondrial citrate transport protein (CTP), we measured, in the presence and absence of inhibitors, the EPR spectra of spin-labeled single-Cys CTP mutants that were reconstituted in liposomes. We selected spin-label locations to report on substrate binding sites 1 and 2 (i.e., 187, 183, and 179), binding site 2 (39), TMDIII pointing away from the transport pathway (118), and a *matrix-facing* hydrophilic loop (47). In the absence of inhibitor, the EPR lineshapes show residue-dependent variations in mobility. Addition of external 1,2,3-benzenetricarboxylate (BTC), the defining inhibitor of the CTP, caused a modest, residue-dependent decrease in the mobile component and a concomitant increase in the immobile component. Addition of compound 792949, a novel, purely competitive inhibitor that we previously identified via high throughput *in silico* screening using the homology-modeled CTP in its *cytosolic-facing* conformation, yielded EPR spectra that contain a *substantial* increase in the immobile component at each location. We conclude that the two inhibitors cause CTP to assume different conformations, which vary significantly in their extent of immobilization.