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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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.

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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.