3988-Pos Board B716
Probing Drug-Binding Pathways in P-Glycoprotein with Ensemble Docking
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P-glycoprotein (P-gp) is one of the key members of the ATP-binding cassette (ABC) transporters family and the major contributor of multidrug resistance against a broad spectrum of drugs in cancer cells. Powered by ATP, P-gp acts as an efflux pump to export a diverse set of hydrophobic molecules, including drugs, out of the cell. These hydrophobic substrates are assumed to enter the translocation pathway of P-gp either via the intracellular side of the cell or directly from the cytoplasmic leaflet of the membrane. In order to characterize the mechanism, residues facilitating drug entry, and translocation, we have taken an approach combining the strengths of molecular dynamics (MD) and molecular docking. A 100 ns MD simulation of membrane-bound inward facing P-gp with Mg^{2+} and ATP was performed and subsequently used in ensemble docking. Doxorubicin, one of the drug substrates of P-gp, was docked into the defined active sites of 10,000 different snapshots representing the conformational flexibility of the protein in the membrane. Clustered docked poses identified the binding sites and yielded a picture of the sequence of binding events of the substrate within the translocation pathway. Interestingly, a few of the highly populated clusters were located within the region that interacts with the cytoplasmic leaflet of the membrane supporting the recruitment of the substrate laterally and directly from the membrane. Further MD simulations and energetic calculations of representative substrate poses are then used to characterize their real-time binding behavior and dynamics within the intracellular lumen and at the cytoplasmic leaflet of the membrane. This study, for the first time, explored and utilized the dynamic ensemble of conformations of P-gp in molecular docking and dissected important and complex features of its translocation pathway.

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Functional Assay for Characterizing Human P-Glycoprotein Transport using the Pore Forming Peptide Gramicidin A
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P-glycoprotein (P-gp), a member of the ATP-Binding Cassette (ABC) transporter family, actively transports chemotherapeutics out of cancer cells, leading to reduced efficacy of these drugs. Thus the development of functional assays for better characterization of P-gp is important in finding ways to combat multidrug resistance (MDR) in cancer treatment. We establish a novel approach that takes advantage of the single molecule sensitivity of the patch clamp technique and provides access to the intracellular environment as well as direct control of the cellular transmembrane potential. Briefly, the assay measures whole cell current in the presence of ion channel forming peptide gramicidin A (gA). Since gA is a substrate of P-gp, changes in gA current report the efflux of gA by P-gp. For instance, gA current in chemosensitive parental cells that did not express P-gp, increased with time in a dose-dependent manner. In contrast, P-gp expressing resistant cells exhibited significantly reduced gA current due to its efflux by P-gp. Consistently, P-gp inhibitors restored gA current in P-gp expressing cells to a level comparable to that in parental cells. HeLa cells transduced with non-functional mutant (E556Q/E1201Q) P-gp displayed large gA currents comparable to control HeLa cells. This can be attributed to the loss of transport activity due to defective ATP hydrolysis by this mutant P-gp. Additionally fitting the gA currents data to a model that quantifies the kinetic constants showed that P-gp efflux activity was correlated with the intracellular ATP concentration. Moreover, to our surprise, membrane depolarization enhanced P-gp activity whereas hyperpolarization reduced its activity. Collectively our findings demonstrate that whole cell gA current can be used to monitor P-gp function.

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Systems Level Study of Bacterial Multi-Drug Resistance from Efflux Machinery
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Bacterial multi-drug resistance efflux pumps are complex molecular machines that expel multiple drugs and antibiotics. They also influence important genetic and cellular processes to confer additional drug resistance. We have developed a mathematical framework to integrate genetic and cellular processes to understand how efflux pumps confer resistance. Our model is validated against multiple lines of experimental evidence, such as time-kill behavior of wild-type cells, as well as mutant strains deficient in efflux pump or genetic regulation mechanisms. Our model exhibits quantitative agreement with the experiments and predicts specific, experimentally testable properties which are important for drug efflux. Also, we describe our current efforts to integrate molecular mechanisms from a data-driven structural model of the P. aeruginosa tripartite MexAB-OprM complex that was constructed based upon the crystal structures of the individual components MexA, MexB, and OprM, energetic calculations of the membrane-bound complex, and distance constraints between components derived from crosslinking studies.

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Plant VDAC Selectivity and Voltage-Dependence are Uncoupled
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The mitochondrial respiration requires the exchange of inorganic ions and metabolites between the cytoplasm and the mitochondrial matrix. This entails the translocation of these solutes through both inner and outer mitochondrial membranes. This function is fulfilled by the voltage-dependent anion-selective channel (VDAC) in the mitochondrial outer membrane. A typical feature of VDAC is its voltage-dependence. At low voltage, typically $|V|<20$mV, it is in its open state and it switches to subconductance states upon increasing the voltage amplitude. It is generally assumed that both selectivity and voltage-dependence of VDAC are coupled: the VDAC is anion selective in the open state but cation selective in subconducance states. However, it was shown that the selectivity of the open state can spontaneously switch between anion and cation selectivity [1]. The origin of this selectivity inversion is not known. We studied the selectivity of the plant PcVDAC32 that belong to the canonical isoform of VDAC including mammalian VDAC1 and yeast VDAC1 with which it shares similar electrophysiological properties as well as secondary structure content [2]. We show that change of selectivity occurs in open and in subconductance states without being related to gating. In the open state the probability of selectivity inversion is regulated by the membrane lipid composition, the ion strength and the magnitude of the ion gradient across the membrane. Furthermore no matter the experimental condition the selectivity inversion is not correlated to a change in channel conductance. Altogether these results indicate that there is no correlation between the VDAC selectivity and its voltage-dependence.
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VDAC3 Interactomic Analysis
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VDACs (voltage-dependent anion channel), are a small family of proteins of the mitochondrial outer membrane, considered as gatekeeper of mitochondrial metabolites and thereby controlling cross-talk between mitochondria and the rest of the cell (1-2). VDAC is also a key player in mitochondria-mediated apoptosis (3). In addition, VDAC appears to be a convergence point for a variety of cell survival and cell death signals mediated by its association with various ligands and proteins. There are many reports about the involvement of VDAC in several diseases (4). Understanding what proteins are able to interact with VDACs (interactome) is critical for deciphering how this channel can perform such a variety of important functions (5).
Since identification of interacting proteins is very difficult due to complexity of cellular protein extracts, we applied TAP-tag technology (Tandem Affinity Purification with tag) to the analysis of VDAC interactomes. In particular we have investigated binding partners of human VDAC3 (human voltage dependent channel isoform 3) (6), the least known and the most intriguing among the various porin isoforms, with a stable HeLa line expressing YFP-tev-HIS-VDAC3. After cell lysis, the proteins interacting with VDAC3 were purified by two affinity chromatographies. Finally, the protein complex was analysed by proteomics.
A list of interacting proteins has thus been defined and this will be used to establish functional roles of VDAC3.
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