very different structural flexibility and fluctuations in their inward-facing states, especially with regard to the separation of the NBDs. Comparing the dynamics of NBDs, it becomes evident that ABC importers fluctuate significantly lower than ABC exporters, suggesting that the conformational ensembel obtained for the former represents a deeper energy well. In contrast, the NBDs of P-glycoprotein are able to reach near dimerized conformations even in the absence of the nucleotide due to the overall higher structural flexibility of the protein architecture. The dynamical difference between the two ABC transporters appears to present a fundamental mechanistic difference between the subfamilies they belong to, which can be described as different energy landscapes along their pathways for NBD dimerization. The difference in energy landscapes is evidenced by their transport activities in that the NBDs of the maltose transporter dimerize only in the presence of its substrate, whereas P-glycoprotein possesses a remarkable substrate-independent ATPase activity.

3350-Pos Board B211
Studying the Conformational Cycle of the Secondary Multidrug Transporter LmrP by EPR Spectroscopy
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LmrP, a Major Facilitator Superfamily (MFS) multidrug transporter from Lactococcus lactis, acts a secondary antiporter, catalysing the extrusion of a large spectrum of hydrophobic drugs by dissipating a proton gradient. This implies that it has evolved to bind and export a series of structurally diverse cytotoxic substances from within the membrane, in contrast to the majority of MFS transporters that are specialized in the transport of a single soluble substrate, captured from the aqueous medium. Its transport mechanism could thus potentially differ from the alternating access model of the lactose permease that currently stands as a paradigm for all MFS transporters. Using Electron Paramagnetic Resonance spectroscopy on a library of cysteine mutants, labeled with either one or two spin probes, we aim at mapping the conformational dynamics of LmrP during its transport cycle. We are using accessibility patterns and intramolecular distance changes to understand the conformational rearrangements involved in the energization and subsequent substrate recognition and extrusion mechanisms of the transporter. Preliminary measurements on a first set of mutants show profound structural changes upon ligand binding or protonation of key acidic residues. In particular, we observe a significant conformational rearrangement of helix VIII upon substrate binding, suggesting that the interface between the N- and C-terminal halves could be key in the controlled access of drugs to the substrate binding pocket. These findings could shed light on the mechanistical divergence with MFS transporters recognizing soluble substrates from the extracellular medium. Based on these first results, we present a blueprint of the catalytic cycle of LmrP.

3351-Pos Board B212
Structure and Dynamics of the MFS Multidrug Transporter EmrD
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Major Facilitator Superfamily (MFS) transporters harness the free energy stored in ion or solute gradients for active transport of a variety of substrates. Although most of the 58 distinct families of MFS transporters are substrate-specific, six families contain multidrug transporters (MDR). EmrD is a MFS-MDR from E.coli whose crystal structure has been determined. Similar to the lac permease, the topology consists of 12 transmembrane helices arranged in two bundles of six helices. However, EmrD has a more compact conformation that occludes access to a hydrophobic cavity/chamber from both sides of the membranes. To define the conformational motion involved in substrate transport, we are using spin labeling and electron paramagnetic resonance (EPR) spectroscopy. Spin labels were introduced at selected sites along transmembrane helices 5, 6 and 8. EPR analysis of mobilities and accessibilities reveal distinct changes in spin label dynamics and exposure to NiEDDA upon protonotation of the transporter. The sites of these changes cluster at the periplasmic side of EmrD suggesting that this end of the transporter undergoes conformational rearrangements at low pH. Double electron electron resonance analysis is in progress to determine the nature and amplitude of the protein conformational motion.

3352-Pos Board B213
Modulation of Substrate Efflux in Bacterial Small Multidrug Resistance Proteins by Mutations at the Dimer Interface
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Bacteria evade the effects of cytotoxic compounds through the efflux activity of membrane-bound transporters such as the small multidrug resistance (SMR) proteins. Consisting typically of ca. 110 residues with four transmembrane (TM) α-helices, crystallographic studies have shown that TM helices 1 through 3 of each monomer create a substrate binding 'pocket' within the membrane bilayer, while a TM4-TM4 interaction accounts for the primary dimer formation. Previous work from our lab has characterized a highly conserved small residue heptad motif in the Halobacterium salinarum transporter Hsmr as 90GLxLIxxGV98 that lies along the TM4-TM4 dimer interface of SMR proteins as required for function. Here, we present our focus on the conserved positions 91, 93, 94, and 98 where we substituted the naturally occurring Hsmr residue for Ala, Phe, Ile, Leu, Met, and Val at each position in the Hsmr TM4-TM4 interface. Large residue replacements were studied for their ability to dimerize on SDS-PAGE, to bind the cytotoxic compound ethidium bromide, and to confer resistance by efflux. Although the relative activity of mutants did not correlate to dimer strength for all mutants, all functional mutants lay within +/− 10% of dimerization relative to WT, suggesting that optimal dimer strength at TM4 is required for proper efflux. Furthermore, non-functional substitutions at the centre of the dimerization interface that do not alter dimer strength suggest a dynamic TM4-TM4 'pivot point' that responds to the efflux requirements of varying substrates. This functionally critical region represents a potential target for inhibiting the ability of bacteria to evade the effects of cytotoxic compounds.

3353-Pos Board B214
All-Atom Molecular Dynamics Simulation of Multidrug Efflux Transporter AcrB
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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 protomer is different conformation, ''binding'', ''extrusion'' and ''access'' state.
Especially, the shapes of the tunnels of drug pathways in porter domains are different. In addition, extrusion state protomer has the unique side chain conformations of residues which are essential for proton translocation, ''proton translation site'' (Asp407, Asp408 and Lys940). These results suggest that drugs are exported by a three-step structural change involved in the proton motive force which related with the conformational change in protonation sites. In the present study, to clarify a relation between the small conformational change of proton translation site and the large structural change of porter domain, we performed all-atom molecular dynamics (MD) simulations of AcrB-membrane-water system.

3354-Pos Board B215
Dynamics of the Dopamine Transporter in Various States in the Presence of an Explicit Sodium Gradient
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The monoamine transporters (MATs) are a family of neurotransmitter transporters located on the presynapse of a neuron. These monoamine transporters regulate the levels of neurotransmitters available in the synapse to bind to post-synaptic receptors. One of the MATs, the dopamine transporter (DAT) is a target of various therapeutic and illicit drugs. The mechanism and conformational states of the DAT is still widely debated. A driving force behind the transport mechanism for DAT is the sodium gradient. We present an all-atom MD study of the DAT at physiological concentrations of Na^+, K^+ and Cl^-. A dual POPE lipid bilayer system was constructed to impose an explicit sodium gradient via two water baths. Six permeations of DAT states were simulated that vary bound sodium ions and dopamine molecules. Trajectory data have been analyzed to gain insight to the role of sodium and substrates in conformational changes of the DAT.

3355-Pos Board B216
Computer Simulations of the Multi-Drug Efflux Pump AcrAB-TolC
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Preventing drug access to the target molecule(s) is one of the major strategies by which bacteria achieve antibiotics resistance. A prominent example of this mode of action is drug expulsion by an over-production of multi-drug efflux pumps like the proton drug antiporter AcrAB-TolC in Escherichia coli. Performing atomistic molecular dynamics simulations of the pump and its components in a membrane / water environment on a 50 ns - 1 μs time scale, we were