classification based on the amino acid frequency yielded an accuracy of 75% or higher. Integrating additional information improved the prediction performance to 90% and higher.

722-Pos Board B522
Indole Transport across Escherichia Coli Membranes
Catalin Chimerel, Ulrich F. Keyser, Silvia Piñero-Fernandez, David K. Summers.
Our understanding of the roles of indole in bacterial signalling has grown rapidly in recent years. The list of processes in which indole participates is long and diverse. It regulates the transition from exponential to stationary phases of growth, it is involved in the control of plasmid stability, and it regulates biofilm formation, virulence and stress responses (including the development of antibiotic resistance). Its involvement is not restricted to bacterial signalling but has recently been shown to include mutually beneficial signalling between enteric bacteria and their mammalian hosts. In many respects indole behaves like the signalling component of a quorum sensing system.
Indole synthesised within the producer bacterium is exported into the surroundings where its accumulation is detected by sensitive cells. The established view in the literature is that export and import are protein-mediated, with the AcrEF-TolC and Mtr transporters implicated in these roles for the indole-positive *E. coli*. However these assumptions are not based upon direct evidence. We have combined *in vivo* and *in vitro* approaches to re-examine the mechanism of indole transport. We conclude that the movement of indole across the *E. coli* membrane is entirely independent of AcrEF-TolC and Mtr.
To monitor the transport of indole through the lipid membrane we developed a deep UV-fluorescence microscope which is capable of imaging the intrinsic fluorescence of indole molecules. In parallel we used electrophysiology to monitor the transport of indole through black lipid membranes. Our measurements provide an unambiguous prove that indole can indeed pass through intact lipid membranes (1,2-Diphytanoyl-sn-Glycero-3-Phosphatidylcholine and E. coli ploar lipid extract). These observations enhance our understanding of indole signalling in bacteria and, perhaps more importantly, provide a simple explanation for the ability of indole to signal between biological Kingdoms.

723-Pos Board B523
FRET Analysis of the Dimer Conformation of the Bacterial Translocon SecYEG
Lukas Winter, Christine Siligan, Andreas Horner, Peter Pohl.
A decisive step in the biosynthesis of many proteins is their partial or complete membrane translocation. It is mediated by a highly conserved protein conducting channel, which is called SecYEG in bacteria. On the basis of its structure, it was proposed that the translocase consists of just one SecYEG complex. If assembly into a dimer occurs, one of the copies remains inactive. According to a contrasting hypothesis, the actual translocase offers a much wider diameter for protein transport than can be attained by a single copy. This is achieved by assembly into dimers with the lateral gates facing each other (front to front configuration). Here we show by the use of Förster resonance energy transfer (FRET) that the purified and reconstituted SecYEG complex forms dimers, whereas in detergent the SecYEG complex remains monomeric. The FRET efficiencies measured for labels introduced at various SecYEG positions indicate that the front to front configuration is highly unlikely.

724-Pos Board B524
Molecular Transport Across Porins: Revealing Fast Kinetics
Mahendran Kozhinjampara, Pratik Raj, Singh Ulrich Kleinekathöfer, Helge Weingart, Mathias Winterhalter.
The permeation of water soluble molecules across cell membranes is controlled by channel forming proteins and particularly the channel surface determines the selectivity. An adequate method to study properties of these channels is electrophysiology and in particular analysing the ion current fluctuation in the presence of permeating solutes provides information on possible interactions with the channel surface. As the binding of antibiotic molecules in the channels of interest is significantly weaker than that of preferentially diffusing nutrients in substrate-specific pores, the resolution of conductance measurements has to be significantly increased to be able to resolve the events in all cases. Due to the limited time resolution, fast permeation events are not visible. Here we demonstrate that miniaturization of the lipid bilayer; varying the temperature or changing the solvent may enhance the resolution. Although electrophysiology is considered as a single molecule technique, it does not provide atomic resolution. Molecular details of solute permeation can be revealed by combining electrophysiology and all atom computer modeling.

725-Pos Board B525
Both Lobes of Maltose Binding Protein are Engaged in Stabilization of the Semi-Open State of the Malk Dimer in the Maltose ABC Transporter
Frances Joan, **D. Alvarez**, Cedric Orelle, Candice S. Klug, Amy L. Davidson.
ATP-binding cassette (ABC) transporters involved in uptake in bacteria are capable of accumulating substrates against a concentration gradient at the expense of ATP. In most systems, high-affinity binding protein located in the periplasmic space or outside the cell scavenges solutes for delivery to and through the transmembrane components. One such system is the maltose transporter in *Escherichia coli*, which is composed of a periplasmic maltose binding protein (MBP), two transmembrane domains, MalF and MalG, and a nucleotide-binding homodimer, $MalK_2$. Interaction of the ligand-bound MBP with the $MalFGK_2$ transporter in the presence of MgATP triggers conformational changes that result in reorientation of transmembrane helices to receive maltose from MBP and closure of the nucleotide-binding interface in $MalK_2$ to hydrolyze ATP. Following ATP hydrolysis, the MalK dimer opens and transmembrane helices reorient, bringing maltose into the cell. Recently, evidence of a semi-open state of $MalK_2$ was found when the transporter was allowed to hydrolyze ATP in the presence of MBP. We have used site-directed spin labeling and electron paramagnetic resonance spectroscopy to further characterize this ''post-hydrolysis'' state. By placing EPR probes in different locations along the interface where MBP is known to interact with MalFGK2, we found that whereas only the N-lobe is bound to the transporter in the resting state conformation, both the N- and C-terminal lobes of MBP are engaged with the transporter under conditions where the semi-open state of $MalK_2$ is stabilized. Interpreted in light of how MBP may stimulate the ATPase activity of the transporter, it appears that both lobes of MBP are involved in dictating the conformation of the MalK dimer on the opposite site of the membrane.

726-Pos Board B526
A Structure-Function Approach to Study ABCB1 (mdr1/P-Glycoprotein) Pharmacogenomics in Drug Disposition
Alana A. White, J.B. Alexander Ross, Erica L. Woodahl.
P-glycoprotein (P-gp) is an efflux drug transporter located in tissues important in drug disposition, including the intestine, liver, kidney, and blood-brain barrier. P-gp transports a wide variety of structurally and functionally different drugs, but the mechanisms for binding and transport are poorly understood. The ABCB1 gene, which encodes P-gp, is highly polymorphic and ABCB1 genetic variation alters P-gp-mediated transport; however, the specific mechanisms are also unclear. Our goal is to characterize the structure-function relationships in ABCB1 pharmacogenomics using biophysical and computational methods. We constructed a wild-type human P-gp homology model based on the mouse crystal structure as a scaffold to study changes in secondary structure due to ABCB1 single nucleotide polymorphisms (SNPs). Our research has focused on nonsynonymous ABCB1 SNPs at nucleotide positions 1199 and 2677 (amino acids 400 and 893, respectively): 1199G>A (S400N), 1199G>T (S400I), 2677G>T (A893S), 2677G>A (A893T), and 2677G>C (A893P). Our variant homology models predict that these ABCB1 SNPs all cause large perturbations in the local secondary structure of P-gp, which may alter P-gp function. To measure alterations in P-gp function, our future goals are to incorporate wild-type and variant P-gp into phospholipid bilayer nanodiscs to study differential substrate binding and changes in conformation using single-molecule fluorescence. Towards this end, we are expressing P-gp in baculovirus-infected Hi5 insect cells, employing a purification scheme involving a thrombin-FLAG tag attached to the C-terminus to produce high-yield high-purity P-gp. We will correlate these results, and those from in vitro transport studies, with homology modeling to further refine our predictions of the functional significance of ABCB1 genetic variation. Data gained from these studies will provide mechanisms for alterations in P-gp function due to ABCB1 genetic variation, and aid our understanding of interindividual variability in drug disposition of P-gp substrates.

727-Pos Board B527
Transport of Small Molecules across the Bacterial Translocation Channel SecYEG
Denis Knyazev, Alexander Lents, Lukas Winter, Nicole Ollinger, Christine Siligan, Peter Pohl.
Many proteins are translocated across the endoplasmic reticulum (ER) membrane or the bacterial plasma membrane through a conserved channel, formed by a heterotrimeric protein complex (called the Sec61p complex in eukaryotes and the SecYEG complex in bacteria and archaea). Cotranslational or posttranslational translocation requires ribosome or SecA binding, respectively. The resting