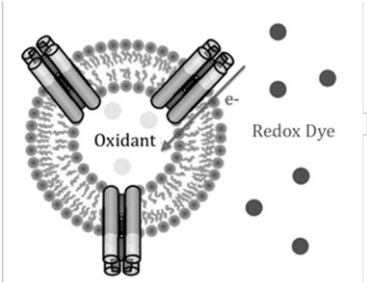
Rather than focusing on the structural details of a specific natural protein, we are designing general protein structural scaffolds (''maquettes'') to accommodate a variety of functions. Here we will present transmembrane electron transfer via AP6, an amphiphilic tetra-helical maquette that binds up to 6 hemes. We demonstrate that AP6 self-assembles with phospholipids into vesicles. Our stop flow experiments confirm that the AP6 maquette significantly increases the electron transfer rates between oxidizing interior and an external redox mediator dye, as shown below.



711-Pos Board B511
Expression and Characterization of Cytochrome C6 from Chlamydomonas Reinhardtii using a Designer Gene
Nicole L. Vanderbush, Brian St. Clair, Marylyn Davis, Dan Davis.
Cytochrome c6 is a lumenal redox carrier in oxygenic photosynthesis. We have constructed a synthetic gene, expressed, purified, and coducted an initial characterization for the cytochrome c6 from Chlamydomonas reinhardtii. The synthetic gene was constructed by the removal introns and the substitution of codons for those best suited for expression in E. coli. The gene was incorporated into a pUCF2 plasmid in place of cytochrome f, downstream of the lac operon and a pelA leader sequence. The protein is expressed by a cotransformation in E. coli with the plasmid containing the c6 gene and the PEC86 plasmid, which contains a set of genes for the covalent attachment of the heme to the protein. The spectral characteristics of the protein were determined using a UV-Vis spectrophotometer and include a reduced α peak at 553nm, β peak at 523nm, and a Soret band at 417nm. The midpoint potential at pH 7 was determined by redox titrations and found to be 365 $\pm$ 5mV. Differential scanning calorimeter experiments also reveal that the folding of the wild-type protein is irreversible and that the Tm for the protein is 78°C. Two mutants of the protein , K29I and K57I, were constructed using site-directed mutagenesis. The redox potential of the K57I mutant was found to be 20mV lower than the wild-type protein. The mutants both fold irreversibly like the wild-type but their Tm's are lower at 70°C for the K29I mutant and 71°C for the K57I mutant.

712-Pos Board B512
Tuning the Intramolecular Electron Transfer in 2[4Fe-4S] Ferredoxin: A Molecular Dynamics Study
Ming-Liang Tan, Yan Luo, Toshiko Ichiye.
The 2[4Fe-4S] ferredoxins are found as a subunit of Photosystem I and of the respiratory complex I ''wire'' and as water-soluble proteins in bacteria. They are generally small (6 kDa) pseudosymmetric proteins containing two [4Fe-4S] clusters. The effects of protein and solvent on the intramolecular electron transfer direction and rate are studied. Interestingly, while the charged side chains overwhelmingly favor the reactant state and the rest of the polar groups of the protein only slightly favor the product state, the solvent and counterions overwhelmingly favor the product so that the net driving force slightly favors the product, in agreement with experiment.

713-Pos Board B513
Electrical Transport along Bacterial Nanowires
Tom Yuzvinsky, Moh El-Naggar, Greg Wanger, Kar Man Leung, Gordon Southam, Jun Yang, Woon Ming Lau, Kenneth Nealon, Yuri Gorby.
Bacterial nanowires are extracellular appendages that have been suggested as pathways for electron transport in phylogenetically diverse microorganisms, including dissimilatory metal-reducing bacteria, photosynthetic cyanobacteria, and thermophilic fermentative bacteria. The presence of bacterial nanowires in organisms across the metabolic spectrum challenges our understanding of extracellular electron transfer in microbial communities and has significant biotechnological implications for renewable energy recovery in microbial fuel cells. To date, several biological assays have demonstrated results consistent with electron transport along bacterial nanowires, but our direct knowledge of nanowire conductivity has been limited to local scanning probe measurements across the width of nanowires. We will present electron transport measurements along the length of individually addressed bacterial nanowires derived from electron-acceptor limited chemostat cultures of the dissimilatory metal reducing bacterium Shewanella oneidensis MR-1. We will also discuss the results of transport measurements on intact biofilms and the contribution of nanowires to their overall conductivity.

Membrane Transport

714-Pos Board B514
Computer Simulation of TolC Ground State Dynamics and Spontaneous Binding of the AcrB Docking Domain
Martin Raunest, Nadine Fischer, Christian Kandt.
In Escherichia coli the AcrAB-TolC efflux pump expels a broad range of drugs and other molecules. While AcrB is the engine in this system, the outer membrane protein TolC functions as an efflux duct interacting with numerous inner membrane translocases. TolC occurs in at least two states, one that is impermeable for drugs and one where drug passage is possible. We performed a series of five independent, unbiased 150ns molecular dynamics (MD) simulations of wildtype TolC 1EK9 embedded in a phospholipid/water environment at 0.15M NaCl concentration. One of these runs was extended to 300ns in three independent copies. Whereas TolC remains closed between a 1st bottleneck region outlined by Asp-374 &371, we observe opening and closing motions in a 2nd bottleneck region near Gly-365. While previous studies reported a frequent binding of potassium ions stabilizing a closed TolC conformation in the bottleneck II region, we observe a frequent passage of sodium ions. However, in one simulation a consecutive binding of two sodium ions occurs between Gly-365 and Asp-374 leading to a closed TolC conformation at the AcrB interface, which was stable for more than 175ns. To gain insight into TolC-AcrB interaction, we performed five independent unbiased 150ns MD simulations of TolC and the AcrB docking domain (AcrB_dd). Initially placed 1 nm away from TolC and identically oriented as in the AcrAB-TolC Symmons docking structure, AcrB_dd spontaneously docks to TolC in one run. Extending this simulation to one micro second, we find an TolC-AcrB_dd docking interface characterized by a larger contact area and a slight asymmetry not present in the Symmons model. At the same time TolC opens in the bottleneck II region. All simulations were performed using GROMACS 4.0.3 and the G53a6-GROMOS96 force field.

715-Pos Board B515
Computational and Experimental Studies of Substrate Binding, Conformational Change and Importance of the Trimeric State in the Glycine Betaine Transporter BetP
Kamil Khafizov, Camilo Perez, Ching-Ju Tsai, Christine Ziegler, Lucy R. Forrest.
The glycine betaine/sodium symporter BetP responds to changes in external osmolality by regulation of its transport activity. A recent X-ray structure of BetP confirms that it is a homotrimer and in this structure each protomer adopts an identical conformation, in which the pathway is occluded from both sides. Despite the availability of a wealth of experimental data for BetP, the structures of the alternate states (e.g., open to the outside of the cell), molecular mechanisms of substrate and Na+ binding and transport, as well as the functional implications of the trimeric state remain poorly understood. To address these questions, we carried out computational studies using a range of techniques to derive hypotheses that were then tested experimentally. First, to identify structural features of the alternate states, we developed a procedure for flexible fitting of the X-ray structure of BetP into a lower-resolution cryo-EM map of BetP in a more native lipid environment, in which the three protomers have different conformations. These results suggest that: (i) the protomers adopt distinct conformational states relevant to the transport cycle; and (ii) there is conformational coupling between the protomers. Second, we performed all-atom molecular dynamics simulations and in silico alanine scanning of BetP trimers in order to identify interface residues crucial for maintaining the trimeric state. Mutations of these residues to alanine were introduced experimentally revealing that the isolated monomers are functional, and that the trimeric state is important for the regulation and higher activity of the protein. Finally, using molecular modeling and biochemical experiments we identified two Na+ binding sites in BetP that could not be resolved in the 3.35 Å resolution X-ray structure.

716-Pos Board B516
Mathematical Model of the Regulatory Cell Volume Decrease
Aleksandr V. Ilyaskin, Galina Baturina, Evgeniy I. Solenov, Aleksandr Ershov, Dmitriy Medvedev, Denis Karpov.
Renal collecting duct principal cells perform vasopressin regulated water reabsorption and form the composition of tubular fluid. The osmotic pressure of the extracellular fluid varies significantly. To maintain viability in hypotonic

conditions cells have mechanisms for regulatory volume decrease (RVD). Despite its importance, very little is known about the volume regulation of renal collecting duct cells.

The purpose of this study was to investigate the time course of cell volume changes in response to hypotonic shock and to create a mathematical model of the renal epithelial cells' reaction to the change of extracellular osmolarity on the basis of the experimental data. The change of extracellular osmolarity in experiments leads to the rapid cell swelling which is followed by regulatory volume decrease (RVD) and recovery of the initial level of cell volume. After the second consequent hypotonic shock the cell behavior is close to the behavior of ideal osmometer.

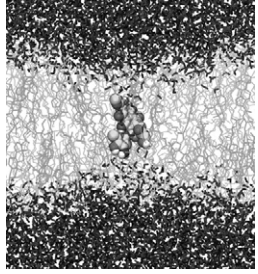
On the basis of foregoing experimental data the mathematical model of cell reaction to the hypotonic shock was created. This model calculates the time dependence of cell volume, transmembrane potential and intracellular amount of osmolytes such as Na^+, K^+, Cl^- and hypothetical organic anions. The quantitative estimation of the intracellular osmolyte loss during the RVD was made along with the calculation of the contribution of different membrane transport systems (channels, cotransporters, Na/K-pump) to this process. Analysis of the model revealed essential features of RVD in collecting duct cells: 1) the increase of membrane K^+ and Cl^- permeability; 2) the activation of KCl-cotransporters; 3) the organic anions efflux; 4) the decrease of membrane osmotic water permeability. The simultaneous activation of all these mechanisms allows the cell to decrease the volume rapidly and saves it from the excessive swelling when the hypotonic shock is repeated.

717-Pos Board B517

Structural Effects and Translocation of Doxorubicin in a DPPC/Chol Membrane: The Role of Cholesterol

Tyrone Yacoub, Allam Reddy, Juan de Pablo, Igal Szleifer.

We use molecular dynamics simulations to characterize the influence of cholesterol (Chol) on the interaction between the anti-cancer drug doxorubicin (DOX) and a dipalmitoyl phosphatidylcholine (DPPC)/Chol lipid bilayer. We calculate the potential of mean force, which gives us an estimate of the free energy barrier for DOX translocation across the membrane. We find free energy barriers of approximately 24kT, 25kT and 31kT for systems composed of 0%, 15% and 30% Chol, respectively. This nonlinear relationship between Chol concentration and the free energy barrier is due to the variable effect of Chol on DPPC acyl chain order. Chain order decreases from 0%-15% Chol and increases from 15%-30% Chol. Our predictions agree with Arrhenius activation energies from experiments using phospholipid membranes, including 20kT for 0% Chol and 29kT for 20% Chol. As Chol concentration increases, the physical barrier changes from the release of DOX into the water, to the movement of DOX over the membrane center. The drug profoundly affects membrane structure by attracting DPPC head groups, curving the membrane and allowing water penetration. Despite its hydrophobicity, DOX facilitates water transport via its polar groups.



718-Pos Board B518

Dissecting the Pathway for Colicin Ia Translocation across the E. coli Outer Membrane

Karen S. Jakes, Alan Finkelstein.

The bacterial protein toxins called colicins all share the common challenges both of binding to and crossing the *E. coli* outer membrane, regardless of their ultimate target in susceptible cells. All colicins have co-opted, as their primary binding receptors, one of a family of outer membrane proteins normally involved in the uptake of essential nutrients, such as siderophore-bound iron or cobalamin. Many colicins then use a porin, such as OmpF, as their translocator across the outer membrane. For another family of colicins, no translocator had ever been genetically or functionally identified. We recently showed that one of those colicins, colicin Ia, uses a second copy of its receptor, Cir, in an entirely different way_as its translocator. Here, we begin to dissect the steps by which the translocation domain of the colicin traverses the outer membrane through the Cir protein. Genetically attaching a folded protein at the C-terminus of the isolated T domain yields a protein that protects sensitive cells from killing by colicin Ia significantly more efficiently than does the purified T domain alone. Therefore, the chimeric T domain protein appears to stop or slow down translocation better than T domain. The effects of the interaction of T domain with the periplasmic protein, TonB, will be reported.

719-Pos Board B519

Kinetics of Precursor Interactions with the Bacterial Tat Translocase Detected by Real-Time FRET

Neal Whitaker, Siegfried M. Musser.

The Escherichia coli twin-arginine translocation (Tat) pathway transports fully folded and assembled proteins across the inner membrane into the periplasmic space. Traditionally, protein translocation studies have been performed using gel based, in vitro transport assays. This technique suffers from low time resolution and the inability to distinguish between different steps in translocation without a mechanism to trap intermediates. To address this, we have developed an in vitro FRET based transport assay that reports on an early step in the translocation process in real-time. The natural Tat substrate SufI was labeled with Alexa532 (donor) and the fluorescent protein mCherry (acceptor) was fused to the C-terminus of TatB or TatC. The colored Tat proteins were easily visible during purification, enabling identification of a highly active inverted membrane vesicle fraction yielding transport rates with NADH almost an order of magnitude faster than we previously reported (Bageshwar & Musser, 2007 JCB 179:87). When SufI is bound to the translocon, FRET is observed for both Tat proteins. The FRET signal is rapidly lost upon addition of nonfluorescent SufI, indicating that the initial binding step is reversible. When the membranes are energized with NADH, the FRET signal is rapidly lost after a short delay. These data suggest a model in which Tat cargos initially associate with the TatBC complex, and then quickly leave their initial binding site, e.g., by migrating to a TatA pore or across the membrane.

720-Pos Board B520

Amphotericin B Channel-Forming Activity Depends on Membrane Dipole Potential

Olga Ostroumova, Ludmila Schagina.

Amphotericin B (AmB) is the most effective polyene antibiotic used in treatment of systemic fungal infections. The activity of AmB results from its interaction with sterol-containing membranes and consequent pore formation. As some sterols influence membrane dipole potential (V_d) one can assume that V_d may regulate the ability of AmB to form ion channels. The aim of the present work was to study the channel-forming activity of AmB as well as the properties of its single pores in planar lipid bilayers of different dipole potentials. AmB (0.1-0.2 μM) was added on both sides of a membrane prepared from diphytanoylphosphocholine (67 mol %) and cholesterol (33 mol %) in 2 M KCl (5 mM Hepes, pH 7.0) solutions. A reduction of V_d by addition of 20 μM phloretin to the bilayer bathing solutions is accompanied by ~3-fold decrease of a single channel conductance and ~20-fold increase of a steady-state AmB-induced membrane conductance. Increasing the membrane dipole potential by adding 5 μM RH421 produces ~2-fold increase of the conductance of AmB channels and practically does not affect the steady-state bilayer conductance. The observed changes in the channel-forming activity of AmB in the presence of dipole modifying agents correspond to ~60-fold increase and ~2 fold decreases in steady-state open channel number in the presence of phloretin and RH421, respectively. The obtained results allow us to speculate that AmB molecule possess a certain dipole moment. Therefore, the decrease of membrane dipole potential produces a reduction of the energy barrier for interfacial accumulation of AmB dipoles. These results may be instructive in understanding of the membrane activity of amphotericin B. The study was supported in part by RFBR (# 09-04-00883), SS-3796.2010.4, the Program ''Molecular and Cell Biology'', RAS, and the State contract # P1372 (MES, FTP''SSEPIR'').

721-Pos Board B521

Classifying Substrate Specificities of Membrane Transporters from Arabidopsis Thaliana

Nadine S. Schaadt, Jan Christoph, **Volkhard Helms**.

Membrane transporters catalyze the active transport of molecules across biological barriers such as lipid bilayer membranes. Currently, the experimental annotation of which proteins transport which substrates is far from complete and will likely remain so for much longer. Therefore, it is highly desirable to develop computational methods that may aid in the substrate annotation of putative membrane transport proteins. Here, we measured the similarity of membrane transporters from Arabidopsis thaliana by their amino acid composition, higher sequence order information, amino acid characteristics, or sequence conservation. We considered the substrate classes amino acids, oligopeptides, phosphates, and hexoses. Substrate