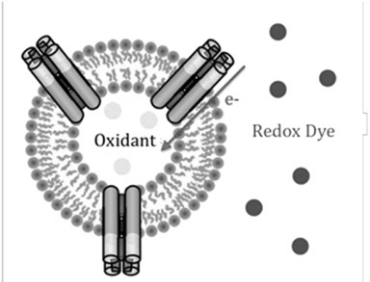
Rather than focusing on the structural details of a specific natural protein, we are designing general protein structural scaffolds (“maquettes”) to accommodate a variety of functions. Here we will present transmembrane electron transfer via AP6, an amphiphilic tetra-helical maquette that binds up to 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