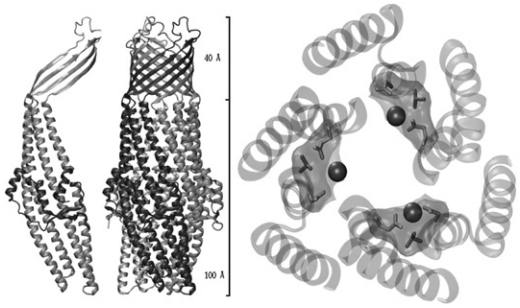
1387-Pos Board B231
Simulating Efflux Pumps: Opening of the Exit Duct TolC
Ulrich Kleinekathöfer, Robert Schulz.
Jacobs University Bremen, Bremen, Germany.
Bacteria, such as E. coli, use multidrug efflux pumps to export toxic substrates through their cell membranes. Upon formation of the AcrAB-TolC efflux pump, the aperture of its outer membrane protein TolC opens and thereby enables the extrusion of substrate molecules. Within this study, TolC was investigated using all-atom molecular dynamics simulations in a system of approx. 200 000 atoms. The stability of the periplasmic aperture was examined by applying double point mutations at the constricting ring which remove some salt bridges and hydrogen bonds. These mutants, which showed partial opening in experiments, did not spontaneously open during a 20 ns equilibration at physiological values of the KCl solution. Detailed analysis of the constricting ring revealed that the cations of the solvent were able to constitute ionic bonds in place of the removed salt bridges, which inhibited the opening of the aperture. To remove the ions from their binding sites, an external force in the form of an electric field was applied. Depending on the direction of the field, the ions were removed from their binding sites and the mutated TolC partially opened in the simulations.



1388-Pos Board B232
The Structure and Dynamics of EmrE in Liposomes
Sepan T. Amadi, Hanane A. Koteiche, Sanjay Mishra, Hassane S. Mchaourab.
Vanderbilt University, Nashville, TN, USA.
Site-directed spin labeling and electron paramagnetic resonance were used to investigate the structure and conformational dynamics of the small multidrug resistance transporter EmrE from *Escherichia coli*. The data set consists of the mobilities, solvent accessibilities of 110 spin labeled residues as well as selected pairwise distances. These parameters were interpreted as constraints on the local steric environment, the orientation of helices in the lipid phase and packing of the transmembrane helices in each monomer as well as across the dimer interface. The data collectively suggest that in liposomes, ligand-free EmrE average structure is not compatible with the crystal structure and an EM-based model. Spin label pairs at the dimer interface located at opposite sides of helix 3 reveal dipolar interaction between the spins separated by less than 15Å which contradicts their inverted relative orientation in the crystal structure. Furthermore, distances between spin labels along helix 4 are not consistent with its tight packing at the dimer interface. The spectroscopic data is supported by an expanded topological analysis of EmrE-GFP chimeras which reveals that the previous interpretation of Rapp et al is not unequivocal. Binding of tetraphenyl phosphonium (TPP^+) to EmrE increases the structural order as manifested by a narrowing of spin label distance distributions but does not lead to major conformational rearrangements. The EPR-based constraints are being used to generate a model of EmrE in liposomes.

1389-Pos Board B233
Coupling of Ca2+ and Substrate Binding in the Outer Membrane Transporter BtuB
James C. Gumbart[1], Michael C. Wiener[2], Emad Tajkhorshid[1].
[1]University of Illinois, Urbana-Champaign, Urbana, IL, USA, [2]University of Virginia, Charlottesville, Charlottesville, VA, USA.
In Gram-negative bacteria, TonB-dependent transporters (TBDTs) in the outer membrane bind large, scarce organometallic substrates with high affinity preceding active transport. Unique among TBDTs, the cobalamin (e.g. vitamin B12) transporter BtuB requires the additional binding of two Ca^{2+} ions to its extracellular loops before high affinity substrate binding can occur. Using the wealth of crystallographic data available for BtuB, we have carried out extensive (over 200 ns) equilibrium molecular dynamics simulations of multiple functional states of BtuB to address the role of Ca^{2+} in substrate recruitment. Simulation of the apo structure of BtuB with Ca^{2+} ions present in solution demonstrated the fast, spontaneous recruitment of Ca^{2+} by BtuB. Once bound, we find that Ca^{2+} both stabilizes and repositions key loops, in order to optimize their interactions with the substrate. Interestingly, replacement by Mg^{2+} abolishes this effect, in accordance with experiments, due to its inability to stabilize the Ca^{2+}-binding loops. We have also performed the first simulation of the substrate-bound form of BtuB using CHARMM forcefield parameters we recently developed for cyanocobalamin. In this simulation, we observe interactions between the substrate and two loops not seen in the crystal structures; however, these two loops have been reported to be important for substrate binding and transport. Based on our results, we suggest that the large size of cobalamin compared to other TBDT substrates explains the requirement of Ca^{2+} binding for high affinity substrate recruitment in BtuB but not other TBDTs. Research supported by NIH Grant 2R01-GM079800-06A2.

1390-Pos Board B234
Three Dimensional Imaging of Clathrin Coat Dynamics in Living Cells and Tissues
Comert Kural, Tomas Kirchhausen.
Immune Disease Institute, Harvard Medical School, Boston, MA, USA.
We generated a tracking algorithm that enables us to map the three dimensional trajectories of fluorescent objects imaged by spinning disc confocal microscopy. We have then used it to follow the formation of clathrin coated structures within the cytoplasm of cells in cultures and within the cells in live Drosophila tissues. In the first step of the analysis, the algorithm extracts the X, Y coordinates of the fluorescent spots from the projected image of a rapidly acquired 3D stack by the use of the Fluorescence Imaging with One Nanometer Accuracy (FIONA) technique. Next, a region of interest (centered at the positions determined by FIONA analysis) is defined and extended to all of the focal planes so as to obtain the fluorescence intensity distribution of each spot along the Z axis. Finally, a single Gaussian curve, fitted to the intensities calculated from the regions of interest, is used to determine the axial position (as described by Watanabe and Higuchi, Biophysical Journal, 2007). Using this tracking scheme we are able to extract for the first time the temporal and spatial behavior of AP-3 containing clathrin structures as they traffic between endosomes and lysosomes of BSC1 monkey cells. We have also managed to dissect the behavior of clathrin coated structures as they form at the plasma membrane and in endosomes of Drosophila embryo and third instar larvae imaginal disc epithelial cells.

1391-Pos Board B235
Epithelial Cell Culturing and Water Transport Measurements in Microfluidic Biochips
Viktoriia Kolotovska[1], Thomas Frühwirth[1], Peter Pohl[2], Alois Sonnleitner[1].
[1]Upper Austrian Research GmbH (UAR), Linz, Austria, [2]Johannes Kepler University, Linz, Austria.
The formation of a dense monolayer of cells is considered a prerequisite for development of tight junctions and cell polarization. For this cells are routinely cultivated on porous membranes to provide sufficient delivery of nutrients to the bottom membrane. Aquaporin-5 (AQP5) a water transporter mediating the rapid exchange of water across the plasma membrane, is found to be polarized when expressed in Madin Darby Canine Kidney (MDCK) cells.
Two types of microfluidic biochips have been developed and characterized for cell growth and compatibility with microscopy. The biochips either incorporate a thin and porous PDMS membrane or commercially available porous polymer membranes (3μm pore size). The biochips consist of a chamber for cell cultivation and channels for a precise control of delivered media at the basal and apical membrane.
The biochips were applied to combine epithelial cell culture under physiological conditions with measurements of water transport in cell monolayers with single cell resolution.

1392-Pos Board B236
Mitochondrial Transport (Carrier) Proteins. Homodimers and Heterodimers
Hartmut Wohlrab[1,2].
[1]Boston Biomedical Research Institute, Watertown, MA, USA, [2]Harvard Medical School, Boston, MA, USA.
Mitochondria are essential for the eukaryotic cell. A primary reason is metabolic reactions taking place within the mitochondrial matrix. These reactions require metabolites from the cytosol and generate metabolites essential for cytosolic reactions. The flux of such metabolites across the mitochondrial membrane can be deleterious to the electrochemical proton gradient with a dramatically negative impact on oxidative phosphorylation. Transport (carrier) proteins in the inner mitochondrial membrane tightly regulate the