1387-Pos Board B231
Simulating Efflux Pumps: Opening of the Exit Duct TolC
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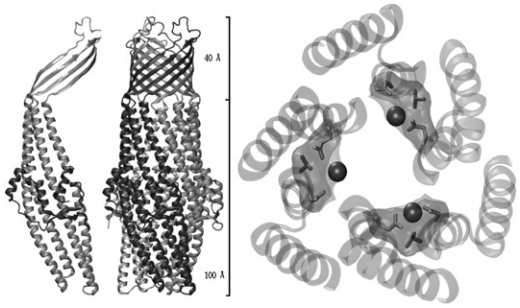
Bacteria, such as E. coli, use multidrug efflux pumps to export toxic substrates through their cell membranes. Upon formation of the AcrAB-TolC efflux pump, the aperture of its outer membrane protein TolC opens and thereby enables the extrusion of substrate molecules. Within this study, TolC was investigated 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.



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The Structure and Dynamics of EmrE in Liposomes
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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
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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
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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
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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
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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

transmembrane flux/exchange of the metabolites. These transporters may possess novel and as yet unidentified characteristics that are in proportion to their critical role in cell metabolism. Evidence has been presented that they function as homodimers *(1)* even though each subunit appears to posses its own metabolite transport path *(2)*. We have expressed some of these transporter subunits as bacterial inclusion bodies. Homodimers but not complexes with more than two subunits can be formed and such dimers are able to catalyze metabolite transport or exchange when incorporated into liposomes. We find now that dimers can also be formed between subunits of different transporters (carriers), i.e. between phosphate and oxaloacetate or between phosphate and dicarboxylate. These experiments suggest that the subunit interface of different transporters must be very much alike. Heterodimers have never been purified from or identified in mitochondria. The homodimers are formed during insertion into the mitochondrial membrane *(3)*. These membrane insertion steps are very important since a heterodimer of different transporter subunits can lead to an inappropriate exchange of metabolites that can be deleterious to oxidative phosphorylation and to the link between metabolic reactions of the matrix and the cytosol.

(1) J. Biol. Chem. (1998) 273 14269.
(2) Nature (2003) 426 39.
(3) Science (2003) 299 1747.

1393-Pos Board B237

Role of Zinc Transporter ZnT5 In PKC Signaling And Cardiac Cell Survival

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Zinc transporter ZnT5 was determined to protect from intracellular zinc overload. We asked whether ZnT5 participates in intracellular signaling and regulation of cell function. Using adult rat cardiomyocytes and cultured atrial cells, HL-1, we investigated interaction of ZnT proteins with protein kinase C (PKC). ZnT5 was found to co-precipitate with PKC isoforms δ and ϵ. Complex formation was zinc-dependent: zinc-depletion with the specific chelator TPEN promoted interaction. PKC activators increased ZnT5 association with PKC ϵ and dissociated PKC δ from the complex. To determine functional significance we assessed translocation of PKC isoforms in HL-1 cells. The cells were transfected with ZnT5cDNA or the mutated dominant negative (DN) form. When ZnT5 expression was modified, localization of both PKC isoforms was altered, particularly pronounced with DN ZnT expression that resulted in disappearance of PKCδ from the Golgi complex. ZnT5 was found to localize in the Golgi in proliferating cultured HL-1 cells but not in adult cardiomyocytes where sarcomeric pattern was observed. Overexpression of ZnT5 enhanced proliferation of HL-1 cells. High demand for ZnT5 in the cells with high rate of proliferation was confirmed in the developing embryos and embryonic bodies. ZnT5 was prevalent in the areas of highly proliferating cells belonging to inner cell mass and not the differentiated ones surrounding basement membrane stained for laminin. Biochemical experiments confirmed that highly proliferating cells in embryonic bodies at stage D2 have higher expression of ZnT5 but not α-actin as compared to D7 stage of development. The expression of α-fetal protein (α-FP) at day 7 indicates differentiation. The data suggest that in addition of controlling zinc homeostasis, ZnT5 zinc transporter plays an important role in signaling. Both functions are likely required for cell survival, proliferation and therefore cardioprotection and embryonic development.

1394-Pos Board B238

Mrp4 Is A Transmembrane Export Pump Acting As An Endogenous Regulator Of Cyclic- Nucleotides Dependent Pathways

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Cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) are second messengers which regulate many biological processes. They can be eliminated by active efflux transporters, namely the multidrug resistance proteins MRP4 and MRP5. To delineate the role of MRP4/5, we studied arterial smooth muscle cells in which the role of cyclic nucleotide levels on proliferation has been well-established.

Methods: Human SMCs were isolated from the coronary artery media from patients. Small interfering RNAs (siRNA) specific for MRP4 mRNA were designed and validated. Adenovirus encoding MRP4 short hairpin RNA (Ad-shMRP4) were used for *in vivo* studies.

Results: MRP4 was over-expressed in serum-induced proliferating SMC as well as in atherosclerotic plaques in human coronary arteries and in neo-intima of injured rat carotid arteries. Inhibition of MRP4 by siRNA blocked VSMC proliferation *in vitro*. In balloon-injured rat carotid arteries, intima/media ratios were significantly lower in Ad-shMRP4-infected arteries than in Ad-shLuciferase-infected arteries (0.65 ± 0.1 *vs* 1.05 ± 0.2; $p<0.03$). *In vitro*, MRP4 inhibition significantly increased intracellular cAMP and cGMP levels. A PKA inhibitor (PKI) but not the PKG inhibitor (KT5823) completely reversed the anti-proliferative effect of MRP4 inhibition. The level of pCREB increased by $329 \pm 18.8\%$ ($p=0.003$) on MRP4 inhibition. **Conclusion**: We provide first evidences that MRP4 acts as an independent endogenous regulator of cyclic nucleotides intra-cellular levels in vascular smooth muscle cells

Calcium Fluxes, Sparks, and Waves I

1395-Pos Board B239

Synchronized Spontaneous Calcium Release Events Throughout The Intact Heart

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Intracellular calcium (Ca) dysregulation associated with cardiac disease (e.g. heart failure) has been linked to mechanisms of ventricular arrhythmias. Such arrhythmias can arise at the sub-cellular level from delayed afterdepolarizations that are due to spontaneous calcium release from the sarcoplasmic reticulum. The mechanisms that govern an aggregate of sub-cellular spontaneous calcium release events at the tissue level (i.e. an SCR) are not well understood. We hypothesize that in tissue, an SCR can be significantly influenced by ryanodine receptor (RyR) function. **Methods:** High resolution optical mapping of Ca (Indo-1AM) from the anterior surface of the Langendorff perfused guinea pig heart (n=4) was performed in hearts under high Ca conditions ($[Ca^{2+}]_e$=5.5mM), with and without caffeine (CAFF, 1mM) to enhance RyR open probability. Endocardial cryoablation was performed to eliminate Purkinje fibers and cytochalasin-D (7μM) was administered to remove motion artifact. Fifteen seconds of rapid pacing (400-160 ms cycle length) followed by a pause was used to induce SCR activity. **Results:** In all preparations**,** synchronized SCR activity was observed across the entire anterior surface of the heart with and without CAFF. SCR activity increased in magnitude and occurred earlier with decreasing pacing cycle length. With CAFF, the amplitude of SCR activity increased (+10.9%, $p < 0.05$) and occurred earlier (+15.6%, $p < 0.05$). CAFF also decreased the spatial heterogeneity of SCR onset across the mapping field (-37.4%, $p < 0.05$), suggesting that increased RyR open probability enhances the synchronization of SCR activity. **Conclusions:** These results demonstrate that sub-cellular spontaneous calcium release events (an SCR in tissue) occur over a broad region of the intact heart and are enhanced when RyR open probability is increased. SCR activity may be an important mechanism of arrhythmogenesis in heart disease associated with calcium dysregulation.

1396-Pos Board B240

Investigating the Ca^{2+}-Cycling Basis of Rhythmicity and Synchronicity in Coupled Cardiomyocyte Monolayers

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Arrhythmia arises from the breakdown of synchronous ion handling. Abnormal intracellular Ca^{2+} cycling has emerged as a primary driver for subsequent arrhythmogenic perturbations in sarcolemmal Na^+ and K^+ fluxes. Consequently, therapeutic manipulation of intracellular Ca^{2+} handling represents a key target for new anti-arrhythmic strategies. In order to better understand the potential modes of modulating Ca^{2+} cycling that may underpin feasible anti-arrhythmic approaches, we focussed on dissecting the relationships between the spatiotemporal aspects of Ca^{2+} release and intercellular (dys)synchrony. Ca^{2+}-dependent fluo-4 signals recorded in spontaneously oscillating, electrically-coupled cardiomyocytes were decoded using the **S**ynchronicity-**A**mplitude-**L**ength and **V**ariability of **O**scillation (SALVO) program that describe 'contractile' and 'non-contractile' aspects of Ca^{2+} handling. In 40 separate experiments (n> 500 cells), intercellular synchronisation of Ca^{2+} release and sequestration was relatively constant ($42.7 \pm 2.5\%$) over wide ranges of Ca^{2+} transients profiles (transient areas of 7 - 117 units) and oscillatory frequencies (0.026 - 2.17Hz). Surprisingly, the relative areas of Ca^{2+} transients (reflecting the amplitude and kinetics of Ca^{2+} release and sequestration) were not linked to the oscillatory frequency. Although high levels of intercellular synchrony persisted despite a remarkable plasticity in both the frequencies and shapes of Ca^{2+} transients, we found that the transients became more uniformly ordered at