716-Pos Effects Of Thiol Reagents And Cu^{2+} On *Escherichia Coli* F0F1-ATPase

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Board B560

Thiol (SH-) groups have been shown can modulate ion transport and ATPase activity of *Escherichia coli* proton-F0F1-ATPase and be significant for an energy transfer by interchange of 2 SH-⋄-S-S- + 2H between F0F1 and the other proteins when the change in the number of SH-groups were determined. A modification of SH-groups in membrane proteins of fermenting *E. coli* (pH 7.5) by 5.5-dithiol-bis-(2-nitrobenzoic) acid or Ellman's reagent and succinimidyl-6-(β-maleimidopropionamido) hexanoate decreased H^+-K^+-exchange, disturbed the K^+-dependent and *N,N'*-dicyclohexylcarbodiimide- and azide-inhibited ATPase activity of F0F1 and the production of H2. But these reagents did not affect these processes upon cysteine in b subunit of F0 substituted by alanine. Moreover, a drop in redox potential caused by H2 produced upon fermentation of glucose or utilization of formate was less in the presence of these reagents. Such effects were not observed with the other thiol reagent - *N*-ethylmaleimide (NEM). Besides, Cu^{2+}, breaking the disulfides of membrane proteins, increased the number of SH-groups independently on K^+ and suppressed the increased level of SH-groups by ATP in the presence of K^+. The increase in the number of SH-groups and the inhibition in ATP-dependent increasing SH-groups number by Cu^{2+} lacked when treated with NEM. Such effects were not observed with Zn^{2+}, Co^{2+} or Cu^+.

The results indicate a role of SH-groups and cysteine in the b subunit of F0 for H^+-K^+-exchange and the production of H2 by *E. coli* those are participating in a dithiol-disulfide interchange within the F0F1-ATPase and in K^+ uptake system and hydrogenases 4 or 3. A difference in properties of thiol reagents causing different effects in *E. coli* and a discrimination between divalent ions are discussed.

Membrane Transport

717-Pos How Do Arginine Rich Peptides Cross The Lipid Membrane? - A Molecular Dynamics Study

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Board B561

The recombinant HIV-1 Tat protein contains a small region which is capable of translocating cargoes of different molecular sizes, such as proteins, DNA, RNA, or drugs, across the cell membrane in an apparently energy independent manner. The pathway that these peptides follow to enter the cell has been the subject of strong controversy for the last decade. This peptide is highly basic and hydrophilic (47YGRKKRRQRR57R). Therefore, a central question that any candidate mechanism has to answer is how can this highly hidrophilic peptide be able to cross the hydrophobic barrier imposed by the cell membrane. We will present a mechanism for the spontaneous translocation of the Tat peptide across a lipid membrane revealed by extensive molecular dynamics simulations. This mechanism explains how key ingredients such as the cooperativity among the peptides, the large positive charge, and the arginine amino acids contribute to the uptake. These results provide a general mechanism that describes how cell penetrating peptides rich in arginine amino acids are able to translocate across the membrane in an energy-independent manner.

718-Pos Do Porins Pass CAPs?

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Board B562

The cationic antimicrobial peptide (CAP) protamine is known to inhibit bacterial survival (Pink et al., *Langmuir **19***, 8852 (2003), and references therein), but the mechanism of attack is as yet undetermined. For Gram-negative bacteria, two pathways have been proposed:

(a) self-promoted uptake, and
(b) passage through porins.

Here, we study the latter possibility, and model part of the outer membrane of a Gram-negative bacterium in an aqueous solution containing multivalent ions and CAPs. The intent is to determine whether CAPs could pass through porins and, if so, what aspects of external (e.g., ionic concentration) and internal (e.g., porin and O-sidechain characteristics) parameters affect their passage. This study is accomplished via Monte Carlo computer simulations of a "minimal model" of the outer membrane of a Gram-negative bacterium with an embedded porin.

719-Pos Drug permeation through the cell membrane and OmpF investigated by Molecular Dynamics simulations

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Board B563

Gram negative bacteria represent a serious threat to human health. In particular, their mechanism of drug resistance is invalidating the currently used antibiotic therapies and new chemical entities are urgently required to fight this ever increasing problem. One promising line of research includes drugs that may act as inhibitors of the Beta-Lactamase enzyme, thus interfering with the metabolic pathway of drug resistance. The crucial problem for these drugs is their translocation through the outer membrane of the bacteria. Here, we

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report the first results of Molecular Dynamics simulations performed on different compounds to analyze qualitatively and quantitatively their passage through a 1-palmitoyl-2-oleoyl-phosphatidilcoline (POPC) lipid bilayer in explicit solvent. In particular, we apply metadynamics to estimate the free energy differences corresponding to drug translocation, to analyze the crucial steps in the translocation process, and to identify the molecular features that affect the drug permeability. In addition, we analyze the role of the bacterial outer membrane porin OmpF in assisting the passage of these small compounds across the POPC bilayer. We report the estimated free energy barriers corresponding to the one-directional passage of specific drugs through OmpF and compare our results with recent single-channel experiments.

720-Pos Ciprofloxacin Transport through OmpF porin by Steered Molecular Dynamics Simulation

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Board B564

OmpF porin is an integral membrane protein located in the outer membrane of Escherichia coli which allows for the passive diffusion of small, polar molecules, such as sugars, water and ions. Computer simulations, with their atomic level of details, could provide valuable information on transport phenomena in channel proteins.

In this study, we have simulated the permeation of an antibiotic, ciprofloxacin, through the OmpF, using the GROMACS simulation package, version 3.3.1. Steered molecular dynamics method has been applied to facilitate and characterize the full permeation of the ciprofloxacin. It can be observed that adhesion force is changing while ciprofloxacin is moving through the porin, according to interactions between the OmpF and ciprofloxacin.

721-Pos How Glycerol Finds Its Way Through Aquaporin GlpF

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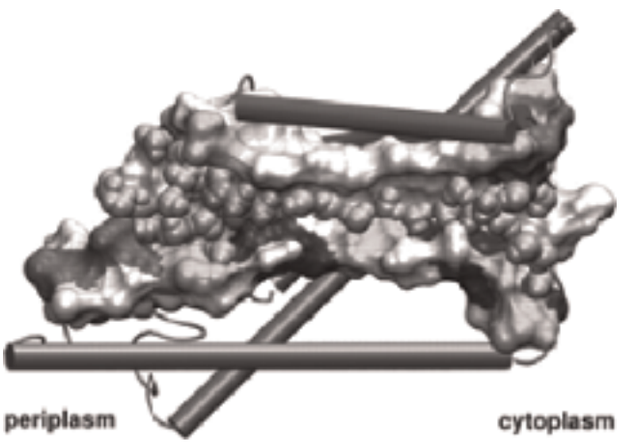
Board B565

The glycerol uptake facilitator, GlpF, a major intrinsic protein found in Escherichia coli, selectively conducts water and glycerol across the inner membrane. The free energy landscape characterizing the assisted transport of glycerol by this homotetrameric aquaglyceroporin has been explored by means of equilibrium molecular dynamics over a time scale spanning 120 ns. In order to overcome the free energy barriers of the conduction pathway, an adaptive biasing force (ABF) is applied to the glycerol molecule confined in each of the four channels. The results illuminate the critical role played by intramolecular relaxation on the diffusion properties of the permeant. The present free energy calculations reveal that glycerol tumbles and isomerizes on a time scale comparable to that spanned by its ABF-assisted conduction in GlpF. As a result, reorientation and conformational equilibrium of glycerol in GlpF constitute a bottleneck in the molecular simulations of the permeation event. A profile characterizing the position-dependent diffusion of the permeant has been determined, allowing reaction rate theory to be applied for investigating conduction kinetics based on the measured free energy landscape.



722-Pos New Insights Into the Gating Mechanism of Plant Aquaporins from Computer Simulations

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Board B566

The gating mechanism of the spinach leaf aquaporin (AQP) SoPIP2;1 has been described based on high-resolution structures of the open and closed conformations. Being a fast, highly selective water transporter isolated from plants, SoPIP2;1 can be incorporated into industrial membranes for water-filtration applications. For this, it is important to quantify channel permeability, and to drive the conformational equilibrium of SoPIP2;1 towards a constitutively open state.

Molecular dynamics (MD) simulations of the closed and open conformations of the SoPIP2;1 tetramer embedded in POPC lipid bilayers were implemented to calculate single channel permeability constants (pf) . The D-loop (residues 181–200) is longer by four residues in SoPIP2;1 compared to mammalian AQPs. It stabilizes the closed conformation by anchoring to the N-terminus by a network of H-bonds mediated by R190 and D191. The double mutant R190A-D191A and a truncated D-loop mutant (TRUNC) of the closed form were tested for increasing equilibrium water permeability. Two copies of each simulation were run.

The calculated ratio of pf (open)/pf (closed) was similar to the ratio of Pf(open)/Pf(closed) measured in swelling experiments in Xenopus oocytes. Both mutants increased the permeability of the

closed conformation to levels of the open conformation. In experiments, single-residue alanine mutants of homologous residues (R194 and D195) in Arabidopsis thaliana PIP2;2 could not be closed by cytosolic pH reduction, which in the wild-type led to 85% activity loss. A persistent H-bond (occupancy: 0.8) between the side-chains of D191 and S36 was detected in the wild-type closed conformation. The D191-S36 interaction was severed in both mutants, driving the D-loop away from the N-terminus of the protein and opening the water channel. The D191-S36 H-bond was not previously implicated in the gating mechanism. Interestingly, S36 is a conserved residue in the PIP2 family.

723-Pos The Cys154 → Gly Mutation In LacY Increases Periplasmic Accessibility Of Sugar To The Binding Site

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Board B567

The lactose permease of *Escherichia coli* (LacY) is a highly dynamic membrane transport protein, while the Cys154 → Gly (C154G) mutant is conformationally crippled. Thus, the mutant binds sugar with high affinity, but catalyzes very little translocation across the membrane. However, the X-ray structures of the mutant and the wild-type proteins are indistinguishable. In order to investigate biochemical differences, fluorescent maleimides were used to examine the accessibility/reactivity of single-Cys mutations primarily on the periplasmic side of the protein in right-side-out membrane vesicles. As shown previously, sugar binding to single-Cys mutants (pseudo wild-type LacY; pWT) induces a marked increase in reactivity of periplasmic mutants and decreased reactivity of cytoplasmic mutants. Thus, the predominant population of pWT LacY in the membrane is in an inward-facing conformation in the absence of sugar, and sugar binding with pWT induces opening of a hydrophilic pathway on the periplasmic side. In striking contrast, the reactivity of periplasmic mutants in the C154G background is very high in the absence or presence of sugar. The findings indicate that an open hydrophilic pathway is present in C154G LacY in the absence of substrate, the accessibility of which is not altered by ligand binding. The conclusion is fully consistent with findings obtained from single-molecule fluorescence and double electron-electron resonance.

724-Pos Gating in the LacY Symporter: Monte Carlo Normal Mode Following Simulations

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Board B568

Lactose permease (LacY) is a typical facilitator, using energy stored in a transmembrane electrochemical proton gradient to drive cytosolic accumulation of galactosides against their concentration gradient. While the 3.5 Angstrom crystal structure of LacY from *E. coli* suggests a physical realization of the alternating access model for the structural differences between its inward- and outward-facing conformations [Abramson et al., *Science,* (2003) ***301***:610], the molecular details of this gating transition remain elusive. We probe large-scale conformational changes in this LacY symporter by Monte Carlo Normal Mode Following, modified using a rotation-translation of blocks approximation. We find that perturbation of this system along the 7th all-atom normal mode initiates opening and closing via global counter-rotation of the intracellular and extracellular domains around the pore axis. The stationary plane relative to which counter-rotation occurs passes through the center of LacY parallel to the membrane. Counter-rotation of the two halves is highly cooperative and concerted. The long TM6-TM7 polypeptide loop, the small helices and the N- and C-termini on the intercellular side undergo large scale rotational motion. Pore size is essentially unaffected by these motions; it remains open toward the cytosol and occluded toward the periplasm. Perturbation along the 8th normal mode leads to large scale expansion and constriction of the intracellular mouth. The N- and C-termini of LacY alternately approach and separate, strongly affecting the size of the intracellular pore mouth. Normal mode following along the lowest frequency eigenvector reveals structural details of the large-scale gating transition in the LacY symporter.

724.01-Pos Evidence For Functional Similarity Between The Lipid A Flippase Msba And The Pgp Multidrug Transporter: Fluorescence Studies Of Substrate And Nucleotide Binding

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Board B568.01

The problem of multidrug resistance (MDR) in human cancer led to the discovery of P-glycoprotein (Pgp, ABCB1), an ABC drug efflux pump. Pgp has remarkably broad substrate specificity for structurally diverse amphipathic drugs. Comparison of the sequences of Pgp and bacterial transport proteins led to the identification of close homologues, the MDR transporter LmrA from Lactococcus lactis, and the lipid flippase MsbA from *Escherichia coli.*

Site-directed labeling with fluorescent probes is a powerful tool to investigate structural rearrangements taking place within ABC proteins. Labeling of purified MsbA at Cys88 was carried out using the fluorescent probe MIANS. Protein ATPase activity, stability, and labeling stoichiometry were characterized using mass spectrometry, optical spectroscopy, and site-directed mutagenesis. Saturable quenching of MIANS fluorescence was observed with both Lipid A and several Pgp substrates, providing strong evidence that MsbA is indeed capable of binding multiple drugs with an affinity (Kd = 0.6–9 μM) comparable to that for the putative physiological substrate, Lipid A (Kd = 1.9 μM). Thus MsbA has overlapping substrate specificity with human Pgp.

Binding of several nucleotides also led to saturable quenching of MIANS-MsbA. The Kd for ATP binding was 0.26 mM, similar to the affinity observed for Pgp. The nature of the inhibition of MsbA ATPase activity by vanadate and beryllium fluoride was character-

ized. Like Pgp, MsbA forms post-hydrolysis and pre-hydrolysis trapped transition state analogues with Vi and BeFx, respectively. Taken together, our data strongly support the idea that MsbA utilizes the same mechanism for substrate binding and transport as Pgp.

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724.02-Pos Characterization of the Nucleotide, Lipid and Drug Interactions of the E. coli MsbA Protein

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Board B568.02

The *E. coli* MsbA protein is a 65 kDa member of the ABC (ATP-binding cassette) superfamily. It exists as a membrane-embedded dimer that is thought to function as an ATP-dependent lipid translocase or flippase that moves lipid A from the inner to the outer leaflet of the cytoplasmic membrane. His6-tagged MsbA was expressed in *E. coli,* purified, and functionally reconstituted into proteoliposomes of *E. coli* lipid. MsbA existed as a homodimer in the detergent dodecyl maltoside, as shown by gel filtration FPLC. The ATPase activity of MsbA was stimulated and inhibited by a variety of lipids, including lipid A, and several drugs that are also substrates for the mammalian multidrug transporter P-glycoprotein (ABCB1), such as Hoechst 33342. The ability of MsbA to bind nucleotides, lipids/lipid-based compounds, and drugs was investigated by monitoring saturable quenching of the intrinsic Trp fluorescence of the protein in detergent solution. MsbA had an apparent binding affinity for ATP of ~2 mM, and also bound AMP-PNP, ADP and AMP. The putative physiological substrate lipid A bound to the protein with an affinity of 6.7 μM. MsbA also interacted with P-glycoprotein drug substrates with an affinity that ranged from ~0.25 to >50 μM. This work represents the first report of quantitation of the MsbA binding affinity for nucleotides, lipids and drugs by quenching of the intrinsic fluorescence of the protein. Our results suggest that MsbA has significant similarities to the P-glycoprotein multidrug transporter.

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725-Pos Copper-Dependent Regulation of hCTR1, the Human Copper Transporter, in Mammalian Cells

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Board B569

The underlying mechanism of how cells maintain copper homeostasis has been better understood since the discovery of the human copper transporter, hCTR1,. It has been reported that hCTR1 is regulated by extracellular copper levels via its copper-dependent removal from the plasma membrane and subsequent degradation. Thus, the uptake of potentially toxic levels of copper would be decreased in the face of elevated extracellular copper levels. We have carried out a comprehensive and quantitative analysis of copper regulation by hCTR1 to determine how cellular copper homeostasis is maintained. We have investigated the effect of varied concentrations of extracellular copper on 64Cu uptake rates and plasma membrane levels of hCTR1 using cell surface biotinylation and imaging techniques in HEK293, MDCK and Caco-2 cells. We have investigated the effects on both endogenous and over-expressed hCTR1. There are good correlations between the decrease in 64Cu uptake rates and the loss of hCTR1 from the plasma membrane. Using biotinylation we found that heterologously expressed hCTR1 plasma membrane levels in HEK293 cells decreased maximally by 30% when cells were exposed to between 2.5–20 uM Cu for 2 hrs, along with a concomitant decrease in the rate of 64Cu uptake. The time-dependent effect of copper on hCTR1 was investigated. The decrease in plasma membrane hCTR1 occurred rapidly, within 10 minutes. We will discuss the kinetics, mechanism and cell specificity of these copper-dependent phenomena.

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726-Pos Annexin A5 Binds To The Intracellular Regulatory Loop Of Na-Ca Exchanger In A Ca- And Phospholipids-dependent Manner

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Board B570

Annexin A5 (AnxA5) is a Ca-dependent phospholipid-binding protein that forms a complex with the Na/Ca exchanger (NCX) and caveolin-3 in human heart. NCX is the main Ca extrusion mechanism of cardiomyocytes, and can also bring Ca into cells (e.g. in heart failure). Failing human myocytes also have increased diastolic [Ca], altered expression of phospholipids (PLs) and increased AnxA5/NCX complex amount. Our aim here is to determine if AnxA5 associates directly with the regulatory domains of NCX (in the intracellular loop) and define whether [Ca] and PLs can facilitate complex formation. The entire NCX regulatory loop (less the XIP region) was purified by C-terminal histidine-tag and used in in vitro assays to pull-down purified Anx A5. Pull-downs showed that Anx A5 co-precipitation increased in dose dependant manner with both NCX loop or [Ca]. In addition, the role of PLs was tested by precipitation in presence of phosphatidylserine (PS-1μg) that has high AnxA5 affinity or PIP2 (0.01 μg) which regulates NCX activity. NCX-loop pull-down showed that PS increased the AnxA5 precipitation, whereas PIP2 had only a marginal effect. Finally, to validate these results, Surface Plasmon Resonance (SPR-Biacore) assays were performed. Injection of NCX loop (0.8 μM) on immobilized AnxA5 confirmed their direct interaction. Moreover, increasing [Ca] (100nM to 3μM) led to an increase of association and slower dissociation. In conclusion, we demonstrated for the first time that AnxA5 interacts with the NCX regulatory loop. In addition our results support a potential Ca- and PL-dependent regulatory role of AnxA5 on cardiac NCX.

727-Pos Na^+-dependent Ca^{2+} Influx and Efflux via Electrogenic Na^+-Ca^{2+} Exchange in Cardiac Mitochondria

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Board B571

We aimed to clarify the role of mitochondrial Na^+-Ca^{2+} exchange (mNCX) at normal and depolarized mitochondrial membrane potential ($\Delta\Theta$). Using fluorescence probes (Rhod-2, TMRE), we measured mitochondrial Ca^{2+} (Ca^{2+}m) and $\Delta\Theta$ in the permeabilized rat ventricular cells. Applying 300 nM cytoplasmic Ca^{2+} (Ca^{2+}c) induced accumulation of Ca^{2+} in mitochondria and this accumulation was attenuated by cytoplasmic Na^+ (Na^+c) when $\Delta\Theta$m is intact (IC50 = 2.4 mM). On the contrary, when $\Delta\Theta$ was depolarized by FCCP, a mitochondrial uncoupler, the $Ca2^+$c-induced Ca^{2+}m accumulation was still observed and Na^+c augmented the accumulation (EC50 = 5.7 mM). In the presence of Na^+c, applying CGP-37157, an inhibitor of mNCX, further enhanced the Ca^{2+}m accumulation at normal $\Delta\Theta$, while it inhibited the Ca^{2+}m accumulation at depolarized $\Delta\Theta$, indicating that the exchange mode was changed from forward to reverse by the $\Delta\Theta$ depolarization. Under the condition that the Ca^{2+} uniporter was suppressed by ruthenium red, applying 600 nM Ca^{2+}c only slightly induced the Ca^{2+}m accumulation at normal $\Delta\Theta$, but depolarization induced by FCCP remarkably enhanced the Ca^{2+}m accumulation. The accumulation was largely suppressed by CGP-37157, suggesting that the reverse mode of mNCX is voltage dependent. The Ca^{2+}m increase via reverse mode of mNCX was suppressed by removing Na^+c. When respiratory chain was suppressed, $\Delta\Theta$ measured with TMRE hyperpolarized upon inducing the Ca^{2+} influx via reverse mode of mNCX, while it depolarized upon inducing the Ca^{2+} efflux via forward mode of mNCX. The above experimental data strongly supported that mNCX is voltage-dependent and electrogenic. A computer simulation study suggested that the Na^+c-dependent increase in Ca^{2+} influx via the reverse mode of mNCX is probably due to the depolarization-induced increase in matrix Na^+. It was suggested that Na^+c and $\Delta\Theta$ dynamically modulate Ca^{2+}m via electrogenic mNCX.

728-Pos Control of the Membrane Potential by Chloride Transport in Skeletal Muscle - Theory and Experiment

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Board B572

We present a model for the control of the transmembrane potential that involves active and passive transport of Na+, K+, and Cl- As we compare the model to experimental measurements on murine skeletal muscle cells we find that the model can account for the observed bistability of the transmembrane potential at low extracellular potassium concentration. We measure and model the effects of a medium with bumetanide, a blocker of the Na,K,2Cl-cotransporter, and a hypertonic medium, which is known to stimulate the Na,K,2Cl-cotransporter. Increased chloride transport has two effects on the interval along the extracellular potassium concentration axis where the system is bistable: the interval is shifted towards higher potassium concentrations and the length of the interval is reduced. By matching the model with experiment, we obtain estimates for the chloride permeability, the transmembrane chemical potential of chloride, and the steady state flux through the Na, K,2Cl-cotransporter.

729-Pos Anion- And Voltage-dependent Gating Of Eaat4 Associated Anion Channels

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Board B573

EAAT glutamate transporters remove glutamate from the synaptic cleft to ensure low resting glutamate levels and to prevent neuronal damage by excessive glutamate receptor activation. EAATs are not only secondary-active glutamate transporters, but also anion-selective channels. EAAT anion channels exhibit voltage-dependent gating transitions that are modified by the concentration of permeant anions and of transporter substrates. We expressed the rat EAAT4 in mammalian cells and studied gating of EAAT4 anion channels through patch-clamping under a variety of conditions. Two different gating processes can be distinguished, one activated by hyperpolarisation of the membrane, in the following denoted as h-gate, and another one activated during membrane depolarisation, denoted d-gate. In the absence of glutamate, only the h-gate opens and closes within a voltage range between −160 mV and +160 mV. Glutamate shifts depolarisation- and hyperpolarisation-induced gating to more negative potentials in a dose-dependent fashion. At saturating glutamate concentrations, both gates are active in a physiological voltage range. The concentration of the permeant anions modifies only the voltage dependence of the depolarisation-induced d-gate, leaving the h-gate unaffected. Depolarisation-induced gating modifies the selectivity of the ion conduction pathway of EAAT4 channels and permits the passage of cations in addition to anions. Conditions activating the d-gate allow EAAT4 anion channels to conduct excitatory currents. Voltage-dependent gating thus appears to determine the role of EAAT channels in regulating cellular excitability.

730-Pos Gating in the LeuT Symporter: A Monte Carlo Normal Mode Following Study

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Board B574

Opening and closing mechanisms of the extra- and intracellular gates of the Na^+/Cl^--dependent neurotransmitter transporters that catalyze the symport of small molecules and ions across membranes remain elusive. Large-scale conformational changes in a bacterial homologue, the LeuTAa symporter, are studied by Monte Carlo Normal Mode Following, modified using a rotation-translation of blocks approximation. Gating is initiated by global counter-rotations of the intracellular and extracellular domains of LeuTAa around the pore axis. As the extracellular half rotates clockwise, the intracellular half rotates anticlockwise, and vice versa. The stationary plane relative to which counter-rotation occurs passes through the center of LeuTAa parallel to the membrane. The counter-rotations of the two halves are highly concerted and cooperative. On the extracellular side, the overall rotation of the peripheral TM helices affects the conformation of five of the inner TM helices: TM1, TM6, TM3, TM8 and TM10. They alternately straighten and bend near their midpoints. Straightening occludes and bending expands the extracellular pore. Loops and small helices on the extracellular surface of LeuTAa undergo large-scale motions. The intracellular domain of LeuTAa rotates concertedly around the pore axis essentially as a single unit. Relative to the pore axis, the radial location of the intracellular ends of TM1a-TM6b and TM3 and TM8 is not affected. Normal mode following along the lowest-frequency eigenvector reveals details of the gating transitions in the LeuTAa symporter.

731-Pos How Do Tricyclic Antidepressants Bind in the Human Serotonin Transporter? Molecular Modelling Studies with Biochemical Validation

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Board B575

Imipramine, a tricyclic antidepressant (TCA), is known to block the re-uptake of monoamine neurotransmitters from the synaptic cleft. It functions by competitively inhibiting the membrane bound human serotonin transporter (hSERT). Although this has been known for years the lack of structural knowledge in atomic details about hSERT has hindered the search for the location of the specific binding mode of TCAs in hSERT. Two years ago the structure of a homolog bacterial transporter, the Leucine Transporter (LeuT), was published. Based on this structure we have built a homology model of hSERT and performed protein flexible docking of TCAs in this structure. This shows a binding site for TCAs located approximately in the middle of the transporter and corresponding to the binding site found for leucine in LeuT and serotonin in hSERT. In contrast, recent studies suggest that TCAs are non-competitive inhibitors of LeuT and five structures were published of LeuT with different TCAs bound in a binding site in the extracellular vestibule of LeuT.

To assess the binding of TCAs in these two different sites molecular docking and dynamics simulations have been performed on both hSERT and LeuT with imipramine in either of the two sites and relative binding affinities have been estimated. The computational results were used in correlation with results from experimental mutagenesis studies on hSERT. From this we suggest that the binding of TCAs in the extracellular vestibule of LeuT represents a secondary binding site, which may not be physiologically relevant, and that the primary TCA binding site in hSERT is coinciding with the one found for serotonin in the central binding site of the transporter.

732-Pos Substrate Binding and Gating in the Glutamate Transporter

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Board B576

The glutamate transporter (GluT) plays a key role in terminating neurotransmission by re-uptaking glutamate from the synaptic space using pre-existing ionic gradients as a source of energy. Recent structures of GluT have put forward a mechanism for the coupling between binding and co-transport of Na^+ ions and the substrate glutamate. However, the details of the transport cycle, e.g., the mechanism of gating, and the binding sequence of the substrate and Na^+ ions to the transporter are not understood. Using more than 100 ns of MD simulations of a membrane-embedded model of GluT (~200,000 atoms), in the Apo and different bound states, we have investigated the dynamics and energetics of the binding sites, and the mechanism of co-transport of Na^+ ions. The results show that the periplasmic access to the substrate binding pocket is controlled by one of the internal loop structures (HP2). Interestingly, the conformation of this loop appears to be controlled by one of the co-transported Na^+ ions (Na-2); in the presence of Na-2, HP2 undergoes a large movement opening the binding pocket to the periplasm. In contrast, the binding site remains closed in the Apo-transporter, and in the substrate-bound form, i.e., after the binding of the substrate. Based on these results, we propose that the binding of Na-2 precedes that of the substrate. Furthermore, we find that the binding of Na-1 to the GluT is significantly stronger (by ~100 kcal/mol) than Na-2, primarily due to D405. Simulation of the system at different protonation states of D405 reveals that the binding of Na-1 is strongly affected by the titration state of this residue. We thus propose that D405 might play a key role in coupling of proton and Na co-transport across the protein.

733-Pos Computational Studies of Substrate-dependent Behavior of the E. coli Cobalamin Transporter BtuB

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Gram-negative bacteria possess specialized transport systems that couple the energy-generating proteins of the inner membrane to a variety of transporters in the outer membrane. One particular system, the TonB-dependent system, functions through the interaction of the inner membrane protein TonB with the outer membrane transporters. TonB is a component of an inner membrane motor complex, with similarity to the bacterial flagellar motor, comprised of TonB, ExbB and ExbD. Molecular dynamics simulations performed previously suggested that the interaction between TonB and the cobalamin (e.g., cyanocobalamin, vitamin B12) transporter BtuB is likely mechanical in nature, with TonB transmitting force to the pore-occluding luminal domain of BtuB. A deficiency of our previous simulations was the lack of substrate, which had not yet been parameterized for the CHARMM forcefield. We have completed parameterization of cyanocobalamin and now include substrate in our simulations. Initial findings indicate a large stabilizing effect of the substrate, especially on the extracellular loops of BtuB; simulations also show a decrease in interaction between the substrate and its binding site during unfolding of the pore-occluding luminal domain. Experimental studies demonstrate that binding of the substrate on the extracellular side of BtuB induces conformational change and an order-disorder transition on the periplasmic side of the transporter. We explored this allostery by comparing correlated motions of residues before and after substrate binding. Also, solvent accessibility can be calculated from structures obtained during simulations. We are utilizing these accessibility results in conjunction with experimental accessibility data being obtained with an *in vivo* assay.

734-Pos Microscopic Rate Analysis Of The Novel Glutamate Transporter Blocker NBI-59159 Reveals Complex Unbinding Kinetics

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Board B578

NBI-59159 is a structurally novel non-transported inhibitor of the human excitatory amino acid transporters (hEAATs) that exhibits nanomolar potencies for blocking glutamate uptake and ~10-fold selectivity for the neuronal transporter hEAAT3 (Curr Top Med Chem. *6* 1897 2006). We recorded currents in voltage-clamped *Xenopus* oocytes expressing hEAATs and performed Schild analysis of NBI-59159, confirming its competitive mechanism of inhibition. We also used concentration jumps to measure binding and unbinding rates of NBI-59159 in the presence or absence of glutamate by monitoring steady state glutamate transport currents or pre-steady state Na-dependent charge movements, respectively. In the presence of 3 μM glutamate, the binding rate of the drug was 4.4 +/− 0.5×10^5 $M^{-1}s^{-1}$. The on-rate was not significantly different in hEAAT1, hEAAT2, or hEAAT3 and was approximately one order of magnitude slower than that of glutamate (6.8×10^6 $M^{-1}s^{-1}$; J. Neurosci. *18* 7650 1998). The selectivity of NBI-59159 for hEAAT3 was found to result from a slower off-rate of the drug (0.031 +/− .002 s^{-1}, 0.028 +/− .003 s^{-1}, and 0.0040 +/− .0002 s^{-1} for hEAAT1, 2, and 3 respectively). The binding and unbinding rates were not voltage-dependent over the range from −80 to −20 mV. Unexpectedly, the rate of recovery from block was slowed 3.3 +/− 0.3 fold when L-Glu was not present during washout of the drug. This result is inconsistent with pseudo first-order unbinding kinetics and suggests a significant probability of drug rebinding subject to competition with glutamate. We propose that the large aqueous central cavity in the trimeric complex (Nature *431* 811 2004) may restrict diffusion near the three ligand binding sites, resulting in an effective dissociation rate significantly slower than the true first-order subunit unbinding rate.

735-Pos Dissecting Lipid- and Protein-Mediated Exchange of Gas Molecules across Biological Membranes

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Board B579

Exchange of gas molecules across the cellular membrane is one of the fundamental processes in aerobic organisms. Although lipid bilayers are generally considered to be gas permeable, several studies have suggested that the lipid composition of the bilayer can significantly reduce its gas permeability. Furthermore, the involvement of membrane proteins, which may take up to 75% of the total area of the membrane, has also been demonstrated in some recent studies, including our earlier report on CO2 and O2 conduction across aquaporins. To further investigate the phenomenon of protein medicated gas permeation, and, more importantly, its physiological relevance, we have conducted extensive molecular dynamics simulations of a variety of lipid bilayers and membrane-embedded models of the human aquaporin-4 (AQP4), and calculated gas permeability of O2, nitric oxide (NO) and CO2 using various computational methodologies. Three different lipids: palmitoyloleoylphosphatidylethanolamine (POPE), dioleoylphosphatidylserine (DOPS) and tetramyristoyl cardiolipin (TMCL) are investigated, from which the last two represent important constituents of the inner mitochondrial membrane. Our simulations reveal that the main barrier against gas diffusion across lipid bilayers is in the head group region and that the charge of this region is the main factor in determining the height of the barrier. Compared with POPE, negatively charged lipids (DOPS and TMCL) exhibit at least 50% higher energy barriers against O2 permeation. We have also calculated a free energy barrier of 2.5 kcal/mol for NO permeation across AQP4, demonstrating that AQP4 can indeed conduct NO. This observation is of important relevance to brain physiology, since it implicates for the first time membrane channels in conduction of signaling molecules across the membrane.

736-Pos Carbon dioxide transport through membranes

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Several membrane channels, like aquaporin-1 and the RhAG protein of the Rhesus complex were hypothesized to be of physiological relevance for carbon dioxide transport. However, the underlying assumption that the lipid matrix imposes a significant barrier to CO2 diffusion was never confirmed experimentally. Here we show that virtually under any experimental conditions, CO2 transport is not limited by movement across lipid membranes but by diffusion through near-membrane stagnant water layers as these layers are unavoidable, ever present and always thicker than 0.1 μm. This holds for biological membranes of different lipid compositions and also for the very tight membranes of epithelial cells. We have determined transmembrane CO2 flux by imposing a CO2 concentration gradient across planar lipid bilayers and detecting the resulting small pH shift in the immediate membrane vicinity with a scanning microelectrode. Membrane CO2 permeability was calculated by an analytical model, which accounts for the presence of both carbonic anhydrase and buffer molecules. For the tightest known lipid membranes containing sphingomyelin and cholesterol it was equal to 3.0 cm/s indicating that a physiologically significant facilitation of CO2 transport by AQP1, RhAG or any other protein is highly unlikely. The conclusion was confirmed by the observation that epithelial cell monolayers exhibited always the same CO2 permeability - whether aquaporin-1 was overexpressed in both the apical and basolateral membranes or not.

737-Pos Ammonium Permeability of Human RhCG Glycoprotein Quantitatively Determined by Expression of HA-tagged Constructs in HEK293 Cells: Effects of Mutations

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In order to compare single channel ammonium permeabilities of wild type or mutated RhCG proteins, we generated stabilized sorted HEK pools expressing RhCG proteins carrying HAtag in the second extracellular loop or substitutions at critical positions. The ammonium transport function of RhCG was investigated (stopped-flow) by comparing intracellular pH variations in these cells in the presence of a 20 meq NH4+ gradient using a pH-sensitive fluorescent probe.

Flow cytometry analysis, using a murine polyclonal ascite anti RhCG, showed a fluorescence level corresponding to RhCG expression at the cell surface which is linearly correlated to the transport activity. Using the anti-HA tag, we determined the site number of membrane incorporated RhCG protein allowing the calculation of a unitary permeability, close to $2.10^{-15}cm^3.s^{-1}$. Unitary permeabilities of different mutants were also calculated and compared to that of the non-mutated RhCG.Based on the 3D structure of the bacterial NH3 channel (AmtB) and on homology modeling of the human RhCG protein, we have analysed the role of residues predicted to be located in the pore or in the external vestibule of the channel. In the pore, some conserved amino acids (F130, F235, H344) are essential for NH3 transport. However, two residues located in the vestibule (F167 and I126) reveal different functional properties, as compared to the role of the corresponding residues in AmtB. This might be explained by an efficient recruitment of NH4+ which is required, at low concentrations, for NH3 movements across the pore in bacteria, whereas in mammals the ammonium concentration is high enough to bring the substrate to the channel. Thus, some different functional properties revealed by this study might be related to different physiological needs of bacteria and mammals for NH3.

738-Pos Channel Geometry Of FhaC Studied By Polymeric Nonelectrolytes Partitioning

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In the Two-Partner Secretion (TPS) pathway, widespread among Gram-negative bacteria, large alpha-helical proteins, called "TpsA" proteins, serving mostly as virulence factors are translocated by their specific "TpsB" transporters across the outer membrane. FhaC is a TpsB transporter that mediates the translocation to the bacterial surface of the major adhesin of *B. pertussis,* the 230-kDa filamentous haemagglutinin (FHA). FhaC has been shown to form ion-permeable pores in lipid bilayers and identified as the FHA secretion channel (Meli *et al.,* 2006, *J Biol Chem 281*, 158). We have just solved the X ray structure of FhaC at 3.15 angstrom resolution (Clantin *et al.*, 2007, *Science*, *317*, 957). FhaC is a monomeric protein that forms a 16-stranded beta-barrel occluded both by an N-terminal alpha-helix, H1, and an extracellular loop, L6. Upstream of the beta-barrel is a periplasmic module made up of two aligned POTRA domains likely contributing to FHA recognition. The FhaC pore identified in the structure does not appear large enough to accommodate FHA even in an extended conformation and thus channel enlargement is necessary. H1, L6 or both may move out of the channel during translocation. In this study, in order to measure the effective diameter of FhaC pores in a functional state, we looked at the effect of water-soluble polymers, polyethylene glycols (PEGs), on FhaC channel conductance. Conductance measurements with symmetrical application of PEGs to FhaC channels allowed us

to determine pore size openings which are in agreement with the ones estimated from the cristallographic data. Asymmetrical application of PEGs to FhaC channels are currently in progress to determine the size and location of the constrictions in the pore lumen. These data will be presented and compared to the pore geometry obtained from the cristallographic data.

739-Pos Study of the Effect of Pharmaceutical Formulations on the Membrane Permeation of Fluvastatin-Na

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Board B583

An important requirement when developing a pharmaceutical formulation for a solid dosage form is choosing excipients that stabilize the active ingredient, but also ensure appropriate bioavailability. As can be expected, excipients that stabilize a formulation sometimes adversely affect the bioavailability. For example, the bioavailability of Fluvastatin-Na (FS-Na), a synthetic inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase has been shown to be affected by the presence of carbonate salts in the composition of the pharmaceutical formulation. The purpose of our work is to identify the mechanism by which the pharmaceutical formulation hampers the membrane penetration and subsequent bioavailability of FS-Na. We performed a permeability study using modified 4-mL Franz cells, with donor and receptor cells separated by artificial membranes consisting of filters previously conditioned in a lipophilic mixture containing different ratios of isopropyl myristate (IPM), isopropyl alcohol (IPA), and water. FS-Na formulations were prepared with different proportions of CaCO3 and NaHCO3, and the permeation through the membrane was measured by HPLC. The results show that the overall permeation of FS-Na is reduced by increased amounts of salts and even further when membranes are made more lipophilic with the addition of paraffin oil. The permeation of FS-Na through membranes was then studied by FTIR spectroscopy using multilamellar vesicles composed of a DMPC-d54/DMPS mixture (molar ratio 9:1) to mimic the composition of enterocyte membranes. The infrared CH2 and CD2 antisymmetric stretching modes were monitored between 15°C and 50°C to study the insertion of FS-Na with and without salts. Our results indicate that the presence of salts would hinder the penetration of FS-Na by possibly affecting its solubility. Complementary results obtained by ^{2}H and ^{31}P solid-state NMR will be discussed, along with the penetration mechanism of FS-Na in relation to its bioavailability.

740-Pos Free Fatty Acid Transport in Murine Cardiac Myocytes

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Board B584

Previously we measured the transport of free fatty acids (FFA) across adipocyte membranes using cells microinjected with ADIFAB, the fluorescent probe of unbound FFA (FFAu). In the present study we have applied this methodology to murine cardiac myocytes. Freshly isolated cardiac myocytes were microinjected with ADIFAB, and the transport of oleate (OA) was determined by monitoring the intracellular FFAu concentration ([FFAi]) using fluorescence ratio microscopy. Transport cycles were carried out at 37°C and started with the cells in media in which the extracellular FFAu concentration ([OAo]) was clamped at zero with fatty acid free bovine serum albumin (BSA). Oleate influx was initiated by rapidly increasing [OAo] using OA-BSA complexes (600 μM BSA), which clamped [OAo] at fixed values. The time course of influx was monitored from the change in [OAi], which rose exponentially to a steady state level with a time constant of approximately 100 s. Once steady state was achieved, efflux was initiated by changing the extracellular media back to zero [OAo]. Efflux was monitored by the decrease in [OAi] which, like influx, revealed exponential behavior with a time constant of approximately 40 s. Moreover, at steady state [OAi] was observed to be larger than [OAo] by as much as a factor of 3. Thus, FFA influx in cardiac myocytes occurs up a concentration gradient, even though the efflux rate constant (when [OAo]=0) is three times faster than the influx rate constant. These results are strikingly similar to the transport characteristics observed in cultured adipocytes. This finding is surprising given the very different functions of these two cell types, especially with regards to fatty acid utilization, and suggests that our understanding of cardiac metabolism is incomplete.

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741-Pos Fatty Acid Transport into Preadipocytes Syringe Loaded with ADIFAB

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Board B585

Whether transport of free fatty acids (FFA) across cell membranes is lipid or protein mediated and, if protein mediated, what the identity of the protein(s) might be is intensely debated. The debate arises principally because of the difficulty in measuring the intracellular FFA concentration. Previously, we demonstrated that FFA concentrations could be measured in the cytosol of adipocytes microinjected with ADIFAB, the fluorescent indicator of unbound FFA (FFAu). Using this system, we have monitored FFA transport in preadipocytes and adipocytes and have found virtually identical transport characteristics in these cells. To overcome the practical challenges involved with microinjection and fluorescence microscopy of individual cells, we have developed a syringe loading method that results in efficient trapping of ADIFAB in several million well-sealed and functioning cells. FFA influx and efflux were measured in suspensions of syringe loaded preadipocytes, and the transport characteristics were similar to those observed with microinjected cells. FFA influx in both syringe loaded and microinjected cells resulted in an

ATP-dependent concentration gradient across the cell membrane. Using the syringe loaded cells, we have discovered that the magnitude of the gradient can be modulated (ratio of the inside to outside FFAu concentration between 1.5 and 6) after sealing the cells by altering the extracellular medium prior to transport, with calcium playing an especially important role. These results provide additional support that FFA transport across the adipocyte cell membrane is mediated by an as yet unidentified protein pump.

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742-Pos Volume Responses of Permeabilized Cells Demonstrate Hydrogel Properties of the Cytosol

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Board B586

Current models describing cell volume regulation almost exclusively consider the cell cytoplasm as a solute surrounded by a semipermeable membrane across which water movement is solely dictated by transmembrane osmotic gradients. However, the cytoplasm is filled with large, charged polymers, which give it a gel-like consistency, and may have profound effects on intracellular ion distribution and cell volume homeostasis. For example, both artificial and natural hydrogels respond with significant volume changes to pH or ionic composition alterations. We examined the volume responses of digitonin-permeabilized epithelial A549 cells and tested if their behaviour resembles that of synthetic hydrogels. Volume changes of single, substrate-attached cells were investigated by a 3D imaging technique developed in our laboratory. Upon membrane permeabilization, the cells swelled 2.2-fold and the addtion of ~200 mM mannitol was required to shrink them back to their initial volume. Permeabilized cell volume showed a bell-shaped pH-dependency with a maximum at pH 7.4 (pH 5 → 1.86-fold, pH 6 → 2.0-fold, pH 7.4 → 2.2-fold and pH 8 → 1.96-fold initial volume). Interestingly, intact cells presented almost identical pH-dependent volume changes with a maximum at pH 7.4. This effect may be attributed to protonation of protein carboxylate anionic groups and reduction of anion-anion electrostatic repulsive forces. Isoosmotic replacement of K^+ by Mg^{2+} resulted in significant shrinkage of permeabilized cells (from 2.2-fold at 5 mM Mg^{2+} to 1.7-fold initial volume at 35 mM and 1.51-fold at 70 mM Mg^{2+}) likely due to increased charge screening and ionic crosslinking effects. We conclude that the cytosol of A549 cells behaves as a hydrogel and contributes to volume responses of intact cells.

(This study was supported by the Canadian Institutes of Health Research and the Natural Sciences and Engineering Research Council of Canada).

743-Pos Optical Detection of Ion-Channel-Induced Proton Transport in Supported Phospholipid Bilayers

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Board B587

The integration of ion-channel transport functions with responses derived from nanostructured and nanoporous silica mesophase materials is demonstrated. Patterned thin-film mesophases consisting of alternating hydrophilic nanoporous regions and hydrophobic nanostructured regions allow for spatially localized proton transport via selective dimerization of gramicidin in lipid bilayers formed on the hydrophilic regions. The adjoining hydrophobic mesostructure doped with a pH sensitive dye reports the transport. The ease of integrating functional membranes and reporters through the use of patterned mesophases should enable high throughput studies of membrane transport.

744-Pos A QM/MM Molecular Dynamics Simulation Modeling the Proton Conduction through the Hv1 Channel

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Board B588

The passive transport of protons across cell membranes is an important mechanism for maintaining intracellular pH. A new class of voltage sensitive, proton-selective transport proteins (Hv) were recently discovered and characterized (Clapham et. al. 2006, Nature *440*: 1213; Okamura et. al. 2006, Science *312*: 589). In light of their sequence similarity to the voltage sensing domains (VSDs) of voltage-gated potassium (Kv) channels, the functional domain of Hv1 is putatively considered to consist solely of a VSD with a similar architecture to the VSDs of Kv channels. Based on a molecular dynamics (MD) simulation of the isolated VSD of the KvaP channel, we have proposed that protons could be conducted along a water wire that forms in the interior of a mutant of the KvAP VSD that lacks an essential, highly conserved, salt-bridge (Freites et. al. 2006, Biophysical Journal *91*: 11). Here we report a QM/MM MD simulation study of proton transport in the KvAP VSD D62S/R133I mutant. The water wire and excess proton were modeled using the self-consistent charge-density functional tight binding (SCC-DFTB) approach (Cui et. al. 2006, J. Phys. Chem. B *110*: 6458), and the surrounding protein and lipid atoms were described using the CHARMM molecular mechanics force fields. Long-range electrostatics and the transmembrane potential were included via a generalized solvent boundary potential (Roux et. al. 2001, J. Chem. Phys. *114*: 2924). Using the umbrella sampling technique, the potential of mean force and diffusion constant of an excess proton were computed as a function of position in the channel. These quantities were then used to estimate single channel proton conductance, which are discussed in relation to experimental measurements.

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745-Pos Dynamics and Regulation of the Sec61 Channel

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Board B589

The electrophysiological and oligomeric properties of active Sec61 complexes in the endoplasmic reticulum (ER) membrane of canine pancreas- and yeast-cells were investigated by the planar bilayer technique and with high resolution confocal fluorescence laser spectroscopy in artificial horizontal bilayers. After activation of the Sec61 complexes by various substrate polypeptides, displayed a large variety of open channel states with two regimes of transient channel openings. These corresponded to internal mean pore diameters of 1.2 and 2.2 nm. The various substrate polypeptides determined the respective pore size distribution and selectivity of the channel. Thus the Sec61 complex contains a highly dynamic substrate-activated channel that, once active, fluctuates between two distinct conformations. In the one state small pores exist, in the other state, a single channel pore with roughly doubled pore size is formed. The size of this channel pores are only compatible with an oligomeric structure of the Sec61 complex. This is in line with the FIDA (fluorescence intensity distribution analysis) of the labeled Sec61 complex in artificial horizontal bilayers which indicated that the tetrameric state of the heterotrimer constituted the most prominent oligomeric fraction of the complex. In addition, the channel is regulated by ribosomes on the cytosolic face and calcium-calmodulin and by BiP on the lumenal face of the membrane. We propose that these interactions are important factors of the regulation in preventing the uncontrolled efflux of calcium and other small ions or non charged solutes from this calcium storage compartment.

Solution NMR

746-Pos Study of Biochemistry of Muscle Disorders using *in-vitro* Proton NMR Spectroscopy

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Board B590

The metabolism in muscle disorders was investigated using *in vitro* proton NMR spectroscopy. Muscle tissues were obtained by open surgical biopsy procedure under regional anesthesia from patients with Mitochondrial Myopathy (MM), Duchenne Muscular Dystrophy (DMD), Limb Girdle Muscular Dystrophy (LGMD) and controls (from orthopaedic surgery). Histological and histochemical methods were used for diagnosis of myopathy. Perchloric acid extracts of muscle tissues were prepared and various 1D and 2D NMR experiments were carried out for assignment of the different metabolite resonances and concentration of metabolites was determined.

Significantly higher levels of Lac, Gln and Ala was observed in MM patients compared to controls which could primarily be due to

(i) accelerated rate of anaerobic glycolysis to maintain ATP concentrations resulting in high Lac, and
(ii) defective oxidative metabolism due to mitochondrion abnormalities.

Higher concentrations of Gln and Ala indicate their utilization to meet the additional glucose requirement of cells through gluconeogenic pathway in MM. However, patients with LGMD and DMD showed lower concentration of Lac compared to controls indicating the reduced rate of glycolysis and energy deficit in these patients. The concentration of Ala, Glu+Gln, GPC and Cho was also lower in LGMD and DMD patients suggesting altered metabolism and degeneration of muscle tissue. Between patients of various muscle disorders, significantly reduced concentration of Lac, Glu+Gln and GPC was observed in LGMD, DMD patients compared to patients with MM indicating underlying metabolic differences in these muscle disorders.

Present results demonstrate the potential usefulness of *in vitro* MRS in understanding the metabolism of muscle disorders and indicate the role of *in vitro* MRS to complement the histological methods in the diagnosis of various muscle disorders.

747-Pos Biochemical Characterization of Involved and Non-involved Tissue from Breast Cancer Patients using *in vitro* NMR Spectroscopy

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Board B591

Breast cancer is the commonest cancer among women worldwide. Results of the comprehensive biochemical characterization of malignant breast tissue and its comparison with the non-involved tissue to determine biomarkers for diagnosis of breast cancer and to understand the metabolism are presented. Malignant (n=15) and non-involved (n=22) breast tissue were surgically obtained from breast cancer patients who underwent either modified radical mastectomy or breast conservation surgery. Histopathological evaluation of tissue was carried out to determine malignancy. 1D and 2D proton MR spectra of the perchloric acid extract of the lymph nodes were recorded at 400 MHz. Forty metabolites including amino acids, organic acids, carbohydrates and membrane components were assigned and absolute concentration of 14 metabolites were determined. Kruskal-Wallis and Mann-Whitney U test were used to compare the concentration between malignant and non-involved tissue.

Malignant tissues showed significantly higher concentration of Lac, Ala, Lys, Ace, Glu, Gln, PCr/Cr, Cho, GPC, mI, Tyr and Phe in comparison to the non-involved tissues. The increase in the concentration was of the order of 5-10 times in ceratin metabolites. Higher concentration of Ala, Gln, Glu and Lys are attributed to increased protein synthesis in fast growing tumor cells, as amino acids serves as building blocks for proteins. Higher concentration of Lac could be due to high glycolytic activity in tumor cells. Higher levels of GPC and Cho characterize the rapidly proliferating nature of tumor cells.