plication induced marked channel activation. In symmetrical 210mM K^+ and 10μM Ca^{2+}, NAADP dose-dependently activated TPC2 channels with an EC_{50} of 500nM. Addition of 200μM *trans* Ca^{2+} significantly increased the sensitivity of TPC2, shifting the EC_{50} to 5nM. We have previously demonstrated that ligand-activation of RyR channels is also highly sensitive to luminal Ca^{2+} and therefore we have investigated how NAADP affects RyR1 and RyR2 in the presence of sensitising levels of luminal Ca^{2+}. Addition of NAADP ($\leq$1μM) did not affect RyR2 Po but slightly activated RyR1 (1μM NAADP increased Po from 0.022 ± 0.035 to 0.106 ± 0.147; SD, n=5). In contrast, larger increases in TPC2 Po (0.001 ± 0.002 to 0.4 ± 0.2; SD, n=3, $P<0.05$) could be elicited with much lower NAADP concentrations (10nM). Our study is the first to show that animal TPCs form functional, Ca^{2+}-permeable ion-channels. We also provide further evidence that TPC2 is capable of mediating NAADP-sensitive Ca^{2+}-release from acidic organelles but do not rule out a role for RyR1.
BHF supported

Membrane Transporters & Exchangers II

3559-Pos

Functional Reconstitution of Influenza a M2 (22-62)

Ted J. Ryser[1], Daniel Inouye[1], Spencer Funk[1], Emily Peterson[1], Mukesh Sharma[2], Timothy A. Cross[2], David D. Busath[1].
[1]Brigham Young University, Provo, UT, USA, [2]Florida State University, Tallahassee, FL, USA.

Amantadine-sensitive selective proton uptake by liposomes is currently the ideal method of demonstrating M2 functionality after reconstitution, validating structural determination of the reconstituted protein such as that carried out using solid state NMR (e.g. with M2 22-62, Sharma et al, this meeting). Under pH and Vm gradients, the truncated construct (which lacked 21 residues at the N-terminus and 35 at the C-terminus) was shown to transport protons at the same rate (21 H+/s at pH 6.5) as a similar construct, M2 (18-60), which elsewhere had been shown to transport similarly to other variants, including the full length protein. 100 μM amantadine was found to reduce transport by ~80%, and 10 μM amantadine or cyclooctylamine reduced transport by 50%. Transport was optimal at protein densities of 0.05-1.0% (weight peptide of weight protein and lipid). At 10%, transport was reduced, presumably due to density-dependent ion leakage. Reduction of pH to 5.0 increased transport. Rundown of total proton uptake after addition of valinomycin and CCCP, as detected by delaying application of valinomycin, indicate M2-induced K+ flux of <1 K+/s and that permeability (flux/concentration) of M2 22-62 to K+, relative to H+, is <10-7. Transport rate, amantadine and cyclooctylamine sensitivity, acid activation, and H+ selectivity are all consistent with full functionality of the reconstituted protein construct.

3560-Pos

***Yersinia* Translocon Complexes are Stabilized in Nanolipoprotein Particles (NLPs)**

Jenny A. Cappuccio[1], Craig D. Blanchette[1], Erin S. Arroyo[1], Angela K. Hinz[1], Feliza A. Bourguet[1], Todd A. Sulchek[2], Brent W. Segelke[1], Paul D. Hoeprich[1], Brett A. Chromy[1], Matthew A. Coleman[1].
[1]Lawrence Livrmore National Laboratory, Livermore, CA, USA, [2]Georgia Tech, Atlanta, GA, USA.

To enter host cells and evade host defenses, many gram-negative bacteria, including the plague pathogen, *Yersinia pestis*, utilize proteins that are able to interact with and enter host membranes. One particular mechanism for invasion is the type III secretion system, which is a highly ordered complex for injecting bacterial proteins into host cells, using a complex referred to as a translocon pore. Our results show cell-free expression of YopB and YopD was enhanced in the presence of liposomes or NLPs. However, liposomes containing YopB/D tended to aggregate and precipitate. In order to enable the study of the type III secretion proteins we have applied cell-free approaches for producing soluble *Y. pestis* membrane associated proteins YopB and YopD that are involved in the translocon pore as a complex supported by nanolipoprotein particles (NLPs). With addition of NLP, the YopB/D complex was rendered soluble. AFM showed that soluble YopB/D complex was associated with NLPs as measured by a height increase compared to NLPs not containing YopB/D. Preliminary AFM results also demonstrated binding between LcrV and YopB/D-NLPs which is indicative of proper folding in the NLP structure. Interaction studies of the YopB/D translocon embedded in a membrane with effectors such as LcrV may elucidate the poorly understood pore-forming event that helps this pathogen to evade the host defenses. Our method is applicable to other membrane proteins and represents a unique solution to solubility and purification problems.

3561-Pos

Location of Transmembrane Segments of Na^+/ca^{2+} Exchanger NCX1 Investigated with Chemical Crosslinkers

Xiaoyan Ren, Debora A. Nicoll, Kenneth D. Philipson.
UCLA, Los Angeles, CA, USA.

The sodium-calcium exchanger (NCX1) is a plasma membrane protein important in regulating calcium in cardiac myocytes. The topological model is comprised of nine transmembrane segments (TMs) and a large intracellular loop, which has two Ca^{2+} binding domains (CBD1 and CBD2), between TMs five and six. CBD1 and CBD2 have been crystallized recently and are important in regulating the function of NCX1. On the other hand, the three dimensional structure of the full length NCX1 is unknown. To gain insights into that 3-D structure, we performed cysteine crosslinking experiments. Pairs of amino acids in different TMs were mutated to cysteine on the backbone of cysteine-less NCX1. The mutated NCXs were expressed in an insect cell line and treated with cysteine-specific chemical crosslinkers followed by SDS-PAGE to determine the proximity of the introduced cysteines. The results allow us to place TMS I, IV and IX into the context of the other TMS. By combining our new results with our previous work (*J Biol Chem. 2006, 281: 22808-14; J Biol Chem. 2001, 276:194-9.*) , we propose that TMs II and VII, which contain a number of hydrophilic residues, are surrounded by the remaining TMs.

3562-Pos

Ca^{2+}-Induced Conformational Changes of Na^+-Ca^{2+} Exchanger Dimers: Role of Ca^{2+} Binding Domains

Michela Ottolia, Bernard Ribalet, John Scott, Kenneth D. Philipson.
Cardiovascular Research Laboratory, UCLA, Los Angeles, CA, USA.

The Na^+-Ca^{2+} exchanger is activated by the binding of cytoplasmic Ca^{2+} to two Ca^{2+} binding domains (CBD1 and CBD2). How binding of Ca^{2+} is translated into exchanger activation is unknown. We investigated Ca^{2+}-dependent movements as changes in FRET between exchanger dimers tagged with CFP or YFP at positions 266 within the large cytoplasmic loop of NCX1.1. The biophysical properties of the fluorescent exchangers are identical to those of the untagged NCX. Fluorescent exchangers were coexpressed in Xenopus oocytes from which plasma membrane sheets were isolated. Upon addition of Ca^{2+}, the coexpressed pair NCX-266CFP + NCX-266YFP showed an increase in FRET in a dose-dependent manner. Similar FRET changes were observed after mutating the Ca^{2+} coordination site in CBD2 (E516L). Exchanger E516L is not Ca^{2+} regulated. In contrast, mutating the Ca^{2+} coordination site in CBD1 (D421A, E451A and D500V) abolished FRET changes. These residues likely disrupt binding of Ca^{2+} to CBD1. Nevertheless, Ca^{2+} regulation of NCX is retained though with a substantial decrease in apparent affinity for Ca^{2+}. These results indicate that the Ca^{2+}-induced conformational changes of NCX dimers arise exclusively from the movement of CBD1. Peptides of Ca^{2+} binding domains, flanked by CFP and YFP, recapitulated the full length exchanger results: CBD1 showed movement upon Ca^{2+} addition while CBD2 did not. A peptide spanning CBD1-CBD2 displayed Ca^{2+}-dependent movement, which was abolished by mutating the Ca^{2+} coordination site in CBD1. Our results indicate the following: 1. Exchanger conformational changes are associated with the occupancy of a high affinity Ca^{2+} binding site exclusively within CBD1. 2. FRET studies confirm that the Na^+-Ca^{2+} exchanger exists as a dimer.

3563-Pos

The Role of Microscopic Interactions for Effective Antibiotic Delivery across the Bacterial Outer Membrane

Brigitte Ziervogel, Balasundaresan Dhakshnamoorthy, Lydia Blachowicz, Benoit Roux.
University of Chicago, Chicago, IL, USA.

Outer membrane protein F (OmpF) allows diffusion of beta-lactam antibiotics across the outer membrane of Gram-negative bacteria. The diffusion limit for translocating molecules is provided by the constriction zone, which defines both the channel diameter at the narrowest region, as well as electrostatic properties due to a unique arrangement of charged residues. Since reduced outer membrane permeability contributes to antimicrobial resistance, it is necessary to identify the role of drug-protein molecular interactions in antibiotic transfer in order to design antibiotics with improved diffusional characteristics. We have co-crystallized *E. coli* OmpF with various antibiotic molecules and observe the density corresponding to the antibiotic inside the OmpF pore. Results of this work give insights into how the charge distribution of the translocating molecule affects binding interactions within the OmpF constriction zone. Furthermore, functional assays and mutational analysis provide evidence that alteration of some key charged OmpF residues has an effect on bacterial cell survival. We are also using computational methods to model the pathways of diffusing antibiotics and measure their residence time in the OmpF pore.

Here we are employing a new approach called the String Method with swarms-of-trajectories to study transition pathways for various zwitterionic and anionic molecules across both WT and mutant OmpF porins. Results of this work will therefore assist in the design of new antibiotics that are more effective in the treatment of bacterial infections. [Supported by NIH grant GM062342].

3564-Pos

Towards a Cell-Free Assay to Investigate Lipid Bilayer Permeation and Efflux Transport of Therapeutic Agents

Denise I. Ilgen, Sara Belli, Klaus Eyer, Vivianne Hermann, Stefanie D. Krämer.
Pharmaceutical Sciences, ETH, Zurich, Switzerland.

Lipid bilayers and efflux transporters like P-glycoprotein (P-gp) represent the most important *in vivo* barriers for therapeutic agents. Driven by ATP hydrolysis, P-gp exports structurally diverse hydrophobic compounds from the cell reducing intestinal absorption and blood-brain barrier passage. P-gp expression has also been linked to the efflux of chemotherapeutic drugs in human cancer cells, contributing to multidrug resistance.

The aim of this project is to adapt a liposomal permeation assay to study lipid bilayer permeation and P-gp transport in parallel. This is achieved by integrating P-gp into liposomes.

Fully functional His-tagged P-gp was overexpressed in HEK293 cells and purified by immobilized metal affinity chromatography. P-gp was reconstituted into liposomes, incorporation was verified by density gradient centrifugation. 90% of the basal ATPase activity originated from P-gp as determined with an anti-Pgp antibody. Cryo-TEM images showed unilamellar vesicles with a homogenous size distribution.

In parallel, we established liposomal assays to investigate membrane partitioning and permeation with the pH-sensitive probe fluorescein, linked to the phospholipid DHPE. A minute pH-shift at the membrane surface due to weak acids or bases entering or crossing the lipid bilayer results in a characteristic change in fluorescence. Well-known P-gp substrates and non-substrates were studied. The resulting fluorescence time-curves followed mono- or biexponential functions and the rate constants of the faster terms were used to calculate the apparent permeation coefficients ($Perm_{app}$). The $Perm_{app}$ values of the compounds investigated do not correlate with their lipophilicity or other physico-chemical parameters, which are generally used to estimate membrane permeation. The data obtained strongly emphasizes the importance of developing a simplified permeation assay.

We thank the OPO Foundation Switzerland and the Electron Microscopy Center Zurich.

3565-Pos

Classification of Human Solute Carrier Superfamily Members Reveals Functional Similarities across Families

Avner Schlessinger, Andrej Sali.
UCSF, San Francisco, CA, USA.

Solute Carrier (SLC) superfamily members are membrane transporter proteins that control the uptake and efflux of solutes, including essential cellular compounds, environmental toxins and therapeutic drugs, across biological membranes. Members of the SLC superfamily can share surprisingly similar structural features despite weak sequence similarities. Identification of sequence relationships among SLC members is needed to enhance our ability to model individual transporters and to elucidate the molecular mechanisms of their substrate specificity and transport.

Here, we describe a comprehensive sequence-based classification of SLC members into families. We classify the proteins using sensitive profile-profile alignments and two classification approaches, including similarity networks. The clusters are analyzed in view of substrate specificity, transport mode, organism conservation, and tissue specificity. SLC families with similar substrates generally cluster together, despite exhibiting relatively weak sequence similarities. In contrast, some families cluster together with no apparent reason, revealing unexplored relationships. We demonstrate computationally and experimentally the functional overlap between representative members of these families. Finally, we identify 4 putative SLC transporters in the human genome.

The SLC superfamily constitutes a biomedically important family of membrane proteins that is highly diverse in sequence. The proposed classification of the superfamily, combined with experiment, reveals new relationships among the individual families and identifies new superfamily members. The classification scheme will inform future attempts directed at modeling the structures of the SLC transporters, a prerequisite for describing their substrate specificity.

3566-Pos

Model-Structure, Mutagenesis and Functional Characteristics of the Human Transporter, NHA2

Maya Schushan[1], Minghui Xiang[2], Etana Padan[3], Rajini Rao[2], Nir Ben-Tal[1].
[1]Tel-Aviv University, Tel-Aviv, Israel, [2]Johns Hopkins University, Baltimore, MD, USA, [3]Hebrew University, Jerusalem, Israel.

Human NHA2 is a novel member of the Cation/Proton Antiporters-2 (CPA2) family, linked to essential hypertension. Using the crystal structure of distant bacterial transporter NhaA as template, producing a model-structure of NHA2 necessitated a composite modeling approach. Through extensive mutagenesis guided by our model, we show that while NHA2 retained some functional and structural core elements of other Na+/H+ exchangers, it exhibited other significant exclusive features. A cluster of highly conserved titratable residues was located in the so-called assembly region, made of two discontinuous helices TM4 and TM11. Whereas in NhaA, oppositely charged residues have been proposed to compensate for partial dipoles generated within this assembly, we demonstrate that in NHA2 uncharged but polar residues suffice. Instead, NHA2 possesses unique, conserved charges predicted to interact with key essential residues. Combining structural data with evolutionary conservation analysis and mutagenesis, we propose a transport mechanism for NHA2, and compare it with mechanisms proposed for NhaA and NHE1. This study illustrates an attractive approach for studying new transporters, starting from structural data to guide initial experimental efforts.

3567-Pos

Functional Reconstitution of a Bicomponent ABC Transporter

Mohammad M. Mohammad[1], David J. Niedwiezki[1], Xianming Lin[2], Richard D. Veenstra[2], Liviu Movileanu[1,3].
[1]Physics Department, Syracuse University, Syracuse, NY, USA, [2]Department of Pharmacology, SUNY Upstate Medical University, Syracuse, NY, USA, [3]Structural Biology, Biochemistry, and Biophysics Program, Syracuse, NY, USA.

The ATP-binding-cassette (ABC) transporters are transmembrane protein nanomachineries present in all living systems. ABC transporters utilize the energy of ATP hydrolysis to transport a variety of solutes across the membrane. *Pseudomonas aeruginosa*, a Gram-negative pathogenic bacterium, employs a bi-component ABC transporter as an active efflux of polysaccharides during the biogenesis of endotoxic lipopolysaccharides. We reconstituted the ABC transporter in various systems, including microsomes, planar lipid bilayers, and transfected mammalian N2a cell lines to obtain a mechanistic understanding of the functional properties of this nanomachinery. We employed single-channel electrical recordings to show that the transmembrane domain (TMD) of the ABC transporter features pore-forming activity. Further, our biochemical characterization of purified components sheds light on the structural assembly and stoichiometry of this bi-component ABC transporter. Our long-term goal is to detect, explore and characterize the translocation of polysaccharides at single-protein complex resolution. The use of a broad range of reconstitution systems enables a comprehensive examination of the ATP-dependent transport kinetics and thermodynamics of the large-size substrates from one side of the membrane to the other. These studies might also contribute to drug design against *Pseudomonas aeruginosa.*

Acknowledgements. This research was supported by grants from the National Science Foundation (DMR-0706517 and HRD-0703452) and the National Institutes of Health (R01 GM088403) as well as by Syracuse Biomaterials Institute (SBI) for Liviu Movileanu and the National Institutes of Health (HL042220) for Richard Veenstra.

3568-Pos

New Immobilized Proteoliposome-Based Biosensor System for Investigating Human ATP-Binding Cassette Transporters

Patrick Seelheim, Michael Seifert, Hans-Joachim Galla.
University of Münster, Münster, Germany.

ATP-binding cassette (ABC) transporters comprise a large family of membrane proteins that transport a variety of organic substrates across cellular membranes. Human ABC transporters are mostly efflux pumps for physiological and xenobiotic substrates related to immune response and cellular detoxification. Nine human ABC transporters play a major role in cellular multi-drug resistance (MDR), thus being called MDR-related proteins (MRPs). So far, the structure of MRPs and human ABC transporters in general is unclear. Sequence analysis and experimental data indicate that functional ABC transporters are composed of two subunits and imply strong positive cooperativity between those entities. To elucidate the transport mechanism and the molecular origin of the cooperativity a solid-supported