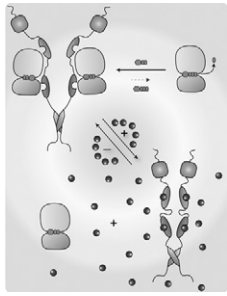
account for either the ability of αCaMKII to self-associate or βCaMKII to be unable to self-associate. Identification of these mutations that disrupt self-association and still allow targeting affords the opportunity to definitively determine the role that self-association plays in the subcellular localization of CaMKII during physiological and pathological conditions.

461-Pos Board B261

Nucleobindin 1 is a Calcium Regulated Guanine Nucleotide Dissociation Inhibitor of GI1

Neeraj Kapoor, Ruchi Gupta, Santosh T. Menon, Ewa folta-Stogniew, Daniel P. Raleigh, Thomas P. Sakmar.

Nucleobindin 1 (NUCB1) is a widely expressed multi-domain calcium-binding protein whose precise physiological and biochemical functions are not well understood. We engineered and heterologously expressed a soluble form of NUCB1 (sNUCB1) and show that sNUCB1 exists as a Calcium binding dimer in solution and binding to Calcium causes conformational changes in sNUCB1. Earlier reports suggested that NUCB1 might interact with heterotrimeric G protein α subunits. We show that dimeric sNUCB1 binds to Gαi1 and that calcium-binding inhibits the interaction. The binding of sNUCB1 to Gαi1 inhibits its basal rate of GDP release and slows its rate and extent of GTPγS uptake. Additionally, our tissue culture experiments show that sNUCB1 prevents receptor-mediated Gαi-dependent inhibition of adenylyl cyclase (AC). Thus, we conclude that sNUCB1 is a calcium dependent guanine-nucleotide dissociation inhibitor (GDI) for Gαi1. To our knowledge sNUCB1 is the first example of a calcium-dependent GDI for heterotrimeric G proteins. We also show that the mechanism of GDI activity of sNUCB1 is unique and does not arise from the consensus Goloco motif found in RGS proteins. We propose that cytoplasmic NUCB1 might function to regulate heterotrimeric G protein trafficking and signalling.

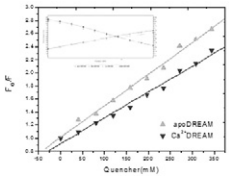


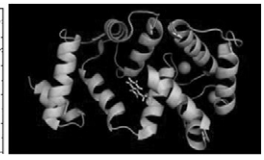
462-Pos Board B262

Conformational Dynamics in Dream Protein

Jaroslava Miksovska.

Downstream Regulatory Element Antagonist Modulator (DREAM), also known as calsenilin or K+ channel interacting protein 3 (KChIP-3) belongs to neuronal calcium sensor proteins that are found predominantly in neuronal cells where they regulate diverse aspects of neuronal function ranging from neurotransmitter release to neuronal growth and apoptosis. DREAM is a highly multifunctional protein that binds to presenilin, acts as a transcriptional repressor for a number of genes including *c-fos* gene and prodynorphin gene in Ca^{2+} dependent manner, and interacts and modulates the activity of A-type of K+ channels. To obtain insight into the Ca2+ signaling mechanism, the impact of binding of metals and DNA on conformational heterogeneity and dynamic motion of DREAM was probed using steady-state and time-resolved fluorescence and anisotropy techniques. In addition the role of the individual EF-hands in the Ca^{2+} signal transductions was determined by investigating DREAM mutants with the impaired EF-hand 3 and EF hand 4.





463-Pos Board B263

The Interaction of S100a1 with Type-2b Regulatory Subunit of Protein Kinase A

Brian Cannon, Erick Hernández-Ochoa, Kristen Varney, Martin Schneider, David Weber.

The S100 family of proteins consists of 24 calcium-activated signaling molecules that are involved in a variety of biological processes. They are expressed exclusively in vertebrates in a tissue-specific manner. One member of this family, S100A1, is involved in several biological processes. In the heart, S100A1 has been shown to be essential for cadiac function (Pleger et al, 2007). In skeletal muscle, S100A1 is known to help modulate excitation-contraction coupling through interaction with the ryanodine receptor (Prosser et al, 2008). Furthermore, in ganglion neurons exogenous S100A1 increases sympathetic output by enhancing Cav1 channel currents (Hernández-Ochoa et al, 2009). This effect is occluded by the inhibition of PKA, suggesting a PKA-dependence of this process. Our laboratory has now used ITC and NMR to demonstrate that S100A1 interacts with the type-2β regulatory subunit of PKA.

464-Pos Board B264

Differential Activation of Intracellular Calcium Oscillations by Multiple Calcium and L-Phenylalanine Binding Sites Located at the Extracellular Domain of Calcium-Sensing-Receptor

Chen Zhang, Yun Huang, Yusheng Jiang, Hing Wong, Xue Wang, Adriana Castiblanco, Ling Wei, Edward Brown, Jenny J. Yang.

Calcium sensing receptor (CaSR), along other members of the family C G protein-coupled receptors (GPCRs), play very important roles in responding to changes in the extracellular calcium concentrations and in circulating levels of amino acids and integrating these extracellular signals into alterations in intracellular signaling pathways. We have reported several potential calcium-binding sites located within the CaSR's extracellular domain using our developed computational algorithms based on geometric factors and surface electrostatic potentials. In the present study, we first report the differential effects of several disease-related mutations located at the predicted calcium binding sites on the inhibition and activation of intracellular calcium responses using both a cell population assay and single cell imaging. We then identify a potential amino acid binding site using computational methods and site-directed mutagenesis. The effect of amino acid binding in altering intracellular calcium responses, especially calcium oscillations, and its synergistic interaction with the effects of extracellular calcium on these parameters are also investigated. A common activation mechanism for CaSR and other family C GPCRs, such as mGluR1 by extracellular calcium and amino acid is been proposed. These results could have important implications for our understanding of how the CaSR integrates information about these two completely different classes of agonists–one an inorganic divalent cation, the other a nutrient–and how the receptor senses these agonists in health and in disease states.

Epithelial Channels & Physiology

465-Pos Board B265

Living on the Edge: Mechanisms of Single Cell Responses at Air-Liquid Interfaces

Nina Hobi, Andrea Ravasio, Thomas Haller.

Many epithelia have contact with air-liquid interfaces. This applies particularly to the lung, where one of the epithelial cell types, the surfactant secreting AT II cells, even project into the air-filled alveolar lumen. This specific environment may be of considerable physiological relevance; however, only few data exist to provide a satisfying description. This is mainly due to the experimental difficulty to manipulate cell-air contacts in a specific way. In previous investigations, using new microscopic approaches, we found that the presence of an air-liquid interface leads to a paradoxical situation: it is a potential threat that causes cell injury, but also a potent stimulus: AT II cells respond promptly, and show sustained Ca^{2+}-signals that activate exocytosis. Exocytosed surfactant, in turn, clearly prolonged the time to irreversible cell damage, and may be an adaptive defense against the harmful nature of surface forces. The strength of this stimulus became also apparent by a rapid and significant change on the transcriptional level: cellular pathways that are involved include e.g. defense response and lipid metabolism, and a Pubmatrix search identified genes associated with several lung diseases and injuries. Furthermore, we found that the signalling mechanisms underlying sensation of an air-liquid interface can be sufficiently explained by mechanical forces. These forces trigger cellular events that are closely related with classical concepts in mechanotransduction. In conclusion, we suggest that an air-liquid interface has to be regarded as a specialized form of an extracellular matrix. This matrix, probably an important constraint in the evolution of air-exposed biological surfaces, exerts distinct physical stimuli which are very well perceived by the cells.

466-Pos Board B266

Electrodiffusion and Osmotic Water Flow and its Variational Structure

Yoichiro Mori, Chun Liu, Robert S. Eisenberg.

We propose a system of partial differential equations (PDE) that describe electrodiffusion and osmotic water flow. From a physical standpoint, this is a far-reaching generalization of the standard treatment of osmosis and electrodiffusion in irreversible thermodynamics to spatially extended systems. As far as we know, this is the first mechanically and thermodynamically consistent model of osmotic water flow and electrodiffusion in systems with deformable cells and membranes with capacitance and conductance. We use an energetic variational approach to enforce consistency and derive a field theory describing the flow, diffusion, and migration of ions, water, and the solution itself. The variational approach is particularly useful because it treats interactions automatically and consistently with a minimal number of arbitrary parameters. Electrodiffusion and osmotic water flow are involved in a wide range of biological functions of organs, tissues, cells, and organelles, including the homeostasis of ions in the brain, fluid secretion by epithelial systems, electrolyte

regulation in the kidney, fluid circulation in ocular systems, gastric protection, water uptake by plants, etc. The field equations can be written with boundary conditions and parameters appropriate for the anatomy of each system. The field equations then form a physically and anatomically consistent model of biological function in the variational framework of modern field theory. The variational approach deals naturally with the many ionic solutions (containing a multitude of interacting components in a wide range of concentrations) and the wide range of conditions and forces used in experiments. Solving the PDEs will help suggest and interpret new experiments to understand the interaction of components, conditions, structure, and forces. In the view of classical physiology and biophysics, these interactions are the essence of biological function.

467-Pos Board B267

Revisiting the Heteromultimeric Structure of ENaC in *XENOPUS Leavis* Oocytes

Rosanna De Nuccio, Miguel van Bemmelen, Ivan Gautschi, Laurent Schild.

The functional epithelial sodium channel (ENaC) is a heteromeric channel formed by three homologous alpha, beta and gamma subunits.

Several functional and biochemical studies have provided evidence that the ENaC is a heterotetramer formed by 2alpha, 1beta and 1gamma subunits. Recently, a high-resolution crystal structure has been obtained from an ortholog of ENaC, the acid-sensing ion channel ASIC1, which showed a homotrimeric channel. This discrepancy between two channels of the same ion channel family, motivated us to revisit the subunit oligomerization of ENaC.

His-tagged ENaC alpha, beta and gamma subunits were expressed in *Xenopus leavis* oocytes. The three ENaC subunits can be co-purified on Ni^{+2}-NTA agarose beads in an alpha beta gamma-ENaC complex. On Western blot, the ENaC subunits showed typical post-translation modifications associated with a functional channel. Using differentially tagged ENaC subunits, we investigated whether the ENaC complex contains more than a single alpha, beta or gamma subunits. Two differentially tagged alpha subunits co-purified with beta and gamma subunits, indicating that ENaC is formed by more than one alpha subunit. The purified ENaC complex channel eluted on Sephadex G 200 column in a fraction corresponding to a molecular weight of 350 kDa, which was higher than expected for a alpha beta gamma-ENaC.

These data confirm previous reports that the functional ENaC channel is a heterotetramer made of 2alpha 1beta, and 1gamma subunits.

468-Pos Board B268

xshroom1 Regulates the Number of ENaC Channels Inserted in the Membrane of Oocytes From *XENOPUS Laevis*

Yanina A. Assef, Luciano Galizia, Gabriela I. Marino, **Basilio A. Kotsias**.

Shroom is a family of proteins linked to the actin cytoskeleton. We studied its effect upon the currents through ENaC channels (INa_{amil}) in oocytes (*X. laevis)* injected with α, β, and γ mENaC and xShroom1 sense or antisense oligonucleotides. We observed a strong reduction in INa_{amil} with the xShroom1 antisense: inward conductances (G_{inward}) (-160 to 0 mV) were 36 ± 12 μS and $1.80 \pm .50$ μS with xShroom1 sense and antisense. Similar results were obtained in oocytes expressing a mutant β-mENaC subunit (β-S518K) with a open probability of 1 (G_{inward} 65 ± 10 μS and 1.80 ± 2.0 μS for oocytes with xShroom1 sense or xShroom1 antisense. The negative effects of xShroom1 antisense can not be reversed with forskolin which reduced the rate of ENaC retrieval: G_{inward} : 124 ± 27 μS and 7.0 ± 1.9 μS with xShroom1 sense or xShroom1 antisense. Trypsin in the range of ng/ml activates the membrane-resident ENaC channels (Bengrine et al.2007), being this effect dependent on activation of G-proteins. Addition of 20 ng/ml of trypsin led to a slow increase in INa_{amil} with xShroom1 sense and it had no effect in most of the oocytes coinjected with ENaC and xShroom1 antisense (2 out of 20). Trypsin were without effects on the endogenous conductances. These data are consistent with the idea that the reduced INa_{amil} when xShroom1 is blocked is most probably due to a lack of functional ENaC channels in the plasma membrane.

Acknowledgements: ENaC cDNAs were provided by Dr M. Carattino (Pittsburgh, Pa). and the set for oocytes was a gift of Dr C. Peracchia (Rochester, NY). Supported by grants MO35 (UBA) and PICT 38181.

469-Pos Board B269

Synthetic Peptide-Based Channels: Candidates for Treatments of Channelopathies

Urska Bukovnik, Monica Sala-Rabanal, Colin Nichols, Bruce Schultz, Jianhan Chen, John Tomich.

Modern approaches for treatments of channelopathies employ mutation-specific pharmacotherapies, use pharmacoagents alone, or a vast variety of ideas for genetic approaches, all with the purpose of restoring defective ion channels or attenuate their negative effects. An alternative to existing approaches is the use of synthetic channel-forming peptides (CFPs) with desirable selectivity, high ion transport rates and overall ability to supersede defective endogenous ion channels. Our synthetic CFPs represent derivatives of a peptide initially reconstituted from the second transmembrane segment of the α-subunit of Glycine receptor (M2GlyR), PARVGLGITTVLTMTTQSSGSRA. Due to poor efficiency of membrane insertion, tendency to form aggregates in aqueous solutions and inconsistent channel forming potentials, parent peptide sequences were modified to alter channel-inducing properties. Resulting NK_4-M2GlyR T19R, S22W, with two threonines replaced by a non-natural amino acid, diamino propionic acid (Dap) (NK_4-M2GlyR T19R, S22W, TT-Dap) represent our best candidates. We examined potential channel pore-lining residues using molecular dynamics studies followed by the sulfhydryl replacement technique. Identified residues involved in ion selectivity filter seem to represent a ring of β-hydroxyls from the threonines at position 17 and 13. Ussing chamber experiments employing MDCK cells and voltage-clamp studies using *Xenopus* oocytes revealed that introduction of Dap substitutions at pore-lining residues yields improved channel conductances. In conclusion, in addition to their ability to form soluble monomers in aqueous solutions, ability to self-assemble into homopentamers, efficient delivery to and insertion into the membranes of tested MDCK cells, NK_4-M2GlyR T19R, S22W, TT-Dap peptides show enhanced ion transport activities at low peptide concentration compared to their parent sequence (NK_4-M2GlyR T19R, S22W).

470-Pos Board B270

Molecular Modeling and Simulation of a Synthetic Peptide Channel

Jian Gao, John Tomich, Jianhan Chen.

Many human diseases, including episodic ataxia, diabetes, epilepsy, cystic fibrosis and Alzheimer's dementia, are related to defective ion channels. A series of channel forming peptides derived from the second transmembran domain of the α1-subunit of the glycine receptor (M2GlyR) have been designed by the Tomich lab with improved anion conduction rate and aqueous solubility. To rationally understand the physiological properties of these synthetic channels and to identify improved designs, we combine NMR, biophysical data, and molecular modeling to provide a structural basis for understanding key physiochemical properties that govern the chloride conductivity and selectivity. Initial structural models were first constructed for one of our lead design, p22-T19R/S22W (KKKKP ARVGL GITTV LTMRT QW), primarily based on the monomer structure from solution NMR, amphipathicity consideration, and the oligomeric state of the channel assembly. Long molecular dynamic simulations in explicit membrane and water were then carried out to characterize the channel structural and dynamic properties. Interestingly, independent simulations from initial constructs with different handiness of helix packing (left, straight, and right) all converge to a similar structural ensemble with left-handed helix assembly. The predicted pore-lining residues are also in excellent agreement with a previous set of cysteine-scanning experiments. Coupled with parallel experimental characterizations in the Tomich lab, the simulation provides important insights into the structural basis of the activity of these synthetic channels.

471-Pos Board B271

Two Genetic Variants of TRPM6 Increase Risk for Hypomagnesemia Associated with Diabetes Mellitus Type 2

Anil Nair, Berthold Hocher, Thiemo Pfab, Martin Konrad, Femke Van Zeeland, René Bindels, Joost Hoenderop.

Diabetes mellitus type 2 (DM2) is characterized by high serum glucose levels, insulin resistance and hypomagnesemia. DM2-related hypomagnesemia has been linked to several chronic diabetic complications. Transient receptor potential melastatin 6 (TRPM6) channel plays an essential role in whole body Mg2+ homeostasis. It has been suggested by conducting nested case control study that two non-synonymous single nucleotide polymorphisms (SNP) of TRPM6, TRPM6(V1393I) & TRPM6(K1584E) might increase risk for DM2 in elderly women. Here, by measuring Total Glycosylated Hemoglobin (TGH) from 997 women in their last weeks of pregnancy as a measure of glucose control, we show that the two SNPs (6.8 %) have higher TGH compared to control subjects (6.3 %). Upon overexpression in a human kidney cell line, whole-cell patch clamp analysis showed that insulin activates (EC50 = 0.16 nM) TRPM6, but not the SNPs. Inability to phosphorylate T1391 was the identified factor for lack of insulin-mediated V1393I activation. We further demonstrate that TRPM6 stimulation is independent of its own kinase activity, but relied on both Phosphoinositide 3-kinase and Rac1. The impaired response of the SNPs to insulin may lead to hypomagnesemia causing insulin resistance. These SNPs could be used as markers to improve diagnosis and identify those at risk for developing DM2.