**3600-Pos**

**PH Domain Mutation Alters Membrane Targeting Specificity of GRP1 and AKT**

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Protein kinase B (AKT1) and General Receptor for Phosphoinositides 1 (GRP1) regulate multiple cell signaling pathways essential for cell function and survival. AKT1 possesses an N-terminal PH domain (AKT1-PH) that binds important target phosphatidylinositol phospholipids (PIPs) such as PI(3,4)P3 and especially PI(3,4,5)P3. During a signaling event, PI(4,5)P2 gets converted to PI(3,4,5)P3, resulting in targeting of AKT1 to the cell plasma membrane by its PH domain. Our recent published work (Landgraf (2008) Biochemistry) revealed that the E17K charge reversal mutation adjacent to the PIP binding site in AKT1-PH alters the PIP specificity of the site, yielding high affinity binding to PI(4,5)P2 and constitutive binding to plasma membrane. The resulting hyper-activation of AKT1 inhibits apoptosis and explains the known oncogenic character of the E17K mutation, which is linked to multiple human cancers. Here we test the hypothesis that glutamate residues often found adjacent to the PIP binding sites of PI(3,4,5)P3 -specific PH domains are essential to PIP lipid specificity. Using a combination of biochemical, structural, and cell biology approaches, we compare the PIP lipid specificities and intracellular targeting of GRP1-PH and E345K-GRP1-PH. The latter mutant possesses a charge reversal mutation at a glutamate position adjacent to the PIP binding site. The preliminary findings, which will be presented at the meeting, indicate that the mutant exhibits high affinity binding to PI(4,5)P2 and constitutive binding to plasma membrane. These findings support the hypothesis that the adjacent glutamate residue plays an essential role in PI(3,4,5)P3 -specificity and PI(3,4,5)P3 -regulated plasma membrane targeting in cells. Finally, we are investigating the structural basis for the specificity change by attempting to solve the crystal structures of the E17K-GRP1-PH and E345K-GRP1-PH mutants bound to PI(4,5)P2.

**3601-Pos**

**Engineered Nanolipoproteins as Biosynthetic Decoys for Pathogen-Binding**

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The ability to exogenously present cell-surface receptors in a synthetic system offers an opportunity to provide host cells with protection from pathogenic toxins. Practical implementations suffer from serious limitations, primarily because of difficulties in mimicking the role of the membrane micro-environment during the complex and dynamic pathogen-receptor interaction. To this end, we have developed reconstituted lipoprotein - nanometer-sized discoidal lipid bilayers of arbitrary composition bounded by apolipoprotein - to serve as a versatile, biocompatible and stable platform to house pathogen-binding receptors in a membrane-like environment. Our approach exploits the notion that a control of biophysical properties of the membrane micro-environment allows to modulate interactions between membrane-embedded receptors and their pathogenic (e.g., bacterial toxin) targets. We demonstrate here, using a Foerster Resonance Energy Transfer (FRET) based assay, that ganglioside GM1 receptors incorporated at controlled concentrations in reconstituted lipoprotein bind cholera toxin with greater affinity than liposome-based systems. Furthermore, fluorescence microscopy investigations of cholera toxin presented to populations of mammalian cells show that GM1-laden lipoprotein can function as decoys without harming healthy cells.

**3602-Pos**

**Crystallization of Calcium Carbonate Vaterite Associated with Liquid Crystal in Embryonic Yolk Sacs**

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Calcium carbonate is often used as an efficient antacid that absorbs and neutralizes stomach acid while providing calcium for healthy bones. Taking advantage of the lack of adverse side effects of calcium, new drug delivery systems consisting of drug-supported spherical microparticles are being developed. We have reported in our previous studies that a natural process producing calcium carbonate microparticles can be found during avian development. These natural systems provide inspiration for designing more efficient microparticle facilitated drug-delivery systems. In this study, the formation and re-absorption of calcium carbonate crystals were tracked during Gallina *N. meleagris* embryogenesis and early postnatal development. The study demonstrated that the formation of calcium carbonate microparticles, as calcium is transferred from the eggshell into the egg sac, is a process of calcium preservation. X-ray diffraction showed that calcium carbonate crystal is mainly preserved in the vaterite isoform. Calcium incorporated into the yolk sac during this process can be easily assimilated as necessary during postnatal development. Eons of evolution have yielded a calcium preservation process that produces an iso-form of crystalline calcium most readily absorbed by the organism. Our previous results indicate that this biological system is likely a lyotropic process, the method that is currently being used for the production of microparticle drug delivery systems. In this work, our data suggests that calcium carbonate crystal can also initiate its crystallization from the center of liquid crystal, recognizable by a chimeric thermal phase transition. Our work provides valuable information for designing more efficient microparticle for drug-delivery.

## Voltage-gated Ca Channels II

**3603-Pos**

**Stim1 Binds to and Inhibits CaV1.2 Voltage Gated Calcium Channels**

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CaV1.2 and other L-type voltage gated calcium channels play a key role in regulating cardiac contraction, synaptic plasticity, insulin secretion and a variety of other cellular events. Phospho-inositide linked receptors like the muscarinic acetylcholine receptor, inhibit L-type calcium channels and this inhibition is important for parasympathetic regulation of heart contraction as well as for learning and memory in the brain. The mechanisms by which PLC coupled receptors inhibit L-type channels are still controversial though several hypotheses including reduction of cAMP and depletion of PIP2 from the cell membrane have been proposed. We report a new and unexpected mechanism by which PLC-coupled receptors inhibit L-type calcium channels in cells. We have found that depletion of ER calcium stores either down stream of muscarinic receptors or following application of the ER calcium ATPase inhibitor, thapsigargin, inhibits CaV1.2 channels. CaV1.2 inhibition depends on binding to Stim1, an ER calcium sensor protein that activates the Orai family of store operated calcium channels. In cells expressing CaV1.2, Stim1 translocates to ER-plasma membrane junctions and co-localizes with clusters of CaV1.2. In vitro and in vivo studies indicate that the CAD domain of Stim1 binds to a coiled coil in the II-III loop of CaV1.2. Stim1 lacking the CAD domain is unable to bind to CaV1.2 and fails to inhibit CaV1.2 currents following depletion of ER calcium stores. These studies support a new mechanism by which phosphoinositide-linked receptors inhibit L-type calcium channels and suggest that Stim1 dynamically regulates the relative contributions of Orai and CaV1.2 channels to calcium influx in excitable cells.

**3604-Pos**

**Oxygen-Sensing of L-type Calcium Channels in Rat Cardiomyocytes: The Possible Role of Hemoxygenase**

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Hemoxygenases (HO)-1 and −2 are enzymes that metabolize the heme group, generating CO, biliverdin and $Fe^{+2}$. HO-2 is believed to be an $O_2$ sensor in the carotid body controlling the release of dopamine as it needs $O_2$ for its function. HO-2 is necessary for the anoxic activation of nociceptive neuron and binds to calmodulin in neurons, and the complex is modulated by $Ca^{2+}$. Previously, we have found that L-type $Ca^{2+}$ channel is suppressed by 22-43% with acute anoxia prior to metabolic impairment or run down of Ica . This property appeared to require the calmodulin-binding IQ motif on the cytoplasmic carboxy-tail of the channel. Here we probe whether HO mediates this rapid Ica-suppressant effect of anoxia thus serving as the $O_2$ sensor of the heart. Freshly isolated rat cardiomyocites were maintained in Tyrode containing (in mM): 135NaCl, 5.4KCl, 10Hepes, 10glucose, 1$MgCl_2$ and 2$CaCl_2$). Internal solution used was (in mM): 15NaCl, 118CsCl, 5.5Glucose, 14EGTA, 3.92$CaCl_2$, 10Hepes, 3MgATP, 0.5$MgCl_2$. Solutions bubbled with 100% $O_2$ or 100% $N_2$ were rapidly (50ms) applied. $I_{Ba}$ was measured replacing $CaCl_2$ for $BaCl_2$. HO inhibitors ZnPP-IX 100nM or SnPP-IX 100nM were added to either 100% $O_2$ or 100% $N_2$ solutions. Both the $O_2$ removal and the inhibition of HO blocked