3628-Pos Board B356
Revealing Transient Interactions Between Phosphatidylinositol-Specific Phospholipase C and Phosphatidylcholine-Rich Lipid Vesicles
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Using *Bacillus thuringiensis* phosphatidylinositol-specific phospholipase C (*Bt*PI-PLC) as a model amphitropic protein, we are investigating how membrane structure and composition affect protein-membrane interactions. Previous work showed that *Bt*PI-PLC specifically binds to phosphatidylcholine (PC)-rich membranes and preferentially interacts with unilamellar vesicles with high curvature. In this work, we monitored single fluorescently labeled *Bt*PI-PLC proteins as they cycled on and off surface-tethered phosphatidylglycerol (PG)/PC small unilamellar vesicles (SUVs) using total internal reflection fluorescence (TIRF) microscopy. The residence times on vesicles along with vesicle size information, based on vesicle fluorescence intensity, reveal the time scales of protein-membrane interactions as well as the curvature dependence. *Bt*PI-PLC residence times on SUVs average 300 ms, similar to published residence times (300-400 ms) for other amphitropic proteins that transiently interact with cell surfaces. The kinetics of PI-PLC/membrane interactions is well explained by a simple two state binding model with dissociation and association rate constants averaging 3 s^{-1} and 0.6 $\mu M^{-1}s^{-1}$ respectively. In addition fluorescence correlation spectroscopy (FCS) measurements indicate that introducing lipid packing defects PG/PC SUVs by incorporating low mole percentages of dioleoylglycerol (DOG) enhances *Bt*PI-PLC binding to SUVs. By combining these single molecule fluorescence results with previous biophysical measurements and molecular dynamics simulations, we have developed a quantitative model showing how the bacterial virulence factor *Bt*-PI-PLC interacts with cell membranes in molecular detail.

3629-Pos Board B357
Interplay of Membrane Lipids Differentially Affects Lipid Binding of Phosphatidic Acid Effectors
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The interaction of phosphatidic acid (PA) with membrane proteins is responsible for a host of cellular functions. To date no PA specific binding domain has been identified. Instead, electrostatic and hydrophobic interactions are likely to work in tandem to regulate PA effector-PA binding. Electrostatic interactions with the PA headgroup are explained by the electrostatic-hydrogen bond switch model, whereas hydrophobic interactions are explained by the negative curvature of (unsaturated) PA. In order to shed light on PA-protein binding we study the interaction of PA with PA effectors in complex lipid mixture, not just phosphatidylcholine (PC) bilayers, using liposome binding assays. Previously we showed that PE differentially affects the binding of PA effectors. We extended this work to now show that the opposite effect is observed in the presence of lyso-phosphocholine (LPC). We also show that under the right conditions diolyeoyl glycerol (DOG) stimulates binding to PA for a well-known and extensively characterized PA-binding protein. PA-effector-PA binding is thus significantly affected by the presence of other membrane lipids. These studies show the need to incorporate other membrane lipids when investigating the interaction of putative PA binding proteins with PA, thereby further our understanding of PA mediated signaling.

3630-Pos Board B358
The Protein that Held Back the Dye: Annexin's Effect on Membrane Permeability
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The membrane is more than a barrier that protects the cell; composed of lipids and protein, the membrane is implicated in signaling, cell stability, and protein interactions. They must be able to respond to stressors that can affect these roles, and employ different membrane components for that purpose. Cholesterol is a common component to both monolayers of the eukaryotic plasma membrane, moving freely and relaying of information such as changes in lipid distribution. The annexin family of membrane-associated proteins constitutes two percent of eukaryotic proteins within the cell. Annexins interact with multiple binding partners including small molecules like Ca^{2+}, phospholipids, and other proteins that are often involved in membrane repair. To determine how binding of annexin impacts the permeability of the membrane, carboxyfluorescein (CF) release assays were performed. CF efflux from vesicles in the presence of annexin a5 without Ca^{2+} showed a slight decrease compared to the control of vesicles alone; however, with the addition of both annexin and Ca^{2+}, the signal decrease was greater. In order to observe the effects of both cholesterol and protein on permeability, CF studies were repeated on vesicles containing increasing mole fractions of cholesterol. Less CF was released from vesicles containing cholesterol, and an even greater decrease was observed with annexin and Ca^{2+} added. This suggests that in the presence of Ca^{2+}, annexin works to reduce the permeability of the membrane, especially for cholesterol-containing vesicles. In previous work with isothermal titration calorimetry (ITC) we also observed a change in the Ca^{2+} binding parameters and stoichiometry of annexin a5 in the presence of cholesterol-containing membranes. This combined data, leads us to hypothesize that through their calcium binding ability, annexins sense the distribution of lipids and help communicate changes in the membrane environment.

3631-Pos Board B359
Membrane Insertion Pathway of the Apoptotic Repressor Bcl-xL: How (DIS)Similar is it to that of Diphtheria Toxin T-Domain?
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The anti-apoptotic repressor Bcl-xL inserts into the mitochondrial membrane as a supposed part of its physiological action. While the exact molecular mechanism of this transition is poorly understood, the structural similarity of the water-soluble state of Bcl-xL with that of the diphtheria toxin T-domain led to the suggestion that their membrane-insertion pathways will be similar as well. Here we test this hypothesis by applying an array of spectroscopic methods to characterize and compare conformational switching and membrane insertion of the two proteins. CD spectroscopy and thermal denaturation measurements indicate that, unlike the T-domain, Bcl-xL is resistant to acid-induced destabilization in solution. FRET measurements between donor-labeled protein and acceptor-labeled vesicles demonstrate that Bcl-xL undergoes reversible membrane association strongly modulated by the presence of anionic lipids. In contrast, initial stages of membrane action of the T-domain are largely lipid-independent, with anionic lipids playing a role only on the later stages of a multi-step insertion pathway. Site-selective attachment of environment-sensitive fluorophore NBD to the helical hairpin of Bcl-xL ($\alpha5$-$\alpha6$) or the T-domain (TH8-TH9) reveals similarities in the topology of the inserted state, but not in the lipid-dependent kinetic regulation of the insertion transition. Taken together our results indicate that while Bcl-xL and the T-domain share structural similarities, their mode of conformational switching and membrane insertion pathways are distinctly different. We suggest that these variations reflect underlying physiological differences: while cellular entry of the toxin via endosomal pathway requires robust insertion of the T-domain, the apoptotic control through the action of Bcl-xL and other members of the Bcl-2 protein family involves multiple levels of regulation, including those modulated by changes in mitochondrial lipid composition.
Supported by NIH GM-069783 (A.S.L.) and Fulbright-CONICYT (M.V.U.).

3632-Pos Board B360
Do Acidic Residues in TH8-TH9 Play a Role in Transmembrane Insertion of the Diphtheria Toxin T-Domain?
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The translocation (T)-domain plays a key role in the entry of diphtheria toxin into the cell, where it inserts into the endosomal membrane and transfers the catalytic domain into the cytosol in response to endosomal acidification. Protonation of the three acidic residues located in the hydrophobic helical hairpin TH8-TH9 (E349, D352 and E362) has been suggested to modulate transmembrane insertion of the T-domain. Here, we test this hypothesis by combining site-directed mutagenesis with assays that test the conformational switching and bilayer insertion in the context of either isolated helical fragments or that of the entire T-domain. The propensity of individual helices to adopt a transmembrane conformation, studied using translocon-assisted insertion, reveal that only the most hydrophobic helix TH8 has a marginally favorable free energy of insertion. The free energy for TH8-TH9 hairpin was more favorable, yet much lower than that for the entire protein, suggesting a cooperative effect for T-domain membrane insertion. While mutations of acidic residues had no effect on insertion of individual helices, they had an effect in the context of the entire protein. E.g., E362Q mutant labeled with the environment-sensitive fluorophore NBD in the middle of TH9 inserts more efficiently than

the WT under mildly acidic conditions, but less efficiently upon further acidification. CD spectroscopy, intrinsic fluorescence and size-exclusion chromatography suggest that this mutant is more susceptible to acid destabilization and is prone to aggregation at neutral and mildly acidic pH. We suggest that the principal role of acidic residues in TH8-TH9 segment is not to modulate pH-dependent insertion directly, but to control the early stages of refolding in solution by protecting hydrophobic surfaces of the protein prior to initiation of membrane interactions. Supported by NIH GM-069783, Fulbright-CONICYT.

3633-Pos Board B361

Crucial Role of H322 in the Folding of Diphtheria Toxin T-Domain into the Open-Channel State

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The translocation (T) domain plays a key role in the entry of diphtheria toxin into the cell. Upon endosomal acidification, the T-domain undergoes a series of conformational changes that lead to its membrane insertion and formation of a channel. Recently, we have reported that the triple replacement of the C-terminal histidines H322, H323 and H372 with glutamines prevents the formation of open channels in planar lipid bilayers. Here, we report that this effect is primarily due to the mutation of H322. We further examine the relationship between the loss of functionality and membrane folding in a series of mutants with C-terminal histidine substitutions using spectroscopic assays. The membrane insertion pathway for the mutants differs from that of the wild type as revealed by membrane-induced red-shift of tryptophan fluorescence at pH 6.0-6.5. T-domain mutants with replacements at H323 and H372, but not at H322, regain wild type-like spectroscopic signature upon further acidification. Circular dichroism measurements confirm that affected mutants misfold during insertion into vesicles. Conductance measurements reveal that substituting H322 dramatically reduces the numbers of properly folded channels in a planar bilayer, but the properties of the active channels appear to be unaltered. We propose that H322 plays an important role in the formation of open channels and is involved in guiding the proper insertion of the N-terminal region of the T-domain into the membrane. Supported by NIH GM069783 and in part by Fulbright Foundation (MVU).

Membrane Receptors and Signal Transduction IV

3634-Pos Board B362

Activation of Inhibitory G Protein Catalyzed by GPCR: Molecular Dynamics Simulations of the Activated Cannabinoid CB2 Receptor/ Gαi1β1γ2 Protein Complex

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The GPCR signaling cascade via the G protein pathway begins with an agonist binding to an inactive GPCR, causing conformational changes that activate the GPCR. Previously, we used molecular dynamics simulations to study the activation of the CB2 receptor (a Class A GPCR), by the endogenous ligand, 2-arachidonoylglycerol (2-AG) via the lipid bilayer (Hurst et al., 2010). In the next step of our study of the G-protein signaling cascade, we studied G-protein activation by an activated GPCR. In this step, GDP is released via the separation of the Gαi1 ras-like and helical domains of (Van Eps et al., 2011). To this end, we used our 2-AG activated CB2 model to produce an initial 2-AG/CB2/ Gαi1β1γ2 complex based on the crystal structure of β2 adrenoreceptor in complex with Gαsβ1γ2 (Rasmussen et al., 2011). The complex was immersed in a fully hydrated 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) bilayer and NPT NAMD2 (Phillips et al., 2005) molecular dynamics (MD) simulations were initiated for four different trajectories of this system. Results from our longest MD simulation (3μs) suggest that the C-terminal α5 helix of Gαi prefers a different orientation in the CB2 activated receptor relative to the orientation seen in the empty state (GDP-GTP less) β2 adrenoreceptor/ Gαsβ1γ2 complex. Initial hydrophobic interactions between P139 on CB2 intracellular loop 2 (IC-2 loop) and a hydrophobic pocket on Gαi consisting of residues V34 (N terminus); F336, T340, I343 and I344 (C terminus); L194 (β1-sheet); and F196 (β2-sheet) stabilize the receptor / Gαi1 protein interface during the first 0.5μs of the simulation. Later, between 1.4 - 1.6 μs, electrostatic interactions between R229 (CB2 IC-3 loop) and Q304/E308 (Gαi1 α4 helix) facilitate hydration of GDP. [Support: RO1 DA003934 and KO5 DA021358 (PHR)].

3635-Pos Board B363

Resolvin D1, a Trihydroxylated DHA Derivative, Displays Anti-Hyperreactive Effects on Human Pulmonary Arteries

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Pulmonary Hypertension (PH) is a rare and progressive disease characterised by an inflammatory status and vessel wall remodeling, resulting in an increased pulmonary arteries resistance. In the last 20 years, pharmacological treatments have been proposed. However PH remains associated with an important morbidity. Recent studies demonstrate that omega-3 fatty acid derivatives, such as docosahexaenoic acid monoacylglyceride (MAG-DHA) displays anti-inflammatory and modulatory electro-physiological effects (Morin et al., 2008). The goal of this project is to evaluate the sensitivity of human pulmonary arteries (HPA) to omega-3 derivatives and to assess the effects of Resolvin D1 (RvD1), on their pharmacological reactivity. Specific Objectives: 1) To asses the effects of RvD1 and docosapentanenoic acid monoacylglyceride (MAG-DPA) on the mechanical tension and Ca^{2+} sensitivity, developed by HPA treated or not with 5 nM endothelin-1 (ET-1). 2) To test the effects of KCl, 5-HT, U-46619 and PDBu Under various experimental conditions. 3) To evaluate the mode of action of RvD1 and MAG-DPA on the expression and phosphorylation level of various proteins. Our results demonstrate that 5 nM ET-1 pretreatment during 24 h increased the reactivity and Ca^{2+} sensitivity of HPA. 300 nM RvD1 pretreatment decreased the hyper-reactivity induced by ET-1 and also decreased the pharmacological responses. 1μM MAG-DPA pretreatment decreased the mechanical tension induced by ET-1 and the agonists. RvD1 and MAD-DPA also decreased the expression of TMEM 16 A and the phosphorylation level of CPI-17 protien. Thus we demonstrate for the first time, that RvD1, a well known antiinflammatory compound displays modulatory effects on HPA contractile properties.

3636-Pos Board B364

Quantitative Analysis of Receptors and Second Messengers Interactions in Pancreatic Beta Cell

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The response of insulin secretion in pancreatic beta cell to nutrient stimuli and hormonal modulators is coordinated by the different receptors, messengers and signaling networks. We present an updated computational model of second messenger interactions in pancreatic beta cell that incorporate modern data on glucose metabolism, plasma membrane potential, G-protein-coupled-receptors (GPCRs), cytoplasmic and endoplasmic reticulum calcium dynamics, cAMP and phospholipase C pathways. Model includes glucagon like peptide 1 receptor, gastric inhibitory polypeptide receptor and adrenoreceptor for cAMP pathway regulation, and the muscarinic acetylcholine receptor and the fatty acid receptor (GPR40) for phospholipase C regulation. The values of most of the model parameters were inferred from available experimental data. Our analysis of the dynamic data provides evidence for a pivotal role for Ca^{2+}-dependent adenylyl cyclase activation in the effect of glucagon-like peptide 1 on pancreatic β-cells. The regulatory properties of various adenylyl cyclase isoforms determine fluctuations in cytoplasmic cAMP concentration and reveal a synergistic action of glucose and glucagon-like peptide 1 on insulin secretion. On other hand, the regulatory properties of phospholipase C isoforms determine interaction of glucose, acetylcholine and fatty acids (that act through the receptor GPR40). We test the hypothesis that activation of specific key beta-cell GPCRs can be in some cases stimulate but in other combinations inhibit glucose-stimulated insulin secretion. The regulation of messenger's pathway interactions may be important pharmacological targets for improving insulin secretion in type 2 diabetes.

3637-Pos Board B365

Role of Akaps in BCAM/Lu Receptor Activation on Normal and Sickle Erythrocytes

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Background: Human normal and sickle red blood cells (RBCs) adhere with high affinity to laminin-5 via the basal cell adhesion molecule/Lutheran (BCAM/Lu) receptor which is implicated in vasoocclusive episodes (VOEs) in sickle cell disease (SCD). BCAM/Lu is activated through the cyclic adenosine monophosphate (cAMP) signaling pathway.