Lipid-modifying enzymes play a vital role in the regulation of lipids as mediators of cell function. One example is the hydrolysis of phospholipids through phospholipase D (PLD), which produces the signalling molecule phosphatidic acid (PA). These processes at lipid membranes can be observed in situ through the application of different biophysical techniques. Thus, the hydrolysis of phosphatidylcholines by PLD was investigated, showing that the enzyme is highly affected in its catalytic activity by the lipid membrane structure. Briefly, by using Langmuir monolayers as a model system, we revealed that PLD activity depends on the segregation of the hydrolysis product PA within the monolayer. Hence, we could describe how the structure of the PA-rich domains is decisive for the activation and inhibition of PLD. This study demonstrates how membrane structure influences the activity of PLD and regulates the concentration of the lipid messenger PA.
The current research project is aiming at describing a toxic component of the venom of brown spiders (Loxosceles), which has a rare enzymatic activity termed sphingomyelinase D (SMD). SMD catalyzes the conversion of sphingomyelin (SM) into ceramide-1-phosphate (Cer-1-P). While the enzymatic substrate SM is an integral constituent of many cell membranes, especially in the vascular epithelium and red blood cells, the reaction product Cer-1-P occurs in very low concentrations. Cer-1-P is suggested to be a novel lipid second messenger in cellular signal transduction events. At present, the precise mechanism of venom action is incompletely understood, but preliminary results show the strong effect of SMD activity on the membrane structure of giant unilamellar vesicles. In summary, the presented work depicts the correlation between membrane structures and the activity of lipid-modifying enzymes. This implements new models for the regulation of cellular processes through distinct structures of biological membranes.

1948-Plat
Action Of The Antimicrobial Peptide Novicidin: Divorcing Folding From Function
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Many small cationic peptides have antimicrobial properties. This is assumed to be linked to their ability to permeabilize bacterial membrane. Membrane binding is usually accompanied by the transition from an unstructured conformation to an α-helical state. To investigate further the link between folding and membrane permeabilization we have studied the effect of acylating the N-terminus of the antimicrobial peptide Novicidin with C8, C12 and C16 chains. Acylation increases the ability to form α-helical structure in the presence of zwitterionic vesicles but *reduces* the ability to permeabilize these vesicles, even at concentrations sufficiently low to prevent formation of peptide micelles mediated by the acyl chains. Laser confocal scanning microscopy studies that show Novicidin's preference for DOPC vesicles among populations of different vesicles. The divorce between folding and function is further emphasized by stopped-flow studies using fluorophor-labelled peptide which indicate that a more superficial mode of binding is more efficient in releasing vesicle contents. Rapid kinetic measurements showed a significant increase in the vesicle disruption lag time as a function of acyl chain length indicating that acylation actually decreases the kinetics of interaction. We suggest that induction of α-helical structure is not a prerequisite for membrane disruption but may in fact inhibit disruption by sequestering the peptide in less membrane-active conformations inserted deeper into the membrane than the non-acylated form. This is corroborated by surface-measurements using Quartz Crystal Microbalances with Dissipation and Dual Polarization Interferometry. Our microscopy studies also reveal multiple modes of interaction between AMPs and simple model membranes, namely fusion, pore-formation and lysis, and indicate that peptide-membrane interactions may be even more varied in the complex environment of live bacterial membranes.

1949-Plat
Membrane Tubulation by Lattices of Amphiphysin BAR Domains
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Membrane compartments of manifold shapes are found in cells, often sculpted by cellular proteins. In particular, proteins of the BAR domain superfamily participate in membrane sculpting processes in vivo and reshape also in vitro low-curvature membrane liposomes into high-curvature tubes and vesicles, achieving their role by binding with their curved, positively charged surfaces to negatively charged membranes. Recent observations revealed that membranes are shaped actually through the concerted action of multiple BAR domains arranged in a lattice. However, information on the dynamics of membrane bending and an explanation of the lattice's role are still lacking. Here we show by means of coarse-grained molecular dynamics simulations totaling over 1 millisecond, how lattices involving parallel rows of amphiphysin BAR domains sculpt flat membranes into tubes. A highly detailed, dynamic picture of the formation of membrane tubes by lattices of BAR domains over time scales of 100 microseconds is obtained. Lattice types inducing a wide range of membrane curvatures are explored. The results suggest that multiple lattice types are viable for efficient membrane bending. The lattices found to be optimal for producing high membrane curvature are composed of protein rows separated by 5 nm, stability of the rows being maintained through electrostatic interactions between BAR domains.

1950-Plat
Probing the Interaction of Charged Lipids with the Potassium Channel KcsA
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The activity of integral membrane proteins has long been known to be tightly coupled to the lipid composition of the surrounding lipid bilayer. More recently though the presence of non-annular lipid binding sites have been shown to play a key role in the regulation of membrane channels. In particular recent fluorescence studies have revealed that gating of the potassium channel KcsA is highly dependent on the binding of anionic lipids to three or more non-annular lipid binding sites at the lipid protein interface[1]. Here we present solid-state NMR studies on KcsA reconstituted into charged lipid bilayers composed of POPC/POPG. These studies are allowing us to investigate the nature of the interaction between the surrounding lipid and these binding sites.
Employing ^{1}H-^{31}P saturation transfer MAS NMR[2] we have been able to probe the proximity and rate of exchange of lipid in close proximity to the KcsA. A significant attenuation of the POPC resonance was observed upon the saturation of amide protons suggesting that POPC populates the annular sites of KcsA and is in relatively fast exchange with the bulk lipid. In contrast no such attenuation was observed for the POPG, which in light of earlier fluorescence studies suggests that the POPG remains resident at the lipid protein interface and does not readily exchange with the bulk lipid. Preliminary heteronuclear correlation spectra in conjunction with T_2 filtering are beginning to provide us with insights into the types of residues involved in this interaction.
1) P. Marius *et al.*, Binding of anionic lipids to at least three nonannular sites on the potassium channel KcsA is required for channel opening. *Biophysical Journal* 2008 (94)1689-98.
2) *O. Soubias et al.*, Evidence for lipid specificity in lipid-rhodopsin interactions Journal of Biological Chemistry 2006 (281)33233-41.

1951-Plat
Folding and Assembly of Membrane Proteins: Coarse Grained Molecular Dynamics Simulations of EmrE
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EmrE is a bacterial drug resistance transporter, from E. coli. It is believed to function as an antiparallel homodimer, each monomer of which contains four transmembrane helices. Coarse-grained molecular dynamics (CG-MD) simulations have been previously used to study the insertion and self-assembly of transmembrane helices, and the formation of transmembrane helix dimers and tetramers in lipid bilayers. Such simulations have used a local modification of the original Marrink CG forcefield [1]. In the current study, these methods are employed to investigate the folding and self-assembly of EmrE. Self-assembly CG-MD simulations of the isolated helices of EmrE suggest that each of the constituent helices inserts into a phosphatidylcholine bilayer to adopt a transmembrane orientation. Helix hairpins and other fragments have been simulated to explore the self-assembly and folding processes of the protein subsequent to helix insertion. Simulations of parallel vs. anti-parallel pairs of EmrE monomers are used to explore formation and stability of the EmrE dimer.
(1) Bond, P.J., Wee, C.L., and Sansom, M.S.P. (2008) Coarse-grained molecular dynamics simulations of the energetics of helix insertion into a lipid bilayer. Biochem. (in press), bi-2008-00642m.R1.

1952-Plat
Molecular Dynamics Simulations of Apolipoprotein A-I Peptide Mimetic 4F
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Coronary heart disease is the leading cause of death in the United States, claiming more lives than the next seven leading causes of death combined. High levels of high density lipoprotein (HDL) have been correlated with lower rates of coronary heart disease. Apolipoprotein A-I (apoA-I), is the principle protein in HDL, is a 243-residue class A amphipathic alpha helix capable of binding a variable number of lipid molecules. ApoA-I mimetic peptides synthesized by Anantharamaiah et al. are 18-residue class A amphipathic helices. Although

they have no sequence homology to apoA-I, the peptides bind to lipids in a manner similar to that of apoA-I (i.e. antiparallel double-belt on the edge of the lipid disc). Molecular dynamics simulations of the lipid-bound peptide mimetic 4F were performed for 30 ns in explicit water using CHARMM22/27 parameters. The peptides were arranged in a stacked and staggered conformation to determine if there was any difference in the stability of the belt structure of the peptides. In the initial model, 16 straight alpha-helical chains of 4F were placed around two leaflets of 108 dimyristoylphosphatidylcholines (DMPC). In both simulations all peptides remain in contact with the lipid. The staggered model gives a more circular shape while the stacked model distorts into an oval shape. The staggered model also has a lower conformational energy than the stacked model, indicating that peptide-lipid complexes in which the peptides are staggered may be the more stable form. Salt bridge analysis shows there are three additional salt bridge interactions formed that are not present in the stacked conformation. These interactions may be a contributing factor for the more stable form of the staggered conformation.

Platform AK: Voltage-gated K Channels - Gating: Gating Motions & Modulations

1953-Plat

The KCNE1 Subunit Modifies S2-S4 Interactions in the KCNQ1 Subunit of the I_{Ks} Channel Complex

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The I_{Ks} channel is formed by the coexpression of KCNE1 with KCNQ1. KCNE1 modifies KCNQ1 to bring about the characteristic I_{Ks} current that is essential for terminating ventricular action potentials. The objective of this study is to examine how KCNE1 modifies channel activation by altering the interactions between S2 and S4 in the voltage sensing domain (VSD) of KCNQ1. S2 and S4 contain a series of negatively (E1, E2) and positively (R1, R2, R4) charged residues conserved across all Kv channels, which are essential for voltage-dependent activation. We tested the accessibility of E1C by MTS reagents and found that E1C can be modified by $MTSES^-$ only when KCNE1 is present, suggesting that KCNE1 changes the packing of E1C. Likewise, E2 interactions are also altered by KCNE1. E2Q generates constitutively open channels with apparent partial inactivation, a phenotype distinctly different from WT KCNQ1. However, coexpressing E2Q with KCNE1 produces channels that are nearly identical to WT I_{Ks} in activation and deactivation, as if the drastic perturbations caused by E2Q in KCNQ1 were inconsequential to the function of I_{Ks}. Consistent with this view, in KCNQ1 a secondary mutation R2E can rescue the non-functional E2R. However, this double mutant remains non-functional in the presence of KCNE1. Therefore, E2 and R2 interact in KCNQ1 but not when the channel is coexpressed with KCNE1. Taken together, our data indicate that the association of KCNE1 either directly or allosterically changes the environment around E1 and breaks the interaction between E2 in S2 and R2 in S4. These findings offer new insight into the impact of KCNE1 on the structure of the VSD in KCNQ1, revealing a novel mechanism by which KCNE1 may modulate voltage-dependent activation in KCNQ1.

1954-Plat

Wild-Type KCNQ1 Modulates the Gating of the LQT1 Mutation R231C

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KCNQ1 mutations are linked to type 1 Long QT Syndrome (LQT1). *KCNQ1* encodes the voltage-gated K^+ channel α-subunit, Kv7.1, and LQT1 mutations typically reduce Kv7.1 current ($I_{Kv7.1}$). The missense mutation, R231C-Kv7.1, is associated with LQT1 and sinus bradycardia (Lupoglazoff et al., JACC 2004), but functional studies suggest that this mutation yields a constitutively activated large $I_{Kv7.1}$ (Rocheleau et al., JGP 2007). To better understand the molecular phenotype of R231C-Kv7.1, we transfected HEK293 cells with cDNA encoding the auxiliary K^+ channel subunit MinK1 and WT-Kv7.1, R231C-Kv7.1, or WT- and R231C-Kv7.1 (since LQT1 follows a dominant inheritance pattern). We measured $I_{Kv7.1}$ by prepulsing cells from -80 to 90mV in 10-mV increments for 5s, followed by a test-pulse to -50mV. $I_{Kv7.1}$ measured from cells expressing R231C-Kv7.1 was maximally activated at all potentials, and, compared to WT $I_{Kv7.1}$, increased the maximal peak tail $I_{Kv7.1}$ by ~350% (n=6-8 cells, $p<0.05$). In contrast, cells expressing WT- and R231C-Kv7.1 reduced the maximal peak tail $I_{Kv7.1}$ by ~50% compared to WT $I_{Kv7.1}$ (n=4-6 cells per group, $p<0.05$). We plotted the peak tail $I_{Kv7.1}$ measured during the test-pulse, as a function of the prepulse for cells expressing WT-Kv7.1 or WT- and R231C-Kv7.1, and described the data with the Boltzmann equation to calculate the midpoint potential ($V_{1/2}$) and slope factor (k) for peak tail $I_{Kv7.1}$ activation. Cells expressing WT-Kv7.1 had a $V_{1/2}$ of 19 ± 2mV and a k of 15 ± 2mV/e-fold change (n=6), and cells expressing WT- and R231C-Kv7.1 had a $V_{1/2}$ of -16 ± 5mV (n=5, $p<0.05$) and a k of 21 ± 2mV/e-fold change, ($p<0.05$). Cells expressing WT- and R231C-Kv7.1 also had a constitutively activated $I_{Kv7.1}$ that was ~22% of the maximal peak $I_{Kv7.1}$. These data demonstrate that WT-Kv7.1 dramatically alters the R231C-Kv7.1 phenotype and emphasize the importance of co-expressing WT-Kv7.1 and LQT1 mutations.

1955-Plat

Gated Motions and Interactions Between the Intra-Cellular Domains of the I_{KS} Channel Subunits

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Kv7.1 α-subunit assembles with the KCNE1 auxiliary subunit to form the cardiac I_{KS} K^+ channel. Mutations in these subunit genes produce the long QT syndrome, a life-threatening ventricular arrhythmia. Here we studied the static interactions and the voltage-dependent molecular rearrangements of the intra-cellular domains of the I_{KS} channel complex. The I_{KS} subunits were tagged with ECFP and/or EYFP and expressed in *Xenopus* oocytes. Simultaneous spectral analysis of the fluorescence resonance energy transfer (FRET) were combined with TEVC recordings of K^+ currents. In the channel closed state, a strong constitutive FRET signal between the C-termini of Kv7.1 and KCNE1 was observed. This static FRET signal was increased by 2-fold with a C-terminal truncation of Kv7.1 (Δ622-676). In addition, a marked FRET signal was observed between C-terminally CFP/YFP labeled Kv7.1 subunits, and between the N- and C-termini of double tagged α-subunits. Upon channel opening at +30 mV, concomitantly with I_{KS} K^+ currents recording, a voltage-dependent FRET elevation was detected between the C-termini of Kv7.1 and KCNE1 and between the N- and C-termini of the doubly-tagged Kv7.1. Notably, both K^+ currents and dynamic FRET changes were abolished by coexpressing the KCNE1 LQT5 mutant D76N along with Kv7.1. Direct interactions between the C-termini of Kv7.1 and KCNE1 were further explored by the use of purified recombinant peptides in a series of *in-vitro* pull-down experiments. These experiments indicated that the KCNE1 C-terminus physically interacts with the coiled-coil helix-C of the tetramerization domain. Thus, we suggest that the tetramerization domain of Kv7.1 possesses an additional function as an intra-cellular docking site for KCNE1. Moreover, we demonstrate that channel gating is propagated to the C-termini of both subunits, and accompanied by a spatial rearrangement of the channel complex.

1956-Plat

Using Voltage Clamp Fluorometry to Track Voltage Sensor Movement in a Mammalian Kv1.2 Channel in the Presence of the Kvbeta1.2 Subunit

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The N-termini of Kv1 α-subunits bind co-translationally with cytosolic Kvβ-subunits, with 1:1 symmetry. Kvβ-subunits of three distinct families have been found in neural and cardiac tissue, and members of the Kvβ1 family confer fast inactivation and slowed deactivation when co-assembled with Kv1 α-subunits. These effects may be due to a blocking action by the Kvβ1 N-terminus. Kvβ1 subunits also cause an apparent hyperpolarizing shift in the activation curve of Kv1 channels, which may be a consequence of block by the Kvβ1 N-terminus, due to premature saturation of deactivating tail currents, or alternately may be due to an allosteric interaction between Kvβ1 and Kv1 α-subunits, modifying voltage sensor movement. Here, we use voltage clamp fluorometry to directly track the movement of the Kv1.2 voltage sensor in the absence or presence of the Kvβ1.2 subunit, or an N-terminally-truncated Kvβ1.2 subunit which does not produce fast inactivation. While Kvβ1.2 led to a spike-and-decay current waveform and a hyperpolarized shift in ionic current activation, the voltage dependence of ON gating charge movements were unaffected. Kvβ1.2 also slowed Kv1.2 fluorescence and current deactivation, implying that the return of the voltage sensor to its pre-activation position followed the closing of the activation gate. These findings suggest that the hyperpolarizing shift in channel activation is a consequence of pore block by the Kvβ1.2 N-terminus, and not an allosteric effect on the Kv1.2 voltage sensor, and that block prevents both closure of the activation gate and the return of the S4 helix upon repolarization.

1957-Plat

LRET Measurements In The Three Major Conformations Of The Shaker K Channel

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