Gramicidin A (gA) channels make an ideal system to test molecular dynamics (MD) of membrane proteins (and ion permeation). In addition to being one of the most highly studied membrane ''proteins'', gA channels are tiny, relatively speaking, allowing for long MD runs and calculations of potential of mean force in tractable time. The structure of gA has been determined by solid-state Nuclear Magnetic Resonance (NMR) and solution-state NMR. The structures are in overall agreement, but differ slightly in backbone pitch and in a few amino acid (AA) side chains orientations. Most of these differences have been understood using MD simulations of gA channels in planar bilayers (Allen et al., *J Am Chem Soc.* 125:9868-77, 2003). Because the AA backbone lines the pore and tryptophan side chains are in close proximity to the permeating ion, the average structure and extent of fluctuations of all atoms in the peptide will greatly influence ion permeation. This raises the question of how well molecular mechanical force fields used in potential of mean force studies of ion permeation can reproduce experimental backbone and side chain structure and dynamics. To examine this we measured the gA channel backbone dynamics using solution state ^{15}N-NMR on gA dimers in sodium dodecyl sulfate (SDS) micelles, in parallel with fully atomistic MD simulations on a gA dimer within an explicit SDS micelle. The methods enable us to examine the robustness of the MD simulations done under different conditions (different tryptophan force fields, with/without CMAP corrections), as well as their ability to predict the NMR observables.

788-Pos Board B667
Assessment of Merocyanine Subpopulations in DPPC Vesicles using Ansitropy and Lifetime Measurements
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The purpose of this study was to further investigate the properties of the fluorescent probe merocyanine 540, which has been used frequently for over two decades to assess membrane phase properties under various conditions. Differences in emission spectrum shape at temperatures above and below the thermotropic phase transition of model membranes have been hypothesized to represent changes in the position and orientation of the probe in the bilayer. This hypothesis suggests specific predictions concerning probe mobility in the membrane as a function of temperature and emission wavelength. We tested the hypothesis using measurements of steady state anisotropy and fluorescence lifetimes in dipalmitoylphosphatidylcholine vesicles. Below the lipid phase transition temperature, steady state anisotropy decreased by 0.2 units across the emission spectrum from short to long wavelength. In contrast, anisotropy was more stable as a function of emission wavelength when measured above the transition temperature. Fluorescence lifetimes showed minimal wavelength dependence at either temperature. Anisotropy experiments were repeated at a variety of probe-to-lipid ratios to assess the role of probe aggregation on the observations. The data supported previous findings from measurements of the quantum yield of merocyanine 540: in the gel phase, two separate populations of the probe (monomers and dimers) fluoresce. The monomers, which emit at short wavelengths, are oriented perpendicular to the bilayer surface, and are limited in mobility by neighboring phospholipids. The dimmers, which emit at long wavelengths, are oriented parallel to the bilayer surface, and are localized in a membrane region where motion is less restricted, perhaps in the region between the membrane leaflets. At higher temperatures, only monomers fluoresce but exhibit higher mobility due to the lower order of the membrane phase.

Membrane Active Peptides I

789-Pos Board B668
High Throughput Methods for Discovering Membrane Active Peptides
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Rational design and engineering of membrane active peptides remains a largely unsatisfied goal. We have hypothesized that this us due, in part, to the fact that some membrane activities, such as permeabilization, and cell penetrating ability are not dependent on specific amino acid sequences or specific three-dimensional peptide structures. Instead they depend on interfacial activity; the ability of a molecule to partition into in the membrane-water interface and to strain the packing and organization of lipids. We are testing that idea by taking a novel approach to biomolecular engineering and design of membrane-active peptides. Several rational combinatorial peptide libraries containing 10-16,000 members have been screened for water soluble members that either permeabilize phospholipid membranes or translocate without permeabilization. Stringent, two-phase, high-throughput screens were used to identify dozens of unique peptides that had potent membrane permeabilizing activity or cell penetrating activity, but were also highly water soluble. These rare and uniquely active peptides did not always share a particular sequence motif, peptide length or net charge, but always share common compositional features, secondary structure and core hydrophobicity. We suggest that they function by common mechanisms that depends mostly on interfacial activity. We demonstrate here that composition-space peptide libraries coupled with function-based high-throughput screens can lead to the discovery of diverse, soluble, and highly potent interfacially active peptides.

790-Pos Board B669
Non-membranolytic, Translocating Peptides Selected From A Peptide Library
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Translocation is a defining characteristic of cell-penetrating peptides and antimicrobial peptides that act intracellularly. Found in nature, these membrane-active peptides are being redesigned as therapeutic agents of drug delivery, gene-therapy, and anticancer activity. Such peptides can only be loosely categorized as small, cationic peptides, and even with that broad definition there are outliers. They are best defined by their function to translocate across membranes where they exert their action. Here we have used a function-based approach to isolate 12 translocating peptides from a 10,000+ member peptide library of rational design, using a high-throughput screen for monitoring the non-membranolytic translocation of peptides across lipid bilayers. The 12 residue framework of the library, designed with translocation in mind, is a series of 9 combinatorial sites followed by a C-terminal alpha-1-chymotrypsin cleavage site, integral to the screen. The resulting residue in each combinatorial site is one of 2 - 4 variable amino acids, with a hydrophobic or cationic residue available in each position. The sequences of the translocating peptides from the screen have no specific motif, but similarities do arise in overall compositional features, hydrophobicity, and general deficiency of an ordered structure. The continued trend in a lack of convergence regarding a structure-function relationship supports function based screening of peptide libraries as the best way to arrive at de novo membrane-active peptides with specific functions of interest.

791-Pos Board B670
PG-1 Orientation in Lipid Bilayers: Insights from Molecular Dynamics Simulations and Calculations of Potentials of Mean Force as a Function of Its Tilt Angle
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Antimicrobial peptides, the so-called host defense peptides (usually 12 to 50 amino acids long), exist in all living organisms and play a key role in host defense and innate immune response. Protegrin-1 (PG-1) is one of such peptides and has the β-hairpin conformations in aqueous solution and membrane environments because of inter-strand disulfide bonds. The oligomer states of PG-1 largely depend on membrane types. PG-1 inserts spontaneously and exists as a monomer in a DLPC membrane. In POPC, the minimum structural unit of PG-1 appears to be a dimer that exists in the membrane. To investigate the PG-1 orientation in lipid bilayers, we have performed comparative molecular dynamics simulations of PG-1 monomer in DLPC and POPC membranes. We have also calculated the potentials of mean force (PMF) of PG-1 monomer (with two different rotation angles) in DLPC and POPC membranes as a function of its tilt angle using the β-hairpin restraint potential that we have recently developed. In this work, we will present the simulation results and the calculated PMFs, along with the comparison of calculated solid-state NMR properties with available experimental data.

792-Pos Board B671
Structure And Dynamics Of Phospholamban In The Context Of SERCA
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Sacroplasmic reticulum Ca^{+2}-ATPase (SERCA) plays an essential role in transporting Ca^{2+} ions from the cytosol to the lumen during contraction of cardiac cells. Phospholamban (PLB) is a membrane-bound peptide involved in SERCA regulation. Unphosphorylated PLB inhibits SERCA while phosphorylation of PLB relieves the inhibitory effect. It has been proposed that phosphorylation of phospholamban causes a structural change in SERCA in a switching mechanism between the two main conformations of SERCA, E2 and E1. Detailed structural information of membrane-bound PLB, especially in the context of SERCA is lacking. Molecular dynamics simulations of PLB with and without SERCA are presented to provide atomistic information about its structure and dynamics as a function of phosphorylation, binding to SERCA, and E2-E1 conformational switch in SERCA.

793-Pos Board B672
Antimicrobial Lipopeptides In Anionic And Zwitterionic Membranes Investigated By Molecules Dynamics Simulations
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The explosive development of new antibiotic resistant strains of bacteria makes it essential for us to develop new antibiotic drugs, preferably one which operate via novel pathways. Compounds based on antimicrobial peptides seem like good candidates, because they are broadly effective and largely resilient to evolved immunity. However, natural peptides tend to be much larger than typical drugs, expensive to synthesize, and tend to be rapidly digested in the bloodstream. Shai and coworkers have recently begun invesitgating a new set of compounds, synthetic antimicrobial lipopeptides, designed to have many of the strengths of natural peptides while avoiding many of their weaknesses. Here we use all-atom molecular dynamics of simulations of two of the peptides they used – C16-KGGK and C16-KGGK – bound to zwitterionic, ''mammlian-like'' membranes (POPC) and anionic, ''bacterial-like'' membranes (POPE:-POPG). The variations in their structure and dynamics suggest new insights into the mechanism of selectivity and function.

794-Pos Board B673

Learning From A Bacillus How To Kill Fungi

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Bacterial lipopeptides are a new, very potent and environmentally safe alternative to classical fungicides used in agriculture.
We have studied peptide fractions from strain QST713 of Bacillus subtilis which is registered and applied as a pesticide active ingredient and produces high levels of three classes of lipopeptides: iturins, agrastatins (plipastatins), and surfactins. These compounds consist of a small cyclic peptide ''head group'' containing 7 or 8 (mostly anionic and hydrophobic) amino acid residues and a lipidic chain. In spite of this surfactant-like design, the lipopeptides are much more effective in disrupting membranes and a few mole percent of bound peptide (corresponding to less than 10 uM free concentration) suffice to induce leakage and lysis of the membrane. We have studied these compounds by ITC, membrane leakage assays and other experiments utilizing time-resolved fluorescence spectroscopy, NIBS light scattering, and other methods. We will address the mechanism of action and the reason for the extreme lytic activity of these compounds, the interplay between the different peptides in the biologically optimized mixture, and the selectivity of the action to different lipid membranes. It should be noted that bacterial lipopeptides are distinct from antibiotic peptides produced by higher organisms but parallels in their principal behaviour suggest that the insights obtained here will also improve our understanding of this new class of antibiotics.

795-Pos Board B674

Short Membrane Active (Lipo)-peptides - Interplay Of Domain Formation, Membrane Curvature Stress And Cellular Leakage

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The effect of short peptides, derived from lactoferricin, a human host defense peptide exhibiting antibacterial activity and their N-acylated derivatives was studied with biological and membrane mimetic systems. The work carried out during the European RTD-Project ''ANEPID'' revealed correlation between biological activity against E.coli and interaction with negatively charged lipid model systems, leading to formation of peptide effected lipid domains. Increase of hydrophobicity by addition of hydrophobic amino acids as well as N-acylation improved activity in model and biological systems, but was limited by loss of selectivity for bacterial systems. Addition of peptides to bacterial mimics caused de-mixing into charged peptide effected domains and neutral mainly unaffected domains. Following induction of membrane curvature stress and leakage of cellular contents at defect lines of these induced domains appear to be the major effects of the studied peptides, which could be proven with E.coli mimetic systems. Major perturbance of cytoplasmic membranes of bacteria was also revealed by electron microscopy indicated by peptide induced formation of large membrane blebs and partially by detection of oversized cells that might reflect peptide induced defects in cell-division.

796-Pos Board B675

Free Energies of Molecular Bound States in Lipid Bilayers: Lethal Concentrations of Antimicrobial Peptides

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The lipid matrix, or the lipid bilayer, of cell membranes is a natural binding site for amphipathic molecules, including antimicrobial peptides, pore-forming proteins, and many drugs. The unique property of pore-forming antimicrobial peptides is that they exhibit a threshold concentration (called lethal concentration or minimum inhibitory concentration) for activity, below which no effect is seen. Without this property, antimicrobial peptides would not be effective self-defense weapons, because they would have harmed all cells at any concentration. The question is what gives rise to this unique property? Here I give a free energy description for the origin of a threshold concentration. The same free energy also explains binding of drugs which shows no threshold concentration. The idea is compared with theories of micellar solutions which require a large oligomerization size (n~15) to achieve a threshold concentration. What makes the phenomena in membranes different is the elasticity of lipid bilayers. Antimicrobial peptides have a large negative free energy for binding to the bilayer interface, but the binding causes membrane thinning. This elastic energy of thinning elevates the energy level of interfacial binding with concentration, hence gives rise to a threshold concentration for forming pores containing as few as 4 peptides.

797-Pos Board B676

Biophysical Parameters Involved in Bacteria Resistance to Antimicrobial Peptides

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The molecular mechanism, by which bacteria sense antimicrobial peptides (AMPs) that promote its virulence is partially known. Bacteria are capable of changing the expression of virulence genes essential to survival and replication, by sensing changes in their microenvironment within the tissues of their host. As a consequence they have the potential to develop resistance to AMPs. In the case of *Salmonella typhimurium*, some of the virulence genes are controlled by the two component regulatory system, PhoP/PhoQ. The sensor protein of this system, PhoQ, is directly activated by antimicrobial peptides (AMPs). PhoQ phosporylates and activates PhoP, a transcriptional regulatory protein, which in turn activates or represses over 40 different genes. The activation of these genes was found to be essential to the survival of these pathogenic bacteria within the host macrophages. However, it is not yet clear whether this mechanism is shared by AMPs in general, or it requires specific biophysical properties for AMPs such as secondary structure, amino acid composition or specific sequence. Our studies reveal that changing the biophysical properties of a peptide that can induce resistance, such as incorporation of D-amino acids, can improve the peptides activity against *Salmonella typhimurium* probably by affecting the two component system. Studes along this line suggest that such peptide modifications can be used in order to overcome the inducible resistance of *Salmonella typhimurium.*

798-Pos Board B677

Antimicrobial Peptide C18G binds to Lipid Bilayers in a Lipid Composition Dependent Manner

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Antimicrobial peptides serve as one of the first lines of defense in the immune systems of higher organisms. These peptides specifically target and neutralize infecting bacteria in the host organism while exhibiting little or no toxic effect on host cells. The peptide C18G is a highly cationic, amphiphilic peptide derived from the C-terminal sequence of the human protein platelet factor 4 (involved in blood coagulation and wound repair) exhibited antibacterial activity against both gram positive and gram negative bacteria. Using a modified C18G sequence that did not affect antimicrobial efficacy (Y3 changed to W), the binding affinity of the peptide to model membranes was performed using fluorescence spectroscopy. As anticipated, the binding of C18G to lipid bilayers allowed the Trp side chain to localize to a more hydrophobic environment resulting in a blue shift of Trp emission lambda max and spectral barycenter, concomitant with a narrowing of the emission spectrum and and increase in the overall emission intensity. Binding to lipid vesicles composed of binary and tertiary mixtures of POPC:POPG and POPC:POPG:POPE showed a dramatic lipid dependence on binding affinity, with the tightest binding to the most anionic compositions. Increasing the POPE composition enhanced peptide binding but to a lesser degree compared to the anionic POPG. Fluorescence quenching experiments using the aqueous quencher acrylamide confirmed the decreased exposure of the Trp to the aqueous milieu. Dye release assays were used to monitor lipid composition effects on the ability of C18G to permeabilize lipid vesicles. Circular dichroism spectroscopy indicated a conformational change from a disordered to an alpha-helical secondary structure when the peptide interacts with detergent micelles or anionic lipid vesicles.