β-like conformations. The antimicrobial activity typically is higher when four arginines are present, yet the activity does not appear to correlate directly with folding or secondary structure.

513-Pos Board B268
Interaction of the Antimicrobial Polymyxin B1 with the Inner and Outer Membranes of E.Coli: Insights into the Mechanisms of Membrane Disruption
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Antimicrobial peptides (AMPs) are small proteins that show antimicrobial activity against bacteria, fungi and viruses. They disrupt bacterial membranes by increasing the membrane permeability and pore formation. AMPs can be found in most living organisms where they have been shown to play an essential part of the innate immunity. With the increasing number of bacterial strains acquiring resistance to current antibiotics, the need for novel antibiotics is urgent. Polymyxin B1 (PMB1) is a small antimicrobial peptide first derived from the bacteria *Bacilus Polymyxa* in 1947. It is a highly active antimicrobial peptide and shows selectivity predominantly to gram-negative bacteria. It has been shown to be successful in treating infections caused by the bacteria Pseudomonas aeruginosa with little development of resistance. After recent studies have shown that its toxicity may have been exaggerated in the past, interest has been renewed in this AMP. The exact mechanism of membrane disruption by PMB1 is not known. To address this we have conducted a molecular dynamics simulation study to investigate the potential structure related properties this AMP possesses that enables it to disrupt bacterial membranes. To study these properties we simulated PMB1 in three different membrane environments, mimics of the outer membrane of Gram-negative bacteria, the inner membrane, and also a symmetric lipid A bilayer. Our results provide the first molecular-level insights of this antimicrobial peptide inserting into realistic, complex bacterial membranes.

514-Pos Board B269
Membrane Interactions with ATRA Peptides
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The ‘‘catastrophic threat’’ of antibiotic resistance has prompted research into biological methods of combating bacterial infection. One such pervasive strategy employs cationic antimicrobial peptides, CAMPs. These peptides use their structure to target and disrupt bacterial membranes. The cationic peptides are specifically attracted to bacteria because of the high anionic lipid content in the outer leaflet of bacterial membranes. Most have already been shown to have broad spectrum activity, adequate potency, and minimal resistance. Considering these peptides have been active against pathogens for millions of years and have not developed any broad resistance, they are of particular interest to study. One class of CAMPs, AHCAPs, forms amphipathic α-helices when in the presence of anionic membranes. The peptide NA-CATH, from the Naja atra snake, and its analog, ATRA-1A, show promising activity. We are currently examining the effect of lipid composition and peptide chirality (L and D isomers) on peptide activity and membrane interactions. Kinetic assays show increased activity with increasing peptide concentration and match the EC50 (50% of maximal dose) trends for zwitterionic/anionic lipid composition.

515-Pos Board B270
Membrane Insertion Potential of Synthetic Cell Penetrating Peptides
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Cell penetrating peptides (CPPs) have been established as excellent candidates for mediating drug delivery into cells. When designing synthetic CPPs for drug delivery applications, it is important to understand their ability to penetrate the cell membrane. In this paper, anionic or zwitterionic phospholipid monolayers at the air-water interface are used as model cell membranes to monitor the membrane insertion potential of synthetic CPPs. The insertion potential of CPPs having different cationic and hydrophobic amino acids were recorded using a Langmuir monolayer approach that records peptide adsorption to model membranes. Fluorescence microscopy was used to visualize alterations in phospholipid packing due to peptide insertion. All CPPs had the highest penetration potential in the presence of anionic phospholipids. In addition, two of three amphiphilic CPPs inserted into zwitterionic phospholipids, but none of the hydrophilic CPPs did. All the CPPs studied induced disruptions in phospholipid packing and domain morphology, which were most pronounced for amphiphilic CPPs. Overall, small changes to amino acids and peptide sequences resulted in dramatically different insertion potentials and membrane reorganization. Designers of synthetic CPPs for efficient intracellular drug delivery should consider small nuances in CPP electrostatic and hydrophobic properties.

516-Pos Board B271
Membrane Regulation and Signal Transduction by Annexin A5
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Annexins are an abundant family of proteins well known for their Ca^{2+}-dependent membrane binding ability. Though they've been implicated in numerous membrane processes their method of action and signal integration is largely unknown. Here we propose a mechanism for annexin to integrate information regarding Ca^{2+} concentration and resting distribution of lipid and cholesterol in one leaflet, and transduce that information across the bilayer. In a system poised near random distribution, such as lipids of the inner leaflet of the plasma membrane, order can be imposed through even weak interactions. We've previously shown annexin's weak Ca^{2+}-independent membrane binding acts to create a cooperative Ca^{2+} binding response. In this work we demonstrate not only annexin's ability to respond to and integrate information of membrane composition; but also its ability change and regulate that distribution. Protein interactions with membranes can induce membrane order and domain formation if the protein has specificity for individual lipids distributed non-ideally, or even randomly throughout the membrane. A protein that preferentially binds a free component of the membrane alters the amount of that component within other membrane domains. Annexin perturbs membrane distribution through its preferential binding and sequestration of phosphatidylserine headgroups (PS). Cholesterol perturbs phospholipid physical properties and preferentially forms complexes with PS. Then the local sequestering of PC-cholesterol complexes, and annexin-induced membrane domain formation upon Ca^{2+} influx, can act as the driving force for cholesterol redistribution across the bilayer. Using ITC as well as fluorescent probes such as DHE (cholesterol analogue) and carboxyfluorescein encapsulating LUVs. We show annexin's binding of cholesterol-PS complexes induces differential Ca^{2+} binding sites, and redistributes cholesterol throughout the bilayer without disrupting membrane permeability. Together this suggests that annexin has the capacity to redistribute cholesterol across the bilayer in a calcium-ion and membrane composition dependent manner.

517-Pos Board B272
Assembling of a Pore-Forming Toxin on a Model Membrane
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The assembling of the sphingomyelin (SM)-binding pore-forming toxin (PFT), lysenin, to SM/cholesterol bilayer was examined by high-speed atomic force microscopy (HS-AFM) [1]. The HS-AFM images of SM/cholesterol bilayer preincubated with lysenin exhibited the hexagonal close packed (hcp) assembly of lysenin oligomers (Fig. 1A). The in-situ AFM images revealed that the formation of the hcp structure took place quickly (Fig. 1B). Before the full coverage of the membrane surface with a stable hcp assembly of lysenin oligomers, most of the oligomers underwent reorganization either by dissociating into monomers or by rapidly diffusing along the membrane in less than a second. The assembling of lysenin oligomers was also followed on SM/DOPC/cholesterol bilayer. Oligomers firstly formed at the edges of the SM-rich domains and covered these domains similarly to the SM/cholesterol bilayer. Our results revealed the dynamic nature of the oligomers of a lipid binding toxin during its assembling on SM-containing membranes.

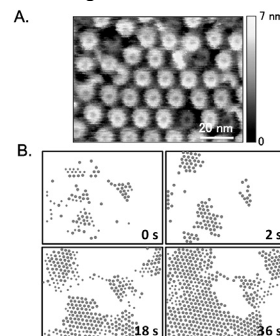

Figure 1. (A) Hexagonal assembly of lysenin oligomers on SM/cholesterol bilayer.
(B) Illustration of assembling of lysenin oligomers on SM/cholesterol bilayer.

Reference
[1] N. Yilmaz, T. Yamada, P. Greimel, T. Uchihashi, T. Ando, and T. Kobayashi, Biophys. J. 105, 1397-1405 (2013).