at concentrations below the aggregation threshold (similar to critical micelle concentration) it forms membrane pores, and at higher concentration it acts like a detergent and solubilizes the membranes of surrounding cells.

1231-Pos Board B123

Mapping the Membrane Topography of Segments TH6-TH7 and TH8-TH9 in the Diphtheria Toxin T-Domain Channel

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The T-domain of diphtheria toxin senses a low pH to insert into a lipid membrane, form a transmembrane channel, and translocate the attached catalytic domain across the membrane. Previous work has identified three transmembrane segments of T-domain in the open channel state, corresponding to TH5, TH8 and TH9 in the aqueous crystal structure; the amino-terminal region, TH1-TH4, was shown to be translocated across the membrane to the trans side. It was also shown that residues near either end of the TH6-TH7 segment are not translocated, remaining on the cis side of the membrane; the intervening 25-residue sequence is too short to form a transmembrane α-helical hairpin, so it was concluded that the TH6-TH7 segment resides at the cis interface. Now we have examined this segment further, using the substituted-cysteine accessibility method. We constructed a series of mutant T-domains with a single cysteine residue at positions in TH6-TH7, monitored their channel formation in planar lipid bilayers, and probed for an effect of thiol-specific methanethiosulfonate reagents on the channel conductance. For at least 12 of the mutants, the reagent caused a change in the single-channel conductance, indicating that the introduced cysteine residue was exposed within the channel lumen. We also compared the reaction rate of reagent added to the cis side vs. that to the trans side in order to estimate the residue's position along the channel axis. This analysis revealed abrupt changes in cis- vs. trans-side accessibility, suggesting that the TH6-TH7 segment forms a constriction that occupies a small portion of the total channel length. The location of this constriction relative to the TH8-TH9 segment was also determined.

1232-Pos Board B124

Interactions of High-Affinity Cationic Blockers with the Translocation Pores of *B. Anthracis*, *C. Botulinum*, and *C. Perfringens* Binary Toxins

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Cationic β-cyclodextrin derivatives were recently introduced as highly effective, potentially universal blockers of binary bacterial toxins: anthrax toxin of *Bacillus anthracis*, C2 toxin of *Clostridium botulinum*, and iota toxin of *Clostridium perfringens*. The binary toxins are made of two separate components: the enzymatic A component and the binding/translocation B component, which forms oligomeric channels in the target cell membrane. Here we studied the voltage and salt dependence of the rate constants of binding and dissociation reactions of two structurally different β-cyclodextrins (AmPrβCD and AMBnTβCD) in the PA_{63}, C2IIa, and Ib channels. With all three channels, the blocker carrying extra hydrophobic aromatic groups, AMBnTβCD, demonstrated stronger binding compared with AmPrβCD. This effect is seen as an increased residence time of the blocker in the channels, whereas the time between blockages stays practically unchanged. Surprisingly, the voltage sensitivity, expressed as a slope of the logarithm of the blocker residence time as a function of voltage, was practically the same for all six cases studied, suggesting structural similarities among the three channels. Also, the more-effective AMBnTβCD blocker shows weaker salt dependence of the binding and dissociation rate constants compared with AmPrβCD. By estimating the relative contributions of the applied transmembrane field, long-range Coulomb, and salt-concentration-independent, short-range forces, we found that the latter represent the leading interaction, which accounts for the high efficiency of blockage. In a search for the putative groups in the channel lumen that are responsible for the short-range forces, we performed measurements with the F427A mutant of PA_{63}. We found that the on-rates of the blockage were virtually conserved, but the residence times dropped by more than an order of magnitude, reducing the difference between the efficiencies of the two blockers.

1233-Pos Board B125

Understanding the Carbohydrate Specificity of Vibrio Cholerae Cytolysin

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Bacterial pathogens secrete a variety of effector molecules to support colonization of the human host. Included in this list are pore-forming toxins (PFTs), which kill host cells by selectively targeting and disrupting the plasma membrane. To improve the efficiency of cell lysis, many PFTs contain recognition motifs that bind protein, lipid, and carbohydrate molecules on the cell surface. The human pathogen Vibrio cholerae secretes a PFT (VCC) that forms heptameric channels in membranes and lyses cells at picomolar concentrations. The toxin is thought to serve as a defensive agent against innate immune cells thus facilitating colonization of the host. To achieve high potency, VCC contains two structural domains with lectin-like folds that target carbohydrate molecules found on mammalian cell surfaces. Disruptive mutations within one of these domains significantly reduces the activity of the toxin against model cell membranes suggesting that carbohydrate-binding is important for targeting membranes at low concentrations. To better understand this process, we conducted screens against a variety of simple and complex carbohydrate ligands and identified glycans recognized by VCC lectin-like domains. Several of these ligands are potential physiological targets of the toxin and we show that they bind VCC with low nanomolar affinity. These glycans decrease toxin activity when added in trans and may serve as a starting point for therapeutic intervention. To aid in this goal, we are pursuing structural characterization of toxin-glycan complexes to better understand the nature of these interactions.

1234-Pos Board B126

A Unique Mechanism of Cholesterol Binding by an RTX Toxin

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The leukotoxin (LtxA) secreted by *Aggregatibacter actinomycetemcomitans* is a member of the repeats-in-toxin (RTX) family, and like the other members of the family, is a virulence protein that destroys host cells. LtxA specifically kills human white blood cells, allowing *A. actinomycetemcomitans* to survive within the host. LtxA has been shown to bind to an alpha-L/beta-2 integrin, lymphocyte function-associated antigen-1 (LFA-1), and upon binding, to form large LtxA/LFA-1 clusters in cholesterol-rich lipid rafts. Here, we have investigated this interaction by studying the binding of LtxA directly to cholesterol. Using surface plasmon resonance (SPR), we showed that the affinity of LtxA for membranes containing 40% cholesterol was four orders of magnitude greater than its affinity for membranes lacking cholesterol ($4.6x10^{-12}$M vs. $1.9x10^{-8}$M). Surprisingly, the affinity was regulated not by an increase in the association rate in the presence of cholesterol but by a decrease in the dissociation rate. We identified two cholesterol recognition/amino acid consensus (CRAC) motifs in the amino acid sequence of LtxA and investigated the role of these cholesterol-binding motifs in the affinity of LtxA for cholesterol. Synthetic peptides corresponding to both CRAC sites interacted strongly with cholesterol, while control peptides did not. However, only the peptide corresponding to the first CRAC site inhibited binding of LtxA to membranes containing 40% cholesterol, and only this peptide inhibited the cytotoxicity of LtxA, demonstrating the requirement for cholesterol binding in the toxic mechanism of LtxA. This represents a unique mechanism of toxin binding to cholesterol in which the toxin's affinity to cholesterol is regulated by the dissociation rate rather than the association rate. The conservation of the first CRAC site among RTX toxins suggests that this is a mechanism shared by this toxin family.

1235-Pos Board B127

Investigating the Mechanism of Action of Antimicrobial Piscidin in Bacterial and Mammalian Lipid Membrane Mimics using Oriented Circular Dichroism

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Antimicrobial peptides (AMPs) are a class of amphiphilic, often cationic peptides, which are able to target and permeabilize the lipid membranes of pathogenic microbes. AMPs are often studied for their potential as novel pharmaceuticals against bacteria, viruses, and even some cancers. Many possible mechanisms for their action have been proposed, but it is believed that at low peptide:lipid ratios (P/L), peptides bind parallel to the membrane surface, known as the ''S-state,'' while at high P/L, peptides insert into the membrane and bind in the ''I-state'' perpendicular to the membrane surface. It is proposed

that peptides in the I-state form pores, while peptides that remain in the S-state use the carpet model of membrane disruption.
Here, OCD is used to investigate the orientation and threshold concentration of Piscidin 1 and Piscidin 3 (P3) in bacterial and mammalian-mimicking lipid systems and thereby determine the mechanism of action of P1 and P3 in these lipid systems. Both peptides are a 22-residue alpha-helical AMPs isolated from the mast cells of hybrid striped sea bass. P1 is both more antimicrobial and hemolytic than P3. We hypothesize that the two peptides behave differently in bacterial versus human cells due to the differences in membrane composition and that P1 initiates its activity at a lower threshold concentration. Mammalian and bacterial membrane mimics have been made using 4:1 PC/CHL (phosphocholine and cholesterol, respectively) and 3:1 PC/PG (phosphoglycerol). The bilayer orientations of piscidin have been investigated over a large range of P/L ratios using OCD. Membrane thinning was studied by x-ray. These studies provide insight into the mechanism of action of an important class of AMPs and may help provide design principles for new drug candidates.

1236-Pos Board B128
Surface and Membrane Binding Properties of the Lipopeptide Daptomycin
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Daptomycin, an antimicrobial lipopeptide used to treat infections caused by Gram-positive bacteria that are resistant to many conventional therapies, acts through calcium-mediated binding to and rapid depolarization of the target bacterial membrane. Convincing evidence has recently been reported suggesting that small daptomycin oligomers form at the membrane surface and that these complexes represent the active state of the drug. Daptomycin's activity is closely correlated with the presence of phosphatidylglycerol (PG) in the target membrane. Although there have no cases of clinical resistance to daptomycin reported, troubling signs are emerging indicating that changes in lipid composition of bacterial membranes cause decreased susceptibility to the drug. It is therefore of interest to gain a more profound understanding of the details of daptomycin's mechanism of activity at the membrane level and the possible causes of potential resistance and their relationship to lipid composition. In the current study, we report on our investigation into the surface and membrane binding properties of daptomycin. From the Gibb's adsorption isotherm, we estimate the molecular area of daptomycin at the air-aqueous interface. Using Langmuir monolayers as membrane models, we also report limiting surface pressures and kinetics for daptomycin insertion to lipid films comprised of pure PG or PG-phosphatidylcholine mixtures. Finally, we attempt to correlate daptomycin's binding behavior in monolayers to that in bilayers, in the form of unilamellar vesicles, by presenting results from isothermal titration experiments. The results represent, for the first time, thermodynamic binding parameters for daptomycin-membrane interactions.

1237-Pos Board B129
Effects of Electrostatic Interactions on Helicity in Model Peptide Antibiotics using 2D NMR and CD Spectroscopies
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Persistent infections caused by antibiotic resistant microbes are a serious public health threat. Peptide antibiotics, which perturb the cell membrane, offer one promising solution. Critical characteristics of both natural and designed peptide antibiotics include the formation of ordered structures such as helices, and amphilicity. We are investigating the effects of electrostatics on the helicity of peptide antibiotic models that are composed primarily of the sterically hindered amino acid Aib, with Lys and Glu residues substituted at various positions in the helix. We report here results for the octameric peptides KK36 (Lys at positions 3 and 6, one 310-helical turn apart), EK36 (Glu and Lys at positions 3 and 6), EK45 (Glu and Lys adjacent in the center of the helix) and KK45 (two Lys adjacent in the center of the helix). NMR resonances were assigned using natural abundance 1H-13C HMBC and HSQC spectra. Distance constraints from 1H-1H ROESY and hydrogen-bonding information from amide temperature coefficients were used to calculate the three-dimensional structures of the peptides using Xplor-NIH. Global structural information was also obtained using CD spectroscopy. We find that KK36 and EK36 are 310-helical, with slightly different helical curvatures both in DMSO-d6 and in methanol-d3. CD spectra also indicate 310-helical structures in TFE and methanol, with a greater ratio of θ220/θ202 in TFE (~0.4 versus ~0.25). KK36 is less well structured in water versus organic solution, as evidenced by poor amide spectral dispersion. However, association of KK36 with lipid vesicles results in complete amide spectral dispersion, and an enhancement of the CD signal. The structures of all four peptides will be presented and interpreted in terms electrostatic effects on helicity in a variety of solvent systems.

1238-Pos Board B130
Channel Crystal Structure and Antimicrobial Mechanism of Dermcidin from Human Skin
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Dermcidin is an anionic antimicrobial peptide (aAMP) derived from the human gene DCD, which encodes a preform that is secreted by eccrine sweat glands and subsequently proteolytically processed into DCD-1L, constituting a vital part of the innate host defense of the dermis. Recently its oligomeric structure was solved by x-ray crystallography and shown to be stabilized by divalent Zn^{2+} ions. Through molecular dynamics simulations, using a novel transmembrane conduction assay, we showed that it forms a conducting channel in a lipid bilayer. Long standing experimental results assign it a selective affinity for negatively charged membranes despite its anionic nature (pI ~5), and in consensus with other amphiphilic AMPs it has a helical structure that only forms upon contact with a hydrophobic interface. The reconciliation of such experimental observations with the mechanistic result of channel formation pose further questions on the selectivity of dermcidin, both for anion conductivity and bacterial membrane adhesion, and for the kinetics of its insertion. Working to understand how the subunits of this channel-forming oligomer interact, we use molecular dynamics simulations to examine the influence and specific role of the Zn^{2+} ions for its stability under conditions relevant to understand it's selectivity for (and insertion into) specific composition lipid bilayers. In extension we examine the effect specific residue mutations which are based on a structure- and sequence-comparison to other known AMPs has for its function, as well as that of lipid bilayer composition.

Membrane Structure I

1239-Pos Board B131
Influence of Divalent Cations on Phosphatidylserine Lipid Flip-Flop
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Phosphatidylserine (PS) lipids are an essential component of the plasma membrane of eukaryotic cells and are known to distribute unequally across the membrane. Changes to this distribution trigger specific cell functions, ranging from blood coagulation to phagocyte recognition, while defects in the distribution are linked to disease. Despite many research efforts, the mechanism to control the PS lipid distribution has proven to be complex and is not yet fully understood. While there are many biological interactions which may contribute to PS distribution, the work presented here investigates the role of divalent cations. Sum-frequency vibrational spectroscopy (SFVS) has been used to study the changes in native lipid behavior induced by the presence of both magnesium and calcium ions. Planar-supported lipid bilayers containing biologically relevant amounts of PS lipids (5-20%) were used as model systems to isolate the impact of these divalent cations on lipid flip-flop and PS distribution.

1240-Pos Board B132
The Thermodynamics of General and Local Anesthesia
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We describe the influence of both local and general anesthetics on the melting transition in lipid membranes. We outline the theory of the interaction and compare it to calorimetric experiments. We found that both local and general anesthetics display very similar effects on membrane melting that can be described by the well-known phenomenon of melting point depression. The partitions coefficients of the anesthetics can be deduced from the calorimetric profiles. We also investigate the influence of hydrostatic pressure on the anesthetic effect. Both classes of anesthetics display pressure reveal, an effect that has been found for general anesthesia but has not been described for local anesthetics. Our findings are in agreement with recent clinical findings by Danish neuroscientists from the Imperial Hospital in Copenhagen (Rigshospitalet) on the excitability of the human median nerve that were performed in collaboration with