

we have developed techniques that use deuterium (^2H) solid state NMR to study AMP-membrane interactions in the context of intact bacteria. In particular we are investigating the correlation between AMP-induced lipid chain disorder in intact bacteria and biological function, as measured by minimal inhibitory concentration assays.

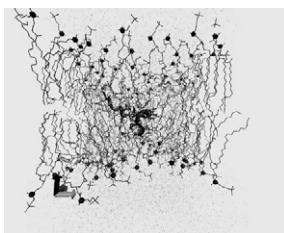
1216-Pos Board B108

Molecular Dynamics Simulations of Cod Antimicrobial Peptide Paralogs in Self-Assembled Bilayers

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Gaduscidin-1 and -2 (GAD-1 and GAD-2) are antimicrobial peptides (AMPs) that are histidine-rich and thus are expected to exhibit pH dependent activity. In order to help explain their mechanism of membrane disruption, we have performed molecular dynamics simulations with the peptides in both histidine-charged and histidine-neutral forms, along with 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) lipid molecules. The simulations employed GROMACS software and an OPLS-AA force-field. Initially, the peptide and lipids were placed randomly in the simulation box and then were allowed to self-assemble. The peptides were found to associate with defects in the bilayer, and to take on a variety of structures and topologies. The observed heterogeneity is consistent with experimental studies with similar peptides, and has potential to help explain the mechanism by which the peptides can incorporate into and disorder lipid bilayers.



1217-Pos Board B109

Modulation of Elastic Properties through Unsaturated Lipid Content as a Mechanism for Inducing Resistance to Amphiphilic Antimicrobial Peptides

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We explore whether changes in fatty acid composition in bacterial membrane models lead to inhibition of amphiphilic antimicrobial peptide-(AMPs) through the modulation of the membrane elastic properties. Specifically, we examined whether changes in the unsaturated/saturated fatty acid ratio, which is controlled by bacteria, inhibit AMP function. In phosphatidylglycerol (PG) lipid bilayers we observe a 10-fold increase in resistance to Magainin-2 (MAG) in POPG (16:0/18:1) vs. DPPG (16:0/16:0) bilayers and a 20-fold increase in resistance in DOPG (18:1/18:1) vs. DPPG bilayers, as measured by calcein release at 45 C (where lipids are in the liquid-crystalline phase). By analyzing the leakage kinetics, we find that the activation energy for pore formation in DPPG/POPG mixtures increases linearly with POPG content, confirming that unsaturation is energetically unfavorable for pore formation. Laurdan polarization (GP) measurements correlate well with MAG resistance, suggesting that head group spacing is an indicator of resistance (increased head-group spacing could allow for more peptides to partition into the head group region, instead of becoming inserted into the bilayer as bilayer-spanning monomers). The changes in potency cannot be explained solely by the increased head group spacing, however. We therefore performed leakage experiments using known modifiers of membrane elastic properties (Triton-X, capsaicin) that both reduce bilayer stiffness but cause opposite changes in curvature. Bilayer softening plays a role in decreasing MAG's potency, whereas the curvature alters only the leakage kinetics, not potency. Based on previous studies, we propose that membrane softening somehow makes it more difficult for MAG to reach a critical concentration necessary to produce pore formation maybe because the energetics of lateral association among bilayer-spanning MAG monomers favors the monomers in softer bilayers. Unsaturated lipids may therefore influence MAG potency by modulating membrane stiffness.

1218-Pos Board B110

Peptide-Induced Bilayer Thinning Structure of Unilamellar Vesicles and the Related Binding Behavior as Revealed by X-ray Scattering

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To quantitatively correlate membrane thinning with peptide binding affinity, we have studied the bilayer thinning structure of unilamellar vesicles (ULV) of a phospholipid 1,2-dierucoyl-sn-glycero-3-phosphocholine (di22:1PC) upon binding of melittin, a water-soluble amphipathic peptide. Successive thinning of the ULV bilayers with increasing peptide concentration was monitored via small-angle X-ray scattering (SAXS). Results suggest that the two leaflets of the ULV of closed bilayers are perturbed and thinned asymmetrically upon free peptide binding, in contrast to the centro-symmetric bilayer thinning of the substrate-oriented multilamellar membranes (MLM) with premixed melittin. Moreover, thinning of the melittin-ULV bilayer saturates at ~4 %, significantly lower than the critical thinning of ~8% (determined via the correspondingly premixed peptide-MLM bilayers) for thermally equilibrated formation of membrane pores, revealing a critical influence of binding affinity for water soluble peptides. Scaling the peptide-ULV bilayer thinning to that of the corresponding peptide-MLM, of a calibrated peptide-to-lipid ratio, we have deduced the number of bound peptides on the ULV bilayers as a function of free peptide concentration in solution. The hence derived X-ray-based binding isotherm allows extraction of a low binding constant for melittin to the ULV bilayers, on the basis of surface partition equilibrium and the Gouy-Chapman theory. Moreover, we show that the ULV and MLM bilayers of di22:1PC may have a same thinning constant upon binding of a same peptide, hence providing a basis in establishing X-ray-based binding isotherms for thermodynamic binding parameters of late stage peptide-membrane interactions prior to pore formation.

1219-Pos Board B111

Molecular Basis of the Blocking Mechanism of Inwardly Rectifying Channels by Tertiapin

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Ion channels are the third largest type of proteins targeted by pharmaceutical industry accounting for 10% of the currently marketed drugs. Recent advances in the 3-D structural determination of ion channel proteins coupled with highly sensitive electrophysiological assays make a compelling case for rational drug design targeting these proteins. High-resolution structure determination of inwardly rectifying Potassium (Kir) channels has thus far yielded two full-length mammalian structures. Specific blockers for Kir channels do not exist but the small bee venom peptide toxin tertiapin (TPN) targets with high affinity two Kir channels: ROMK1 and GIRK channels. Mutations of ROMK1 in the kidney cause hypotension, while, malfunctions of GIRK in the heart are associated with chronic atrial fibrillation. Modification of TPN to specifically target one or the other channel bears great therapeutic potential. We have produced structural models of TPN binding to ROMK1 and GIRK2 channels and have quantified the energies involved for each channel-toxin interaction pair. *In silico* mutagenesis has produced remarkably similar changes to the experimental energies of interaction between the ROMK1 channel and TPN, lending strong validation to the structural model employed. Similar studies in GIRK2-TPN interactions suggest differences in specific interactions of the toxin with each of the two channels. These models make specific predictions that are being pursued for further experimental validation. The underlying hypothesis of our approach is that differences in the interaction energies we compute can guide the design of specific TPN for different Kir channels.

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Membrane Permeability Induced by Stereo and Retro Analogs of Histatin 5

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Candida species combine to be the major cause of fungal infections in humans. These infections are becoming increasingly difficult to treat due to resistance to current common drug therapies or due to the toxicity of other therapeutic compounds. Antimicrobial peptides, short peptides of less than 100 amino acid residues, are known to kill bacterial and fungal pathogens, with the potential to be a more reliable means of treatment for *Candida* infections than the drugs currently available. Humans naturally produce the 24 amino acid cationic histatin 5 peptide. Variants of a 16 amino acid peptide derived from histatin 5 were chemically synthesized to delineate the structural determinants of their antifungal activity. *In-vitro* acridine orange leakage assays were used to evaluate the activity of histatin 5 derivatives with various synthetic membrane bilayers (liposomes) composed of differing lipid and sterol compositions. The variants of histatin 5 were synthesized with alternative stereochemistry and polarity, and then evaluated with liposomes comprised of either Soy PhosphatidylCholine (Soy PC), Soy PC + cholesterol or Soy PC + ergosterol. The results indicate that neither the D or L amino acid stereochemistry nor the N- to C- terminal