fourier transform IR Spectroscopy) as a function of temperature for DMPC and DMPG liposomes in the presence and the absence of R,R4,R7 peptides. Spectra revealed a significant shift of the DMPG transition temperature for ARG4 and ARG 7 reflecting significant changes in the membrane order and the motional freedom of the methylene groups whereas the same peptides did not affect significantly DMPC transition. No changes were observed with arginine alone for both lipids. Molecular modelling showed insertion of part of R7 deeply in the DMPG bilayer that was not observed with free arginine.
Overall the data demonstrate that R7 penetrates into and destabilise the DMPG bilayer which could explain in molecular terms the cell uptake of these arginine oligopeptides. The fact that such a destabilising effect was not observed with the lysine peptides also suggest that the arginine -lipid interaction is quite specific in agreement with the phosphate_guanidine interaction identified by molecular modelling.

440-Pos
Molecular Electroporation and the Transduction of Oligoarginines
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Certain short polycations, such as TAT and polyarginine, rapidly pass through the plasma membranes of mammalian cells by an unknown mechanism called transduction as well as by endocytosis and macropinocytosis. These cell-penetrating peptides (CPPs) promise to be medically useful when fused to biologically active peptides. I offer a simple model in which one or more CPPs and the phosphatidylserines of the inner leaflet form a kind of capacitor with a voltage high enough to create a molecular electropore. The model is consistent with an empirical upper limit on the cargo peptide of about 50 amino acids. More importantly, it fits experimental data on how the transduction of a polyarginine-fluorophore into mouse C2C12 myoblasts depends on the number of arginines in the CPP and on the CPP concentration. The model makes three testable predictions.

441-Pos
Influence of Lipid Composition on the Orientational State of the Antimicrobial Peptide MSI-103 in Membranes. a Solid-State NMR Study
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The antimicrobial peptide MSI-103 is known to undergo a functionally relevant re-orientation in membranes from a surface-aligned S-state to a tilted T-state depending on the peptide concentration and lipid phase. Here, we have used solid-state NMR on the ^{2}H-labeled peptide to determine its orientational state in membranes composed of different types of lipids.
In phosphatidylcholine (PC) bilayers with different acyl chains, there is no effect of the chain length on the peptide orientation. However, a distinct difference is observed in the peptide response to saturated and unsaturated acyl chains. In unsaturated lipids, the peptide always remains in the surface-bound S-state, with its alpha-helical axis perpendicular to the bilayer normal at a tilt angle close to 90°. Only in saturated lipids it is able to insert into the membrane in a tilted T-state, with an angle of around 125°. Interestingly, when lyso-PC is added, the T-state is found to be stable also in unsaturated lipids. These results can be explained by the shape of the lipids; especially the relative area of head group and acyl chains, as will be discussed in detail. It is known that the presence of anionic lipids leads to a higher affinity of the cationic peptide towards bacterial membranes, but such electrostatic effects *per se* do not suffice to induce any change in peptide orientation. Interestingly, we found that the presence of cholesterol prevents MSI-103 from binding to the membrane in any ordered state, but rather induces the formation of immobilized peptide aggregates. This observation can essentially explain the selective membrane-permeabilizing action of MSI-103 on bacteria compared to eukaryotic cells which contain cholesterol.

442-Pos
Understanding the Importance of Residue 13 and the C-terminus on the Structure and Activity of the Amphibian Antimicrobial Peptide, Aurein 2.2
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Previous studies on the cationic antimicrobial aurein 2.2 and 2.3 peptides in DMPC/DMPG and POPC/POPG membranes have shown that bilayer thickness and PG content have significant impact on the interaction of these peptides with membrane bilayers, in a concentration- and peptide sequence-dependent manner [1]. In addition, DiSC$_3$5 assay results have indicated that aurein 2.2 induces greater membrane leakage than aurein 2.3 in *S. aureus* C622 [1]. The difference between aurein 2.2 and aurein 2.3 is a L13I mutation at residue 13.
In order to understand the importance of the nature of the residue at position 13, we have further studied L13A, L13F, and L13V mutant aurein 2.2 peptides. In addition, we have investigated a number of peptides with truncations at the C-terminus. Solution CD results demonstrate that the L13F mutation and truncation of the C-terminus by 6 residues result in decreased helical content, while the L13A or L13V mutation and truncation of the C-terminus by three residues shows no effect on the structure. Oriented CD and ^{31}P NMR spectroscopy results show that only an extensive C-terminal truncation reduces the ability of the peptide to insert into the lipid bilayers and to disorder the headgroups at lower peptide concentrations. The implication of these results in terms of antimicrobial activity will be discussed.
[1] Cheng, J.T.J. *et al.* 2009. *Biophys. J.* 96: 552-565.

443-Pos
Temperature Dependence of the Interaction of Antimicrobial Peptides With Mixed Lipid Bilayers
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The interactions of two α-helical antimicrobial peptides, aurein 1.2 (13 residues) and maculatin 1.1 (21 residues), with model membranes have been examined using solid-state NMR and surface plasmon resonance techniques. P-31 NMR of multilamellar (MLV) dimyristoylphosphatidycholine (DMPC) vesicles with aurein 1.2 revealed minor disruptions in the bilayer above the gel-liquid phase transition. However, below the phase transition temperature an isotropic signal was observed, indicating that the peptide disrupted the bilayer and formed small, rapidly tumbling aggregates ~ 22 nm in diameter as determined by light scattering measurements. However, the isotropic signal was not seen with the longer peptide. Additional experiments conducted using different lipid compositions revealed that both fluidity and temperature influence the peptide interaction. Gel phase lipid bilayers were more strongly affected by the peptide although similar effects were observed at lower temperatures in unsaturated chain lipid bilayers in the liquid crystalline state.
A preliminary study on membranes mimicking the lipid composition of S. aureus has demonstrated a disruptive effect on the bilayer organization by addition of maculatin 1.1, a potent antibacterial peptide. As revealed in P-31 static NMR spectra of MLV composed of dimyristoylphosphatidylglycerol (DMPG) and tetramyristoylcardiolipin (TMCL), the peptide promoted formation of a dominant isotropic phase at 15°C, well below the liquid-crystalline transition temperature; while the lamellar organization was mainly restored above 50°C and an intermediate state was observed at 30°C. Interestingly, relaxation experiments on MLV without peptide indicated coexistence of two populations in the temperature range 30-50°C, most likely composed of fluid DMPG and rigid TMCL. The antimicrobial peptide may insert preferentially at domain boundaries, using defects in membrane packing to lower energy costs. Further experiments are ongoing to determine the nature of the isotropic phase and its relevance to antimicrobial activity.

444-Pos
Determination of a High-Definition Structure of Antimicrobial Piscidin-3 At the Water-Bilayer Interface
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Piscidins are a family of naturally occurring host-defense antibiotics that are short, cationic, and amphipathic in structure. Extensive NMR studies of membrane-bound Piscidin 1 (p1), a 22-mer, have shown that the peptide is composed of two alpha-helical segments that lie in the plane of the lipid bilayer. These segments are joined by a kink at residue glycine 13 (G_{13}). Previous studies of Piscidin 3 (p3), another isoform of piscidin, have revealed decreased antimicrobial and hemolytic activity when compared to p1. The goal of this research is to create a high-definition backbone structure of membrane-bound p3 in order to discern the atomic-level structural features that account for the differences in activity of the two peptides. Understanding the mechanistic differences is critical for the development of novel antimicrobial drugs.
Circular dichroism has previously shown that p3 adopts an alpha-helical structure in the presence of micelles and phospholipid bilayers. Using hydrated, oriented lipid bilayers that mimic bacterial cell membranes and 2D HETCOR (Heteronuclear Correlation) solid-state NMR experiments, high-resolution ^{15}N and ^{1}H Chemical Shifts (CS), and ^{15}N-^{1}H Dipolar Couplings (DC) have been obtained from selectively ^{15}N-backbone labeled p3. Spectra collected at high and ultra high magnetic field have been analyzed to obtain the backbone structure and orientation of membrane-bound p3. This analysis has revealed that p3 also consists of two alpha-helical segments kinked at G_{13}. Interestingly, the rotational angles of p1 and p3 about their own helical axes within the plane

of the bilayer differ significantly. This difference may alter the magnitude of the peptide's side chain implantation in the membrane and thus its activity. The solid-state NMR data collected on p1 and p3 will be used to create a high-definition structure using structure determination programs such as XPLOR.

445-Pos

Structural Studies of An Immune Modulating and Direct Antimicrobial Peptide

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The structure and function of the innate defence regulatory peptide 1018 was investigated. This peptide, whose sequence is distantly related to that of the 12 residue linear antimicrobial peptide Bac2A, a synthetic peptide derivative of the bovine cathelicidin Bactenecin, has both innate immune regulatory and direct antimicrobial activities. We present the solution state NMR structure of 1018 in DPC micelles, as well as its secondary structure in SDS and POPC/PG (1:1 molar ratio) from CD measurements. These structures reveal that 1018 can adopt a variety of folds, tailored to its different functions. The structural data is discussed in light of the ability of 1018 to induce cytokine and chemokine responses, to reduce the LPS-induced TNF-α response, and finally, to directly kill both Gram positive and Gram negative bacteria.

446-Pos

Determining the Charge State of Histidine Side Chains in Antimicrobial Piscidin By Nuclear Magnetic Resonance

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Piscidins constitute a family of three antimicrobial peptides discovered in the mast cells of hybrid striped bass. These peptides, which are highly cationic, contain several arginine and histidine residues. While piscidin 1 is the most antimicrobial and hemolytic isoform, piscidin 3, which has slightly lower antimicrobial activity, is significantly less hemolytic. One of the most striking differences between piscidin 1 and 3 is the substitution of glycine for the histidine at position 17 in piscidin 1.

As part of its mechanism of action, piscidin recognizes negatively charged microbial membranes. Therefore, studying the interactions of the piscidin with lipids can help us better understand the chemical basis of its antimicrobial and hemolytic effects. Because physiological pH is around 7.4, and the average pKa of histidine side chains is around 6.0, a detailed study of the histidine side chains in piscidin 1 and 3 is needed to discern the charge state of the peptides under physiological conditions. In this research, we used solution nuclear magnetic resonance to obtain the pKa of the histidine side chains of piscidin bound to sodium dodecyl sulfate micelles. Heteronuclear multiple quantum coherence experiments were performed on piscidin 1 and 3 containing ^{15}N-side chain labeled histidines. ^{15}N and ^{1}H chemical shifts were recorded as a function of pH to determine the titration curve of each histidine residue. The results will be discussed in the context of structure-function relationships in membrane-active peptides. The knowledge gained from these studies can help identify common principles that will facilitate the design of pharmaceuticals with broad-spectrum antibacterial activity, minimum induction of bacterial resistance, and low toxicity to mammalian cells.

447-Pos

Interaction of the Cationic Peptide Bactencin With DDPC/DMPG Phospholipid Mixtures At the Air-Water Interface

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In this work we show the results of the interaction of the cationic antimicrobial peptide bactenecin (Arg-Leu-Cys-Arg-Ile-Val-Val-Ile-Arg-Val-Cys-Arg) with DPPC/DMPG (X_{DPPC}= 0.5, X_{DMPG}= 0.5) mixtures using the Langmuir Through. The -A compression isotherms exhibit differences compared to those with DPPC alone, remaining the area per molecule, near 50 Å . The results obtained with atomic force microscopy indicate that mixed monolayers show a height near to 1.7 nm. Penetration of the dodecapeptide into the DPPC/DMPG mixtures at various surface pressures were investigated to determine the ability of this lipid monolayer to host the bactenecin. The higher penetration of peptide into phospholipids is attained when the monolayer is in the LC phase due to the control pressure applied (10, 15, 20 mN/m) and a greater interaction is allowed when DMPG is added in comparison with those monolayers of pure DPPC. The effect of bactenecin at the phospholipids' mixed monolayer was the shift of the LE phase at higher area per molecule. Circular dichroism of monolayers and multilayers of bactenicin/phospholipids were performed to investigate the peptide conformation.

448-Pos

LFampin Derived Antimicrobial Peptide: Biophysical Characterization and Biological Implications of Composition and Structure

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The innate immunity factor lactoferrin harbours two antimicrobial sequences situated in close proximity in the N1-domain, Lactoferricin (LFcin) and Lactoferrampin (LFampin). The more recently discovered LFampin by Jan Bolscher's group contains residues 268-284 from the N1 domain of Lactoferrin. Thereafter, a new family of antimicrobial peptides was obtained from LFampin by extension and/or truncation at the C- or N-terminal sides, keeping the essential characteristics, in order to unravel the main structural features responsible for antimicrobial action. These related synthetic peptides show broad-spectrum bactericidal activities against a range of Gram-positive and Gram-negative bacteria, as well as fungus. Bioactivity was tested towards pathogenic yeast *Candida albicans*and model bacteria strains.

The biophysical interaction with model membranes was studied by Differential Scanning Calorimetry (DSC), Isothermal Titration Calorimetry (ITC), Fluorescence Spectroscopy, Circular Dichroism, Zeta Potential and SAXD measurements.

Results will be presented for one of the peptides of this family, Lfampin 265-284, both regarding bioactivity and interaction with liposomes of DMPC, DMPG and DMPC:DMPG (3:1) as model membranes. Furthermore, the biophysical and biological implications of composition and structure will be discussed.

449-Pos

Roles of Lys and Arg in the Activity of Antimicrobial Peptides

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Antimicrobial peptides (AMPs) play a pivotal role in innate immunity. Most peptides kill microorganisms by permeabilizing cell membranes (e.g., magainin 2), although there are peptides targeting intracellular macromolecules, such as DNA (e.g., buforin 2). A common property of AMPs is polycationicity that enables the peptides to selectively interact with negatively charged bacterial surface. Some peptides (e.g., magainin 2) mainly contain Lys, and others (e.g., buforin 2) use Arg as a basic amino acid. To understand the roles of these amino acids in the activity of AMPs, we synthesized the magainin 2 and buforin 2 analogues.

The interaction with lipid bilayers were slightly enhanced by the K-to-R substitution because of a marginally larger hydrophobicity of Arg, and vise versa. In contrast to the membrane interaction, the substitutions significantly affected interaction with DNA. The Arg-containing peptides MGR and BF exhibited much stronger affinity for DNA than the Lys-containing counterparts. The antimicrobial activity of the membrane-acting magainin was not influenced by the K-to-R substitution, whereas that of the DNA-targeting buforin was lost by the R-to-K substitution.

450-Pos

Characterization of Indolicidin-Membrane Interactions By Simultaneous Attenuated Total Reflection Fourier-Transform Infrared Spectroscopy-Atomic Force Microscopy

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A detailed understanding of how antimicrobial peptides interact with bacterial membranes is a key step towards the effective design of novel antibiotics to treat infection. These interactions may include membrane-induced conformational changes to the peptide, membrane disordering, as well as peptide aggregation. To understand the effect of both membrane composition and peptide sequence on these phenomena, we applied simultaneous attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR)-atomic force (AFM) microscopy to directly visualize and characterize the interactions of the model antimicrobial peptide, indolicidin, with a series of supported planar lipid bilayers. This approach allows us to directly interrogate how peptide association, aggregation, and insertion alter the structure of the bilayer. It also allows us to directly assess changes to the secondary structure of the peptide as a consequence of both specific peptide-membrane interactions as well as peptide-peptide interactions. Simultaneously acquired AFM images provide direct confirmation of the effect of the peptide on membrane integrity, evidence of domain targeting, as well as the kinetics and structure of putative peptide