visualize and characterize the interactions of two different classes of model membranolytic peptides with supported planar lipid bilayers. Our studies provided direct evidence of membrane rearrangement and peptide aggregation by a *de novo* cationic antimicrobial peptide designed to adopt a helical motif in bacterial membranes. We also obtained *in situ* evidence of specific secondary structure motifs associated with a membrane-induced fibrillization of peptides derived from haemolytic proteins obtained from sea anemone. This coupled approach provides a unique opportunity to directly link spectroscopic details associated with peptide-membrane interactions with in situ structural insights obtained on nanometer length scales.

1900-Plat
Interactions of Membrane Active Peptides with Planar Supported Bilayers: an Impedance Spectroscopy Study
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Membrane active peptides are a class of amphipathic peptides that destablize or penetrate cell membranes via a mechanism that is not well understood. Here, peptides identified from a combinatorial library using high throughput screens were deposited over a supported lipid bilayer platform constructed on single crystal silicon and their activity was characterized by electrochemical impedance spectroscopy (EIS). By monitoring the bilayer's impedance with time, we determined the kinetics of the interaction as a function of peptide sequence, concentration, and lipid composition. These measurements yield new insights into the activity of membrane active peptides.

1901-Plat
Insertion of Amphipathic Helices into Membranes Depends on the Spontaneous Curvature of the Lipid System
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The antimicrobial peptide MSI-103 forms an amphipathic α-helix when binding to a lipid membrane. The orientation of the helix in the membrane can be determined with high accuracy using ^{2}H solid-state NMR. In the present study the orientation of MSI-103 was determined in a wide range of lipid systems with varying properties.
In phosphatidylcholine (PC) bilayers with different acyl chains, there was no correlation between chain length and peptide orientation. However, a distinct difference was observed in the peptide response to saturated and unsaturated acyl chains. In unsaturated lipids, the peptide always remained in the surface-bound S-state, with its α-helical axis perpendicular to the bilayer normal at a tilt angle close to 90o. Only in saturated lipids it was able to insert into the membrane in a tilted T-state, with a tilt angle of around 125o. Interestingly, when lyso-PC was added, the T-state was found to be stable also in unsaturated lipids.
These results can be explained by the shape of the lipids; especially the relative cross-sectional area of head groups and acyl chains, which is related to the spontaneous curvature. In systems with negative spontaneous curvature, the head group area is small relative to the acyl chain area, and in such systems, peptides are always found in the S-state. In systems with positive spontaneous curvature, peptides are found in a T-state at higher concentration.
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.

1902-Plat
The Interfacial Behaviour of Peptides Over Long Time Scales
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Peptides are frequently used as models for membrane proteins due to their accessibility and low molecular weight, both of which facilitate the interpretation of complex spectroscopic data. Membrane proteins are recycled in vivo with half-lives ranging from minutes to days. It is therefore of interest to examine the behaviour of peptides in model membranes over extended periods in order to determine whether these systems reach equilibrium and remain stable. This timescale is seldom examined in studies using model peptides. We have examined kinetics of defensin HNP-2 binding to aligned membranes by both fluorescence spectroscopy and linear dichroism spectroscopy. Changes in membrane alignment were found to occur over a period of hours to days. The reactivity of peptides towards membranes has been examined using melittin. Acyl transfer from phospholipids to the peptide was found to occur over a period of several days. Some of these observations challenge preconceptions concerning the behaviour of peptides at the membrane interface.

1903-Plat
Phospholipid Fatty Acid Structure and the Activity of Membrane-Active Peptides
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Virtually all studies on the interaction of antimicrobial peptides with model bilayers have focused on phospholipid headgroup composition, using unsaturated phospholipids to model bacterial cell membranes. The use of unsaturated lipid ensures fluid-phase membranes at room temperature, but unsaturated lipids are rarely found in cell membranes of gram-(+) bacteria. In *Staphylococcus aureus*, for instance, the major membrane lipids are saturated iso- and anteiso-branched phospholipids with phase transition temperatures well below room temperature. We found previously that increasing the degree of lipid acyl chain unsaturation markedly reduces the susceptibility of vesicles to delta-lysin, a potent, membrane-active peptide. We thus postulated that the acyl chain structure of bacterial lipids would play a role in the sensitivity of bacteria to antimicrobial peptides. The idea is corroborated by the observation that in methicillin-resistant *S. aureus* (MRSA), the fraction of anteiso-branched acyl chains is elevated relative to susceptible strains, pointing to a relation between fatty acid structure and bacterial resistance. In the work presented here, we tested this hypothesis by synthesizing asymmetric, saturated phospholipids containing an iso- or anteiso-branched fatty acid in the *sn*-2 position. We then examined the effect of the acyl-chain structure on peptide activity by constructing lipid vesicles and measuring the kinetics of dye release induced by delta-lysin. The results were compared with those obtained from lipid vesicles composed of natural staphylococcal phospholipid extracts enriched in either iso- or anteiso-branched phospholipids. Acyl chain structure was shown to indeed have a dramatic effect on membrane stability and the susceptibility to membrane-active peptides. In general, vesicles constructed from bacterial lipid extracts were considerably more stable and more resistant to peptide attack than those made from synthetic, branched lipids.

1904-Plat
Resolution of Sample Heterogeneity in Solid-State NMR Dipolar Recoupling and Magic Angle Spinning Sideband Analyses
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We have engineered algorithms to characterize heterogeneous distributions of chemical shift tensor parameters from magic angle spinning sideband intensities, as well as distances from rotational-echo double-resonance (REDOR) data. In the case of REDOR data, the method can reveal multiple distances with relatively few data points, which is of particular benefit in application to biological systems. This reverses the common practice of comparing REDOR data to dephasing curves simulated for several preconceived structural models, by providing the information necessary to construct models based on unbiased data analysis. In the case of chemical shift tensor analysis, it allows arbitrarily complex phospholipid mixtures to be analyzed for subtle perturbations, for example by association with antimicrobial peptides.
The method uses an adaptation of Boltzmann statistics maximum entropy, and provides for a model-free approach to data analysis, as one need not assume the presence of any specific number of different chemical shift tensors or distances that contribute to observed signal. The strategy differs from the more common practice of including a single weighted term in a fitting procedure, as each datum constitutes additional "information" and independently adds to or subtracts from the entropy of the distribution. A constrained optimisation problem with 100s of unknowns (the number of points used to approximate a continuous probability distribution of the desired parameter(s)) is thereby turned into an unconstrained optimisation problem with one coefficient for each data point.
The Boltzmann Statistics method also offers intriguing philosophical implications – it gives the broadest, and perhaps most "honest" probability distribution consistent with the data, but also helps to determine which data points hold the greatest information content, such that experimental time can be focussed on gaining the best signal-to-noise where it is likely to benefit most.