bacterial cells incubated with these peptides. Based on the similarity between the absorbance versus time trend for the designed peptides with other DNA-binding antimicrobial peptides, such as BF2 and indolicidin, molecular dynamics simulations were used to model the peptides' interactions with nucleic acids. MM-GBSA analyses of the simulations were used to calculate DNA binding energies of individual peptide residues. We used these analyses to create mutant versions of the designed peptides that were predicted to have altered DNA binding. Experimental measurements of the DNA binding and antimicrobial properties of these variants will help us determine whether nucleic acid interactions are important in the bactericidal mechanism of the designed peptides. Ongoing work on the designed peptides is aimed at investigating their translocation behavior *in vitro* with lipid vesicles and i*n vivo* with bacterial cells using confocal microscopy and fluorescently tagged peptides.

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Investigating the Effects of Acylated Lactoferricin Peptides on the Properties of Lipid Bilayers Using Gramicidin A Channels as Probes

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Lactoferricin is an anti-bacterial peptide that is released from the iron-binding glycoprotein lactoferrin through enzymatic cleavage by pepsin. Lactoferricin is found mainly in milk and secreted fluids such as tears, saliva, bronchial mucus and seminal fluid, and it plays an important and multi-functional role in host defense, as it is part of the body's primary defense against bacteria, fungi, protozoa and viruses. It also has antitumor and immunological effects. Previous studies show that lactoferricin may inhibit bacterial growth by two different mechanisms: by sequestering the iron necessary for bacterial nutrition; and by adsorbing to bacterial plasma membranes, which may disrupt the membrane barrier properties or some other membrane function. Lactoferricin indeed permeabilizes bacterial membranes, but it remains unclear whether this is the primary mechanism by which it exerts its anti-bacterial activity. We therefore explored whether lactoferricin analogues could alter other bilayer properties, using gramicidin A (gA) channels of different lengths as probes. Specifically, could the lactoferricins alter lipid bilayer elasticity or intrinsic curvature. We tested two amino acylated lactoferricin derivatives, NC2-LfB-1MeTrp5 and NC4-LfB-1MeTrp5 (with the sequences Ac-R-R-W-Q-MeW-R-NH_2 and Bu-R-R-W-Q-MeW-R-NH_2). Both compounds increase gA channel appearance rates and lifetimes, meaning that they decrease bilayer stiffness, at concentrations (1-10 μM) where they do not cause a breakdown of lipid bilayer barrier properties. Because they had similar effects on the lifetimes of the long and short channels, we conclude that the lactoferricins alter lipid intrinsic curvature.

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On the Role of Helix-Disrupting Amino Acid Residues in Supporting the Activity of Helical Antimicrobial Peptides Isolated from Australian Tree Frogs

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The peptides Aurein 1.2, Citropin 1.1, Maculatin 1.1 and Caerin 1.1 are members of four structurally related families of antimicrobial peptides produced in the skin secretions of Australian tree frogs. Although largely unstructured in aqueous solution, these peptides exhibit a high propensity for folding into amphipathic alpha helices when partitioned into lipid bilayers or dissolved in membrane mimetic media. Some of the distinguishing features of these families of antimicrobial peptides are that they usually form alpha helical structures with large hydrophobic surfaces (hydrophobic angle ~200-240°), and the amino acid sequences of many of the larger members (i.e. those with sequence lengths >~18 aa residues) usually contain significant amounts of helix-disrupting residues such as glycine and proline, the presence of which seems to be essential for the retention of antimicrobial activity. These helix-disrupting amino acid residues seem to be preferentially located in the C-terminal regions of the peptide where they tend to disrupt the break up the helical rod into two or more helical sections separated by disordered ''flexible hinge'' regions. The role of these ''flexible hinges'' has been the subject of considerable study and speculation. Our studies show that because of their large hydrophobic surfaces, they form helices with a high propensity for self association in aqueous media, and this property markedly diminishes the aqueous monomeric solubility of such peptides. Our results suggest the helix-disrupting amino acid residues may be essential for maintaining the aqueous solubility of these antimicrobial peptides

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Fine-Tuning of Acyl-Lysine Antimicrobial Peptide Mimics

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Non-natural mimics of antimicrobial peptides are excellent candidates for anti-infectious agents due to their stability towards enzymatic degradation and broad adjustability of physicochemical properties. Acyl-lysine oligomers have demonstrated capability to be fine-tuned to high antimicrobial activity and negligible toxicity towards human cells. In this work we examine the effect of amino group or a double bond on the N-terminal acyl on interactions of the oligomer with model lipid monolayers using the liquid surface X-ray scattering techniques of X-ray reflectivity and grazing incidence X-ray diffraction. Lipid monolayer formed at the air-liquid interface mimics the membrane interface where antimicrobial peptides approach the outer leaflet of a target cell membrane. Simplified model of an outer leaflet of a bacterial membrane was represented either with DPPG or Lipid A, while mammalian cell membrane was mimicked with zwitterionic DPPC. The peptides were subsequently injected into the aqueous subphase and allowed to interact with the lipid layer. In addition to X-ray experiments, the lipid phase morphology before and after peptide mimics insertion for each lipid film was visualized by epifluorescence microscopy. Significantly higher insertion of the peptide mimics into anionic rather than zwitterionic lipid monolayers strongly supports the activity trends observed in previously reported antimicrobial and hemolytic assays. Although removing of the double bond notably increases peptide's selectivity and introduction of the amino group increases peptide's potency against bacteria, both of the modifications substantially increase MIC of the oligomers.

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Investigation of Antimicrobial and Lipid Perturbing Properties of Lactoferrin Peptides

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An increase in bacterial resistance to conventional antibiotics has led to an intense search for alternative treatments. Lactoferricin B (**FKCRRWQWRMKKLGAPSITCVRRAF**), a peptide with potent broad-spectrum antimicrobial activity, is released by pepsin from bovine lactoferrin. A smaller amidated peptide, (LfB6; **RRWQWR**-NH_2), has been identified as having the core antimicrobial activity (Tomita et al. (1994) *Acta Paediatr Jpn.* 36:585-91). The exact mechanism by which antimicrobial peptides interact with bacterial cell membranes is not well understood, but it is proposed to depend on lipid composition. In contrast to mammalian membranes which are comprised primarily of neutral lipids, bacterial membranes contain a significant (~20-25%) fraction of negatively charged lipids. In the case of LfB6, the presence of two tryptophans (W; Trp) and three arginines (R) are thought to promote selective interaction with bacterial cell membranes. Recently, we have shown that the antimicrobial activity of LfB6 peptides is increased by N-acylation and Trp-methylation (Greathouse et al. (2008) *J. Pept. Sci.* 14:1103-1110).

To ascertain whether LfB peptides perturb lipids with negatively charged head groups, macroscopically aligned bilayers composed of lipids to mimic bacterial cell membranes have been prepared in the absence and presence of peptide. The samples are composed of neutral (POPE) and anionic (POPG) lipids (3:1), containing either s*n-1* chain perdeuterated POPE-d_{31} *or* POPG-d_{31}. The effects of LfB6 and amino acylated LfB peptides on lipid dynamics are being investigated by solid-state deuterium NMR spectroscopy and differential scanning calorimetry. The ^{2}H NMR spectra reveal that the addition of LfB6 results in slight but specific changes in the outer quadrupolar splittings, which result from the methylene groups closest to the lipid head groups. Antimicrobial assays against *S. aureus* and *E. coli* demonstrate that the activity of N-acylated LfB peptides increases with acyl chain length.

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Interaction And Unfolding Of A Model Exchangeable Apolipoprotein, ApolpIII, At Lipid Model Membranes

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Transport of fat in the bloodstream is mediated by lipoprotein particles. These hydrophobic particles are covered by a (phospho-)lipid monolayer and further stabilized by exchangeable apolipoproteins. Their amphipatic helix bundle is thought to unfold upon interaction with the (phospho)lipid monolayer. The dynamics of this interaction at lipid interfaces has not been directly shown experimentally. Here we report on the structure of monolayers formed by

a representative exchangeable apolipoprotein, apoLpIII from Locusta Migratoria, using surface-sensitive X-ray techniques. This model exchangeable apolipoprotein contains a 5-helix bundle and shows high structural homology to the 4-helix bundle in the N-terminus of apoE, a major mammalian exchangeable apolipoprotein. We found that the structure of two diacylglycerols, the proposed binding partner of apoLpIII, dioleoylglycerol (DOG) and 1-palmitoyl, 2-oleoylglycerol (POG), showed great resemblance to other (phospho)lipid monolayers except for details due to differences in the headgroup region. Despite their near identical chemical structures small differences in organization as monolayers were observed between DOG and POG. For the pure apoLpIII monolayer we observed that the unfolded protein was best represented by two distinct regions. This surprising result may originate from the high degree of glycosylation of apoLpIII. The interaction of apoLpIII underneath a densely packed diacylglycerol monolayer causes the surface pressure to increase rapidly. The analysis of the X-ray reflectivity of the lipid/protein system, right after the injection of apoLpIII, shows a diffuse layer underneath the lipid monolayer due to the binding and unfolding of apoLpIII. This may be the first direct visualization of the dynamic unfolding of an exchangeable apolipoprotein at a lipid interface. Our data suggest that the initial interaction occurs when the protein is tilted with respect to the lipid monolayer, a process that would favor the opening of the helix bundle.

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Folding Of Lipid Monolayers Containing Lung Surfactant Proteins SP-B1-25 and SP-C Studied via Coarse-grained Molecular Dynamics Simulations

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To explore the role of lung surfactant proteins SP-B and SP-C in storing and redelivering lipid from lipid monolayers during the compression and re-expansion occurring in lungs during breathing, we simulate the folding of lipid monolayers with and without these proteins. We utilize the recently developed MARTINI coarse-grained force field to simulate monolayers containing pure dipalmitoylphosphatidylcholine (DPPC) and DPPC mixed with palmitoyloleoylphosphatidylglycerol (POPG), palmitic acid (PA), and/or peptides. The peptides considered include the 25-residue N-terminal fragment of SP-B (SP-B_{1-25}), SP-C, and several SP-B_{1-25} mutants in which charged and hydrophilic residues are replaced by hydrophobic ones, or vice versa. Most of these peptides facilitate folding of the monolayer during compression by a ''zipper'' mechanism, which is dependent on the formation of a peptide aggregate. However, we find that if the number of hydrophobic residues is decreased significantly monolayer folding does not occur via the ''zipper'' mechanism. During the re-expansion of folds formed via the ''zipper'' mechanism, the folds are observed to unfold with the lipids re-entering the monolayer before the peptide aggregates. Our results show several key trends. The addition of POPG to the DPPC monolayer has a fluidizing effect, which assists monolayer folding. In contrast, the addition of PA has a condensing affect. The addition of peptides fluidizes the monolayer and accelerates the folding processes. If the peptides are allowed to aggregate, the peptide aggregate nucleates a defect in the monolayer, further assisting the folding process. The results also show a clear system-size dependence that affects the folding mechanism observed. If the system size is large enough peptide containing monolayers can fold without the formation of a peptide aggregate.

Membrane Structure II

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Structural Changes in DMPG upon changes of ionic strength and pH - What to learn from SANS, DSC, FCS, Flourescence Microscopy, FTIR and Viscosity Measurements

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Commonly pure phospholipid membranes are used as models for the more complex real biological membranes. Self-assembling the phospholipids can exhibit a number of different lamellar and nonlamellar phases. They are undergoing a cooperative melting reaction, which is linked to loss in conformational order of the lipid alkyl chains. In certain cases, like for aqueous dispersions of dimyristoyl phosphatidylglycerol (DMPG) a negatively charged phospholipid, this is resulting in an extended network system. The transitions associated are depending on temperature, pressure, lipid concentration and sample environment, such as ionic strength and the pH value (Schneider, M. F. et al., PNAS, 96 (1999) 14312; M.T. Lamy-Freund, K.A. Riske, Chem. Phys. Lipids, 122 (2003) 19).

We studied DMPG dispersions under different pH and ionic strength conditions using methods gaining complementary information about the changes in material properties. In particular we studied the structural changes using small angle neutron scattering (SANS) as well as fluorescence microscopy and the thermodynamical by differential scanning calorimetry (DSC). In addition rheology, Fluorescence Correlation Spectoscopy (FCS) and Fourier-Transform Infrared Spectroscopy (FTIR) was performed. This results in a comprehensive model, taking into account the thermodynamic and structural changes below, in and above the region of the phase transition.

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Formation of Block Liposomes is a General Phenomenon of Charged Membranes

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We have recently reported on the discovery of block liposomes (BLs), a new class of chain-melted (liquid) vesicles, formed in mixtures of curvature-stabilizing hexadecavalent cationic lipid MVLBG2, DOPC and water (Zidovska et al., *Langmuir*, 2008). BLs consist of distinctly shaped, yet connected spheres, pears, tubes, or rods. Unlike typical liposome systems, where spherical vesicles, tubular vesicles, and cylindrical micelles are separated on the macroscopic scale, within a BL, shapes are separated on the nanometer scale. We carried out structural studies of BLs with differential-contrast-microscopy (DIC) and cryo-transmission-electron-microscopy (cryo-TEM) and identified membrane charge and spontaneous membrane curvature as key parameters controlling the BL-formation. BL-formation was believed to be a special capacity of MVLBG2, a newly synthesized highly charged (16+) lipid (Ewert et al., *JACS,* 2006) with giant dendrimer-like headgroup leading to a conical molecular shape resulting into high spontaneous membrane curvature, when incorporated into lipid bilayer. In this work we report formation of BLs for other cationic lipids, demonstrating that BL-formation is a general phenomenon of all charged membranes. We carried out systematic study of binary lipid mixtures comprised of DOPC and a cationic lipid, varying the headgroup size and charge of cationic lipid from 1+, 3+ to 5+. We find that all cationic lipids form BLs on the micrometer and nanometer scale. We have also found that pure DOPC forms BLs in presence of monovalent salt, which is known to cause zwitterionic DOPC to become negatively charged. This latter finding confirms that BL-formation is a general capacity of charged membranes independent of the charge nature. Block liposomes may find a range of applications in chemical and nucleic acid delivery and as building blocks in the design of templates for hierarchical structures. Funding by DOE DE-FG-02-06ER46314, NIH GM-59288, NSF DMR-0503347.

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Lipid Bilayer Pre-Transition as the Beginning of the Melting Process: a Periodic Melting

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We investigate the bilayer pre-ransition, which some lipids present at temperatures below their main phase transition, and which is generally associated to the formation of periodic ripples. Experimentally, we focus on the ubiquitous dipalmytoylphosphatidylcholine (DPPC) and on its charged analog dipalmytoylphosphatidylglycerol (DPPG) at different ionic strengths. Analysis of the excess heat capacity of DPPC and DPPG shows that both pre- and main transitions are part of the melting process. The cooperativeness of DPPG is lower at low ionic strength. Electron spin resonance of spin labels located at the bilayer center reveals the coexistence of gel and fluid domains at temperatures between the pre- and main transitions. Excitation generalized polarization of Laurdan also suggests microphase coexistence in the ripple phase of both lipids. To broaden the knowledge on the ripple phase, we introduce a new statistical model where a next-nearest-neighbor competing interaction is added to the usual two-state model. For the first time, modulated phases, with ordered and disordered lipids naturally appearing in a periodic fashion, are obtained between the homogeneous gel and fluid phases. A better understanding of the different interactions among lipids in a bilayer is of fundamental importance to the full knowledge of the biophysics of natural membranes.

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Phase Transitions In Charged-lipid Membranes: A Statistical Model

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