The explosive development of new antibiotic resistant strains of bacteria makes it essential for us to develop new antibiotic drugs, preferably one which operate via novel pathways. Compounds based on antimicrobial peptides seem like good candidates, because they are broadly effective and largely resilient to evolved immunity. However, natural peptides tend to be much larger than typical drugs, expensive to synthesize, and tend to be rapidly digested in the bloodstream. Shai and coworkers have recently begun invesitgating a new set of compounds, synthetic antimicrobial lipopeptides, designed to have many of the strengths of natural peptides while avoiding many of their weaknesses. Here we use all-atom molecular dynamics of simulations of two of the peptides they used – C16-KGGK and C16-KGGK – bound to zwitterionic, ''mammlian-like'' membranes (POPC) and anionic, ''bacterial-like'' membranes (POPE:-POPG). The variations in their structure and dynamics suggest new insights into the mechanism of selectivity and function.

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Learning From A Bacillus How To Kill Fungi

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Bacterial lipopeptides are a new, very potent and environmentally safe alternative to classical fungicides used in agriculture.
We have studied peptide fractions from strain QST713 of Bacillus subtilis which is registered and applied as a pesticide active ingredient and produces high levels of three classes of lipopeptides: iturins, agrastatins (plipastatins), and surfactins. These compounds consist of a small cyclic peptide ''head group'' containing 7 or 8 (mostly anionic and hydrophobic) amino acid residues and a lipidic chain. In spite of this surfactant-like design, the lipopeptides are much more effective in disrupting membranes and a few mole percent of bound peptide (corresponding to less than 10 uM free concentration) suffice to induce leakage and lysis of the membrane. We have studied these compounds by ITC, membrane leakage assays and other experiments utilizing time-resolved fluorescence spectroscopy, NIBS light scattering, and other methods. We will address the mechanism of action and the reason for the extreme lytic activity of these compounds, the interplay between the different peptides in the biologically optimized mixture, and the selectivity of the action to different lipid membranes. It should be noted that bacterial lipopeptides are distinct from antibiotic peptides produced by higher organisms but parallels in their principal behaviour suggest that the insights obtained here will also improve our understanding of this new class of antibiotics.

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Short Membrane Active (Lipo)-peptides - Interplay Of Domain Formation, Membrane Curvature Stress And Cellular Leakage

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The effect of short peptides, derived from lactoferricin, a human host defense peptide exhibiting antibacterial activity and their N-acylated derivatives was studied with biological and membrane mimetic systems. The work carried out during the European RTD-Project ''ANEPID'' revealed correlation between biological activity against E.coli and interaction with negatively charged lipid model systems, leading to formation of peptide effected lipid domains. Increase of hydrophobicity by addition of hydrophobic amino acids as well as N-acylation improved activity in model and biological systems, but was limited by loss of selectivity for bacterial systems. Addition of peptides to bacterial mimics caused de-mixing into charged peptide effected domains and neutral mainly unaffected domains. Following induction of membrane curvature stress and leakage of cellular contents at defect lines of these induced domains appear to be the major effects of the studied peptides, which could be proven with E.coli mimetic systems. Major perturbance of cytoplasmic membranes of bacteria was also revealed by electron microscopy indicated by peptide induced formation of large membrane blebs and partially by detection of oversized cells that might reflect peptide induced defects in cell-division.

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Free Energies of Molecular Bound States in Lipid Bilayers: Lethal Concentrations of Antimicrobial Peptides

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The lipid matrix, or the lipid bilayer, of cell membranes is a natural binding site for amphipathic molecules, including antimicrobial peptides, pore-forming proteins, and many drugs. The unique property of pore-forming antimicrobial peptides is that they exhibit a threshold concentration (called lethal concentration or minimum inhibitory concentration) for activity, below which no effect is seen. Without this property, antimicrobial peptides would not be effective self-defense weapons, because they would have harmed all cells at any concentration. The question is what gives rise to this unique property? Here I give a free energy description for the origin of a threshold concentration. The same free energy also explains binding of drugs which shows no threshold concentration. The idea is compared with theories of micellar solutions which require a large oligomerization size (n~15) to achieve a threshold concentration. What makes the phenomena in membranes different is the elasticity of lipid bilayers. Antimicrobial peptides have a large negative free energy for binding to the bilayer interface, but the binding causes membrane thinning. This elastic energy of thinning elevates the energy level of interfacial binding with concentration, hence gives rise to a threshold concentration for forming pores containing as few as 4 peptides.

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Biophysical Parameters Involved in Bacteria Resistance to Antimicrobial Peptides

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The molecular mechanism, by which bacteria sense antimicrobial peptides (AMPs) that promote its virulence is partially known. Bacteria are capable of changing the expression of virulence genes essential to survival and replication, by sensing changes in their microenvironment within the tissues of their host. As a consequence they have the potential to develop resistance to AMPs. In the case of *Salmonella typhimurium*, some of the virulence genes are controlled by the two component regulatory system, PhoP/PhoQ. The sensor protein of this system, PhoQ, is directly activated by antimicrobial peptides (AMPs). PhoQ phosporylates and activates PhoP, a transcriptional regulatory protein, which in turn activates or represses over 40 different genes. The activation of these genes was found to be essential to the survival of these pathogenic bacteria within the host macrophages. However, it is not yet clear whether this mechanism is shared by AMPs in general, or it requires specific biophysical properties for AMPs such as secondary structure, amino acid composition or specific sequence. Our studies reveal that changing the biophysical properties of a peptide that can induce resistance, such as incorporation of D-amino acids, can improve the peptides activity against *Salmonella typhimurium* probably by affecting the two component system. Studes along this line suggest that such peptide modifications can be used in order to overcome the inducible resistance of *Salmonella typhimurium.*

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Antimicrobial Peptide C18G binds to Lipid Bilayers in a Lipid Composition Dependent Manner

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Antimicrobial peptides serve as one of the first lines of defense in the immune systems of higher organisms. These peptides specifically target and neutralize infecting bacteria in the host organism while exhibiting little or no toxic effect on host cells. The peptide C18G is a highly cationic, amphiphilic peptide derived from the C-terminal sequence of the human protein platelet factor 4 (involved in blood coagulation and wound repair) exhibited antibacterial activity against both gram positive and gram negative bacteria. Using a modified C18G sequence that did not affect antimicrobial efficacy (Y3 changed to W), the binding affinity of the peptide to model membranes was performed using fluorescence spectroscopy. As anticipated, the binding of C18G to lipid bilayers allowed the Trp side chain to localize to a more hydrophobic environment resulting in a blue shift of Trp emission lambda max and spectral barycenter, concomitant with a narrowing of the emission spectrum and and increase in the overall emission intensity. Binding to lipid vesicles composed of binary and tertiary mixtures of POPC:POPG and POPC:POPG:POPE showed a dramatic lipid dependence on binding affinity, with the tightest binding to the most anionic compositions. Increasing the POPE composition enhanced peptide binding but to a lesser degree compared to the anionic POPG. Fluorescence quenching experiments using the aqueous quencher acrylamide confirmed the decreased exposure of the Trp to the aqueous milieu. Dye release assays were used to monitor lipid composition effects on the ability of C18G to permeabilize lipid vesicles. Circular dichroism spectroscopy indicated a conformational change from a disordered to an alpha-helical secondary structure when the peptide interacts with detergent micelles or anionic lipid vesicles.