why does this peptide attack charged membranes mimicking the bacterial envelope almost as efficiently as neutral and cholesterol-containing membranes mimicking eukaryotic cells?

We have devised a dye release assay to investigate the affinity of maculatin 1.1 towards a particular lipid composition in a competitive environment. The use of large unilamellar vesicles loaded with calcein and mixed with non-encapsulated vesicles of a different lipid composition has allowed determination of differential affinities and/or activities of the peptide for varying lipid compositions. We also have demonstrated that a distinct secondary structure of maculatin 1.1 is not essential for its lytic activity and have data supporting a pore mechanism, the size of which is likely regulated by the lipid composition of the model membrane system.

110-Plat
Host Defense Peptides: Molecular Details of Attack on Bacterial and Neoplastic Mammalian Model Membranes

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Membrane-active host defense peptides constitute a key part of the initial immune response in multicellular organisms. They interact with anionic lipids of bacterial membranes and are also active against neoplastic mammalian membranes. The non-specific nature of these interactions reduces the propensity for developing resistance to antimicrobial and anticancer therapies but makes development of highly effective peptides a challenging task. Various mechanisms of peptide-membrane activity were proposed, but the molecular picture is still incomplete. We employ our novel highly mobile membrane mimetic (HMMM) model with enhanced lipid mobility, combined with all-atom molecular dynamics simulations to investigate structural and dynamic properties determining initial lipid-peptide interactions of magainin 2 (MAG2) and its highly charged variant, paxiganan (MSI-78). Extended multiple simulations were performed of MAG2 and MSI-78 monomers with HMMM binary membranes representing bacterial (PE/PG) and neoplastic (PC/PS) membranes. Spontaneous association of the peptides with the membranes lead to significant clustering of anionic lipids in both membrane models, lending support to the lipid clustering model as a potential first stage of the interaction. Interestingly, membrane surface rupture was observed as a result of lipid rearrangement. PE/PG membranes appear to be more susceptible than PC/PS to rupture by both peptides, which correlates well with recent experimental observations. PE/PG appears to offer easier entry for the charged residues due to hydrogen bonding and lack of positive charges on the PE head groups that may repel the cationic peptides. We propose a ''sweep-and-anchor'' mechanism that initiates membrane rupture: initial clustering of charged lipids by the charged residues is creating more loose domain boundaries in which hydrophobic residues can penetrate, creating a pore in membrane leaflet. Prior to the development of the HMMM method these inquiries were out of reach of atomistic simulations.

111-Plat
Quantitative Studies of Antimicrobial Peptide Pore Formation in Large Unilamellar Vesicles by Fluorescence Correlation Spectroscopy (FCS)

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In spite of intensive research efforts over the past decades, the mechanisms by which membrane-active antimicrobial peptides interact with phospholipid membranes are not yet fully elucidated. New tools that can be used to characterize antimicrobial peptide-lipid membrane interactions are therefore highly warranted. Fluorescence correlation spectroscopy is a biophysical technique that can be used to quantify leakage of fluorescent probes of different sizes from large unilamellar vesicle, thereby potentially becoming such a new tool. However, the usage of fluorescence correlation spectroscopy to quantify leakage from large unilamellar vesicles is associated with a number of experimental pitfalls. Based on theoretical and experimental considerations, we discuss how to properly design experiments to avoid these pitfalls. Subsequently, we apply fluorescence correlation spectroscopy to quantify leakage of fluorescent probes of different sizes through transmembrane pores formed by each of the three representative antimicrobial peptides: melittin, magainin 2, and mastoparan X. The experimental results demonstrate that leakage assays based on

fluorescence correlation spectroscopy offer new and detailed insight into the size and cooperative nature of transmembrane pores formed by antimicrobial peptides that is not available from the conventional quenching-based leakage assays.

112-Plat
How to Correlate Membrane Interaction Kinetics with Structural Measurements

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Studies of membrane problems are often controversial because there is no ideal experimental method for membrane studies. Membrane studies in solution are typically macroscopic observations containing no molecular level information. On the other hand samples that produce molecular level information are not single membranes in solution. Thus there is no direct link between the microscopic structural measurements and the actions in a membrane. Here we show that a combination of aspirated GUV and X-ray diffraction experiments is capable of providing a correlated link between macroscopic observation and molecular measurement. The action of peptides or drugs on a membrane can be observed by the membrane area change in an aspirated GUV. The bound states of peptides or drugs cause membrane thickness changes that can be measured precisely by X-ray diffraction. The incompressibility of the hydrocarbon chains equates the fractional membrane area change $\Delta A/A$ to the fractional membrane thickness change $\Delta h/h$. The equality directly links these two experiments, one on the micron scale and another on the Angstrom scale. We demonstrate the utility of this method by the action of melittin, a prototype AMP. Melittin bound to a lipid bilayer causes membrane thinning in proportion to P/L until P/L reaches a P/L*; further binding has little effect on membrane thickness. In DOPC, P/L*~1/75 and the thinning at P/L* is $-\Delta h/h=5.6\%$. Neutron in-plane scattering and OCD showed membrane pores were formed when P/L exceeded P/L*. In the aspirated GUV experiment, we monitored melittin binding, membrane area change and molecular leakage all at the same time. Spontaneous melittin binding to the GUV caused its membrane expansion. Molecular leakage occurred when $\Delta A/A=5.7\%$. Many conclusions can be drawn from the consistency between the kinetic experiment and equilibrium experiment. Examples on other peptides and drugs are also shown.

113-Plat
Structural Characterization of the Helicobacter Pylori VacA Toxin by Single Particle Em and X-Ray Crystallography

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Helicobacter pylori is a Gram-negative bacterium that infects the human stomach and contributes to the pathogenesis of peptic ulceration, gastric adenocarcinoma and gastric lymphoma. H. pylori secretes an exotoxin called vacuolating toxin (VacA), known for its ability to induce vacuolation in the cytoplasm of mammalian cells. VacA can cause depolarization of membrane potential, alteration of mitochondrial membrane permeability, apoptosis, activation of mitogen-activated protein kinases, inhibition of T cell activation and proliferation, and autophagy. The mechanisms by which these processes occur are not yet fully understood but many of these toxic effects depend on the capacity of VacA to form anion-selective membrane channels. VacA is an 88 kDa protein that contains two distinct domains, p55 and p33. The 88 kDa monomers can assemble into large water-soluble oligomeric ''flower''-shaped structures. Using single particle electron microscopy and the random conical tilt approach, we have determined three-dimensional (3D) structures of six distinct VacA oligomeric conformations at ~15 Å resolution. This analysis shows that VacA can organize into a number oligomeric conformations that include both single and double layer hexamers and heptamers. The structures, regardless of oligomeric type, contain two prominent features: extended straight ''legs'' with a slight kink at the distal end and a central ''spoke-like'' density that contains two distinct globular domains separated by a thin connecting density. We have also generated structures of three VacA mutant proteins that all form oligomers but differ in activity. Overall, these studies provide the most detailed analysis of p33 structure to date and also provide a more thorough understanding of how VacA forms oligomers.