that arginine binding to phosphatidylglycerol is more favorable by only ~1 kcal/mol, suggesting that lipid binding domains and antimicrobial peptides likely require many charged side chains acting together to promote membrane localization.

434-Pos

Physical Modeling of Membrane-Lytic Antimicrobial Peptides: Toward Optimizing Their Membrane Disrupting Activity

Sattar Taheri-Araghi, Bae-Yeun Ha.
University of Waterloo, Waterloo, ON, Canada.

Antimicrobial Peptides (AMPs) are fast microbe-killing molecules found in virtually-all living organisms. Membrane-active AMPs are of particular interest, since they do not easily induce bacterial resistance. Accordingly, these peptides offer promising design principles for developing potent peptide antibiotics, especially for fighting conventional antibiotic-resistant bacteria. Here, we present a physical basis for optimizing the selective membrane-disrupting activity of cationic AMPs. Our approach explains the vital feats of the peptide, shedding quantitative insights into their design principles: Threshold peptide coverage on the membrane surface required for disruption can easily be reached for microbes, but not for the host cell - large peptide charge (> 4) is shown to be the key ingredient for determining the optimal activity-selectivity of AMPs (in an ambient-salt dependent way). Our results also illustrate how reduced fluidity of the host cell membrane by cholesterol enhances the selectivity.

435-Pos

The Role of Hydrophobicity in Peptide-Membrane Interactions: Insights Through Coarse-Grained Molecular Dynamics Simulations

Paraskevi Gkeka, Lev Sarkisov.
University of Edinburgh, Edinburgh, United Kingdom.

Peptide-membrane interactions are complex and diverse phenomena, highly important for various biological processes, such as antimicrobial defence mechanisms, viral translocation, membrane fusion and different functions of membrane proteins. Despite the extensive theoretical and experimental on-going research in the area of these interactions, the underlying mechanisms remain unclear. Here, we will present the latest results of our study on simulations of peptide-membrane interactions, based on the coarse-grained MARTINI force field [1, 2]. We will discuss about the possibility of classifying α-helical peptides into groups according to their hydrophobicity and predicting their interaction with cell membranes [3, 4]. Structural and dynamical properties related to various interaction patterns will be presented. Moreover, results of the potential of mean force (PMF) for peptide translocation across the lipid bilayer calculated for each class of peptides will be presented and compared. Finally, we will examine the possibility of simulating stages of the endocytic pathway and discuss about the reliability as well as wider implications of these results.

[1] Marrink, S. J. ; Risselada, H. J. ; Yefimov, S. ; Tieleman, D. P. and de Vries, A. H., *J. Phys. Chem. B*, 2007, 111, 7812-7824.
[2] Monticelli, L. ; Kandasamy, S. K. ; Periole, X. ; Larson, R. G. ; Tieleman, D. P. ; Marrink, S. J., *Chem. Theory Comput.*, 2008, 4, 819-834.
[3] Gkeka, P; Sarkisov, L; *J. Phys. Chem B.* (submitted)
[4] Gkeka, P; Sarkisov, L; *J. Phys. Chem B.*, 2009, 113, 1, 6-8.

436-Pos

A Comparative Study on the Effect of Hydrophobicity and Net Positive Charge on the Antibacterial and Anti- Endotoxin Activities of Antimicrobial Peptides

Yosef Rosenfeld, Naama Lev, **Yechiel Shai**.
The Weizmann Institute of Science, Rehovot, Israel.

The bacterial outer membrane, lipopolysaccharide (LPS), can serve as a barrier that protects bacteria from antimicrobial peptides (AMPs). However, LPS can also activate immune cells that in sever cases may cause death. This activity can also be neutralized by several AMPs. However, it is difficult to determine common denominators required for antimicrobial and LPS neutralizing activities. To this end, we synthesized and investigated a series of 12-mer D,L-amino acid peptides and their fatty acid-conjugated analogs composed of Leu and Lys with increasing number of positive charges and decreasing hydrophobicities, and with preserved positions for the D-amino acids. The overall altered helical structure in the membrane is similar for all of them as determined by FTIR spectroscopy. All the peptides were tested for their antibacterial and hemolytic activity, their ability to permeate LPS vesicles, to neutralize LPS activation of macrophages, as well as their effect on LPS morphology, determined by negative staining electron microscopy. The data reveal that whereas antimicrobial activity increases linearly with the increase in the peptides' hydrophobicity, peptides with different hydrophobicities are endowed with similar LPS neutralizing activities. Besides its importance to the understanding of antimicrobial and LPS neutralizing activities, this study suggests the use of such diastereomers as potential templates for the development of simple molecules that carry out both types of functions.

437-Pos

Cationic Antimicrobial Peptides: A Physical Basis For Their Selective Membrane-Disrupting Activity

Bae-Yeun Ha, Sattar Taheri-Araghi.
University of Waterloo, WaTERLOO, ON, Canada.

Antimicrobial peptides (AMPs) are not only fast microbe-killing molecules deployed in the host defense of living organisms but also offer valuable lessons for developing new therapeutic agents. While the mode of action of AMPs is not clearly understood, membrane perturbation has been recognized as a crucial step in the microbial killing mechanism of many AMPs. Here, we present a physical basis for the selective membrane-disrupting activity of cationic AMPs. In particular, we calculate the surface coverage of peptides embedded in the lipid headgroup-tail interface and the resulting membrane-area change, in terms of peptide and membrane parameters (e.g., peptide charge and the fraction of anionic lipids). We find threshold peptide coverage on the membrane surface required for disruption can easily be reached for microbes, but not for the host cell - large peptide charge > 4) is shown to be the key ingredient for the optimal activity-selectivity of AMPs (in an ambient-salt dependent way). Intriguingly, we find that in a higher-salt environment, larger charge is required for optimal activity. Our results also illustrate how reduced fluidity of the host cell membrane by cholesterol is implicated in the selectivity.

438-Pos

Energy Barriers and Helix Plasticity in the Membrane Insertion of pHLIP

Francisco N. Barrera[1], Monika Musial-Siwek[1], Oleg A. Andreev[2], Yana K. Reshetnyak[2], Donald M. Engelman[1].
[1]Yale University, New Haven, CT, USA, [2]University of Rhode Island, Kingston, RI, USA.

The pH (low) insertion peptide (pHLIP) is a 36-aa monomeric peptide which is both soluble in water and able to insert as a transmembrane helix in lipid membranes at low pH. Thus, pHLIP has three major states with characteristic secondary structure: it is unstructured in solution (state I) and when bound to the surface of lipid membranes at neutral pH (state II). However, it forms a transmembrane helix in membranes at acid pH (state III), with a pKa of insertion between states II and III of 6.0. The lipid insertion of pHLIP is mediated by the protonation of at least two Asp residues. Thus, pHLIP has to deal with the translocation of acidic residues through the membrane to insert.
Here, we designed several mutant peptides where the number of aspartic residues in the hydrophobic region of pHLIP was modified. Some mutations altered peptide behavior in solution and their interaction with lipid. At the same time, we observed that there was an apparent linear relationship between the number of Asp and both the observed pKa and the cooperativity of the insertion and/or folding in the membrane.
In order to study the role of transmembrane helix formation in the lipid insertion of pHLIP, we designed a mutant peptide where the key residue Pro20 had been mutated to Gly. This peptide retained the overall properties of pHLIP, however both the interfacial (II) and the transmembrane (III) states had a higher helical content than wt pHLIP, and the pKa of insertion was also higher.
Our data suggest that i) the number of Asp and their location at the water-lipid interface affect the pKa and/or cooperativity of the transition and ii) the formation of the membrane interfacial helix promotes peptide insertion into the membrane.

439-Pos

Lipid Membrane Destabilisation By Arginine Peptides Is Chain Length Dependent

Ana Bouchet[1], Marc Lensink[2], Fabiana Lairion[1], Anibal Disalvo[1], Erik Goormaghtigh[3], Jean-Marie Ruysschaert[3].
[1]Universidad de Buenos Aires, Buenos Aires, Argentina, [2]Genome and Network Bioinformatics, Brussels, Belgium, [3]Structure and Functions of Biological Membranes, Brussels, Belgium.

The intracellular delivery of proteins and other bioactive molecules using membrane-permeable carrier peptide vectors is a way to elucidate and control cell functions with therapeutic potentials. One of the most typical peptide vector is a short arginine-rich peptide segment derived from the human immunodeficiency virus (HIV)-1Tat protein as well as various arginine-rich oligopeptides. These peptides seems to translocate with their cargo into eukaryotic cells through a physical mechanism which is neither receptor-mediated and does not implicate endocytosis. Other studies have however implicated an endocytic pathway involving macropinocytosis. We provide here evidence that arginine peptides induce membrane destabilisation in DMPC and DMPG liposomes which is dependent of the arginine peptides length. Evolution of the CH_2- vibration of lipids was monitored by ATR-IR (Attenuated Total Reflection

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
Kevin E. Cahill.
University of New Mexico, Albuquerque, NM, USA.
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
Erik Strandberg[1], Deniz Tiltak[2], Sebastian Ehni[1], Parvesh Wadhwani[1], Anne S. Ulrich[1,2].
[1]Forschungszentrum Karlsruhe, Eggenstein-Leopoldshafen, Germany, [2]University of Karlsruhe, Karlsruhe, Germany.
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
John T.J. Cheng, John D. Hale, Havard Jessen, Melissa Elliot, Robert E.W. Hancock, Suzana K. Straus.
University of British Columbia, Vancouver, BC, Canada.
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
David I. Fernandez, Marc-Antoine Sani, John D. Gehman,
Frances Separovic.
University of Melbourne, Melbourne VIC, Australia.
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
William Wieczorek[1], Sudheendra U.S.[1], Riqiang Fu[2], Myriam Cotten[1].
[1]Hamilton College, Clinton, NY, USA, [2]National High Magnetic Field Laboratory, Tallahassee, FL, USA.
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