generate sufficient traction forces is a prerequisite for tumor cell migration in a 3D connective tissue matrix. Using traction microscopy, we found that wildtype exerted 3-fold higher tractions on fibronectin-coated polyacrylamide gels compared to vin−/− cells. These results show that vinculin controls two fundamental functions that lead to opposite effects on cell migration in a 2D vs. a 3D environment: On the one hand, vinculin stabilizes the focal adhesions (mechano-coupling function) and thereby reduces motility in 2D. On the other hand, vinculin is also a potent activator of traction generation (mechano-regulating function) that is important for cell invasion in a 3D environment.

147-Plat The Conformation and Dynamics of Formin- and Tropomyosin-Bound Actin Filaments

Andrea Vig, Zoltán Ujfalusi, Gábor Hild, Miklos Nyitrai

University of Pecs, Pecs, Hungary.

Formins are conservative proteins and play important roles in the regulation of the microfilament system in eukaryotic cells. They have several domains involving FH1, FH2, GPB and DAD domains. In the interaction between actin and formin the FH2 domain plays a key role. This domain builds antiparalell dimers. The 'mammalian Diaphanous-related 1' constitutes one of the subfamilies of the formins. Previous studies showed that the FH2 fragments of mDia1 modified the conformational properties of actin filaments by making the filaments more flexible. The FH2 effect was strongly concentration dependent. To understand the potential role of the flexibility of the actin filaments we studied here how an abundant actin-binding protein, tropomyosin, affects the conformation of formin-bound actin filaments. For this purpose we applied Förster-type resonance energy transfer (FRET) and fluorescence anisotropy decay methods. According to our results the binding of tropomyosin stabilised the flexible formin-bound actin filaments. The effect of tropomyosin on the actin filaments was independent of the concentration of KCl, but depended on the $MgCl_2$ concentration. These observations indicate that tropomyosins stabilise the conformation of actin filaments and can play an important role in the regulation of the actin cytoskeleton in synergic interactions with formins.

Platform O: Interfacial Protein-Lipid Interactions

148-Plat Surface Acoustic Wave Biosensor as a new Tool to Study the Interaction of Antimicrobial Peptides with Biomembrane Mimetics

Jörg Andrä[1], Arne Böhling[1], Thomas M. A. Gronewold[2], Ulrich Schlecht[2], Thomas Gutsmann[1]

[1] *Biophysics, Research Center Borstel, Leibniz-Center for Medicine and Biosciences, Borstel, Germany*

[2] *Caesar, Center of Advanced European Studies and Research, Bonn, Germany.*

Antimicrobial peptides provide an effective first line of defence against invading pathogens on epithelial surfaces and as part of the armament of immune cells. Consequently, they have attracted attention as lead structures for drug development. Their primary target is the cell envelope of bacteria. The precise mode of action is dependent on the peptide and the chemical nature of the target membrane and still far from being set.

We are interested in the interaction of antimicrobial peptides originated from worms to humans (e.g. arenicin, NK-2) with biomembranes. Among other methods, we utilized a surface acoustic wave (SAW) biosensor. Binding of compounds is monitored by variations of phase and amplitude of an acoustic wave. We developed a multistep procedure to immobilize lipid bilayers on the gold surface of the chip involving a self-assembled monolayer, chemical crosslinking of dextran, and coverage of the dextran layer with poly-L-lysine. Subsequently, membrane lipids of bacteria, i.e. phospholipid liposomes or lipopolysaccharide aggregates, were applied in continious flow using a microfluidic system to form a stable and homogeneous lipid bilayer. The addition of peptides affected the properties of the membrane bilayer. These changes were lipid-dependent and correlate with the biological activities of the peptides. Binding of peptides resulted in a phase shift which is associated with a mass increase on the surface. Additionally, it affected the amplitude of the acoustic wave, which is a measure of the viscoelasticity of the surface. When added to a LPS bilayer, peptides NK-2 and arenicin led to a similar increase in phase shift, but to an increase and decrease of the amplitude, respectively. Thus, though both peptides kill bacteria rapidly at similar concentrations, they apparently utilize different modes of action.

149-Plat Enhanced Selectivity via Structural Perturbation of Linear Amphipathic Beta-Sheet Antimicrobial Peptides

Jack Blazyk, Michelle Pate, Janet Hammer, Jing He, Anne Hansen, Julie Bellissimo

Ohio University, Athens, OH, USA.

We have explored the structural and functional properties of small linear peptides with the potential to form amphipathic beta-sheet structure. These peptides possess antimicrobial activity as good, or better, than their amphipathic alpha-helical counterparts, but appear to be more lytic to bilayers containing bacterial lipids vs. mammalian lipids, thereby offering a selective advantage in targeting bacterial cells. We have studied small 10- or 11-residue peptides based on a KL-repeat sequence. Each peptide includes a single L-to-W replacement, with the position of the replacement having dramatic effects on antimicrobial and hemolytic activity. We report here on our progress in maximizing antimicrobial potency while minimizing lytic activity toward mammalian cells. Strategic single amino acid replacements, including the use of proline or cyclohexylalanine in either the L- or D-enantiomers, resulted in significant changes in activity and selectivity. A mechanism for the membrane-disrupting action of these peptides is proposed based on the results

of systematic substitutions in the parent peptides. Structural studies to test the validity of this mechanism currently are under way.

150-Plat Structure-Function Relationships In Antimicrobial Peptides: A Molecular Dynamics Simulation Study Of Indolicidin And Indolicidin Analogues With Model Lipid Bilayers

Jenny C. Hsu, Christopher M. Yip

University of Toronto, Toronto, ON, Canada.

The mechanisms by which an antimicrobial peptide exerts its action are thought to involve an attack on the intruder cells by solubilizing their membrane lipids or forming pore defects. We report here a comparative molecular dynamics simulation study of the interactions between the 13-residue cationic antimicrobial peptide indolicidin (IL: ILPWKWPWWPWRR-NH2) and its analogues with palmitoyloleoyl-phosphatidylethanolamine (POPE) lipid bilayers. By replacing tryptophans with phenylalanines (ILF) or histidines (ILH), and prolines with alanines (ILA), we address the effect of aromaticity, electrostatics, and helicity on peptide structure and association with the lipid environment. Simulation analyses suggested that backbone conformations of IL had the lowest root mean square deviation with respect to their starting structures, thereby attributing structural stability to cation-ϕ interactions occurring between the end arginine and indole rings of the tryptophan residues. With regard to analogue conformations, alanine substitutions in IL to ILA increased the peptide's turn propensity, resulting in structures most similar to the coiled wild-type. In contrast, conformations of ILF and ILH extended linearly, with little evidence of intramolecular hydrogen-bonding. In all cases, the peptides resided in the interfacial region of the bilayer, with IL having the greatest association with carbonyls of the lipid headgroups. Strong electrostatic interactions between ILH and phosphate headgroups did promote the initial attraction of the peptide towards the membrane surface, but subsequent insertion was impeded due to unfavourable burial of the highly positively charged residues in the membrane. Association of the wild-type and analogue peptides to the membrane surface resulted in increased lipid recruitment over time, suggesting membrane distortion as a possible mode of action for antimicrobial peptides.

151-Plat From Sequence to Thermodynamics: a New Approach to the Mechanism of Antimicrobial and Cytolytic Peptides

Paulo F. Almeida, Antje Pokorny

Univ North Carolina Wilmington, Wilmington, NC, USA.

Antimicrobial or cytolytic peptides are similar in length and amino acid composition, but vary significantly in efficiency and specificity to their target membranes. We have developed a new approach to understand this problem, by shifting the focus from sequence to thermodynamics. We found that the mechanism of a peptide is related to its Gibbs energy of insertion, that is, the transfer from a bound state, at the membrane/water interface, to the bilayer hydrophobic interior.

We propose the following hypotheses:

1. For a peptide to translocate across the membrane, the Gibbs energy of insertion into the bilayer hydrophobic core must be less than a threshold, roughly 20 kcal/mol. This is the maximum activation energy compatible with fast insertion and translocation. Peptide translocation will be the default permeation mechanism because it entails the smallest amount of lipid rearrangement. If the Gibbs energy of insertion is above the threshold, a different mechanism of bilayer disruption is required. For peptides that cause graded release the Gibbs energy of insertion is below the threshold, and for peptides that cause all-or-none release the Gibbs energy of insertion is above that threshold.
2. Formation of salt bridges can determine the ability of peptides to bind and translocate across bilayers. In the simplest case the salt bridges are intramolecular. However, if intermolecular salt bridges are possible and necessary to reduce the Gibbs energy of insertion to a value below the threshold, peptide oligomerization will be coupled with translocation across the membrane.

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152-Plat Design Of Short Lipo(peptides) With Antimicrobial And Endotoxin Neutralizing Activity

Lohner Karl[1], Günter Deutsch[1], Dagmar Zweytick[1], Guillermo Martinez de Tejada[2], Susana Sanchez-Gomez[2], Ignacio Moriyon[2], Sylvie E. Blondelle[3], Jörg Andrä[4], Klaus Brandenburg[4], Bostjan Japelj[5], Mateja Zorko[5], Roman Jerala[5]

[1] *Austrian Academy of Sciences, Graz, Austria*
[2] *University of Navarra, Pamplona, Spain*
[3] *Torrey Pines Institute for Molecular Studies, San Diego, CA, USA*
[4] *Research Center Borstel, Borstel, Germany*
[5] *National Institute of Chemistry, Ljubljana, Slovenia.*

Application of conventional antibiotics at the advanced stage of infection of Gram-negative bacteria may cause the release of bacterial cell components such as lipopolysaccharides (LPS, also termed endotoxin). These lipid molecules can induce excessive and uncontrolled release of inflammatory mediators, which can lead towards septic shock, which accounts for more than 200.000 deaths in the U.S. every year. Thus, neutralization of endotoxin in addition to destroying the bacteria is needed.

In three iterative cycles we designed (lipo)peptides derived from a fragment of human lactoferricin, which exhibited more than 100 fold improved antimicrobial and endotoxin neutralization activity as compared to the parent peptide, LF11. QSAR analysis based on the 3D-determination of peptide structure, biophysical experiments on the interaction of peptides with membrane mimetic systems as well as LPS, and biological assays, resulted in (lipo)peptides with a steady improvement of biological activity. The short (8–10 residues) linear peptides were unstructured in aqueous environment, but folded into a defined conformation upon binding to their target lipids. Interestingly, the N-terminal acyl chain of lipopeptides acts

as a structural organizer, which is tightly intertwined with the hydrophobic amino acid residues of the peptide. The best peptides had a broad specificity against both Gram-negative and Gram-positive bacteria and protected mice against endotoxaemia better than polymyxin B, which also contains an acyl chain shown to be essential for antimicrobial and endotoxin neutralizing activity. Since the (lipo)peptides showed negligible toxicity towards human cells at the therapeutic level, these compounds will be good candidates for the development of novel antimicrobial and antiseptic agents.

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153-Plat Molecular Features Of Membrane-Peptide Interactions By The Example Of Antimicrobial Peptides

Yegor Tourleigh

Moscow State University, Moscow, Russian Federation.

The main idea of the study was to reveal molecular mechanisms of antimicrobial effects of natural antimicrobial peptides, AMPs. The approach used was based on molecular dynamics (MD) simulations techniques with full-atomic force fields. One of the AMPs under study was magainin 2. It is supposed that magainins interact directly with biological membrane and do not need specific receptors. Magainins form alpha-helices with a certain hydrophobic momentum in lipid membranes. It was shown that magainins perturb the structure of lipid matrix of the membranes. The extent of these perturbations depends on the lipid composition of the membrane. Another example of antimicrobial peptides, of non-lytic character, is buforin 2 from the toad *Bufo Bufo*.

In the simulations of the AMPs it was shown that the initial stage of their interaction with the membrane, that is the formation of an alpha-helix at the surface of the membrane, is facilitated though the tuning of the bilayer relief and the outer surface of the peptide (seemingly, spatial superposition of charged groups takes place). Cooperation between magainin 2 molecules appeared in coordinated approach and less deviation from alpha-helix then in the case of a single molecule. Application of a steered force revealed that the peptides slightly deform the membrane structure at accelerated penetration, mainly around the formed pore. The pore formation and coordinated rearrangement of lipid molecules take place in close cooperation with the simultaneous embedding of several magainin 2 molecules, both in simulations and in experiment.

Biological activity of these peptides is therefore determined by the balance of stability of their own structures and penetration activity. In case of magainin 2 it is also connected with spontaneous aggregation activity, which was not possible to study in the framework of non-equilibrium steered MD approach.

154-Plat Mechanism of Membrane Disruption by Antimicrobial Peptide Protegrin-1

Kin Lok H. Lam[1,2], Ting Ann Siaw[1,2], Yuji Ishitsuka[3], Alan J. Waring[4,5], Robert I. Lehrer[6], Ka Yee C. Lee[1,2]

[1] *University of Chicago, Chicago, IL, USA*
[2] *The Materials Research Science and Engineering Center, Chicago, IL, USA*
[3] *University of Illinois at Urbana-Champaign, Urbana, IL, USA*
[4] *University of California Los Angeles School of Medicine, Torrance, CA, USA*
[5] *Harbor-University of California Los Angeles Medical Center, Torrance, CA, USA*
[6] *University of California, Los Angeles, Los Angeles, CA, USA.*

PG-1, a cationic antimicrobial peptide, kills bacteria by forming pores which increase membrane permeability to ions or larger molecules. It has been proposed that PG-1 selectively disrupts bacterial membrane over mammalian membranes. To study the mechanism of action of PG-1, we directly visualize the topological changes induced by PG-1 in model membranes via atomic force microscopy (AFM). PG-1 induces structural transformations in supported lipid bilayers, progressing from fingerlike instabilities at bilayer edges, to the formation of surface-defects, and finally to a network of stripe-like structures in a zwitterionic dimyristoylphosphatidylcholine (DMPC) model membrane with increasing PG-1 concentration. While DMPC bilayers exhibits surface defects with the addition of PG-1, in the presence of an anionic lipid,1,2-Dimyristoyl-sn-Glycero-3-[Phospho-rac-(1-glycerol)], surface defects are not observed at the intermediate stage of membrane disruption. These results indicates the formation of surface defects depends on the phase and charge states of the lipid species, and understanding these effects would help elucidate the mechanism by which PG-1 uses to discriminate between bacterial and mammalian cells.

155-Plat Mechanism and Kinetics of Pore Formation in Membranes by Small Amphiphilic Peptides

Ming-Tao Lee[1], Wei-Chin Hung[2], Fang-Yu Chen[3], Huey W. Huang[4]

[1] *National Synchrotron Radiation Research Center, Hsinchu, Taiwan*
[2] *Chinese Military Academy, Fengshan, Taiwan*
[3] *National Central University, Chung-Li, Taiwan*
[4] *Rice University, Houston, TX, USA.*

Pore formation in membranes by amphipathic peptides has been a subject of active research for three decades, yet there is no agreement on the molecular mechanism of this important membrane process. This is in part due to the difficulty in reconciling the results of kinetic experiments with the material properties of peptide-lipid mixtures. We have resolved this problem by observing individual giant unilamellar vesicles (GUVs) exposed to various concentrations of melittin. Only at high melittin concentrations, pores prob-

abilistically appear in a GUV. In such cases, the GUV first expanded its surface area at constant volume then suddenly changed to expanding its volume at constant area. Pore formation in individual GUVs exhibits three effects that have been deduced from the material properties of peptide-lipid mixtures:

1. binding of amphipathic peptides to the membrane interface stretches the membrane area,
2. when the membrane is stretched beyond a threshold value of fractional area expansion, pores are formed, and
3. peptide-induced pores are stable and have a well-defined size.

The statistical manner by which pores appeared in peptide-bound GUVs is consistent with pores occurred in stretched vesicles of pure lipids; both are dictated by nucleation of precursor defect. Knowing these principles will allow us to design molecules that can open pores in membranes in a controllable fashion for gene transfer and drug delivery.

Workshop 1: Modeling the Membrane

156-Wkshp Thermodynamics of Lipid Bilayer Perturbations

D. Peter Tieleman

University of Calgary, Calgary, AB, Canada.

Computer simulations have reached a point where thermodynamic properties of processes such as pore formation, lipid flipflop, and partitioning of small molecules can be accurately calculated. Simulation of pore formation by mechanical force or electric fields has revealed the molecular architecture of pores and the determinants of pore formation and stability. Free energy calculations on lipid flipflop show that the energy barrier in the process corresponds to the formation of small water-filled headgroup-lined pores. From this barrier, accurate flipflop rates and passive diffusion rates for ions can be calculated. Defects also play a key role in the energetics of partitioning of polar and charged amino acid side chains. Detailed simulations are required to calculate the energies involved in these processes; continuum models break down because they cannot take into account the flexibility of a lipid bilayer at the molecular scale. The results of systematic detailed simulations are useful in developing next-generation coarse grained models to simulate membranes at much larger length scales and much longer time scales.

157-Wkshp Concerted Simulation and Experimental Studies of Membrane Structure and Dynamics

Doug Tobias

University California, Irvine, Irvine, CA, USA.

Over the past decade and a half, molecular dynamics (MD) simulations have emerged as a valuable tool for filling in details of the molecular structure, interactions, and dynamics of membranes that are not available from experiments. The images produced by MD simulations not only underscore the inherent disorder in membranes and the breadth and chemical heterogeneity of the membrane-water and membrane-protein interfaces, but they also offer the possibility of identifying and characterizing the myriad of interactions taking place, and dynamical processes playing out, at the atomic level. The exquisite detail afforded by MD simulations has also proven useful in the validation or refinement of models used in the interpretation of data from a variety experiments probing membrane structure and dynamics. In this talk I will demonstrate the power of a concerted simulation and experimental approach to studying membranes using four examples. With the first example I will show how neutron diffraction experiments inspired by MD simulations have revealed a greater degree of disorder in lipid acyl chains than was previously imagined. Next, based on MD simulations restrained using electron spin resonance and fluorescence quenching data, I will suggest that peripheral membrane proteins sculpt lipids to construct self-induced docking sites in membranes. In the third example, which is relevant to voltage sensing in voltage-gated ion channels, I will use MD simulations to rationalize the remarkably small energetic penalty, measured by translocon-mediated insertion experiments, for placing helical peptides containing arginine residues in transmembrane helical configurations. In the final example, I will show how we are using a combination of neutron spectroscopy and MD simulations to unravel the complex web of dynamical couplings between a membrane protein and its lipid and aqueous surroundings.

158-Wkshp Synthetic Peptides As Models For Intrinsic Membrane Proteins

J. Antoinette Killian

Univ Utrecht, Utrecht, The Netherlands.

The aim of our research is to determine how the lipid environment can influence the structure and organization of membrane proteins by affecting their transmembrane segments. For this purpose we employ designed model peptides that mimick transmembrane parts of proteins. These peptides are incorporated into well-defined synthetic lipid bilayers of varying composition, and the systems are studied by a range of biophysical approaches, including solid state NMR methods, fluorescence spectroscopy, and mass spectrometry. This allows us to analyze in detail the influence of lipids on structural properties of transmembrane protein segments, such as structure and dynamics of the peptide backbone, helix tilt, and direction of tilt. In particular we focus on how these properties are affected by the extent of hydrophobic matching and what the role is of interfacial anchoring interactions of amino acids that flank the hydrophobic transmembrane segments. Results of these studies and