aggregates. This coupled approach provides a unique opportunity to directly link spectroscopic details associated with peptide-membrane interactions with structural insights obtained on nanometer length scales.

451-Pos
Kinetics of Mastoparan X Binding To Lipid Bilayers
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Mastoparan X, a 14 residue peptide with the sequence INWKGIAAMAKKLL-amide, is found in the venom of the Japanese hornet, *Vespa xanthoptera*. The peptide interacts preferentially with anionic lipid bilayer membranes and forms an amphipathic α-helix when bound at the membrane-water interface. We previously studied the interacton of mastoparan X with lipid bilayers. Peptide binding was measured through fluorescence energy transfer from the intrinsic Trp residue in the peptide to the acceptor fluorophore embedded in the membrane at low concentrations. The kinetics of binding were obtained by monitoring the increase in emission from the acceptor fluorophore by stopped-flow fluorescence. At low peptide and lipid concentrations, the peptide is monomeric in solution and the binding kinetics are well described by a single exponential function. We now extended this study to investigate the kinetics of mastoparan X binding to lipid vesicles as a function of both peptide and lipid concentration. The data were analyzed with an exact kinetic model to test if other processes, such as peptide aggregation or conformational changes, influence the observed binding kinetics at higher concentrations.

452-Pos
Cyanylated Cysteine Used To Map Membrane Binding and Inter-Peptide Contacts in a Model Antimicrobial Peptide
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Using single-cysteine mutants of the potent antimicrobial peptide CM15 as a model system for binding at the membrane surface, we are developing an infrared probe to characterize site-specific side chain solvent exposure and the ps-time scale dynamics of both membrane-peptide interactions and peptide-peptide contacts. The selective cyanylation of a mutated cysteine residue covalently attaches a nitrile vibrational probe at the chosen site. The frequency and lineshape of the CN stretching vibration are sensitive to both solvent exposure and peptide aggregation. These sensitivities are applied at multiple label sites to reveal information about the structural aspects of CM15's perturbation of E. coli lipid bilayers.

453-Pos
Fine-Tuning the Activity of Linear Amphipathic Beta-Sheet Antimicrobial Peptides
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It is relatively simple to design highly amphipathic linear cationic beta-sheet peptides containing 10-to-11 amino acids that possess potent antimicrobial activity. Usually, however, these peptides also are quite hemolytic, so that there is insufficient selectivity between bacterial and human cells. Peptides with little or no hemolytic (or other toxic) activity toward host cells at 100 or more times the minimum inhibitory concentrations toward bacterial cells might be potential candidates for clinical use as antimicrobials. We have used two strategies to separately attenuate lytic activity toward host cells while maintaining potent antimicrobial activity. Both strategies involve introducing a structural perturbation in the amphipathic beta sheet. First, a hydrophobic amino acid residue can be substituted by proline. Depending upon the location of the substitution within the peptide, it is possible to nearly eliminate hemolytic activity while retaining potent antimicrobial activity. A similar outcome can be achieved by replacing a hydrophobic amino acid residue with a D-amino acid. Here again, the location of the substitution within the peptide is critical for the desired balance of activities. We show here 10- and 11-residue peptides consisting of alternating lysine and leucine in which a single leucine has been replaced by either proline or a D-amino acid. The effects of these substitutions on antimicrobial and hemolytic activities, secondary structure, and ability to induce leakage in lipid vesicles and bacterial cells are compared. The most promising peptides will be tested in vivo to determine their suitability as either topical or systemic antimicrobial agents.

454-Pos
Towards Design of Novel Antimicrobial Agents: Role of the Conformational Rigidity
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Non-natural mimics of antimicrobial peptides (AMPs) are excellent candidates for anti-infectious agents due to their stability towards enzymatic degradation and broad adjustability of physicochemical properties. Conformationally flexible acyl-lysine oligomers (OAKs) and restrained arylamide foldamers have demonstrated capability to be fine-tuned to high antimicrobial activity and negligible toxicity towards human cells. In the present work we examine how structural rigidity affects interactions of the AMP analogs with model lipid monolayers at the air-liquid interface by constant-pressure insertion assays, epifluorescence microscopy (EFM), X-ray reflectivity (XR) and grazing incident-angle X-ray diffraction (GIXD) using synchrotron radiation. Simplified models of the outer Gram-negative and cytoplasmic Gram-positive membranes were represented Lipid A and DPPG monolayers, respectively, while mammalian plasma membrane was mimicked with zwitterionic DPPC/Cholesterol 6/4 monolayer mixture. Insertion assays show that both AMP analogs readily incorporate into the bacterial, but not mammalian, membrane mimics. Membrane-insertion of OAK and arylamide was accompanied by rapid deterioration of the structural order in lipids. Interestingly, flexible OAK was more efficient in disrupting Gram-negative rather than Gram-positive bacterial model membrane. Electron density profiles across the film, derived from XR data, demonstrate that after insertion the hydrophobic cores of OAK and arylamide were located within lipid acyl chains, inducing negative and positive local curvatures, respectively. Moreover, concentration of flexible OAK within Lipid A was higher than within DPPG, as opposed to restrained arylamide, as well as to natural AMPs we characterized previously, including LL-37, SMAP-29, and PG-1.

455-Pos
Membrane-Active Peptides: Stable Pore-Forming or Cell-Penetrating Peptides Selected With Orthogonal High-Throughput Screening
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There are numerous distinct mechanisms by which a peptide can interact with a lipid bilayer membrane and affect its structure or function. Interfacially-active peptides (i.e. antimicrobial or cell penetrating) partition into the interface and drive rearrangements in the lipids, such that the segregation between the hydrocarbon core and the interfacial zone is broken down. Stable pore-forming peptides assemble into long-lived transmembrane pores using the constraints imposed by the bilayer to direct self-assembly. (Only a few examples of true stable pore forming peptides are known.) We have developed two high throughput screens using lipid bilayer vesicles that simultaneously allow for detection of different membrane activities (i.e. orthogonal screening) and have successfully used them to screen combinatorial peptide libraries for very specific membrane activities. In our **translocation screen**, we simultaneously measure leakage from lipid vesicles, and the ability of a peptide to be cleaved by a vesicle-entrapped protease. Using this screen we identified 12 very potent membrane-penetrating peptides from a library of 13,000 members. These peptides, which share a common sequence motif, spontaneously and rapidly translocate across bilayers without inducing leakage of entrapped contents. These peptides also rapidly translocate across the plasma membranes of living cells without cell permeabilization or toxicity. In our **stable pore screen** we measure immediate leakage of vesicle contents upon addition of peptides, and then also for the continued existence of pores in the same vesicles after overnight incubation. The vast majority of so called ''pore forming peptides'' do not form stable pores in membranes; leakage is a transient phenomenon. However, using this screen we have identified stable pore formers among known peptides, including melittin. This orthogonal screen has also been used to identify true stable pore forming peptides in several peptide libraries.

456-Pos
Interactions of Antimicrobial Peptide Latarcin With Model Cell Membrane
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Latarcins are linear antimicrobial peptides purified from the venom of the Lachesana tarabaevi spider. They are highly active against Gram-positive and Gram-negative bacteria with minimum inhibitory concentrations (MIC) at the micromolar level and low hemolytic activity (1, 2). In the present work, a 26 residue peptide Latarcin 2a that adopts a helix-hinge-helix conformation in a membrane mimetic environment (1, 2) was studied as well as a derivative obtained by replacing the Guanine 11 by with Alanine. The interaction of the peptides with phospholipid mono and bilayers were investigated using Langmuir-Blodgett monolayer technique, Atomic Force Microscopy (AFM),

calcein leakage assay and UV resonance Raman spectroscopy. Effect of small changes in the primary structure of the peptide on the membrane rupturing activity is discussed.

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457-Pos

Simultaneous Single-Channel Recording and Fluorescence Imaging of Calcium Flux Reveals the Behaviour of Individual Antimicrobial Peptide Pores

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We have used simultaneous single-channel recording and total internal reflection fluorescence (TIRF) microscopy to investigate the behaviour of antimicrobial peptides in artificial droplet-on-hydrogel lipid bilayers (DHB).

These pore-forming peptides play an important role in many organisms by providing resistance to infection. An improved understanding of their mechanism of action is essential in the development of new antibiotics.

Our study focuses on two peptides thought to follow different pore-formation mechanisms. Alamethicin is produced by the fungus *Trichoderma viride* and is understood to form barrel-stave pores. Magainin II is found in the skin of the African clawed frog *Xenopus laevis* and is thought to follow a toroidal-pore model.

Using a fluorescent calcium indicator we are able to detect the ion flux through individual alamethicin and magainin II pores and can monitor multiple pores at once. We observe multiple conductance states from single alamethicin pores and see that magainin II forms stable pores.

458-Pos

Peptide-Induced Domain Formation in Supported Lipid Bilayers: Direct Evidence By Combined Atomic Force and Polarized Total Internal Reflection Fluorescence Microscopy

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Direct visualization of the mechanism(s) by which peptides induce localized changes to the structure of membranes has tremendous potential for understanding the structure-function relationship in antimicrobial and cell-penetrating peptides. We have applied a combined imaging strategy to track the interaction of a model amidated antimicrobial peptide, PFWRIRIRR-amide, with bacterial membrane-mimetic supported phospholipid bilayers comprised of POPE:TOCL. Our in situ studies revealed rapid reorganization of the POPE:TOCL membrane into localized TOCL-rich domains with a concomitant change in the organization of the membranes themselves, as reflected by changes in fluorescent membrane probe order parameter, upon introduction of the peptide.

459-Pos

Time-Resolved, Single-Cell Study of the Attack of the Antimicrobial Peptide Ll-37 on Live E. Coli Cells

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Human LL-37 is an antimicrobial peptide whose amphiphilic helices selectively degrade bacterial membranes by a mechanism that is poorly understood. We are using single-cell, two-color fluorescence microscopy to directly observe the attack of rhodamine-LL-37 on live E. coli cells in real time. The cells express either periplasmic or cytoplasmic GFP. This enables quantitative correlation of the extent of LL-37 adsorption with leakage or lysing of GFP from the two different compartments, while simultaneously monitoring cell growth. At 15 uM, LL-37 lyses the periplasm to GFP and halts growth in 2-4 min, long before the cytoplasm lyses to GFP at 20-30 min. At 6 uM, rhodamine-LL-37 binding occurs in three distinct waves, with Wave 2 correlating in time with the halting of cell growth (t = 7-10 min). Wave 1 coats the cell periphery uniformly, but Wave 2 preferentially attacks the septal region and slowly spreads outward towards the poles. This suggests that the cell division machinery may be a target of LL-37-induced cell death. We will use FRET to discern the penetration depth of LL-37 during the different waves of attack and a variety of mutant strains to correlate the LL-37 attack with formation of the Z-ring and additional parts of the divisome. These methods will enable quantitative comparison of antimicrobial attack on real bacterial membranes with studies of lysing of synthetic lipid bilayers. They will be applicable to a wide variety of antimicrobial agents and bacterial species.

Interfacial Protein-Lipid Interactions I

460-Pos

Membrane Diffusion of PH Domain-PIP3 Complexes: the Effects of Target Lipid Stoichiometry on Diffusion Constant Probed Using Single-Molecule Fluorescence Microscopy

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Pleckstrin homology (PH) domains are recruited to specific membrane surfaces during a variety of cell signaling events, including those occurring at the leading edge of chemotaxing cells. This recruitment is often driven by molecular recognition of specific phosphatidylinositol phosphate lipids, such as phosphatidylinositol-(3,4,5)-trisphosphate (PIP3). Using single-molecule fluorescence microscopy, we have recently shown that when bound to PIP3 on the surface of a supported lipid bilayer, the PH domain of GRP1 diffuses at the same rate as an individual phospholipid molecule diffusing in the same type of bilayer. More generally, we hypothesize that protein lateral diffusion constant will decrease as the number of lipid molecules tightly bound to the protein increases. Here, we probe the effects of PIP3 stoichiometry on the diffusion constants of constructs containing one, two, or three GRP1 PH domains coupled by flexible linkers. To a first approximation, we find that the lateral diffusion constants of these engineered PH domain constructs are inversely proportional to the number of bound PIP3 molecules. This observation suggests that the frictional contributions of multiple, tightly bound lipids are additive, at least when the binding sites are located on separate domains. At the meeting we will present our latest diffusion constant measurements, which will shed light on the mechanisms of peripheral membrane protein diffusion, and will provide useful calibration points in molecular dynamics simulations of proteins docked to membranes. Overall, single molecule diffusion methods provide a new, much needed window into the lipid interactions of membrane targeting proteins.

461-Pos

The Autism-Related H93R PTEN Mutant Shows Enhanced Plasma Membrane Binding But Reduced Activity

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The tumor suppressor, phosphatase and tensin homologue deleted on chromosome 10 (PTEN), is a phosphoinositide (PI) phosphatase specific for the 3-position of the inositol ring. PTEN has been implicated in autism for a subset of patients with macrocephaly. Various studies identified patients in this subclass with one normal and one mutated PTEN gene. We characterize the binding, structural properties, activity and subcellular localization of one of these autism-related mutants, H93R PTEN, using fluorescence quenching, SPR and ITC. The membrane association of the mutant protein with solid-supported membranes (tBLMs) is investigated with neutron reflection. We observe that H93R PTEN shows enhanced binding to phosphatidylserine (PS)-containing membranes, in contrast to *wt* PTEN. On the other hand, binding to membranes that contain $PI(4,5)P_2$, a requirement for allosteric activation of PTEN, was strongly reduced for the H93R mutant. H93R and *wt* PTEN share the same secondary structure. However, while α-helical content increases in *wt* PTEN upon binding to $PI(4,5)P_2$, this is not observed for the H93R mutant. Consistent with the increased affinity of H93R PTEN to PS, we find in cell-based studies that the association of the mutant with the plasma membrane is strongly enhanced in comparison to *wt* PTEN. Unexpectedly, this does not enhance $PI(3,4,5)P_3$ turnover, but instead reduces enzyme activity significantly. We hypothesize that the tight binding of H93R PTEN to PS prevents $PI(4,5)P_2$ from interacting with the protein, thereby inhibiting allosteric activation, which is a requirement for binding to and turnover of the substrate, $PI(3,4,5)P_3$.

462-Pos

Unusual Thermal Stability of Human Secreted Phospholipase A2 Enzymes

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Analysis of the thermal stability of proteins in general and enzymes in particular is important for understanding their molecular mechanisms and for their analytical or industrial exploitations. While enzymes with extreme