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),