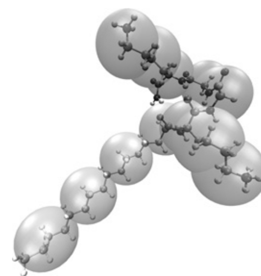
a non membrane-permeable quencher of dye-labeled lipids. Using these assays we have observed a significant difference between melittin and other lytic peptides, such as alamethicin. Even at very high lipid concentrations (peptide:lipid < 1:2000) alamethicin forms long-lived pores, which release ~100% of entrapped contents and give the quencher 100% access to the inside of a vesicle after overnight incubation. Melittin's activity diminishes significantly at lipid concentrations larger than P:L = 1:500. To learn what factors modulate melittin's activity, we have designed a 7,776-member, melittin-based combinatorial peptide library in which we vary critical residues in its natural sequence. We also incorporate self-associating motifs that are found in alpha-helical membrane proteins. Library members were screened using the orthogonal assays at very high and very low stringency. Selected positives from the highly stringent assay (i.e. gain in activity sequences) show a high frequency of alanine substitutions at specific polar and basic residues. Selected negatives from the low-stringency assay (i.e. loss of activity sequences) show two key nonpolar-to-glycine replacements as well as a substitution of the proline residue. Selected peptides from both screens have been contrasted to melittin's activity by using biophysical techniques, antimicrobial and hemolytic assays.

2699-Pos Board B685
Inhibition of Melittin Activity by Cholesterol, Unsaturated Lipid, and Negatively Charged Lipids Studied by Molecular Dynamics Simulation
Jian Dai, Mohammad Alwarawrah, Juyang Huang.
Melittin is shown to cause membrane lyses. However, its lytic activity depends on the lipid composition of the membrane. Experiments have shown that the presence of some lipid components in the membrane can inhibit melittin's lytic power against the membrane. For the sake of atomistic details, molecular dynamics simulations was used to investigate the inhibition of melittin activity by cholesterol, unsaturated phospholipid (POPE), and negatively charged phospholipid (POPS). A pure DPPC lipid bilayer with melittin was simulated as a control, and significant disturbance of the bilayer by melittin was found. The order parameter of DPPC changed dramatically and a large curvature was observed in one of the membrane leaflets, probably leading to a future membrane rupture. The DPPC bilayer with cholesterol showed strong resistance to melittin: Melittin can hardly bind to the membrane surface. In the simulation with unsaturated lipid, melittin can only bind either its N or C terminal region to the bilayer, but the interaction between the body of melittin and the lipids is weak. Melittin binds strongly to negatively charged lipids; however, it cannot induce membrane curvature nor disruption. In above three simulations, C termini of melittin were observed to be able to bind to the membrane more easily than N termini. A deep and stable adsorption of melittin to the membrane requires the binding of both N and C-terminal regions to the membrabe. The tendency of melittin to aggregate was observed in all simulations, especially in the simulation containing cholesterol. This study provides insight into the possible mechanisms of the inhibition of melittin's lytic activity by different membrane components.

2700-Pos Board B686
Melittin vs E.coli: Insights from Molecular Dynamics Simulations
Syma Khalid, Thomas J. Piggot.
The interactions of antimicrobial peptides with eukaryotic cell membranes have been well-studied by experimental and computational methods. However, the molecular-level interactions of these peptides with Gram-negative bacteria such as E.coli are more difficult to study, largely due to the complex nature of the asymmetric outer membrane of Gram-negative bacteria. While cell lysis is thought to proceed via destruction of the inner membrane, it is the outer membrane that is the first barrier encountered by antimicrobial peptides as they attempt to reach their target site.
In the present work, we employ atomistic molecular dynamics simulations to study the interaction of melittin, the major component of honey bee toxin, with detailed models of the E.coli outer membrane. Experimental data (ref), has indicated that upon interaction, melittin induces reorganisation of the lipopolysaccharide (LPS) component of the bacterial outer membrane. We have simulated multiple copies of melittin in lipid bilayers composed of varying depths of LPS in the outer leaflet, and realistic mixtures of phospholipids in the inner leaflet. Our simulations reveal details of the melittin-outer membrane recognition process and the structural stability and dynamics of melittin as it interacts with the outer membrane. Furthermore, our simulations provide specific, molecular-level details of the interaction of melittin with the E.coli outer membrane that may aid the future design of novel antimicrobial agents.

2701-Pos Board B687
Characterization of Potent Antimicrobial Lipopeptide via All-Atom and Coarse-Grained Molecular Dynamics
Joshua N. Horn, Jesse Sengillo, Alan Grossfield.
The prevalence of antibiotic resistant pathogens is a major medical concern, prompting increased interest in the development of novel antimicrobial compounds. To characterize a potent, synthetic lipopeptide, C16-KGGK, microsecond time-scale all-atom simulations with the CHARMM forcefield are utilized. This lipopeptide targets the bacterial membrane, but the binding and reorganization processes are extremely slow. To increase simulation speed, supplemental coarse-grained simulations with the MARTINI forcefield are used. This combination provides insights into the structure, dynamics, binding and mechanism of antimicrobial action.



2702-Pos Board B688
Electrostatic Interactions between Antimicrobial Peptides and Anionic Membranes: Insights from an Implicit Membrane Model
Yi He, Lidia Prieto, Themis Lazaridis.
Electrostatic interactions between antimicrobial peptides (AMPs) and anionic bacterial plasma membranes are crucial for their activity and selectivity. In previous implicit models of anionic membranes, the effects of dipole potential and the presence of pores were not included. In this work, we studied the electrostatic interactions of AMPs with the anionic lipids using a membrane model where double layers of charges are used to represent the head group dipole. The electrostatic interactions between AMPs and the anionic membranes and their influence on pore formation are discussed considering the contributions from the dipole potential and the surface potential. We found that the dipole potential had a strong effect on peptide binding and insertion: their location and orientation changed as a function of the strength and the sign of dipole potential. The surface potential, which is caused by the excess charge of anionic lipids however, has influence only on binding. Alamethicin, which is known to form barrel-stave pores, favors binding to cylindrical shaped pores. Melittin and magainin, on the other hand, strongly favors binding to parabola shaped pores, in agreement with the experiments. For these two peptides, increased anionic lipid content enhances this trend while increased dipole potential has the reverse effect.

2703-Pos Board B689
Molecular Dynamics Simulations of Influenza Fusion Peptide: A Correlation between Flexibility and Fusogenicity
Sébastien Légaré, Patrick Lagüe.
A flu infection starts with the entry of the influenza virus into an host cell. Entry requires endocytosis of the virus by the host cell and subsequent fusion between the viral and endosomal phospholipid membranes. This important membrane fusion step is catalysed by the influenza fusion peptide whose action mode remains unsung. Detailed knowledge on the fusion peptide is essential for the development of therapeutics as well as to understand many biological processes since membrane fusion is ubiquitous to life.
Previous experiments on fusion peptides from various influenza strains and their respective mutants aimed to unveil a structure-function relationship without general agreement. The peptides were shown to adopt a helix-hinge-helix motif essential for fusogenicity, but results diverged on the hinge region which strongly impacts on the peptide structure. The hinge region was sometimes shown as flexible (Dubovskii, Prot. Science 9:786), as a fixed kink (Han, Nat. Struc. Biol. 8:715) or as a tight hairpin (Lorieau, PNAS 107:11341). Similar disagreement occurred in modelling studies (Jang, Proteins 72:299 ; Li, J. Phys. Chem. B 114:8799 ; Panahi, J. Phys. Chem. B 114:1407).
In this work, the structure-function relationship of the fusion peptide from type three hemagglutinin and two mutants (F9A and W14A) were studied from extensive explicit solvent molecular dynamics simulations. Our results show that the hinge region of the fusion peptide is flexible and allows it to be in an equilibrium between kinked and helical conformations. The two mutants also show a different flexibility than the wild-type. Moreover, a correlation between peptide flexibility, lipid protrusion (proposed as the membrane fusion catalysis mechanism (Kasson, PLoS Comput. Biol. 6:e1000829)) and fusogenicity reported in the literature was observed.