The molecularly thin layer of water in direct contact with bio-molecules in a physiological environment plays a major role in determining their properties and functions. In this context, water is a shorthand notation for ''*water electrolyte solution*'', since almost without exception a variety of ions dissolved in water are needed to ensure the stability of bio-systems, greatly contributing to their complex behaviours.
In recent years, the development of compounds of the so-called room-temperature ionic liquid (RTIL) family has enormously expanded the number of ionic systems that could be used to modify the properties of the interfacial water, and thus to affect the behaviour of bio-systems.
Our study concerns the microscopic mechanisms underlying RTIL effects on biosystems (e.g. phospholipid bilayers, proteins, and nucleic acids) through their hydration water, and relies on the combination of neutron scattering and molecular dynamics (MD) simulations.
We present the results for the interaction of imidazolium-based RTILs with phospholipid bilayers [1,2]. Neutron reflectometry and MD simulations confirm the tendency of cations to be absorbed into the lipid phase, enhancing the penetration of water into the bilayer. Neutron scattering and MD reveal apparent changes in the relaxation time of water in close contact of the lipid head upon addition of RTILs, that reflect phase changes in the structure and dynamics of the system.
[1] A. Benedetto, et al. J. Phys. Chem. B, 2014, 118, 12192.
[2] A. Benedetto, et al. J. Chem. Phys., 2015, 142, 124706.

Membrane-active Peptides and Toxins II

2049-Pos Board B193
Studying Antibiotic-Membrane Interactions via X-Ray Diffraction and Fluorescence Microscopy
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Antibiotic drug resistance is a serious issue for the treatment of bacterial infection. Understanding the resistance of antibiotics is a key issue for developing new drugs. In this study, we used penicillin and sulbactam as the model antibiotics to interact with model membranes. Cholesterol was used to target the membrane for comparison with the well-known insertion model. Lamellar X-ray diffraction (LXD) was used to determine membrane thickness using successive drug-to-lipid molar ratios. The aspiration method for a single giant unilamellar vesicle (GUV) was used to monitor the kinetic binding process of antibiotic-membrane interactions in an aqueous solution. Both penicillin and sulbactam are found lying outside the model membrane, and cholesterol inserts perpendicularly into the hydrophobic region of the membrane in aqueous solution. This result provides structural insights for understanding the antibiotic-membrane interaction and the mechanism of antibiotics.

2050-Pos Board B194
Lancing Liposomes: Unlocking the Mechanism of Short Amphiphilic Beta Sheet Forming Peptides
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Antimicrobial peptides (AMPs) are part of the innate immune system in humans and many other forms of life, exhibiting selective activity against bacterial cells, while leaving host cells unaffected. Of particular importance for this action is the difference in phospholipid composition of bacterial cells from cells of human tissue. Because of their antimicrobial and microbicidal characteristics, AMPs are of great interest for applications in human health, and non-natural AMPs have been devised to optimize the microbicidal characteristics of natural AMPs. It has been found that when dyad repeats are used in the pattern (XY)n, where X is a hydrophobic amino acid residue and Y is a cationic residue, a β-sheet secondary structure forms, and that these peptides are capable of killing bacteria, biofilms, and fungi. In this work, we design a synthetic 8 residue peptide using the (XY)¬¬n motif and conduct a series of biophysical tests on membrane mimicking lipid vesicles to elucidate a mechanism of antimicrobial action. A battery of biophysical tests is used to elucidate the significance of phospholipid membrane composition and the mechanism by which this peptide acts against bacterial cells. These studies include vesicle turbidity, kinetic vesicle leakage, and membrane fusion FRET assays. Taken together with previously performed biological assays, these results can aid in the elucidation of critical components of AMPs and in the design of stronger, more selective, AMPs.

2051-Pos Board B195
Towards the Delivery of Cargo Across Biological Barriers
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Limited knowledge exists on the utility of cell-penetrating peptides for transcellular delivery of cargo across biological barriers. Previously, we reported that a short peptide, referred to as the CL peptide, enhanced permeability of a covalently-conjugated small-molecule cargo across a monolayer of cells in vitro. In this study we are investigating the mechanisms by which the cargo is internalized into the cells and delivered across the cell monolayers. This knowledge can guide us in designing future peptide-based drug delivery systems that can bridge biological barriers.

2052-Pos Board B196
Structural and Mechanistic Studies of Andropin, A Membrane-Selective Antimicrobial Peptide
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Andropin is a 34 residue peptide, which originates from the testes of Drosophilia melanogaster and functions as an antimicrobial for the male reproductive tract. Andropin has activity against gram-positive bacteria, likely by selective disruption of the membrane wall, which causes cell lysis. The biological properties of this peptide have characteristics that are similar to both Cecropin and ALL38, but Andropin's structural properties and mechanistic details for membrane disruption have not yet been examined. Here, we examined Andropin's structural properties and its mechanism to selectively disrupt lipid membranes by using model membrane mimics of gram-positive, gram-negative, and mammalian cellular membranes. Turbidity measurements, as well as fluorescence leakage, FRET, and fluorescence anisotropy measurements were used to determine Andropin's effectiveness to disrupt these model membrane mimics. The secondary structure of the peptide was also probed with and without membrane environments using FTIR, CD, and NMR spectroscopies. Our preliminary results indicate that lipid head groups, particularly negatively charged species, drastically change Andropin's rate of membrane disruption. DPH anisotropy, combined with FRET measurements, indicates that Andropin functions either through a torodial pore or barrel-stave mechanism to pierce the membrane surface. Pore formation is supported by preliminary FTIR data that reveals a transition from a helix-turn-helix structure, when Andropin is free in solution, to a helix bundle upon interaction with negatively charged membranes. Ultimately, we plan to further characterize the high-resolution structure of Andropin and its membrane penetrating properties using an array of spectroscopic techniques. These results will help us understand how antimicrobial peptides can be designed to obtain properties for selective disruption of cellular membranes.

2053-Pos Board B197
Characterization of PA Channels in Anthrax Toxin using Trp Peptides
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Anthrax toxin is a three-protein virulence factor that utilizes transmembrane protein translocation as its mechanism to intoxicate cells during anthrax pathogenesis. The protective antigen (PA) assembles into a prepore state on the host cell surface. Upon endocytosis, acidic endosomal pH conditions drive PA to form a transmembrane channel, through which the lethal factor and edema factor are translocated into the host cytosol. Translocation is catalyzed by two key nonspecific polypeptide binding sites - the α-clamp and Φ-clamp. However, since the exact molecular mechanism of protein translocation has not been elucidated and electron microscopy structure revealed that the Φ clamp is narrow, we are testing the hypothesis that the clamp changes state to a more open state to accommodate large sterically bulky peptide sequences. A 10-residue, sterically bulky peptide, 'KKKKKWWSWW', was used to functionally probe polypeptide-clamp dynamics within wild-type (WT) and mutant PA channels. The peptide was synthesized with all L-stereoisomers (L-Trp), all D-stereoisomers (D-Trp) and alternating D- and L-stereoisomers (DL-Trp). Ensemble and single-channel assays were performed using planar lipid bilayer electrophysiology. Overall DL-Trp translocated less efficiently than D-Trp and L-Trp. In single-channel experiments, sub-conducting intermediates were observed for each peptide isomer. These intermediates were populated on pathway with the translocation process. A series of different intermediates was observed during an individual translocation event through the Φ-clamp mutant (F427A)