how these cholesterol-containing complexes improve transfection in-vivo.) Our studies on CL-DNA complexes employs synchrotron x-ray diffraction to reveal structure, confocal microscopy to reveal CL-DNA pathways and interactions with cells, and transfection efficiency measurements. The combined data indicate that the mechanism of gene release from complexes in the cell cytoplasm is dependent on their precise liquid crystalline structural nature and the physical and chemical parameters (e.g., the membrane charge density, membrane composition) of the complexes. The talk will describe results on cationic complexes with and without cholesterol emphasizing the differences in the interactions between the membranes of complexes with endosomal membranes leading to fusion and release into the cytoplasm. Funding provided by NIH GM-59288.

2251-Plat
Biophysical Studies of Peptides that Translocate through Cell Membranes: Induced Structures and Membrane Interactions
Astrid Graslund.
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Biophysical techniques such as high resolution NMR, CD, linear dichroism and fluorescence spectroscopy have been used to investigate the interactions of cell penetrating peptides (CPPs) with various membrane mimetic solvent systems. A pH gradient appears to be required to drive the peptide across a unilamellar phospholipid vesicle bilayer. Membrane leakage induction by CPPs (possibly associated with transient pore formation) in unilamellar vesicles has been studied in parallel with peptide translocation. The membrane perturbation caused by the TP10 peptide depends on the type and size of cargo attached to the peptide, and the potent leakage caused by the peptide alone is lost when the peptide is attached to a large cargo. Effects of the hydrophobic negatively charged counter-ion pyrene butyrate on membrane leakage has been studied for selected CPPs and compared to its CPP-enhancing efficiency using biological assays. The different proposed mechanisms for CPP activities will be discussed based on these observations.
We have also studied native peptide sequences which have CPP activities that may be related to a biological function. These are peptides derived from the N-terminal sequence of prion proteins (including the signal sequence) from mouse or cow. The prion protein derived peptides have shown an unexpected activity in counteracting scrapie infections in a neuronal cell system. We hypothesize that the CPP activity of the peptides may guide them into a specific cellular compartment where they may interfere with the prion protein aggregation and structure conversion into the scrapie form.

2252-Plat
Endosome Entrapment of CPP-ON Conjugates : Is there a Way to Overcome this Limitation ?
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Cell penetrating peptides (CPP) have been proposed as vectors for the delivery of biomolecules such as nucleic acids since poor translocation across membrane barriers is a major limitation for most of their clinical applications.
Direct translocation across the plasma membrane has been proposed initially but an endocytotic mechanism of cell import is now favored at least at low CPP concentrations. Allowing escape from endocytotic compartments and avoiding degradation of the transported cargo are now considered as the major limitations, problems in common with most non-viral delivery strategies.
Our group has focused on the CPP delivery of steric-block ON (using a splice redirection assay as end point) and more recently of apoptosis-regulating peptides. Although biological responses at submicromolar concentrations can be monitored, endosome escape remains limiting with arginine-rich CPPs. In keeping with these observations, cell permeabilization or endosomolytic treatments strongly lowers the active ON concentration.
Assays to monitor endosomal release as well as SAR studies aiming at improving CPPs in this respect will also be described.

2253-Plat
Breaching the Membrane Barrier with Antimicrobial Agents that Cluster Anionic Lipids
Richard M. Epand, Raquel F. Epand.
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The bacterial membrane plays an important role in the action of antimicrobial agents. The presence of a larger exposed fraction of negatively charged lipids in bacterial membranes contributes to the higher toxicity against bacteria. The mechanism of action of many antimicrobial agents is thought to be by damaging the bacterial membrane. Several mechanisms exist that result in such damage. The membrane also must be breached in order for the agent to reach an intracellular target. One recently recognized contribution to membrane damage by certain antimicrobial agents is their ability to cluster anionic lipids from zwitterionic lipids. This results in the formation of membrane domains enriched in the antimicrobial agent and the anionic lipid. Such lipid clustering has been demonstrated by DSC, FTIR, ^{31}P-MAS/NMR, ^{2}H-NMR, freeze fracture transmission electron microscopy and AFM combined with polarized fluorescence microscopy. In cases where this is the principal mechanism of membrane damage, it predicts that those species of bacteria whose membrane is composed largely of anionic lipids are more resistant to these agents, while other bacterial species that contain both anionic and zwitterionic lipids in their membrane exhibit greater susceptibility. The smallest active antimicrobial fragment of LL-37 (KRIVQRIKDFLR) is capable of inducing clustering of anionic lipids and is toxic against *E. coli* that has a high PE content but not against *S. aureus* that is composed largely of anionic lipids. The loss of both lipid clustering ability and antimicrobial action that occurs on removal of two cationic residues to make RI-10, gives further support to the role of lipid clustering in the antimicrobial activity. These predictions also hold well for certain antimicrobial oligo-acyllysines and also for the peptide PFWRIRIRR-amide and its analogs against several Gram positive bacterial strains having different membrane compositions.

2254-Plat
Different Scenarios of Membrane Permeabilization by Bacterial Lipopeptides
Hiren Patel[1], Mozhgan Nazari[1], Clemens Tscheka[2], Katarina Edwards[3], Goran Karlsson[3], **Heiko Heerklotz[1]**.
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The fungicidal activity of Bacillus subtilis QST 713, based mainly on the production of fengycin (FE, including several agrastatins and plipastatins), surfactin (SF), and iturin (IT) lipopeptides, has been utilized for a highly effective and environmentally safe protection of crops against a variety of pathogens. Here we use a new assay, lifetime-based calcein leakage, to study their activity, selectivity, and mechanism of membrane permeabilization. SF permeabilizes monounsaturated POPC vesicles essentially like an extremely potent detergent: It causes graded leakage starting at about Re = 0.05 peptides/lipid in the membrane and releases all dye already below the concentration required for lysis to micelles. FE shows a totally different behaviour; leakage is all-or-none and reaches a plateau after opening of 15% of the vesicles; further progress of leakage is very weak up to high peptide concentrations. Further information is obtained from ITC, fluorescence spectroscopy, light scattering, and cryo-TEM. We explain this very unusual behaviour of FE analogously to the phenomenon of detergent-resistant membranes, although no such resistance has been described so far for a cholesterol-free membrane of an unsaturated lipid. These surprising findings have major consequences for the biological activity and possible technical applications of the lipopeptides.

2255-Plat
Discovery of Transdermal Penetration Enhancers for Drug Delivery
Samir Mitragotri.
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Transdermal drug delivery is an excellent alternative to conventional methods including injections and pills. However, applications of transdermal drug delivery are limited to a handful of molecules due to excellent barrier properties of the skin. Several chemicals offer potential in overcoming this barrier to enhance transport of drug molecules across the skin. However, current chemicals are limited in their effectiveness in permeabilizing the skin barrier. Further, these chemical are usually known to cause skin irritation. Our research focuses on identification of novel chemicals including peptides and amphiphilic molecules to enhance skin permeability. I will also discuss methods for discovery of such enhancers.

Platform AR: DNA Replication, Recombination, & Repair

2256-Plat
Conformational Changes in DNA Polymerase I Revealed by Single-Molecule FRET
Yusdi Santoso[1], Catherine M. Joyce[2], Olga Potapova[2], Ludovic Le Reste[1], Johannes Hohlbein[1], Joseph P. Torella[1], Nigel D.F. Grindley[3], **Achillefs N. Kapanidis[1]**.
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The remarkable fidelity of most DNA polymerases depends on a series of early steps in the reaction pathway which allow the selection of the correct nucleotide substrate, while excluding all incorrect ones, before the enzyme is committed to the chemical step of nucleotide incorporation. The conformational transitions that are involved in these early steps are detectable with a variety of