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
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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
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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
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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

fluorescence assays and include the fingers-closing transition that has been characterized in structural studies. Using DNA polymerase I (Klenow fragment) labeled with both donor and acceptor fluorophores, we have employed single-molecule fluorescence resonance energy transfer (smFRET) to study the polymerase conformational transitions that precede nucleotide addition. Our experiments clearly distinguish the open and closed conformations that predominate in Pol-DNA and Pol-DNA-dNTP complexes, respectively; minor conformations (corresponding to the closed conformation in the Pol-DNA complex, and the open conformation in the Pol-DNA-dNTP) are also present. By contrast, the unliganded polymerase shows a broad distribution of FRET values, indicating a high degree of conformational flexibility in the protein in the absence of its substrates; such flexibility was not anticipated on the basis of the available crystallographic structures. Real-time observation of conformational dynamics showed that most of the unliganded polymerase molecules sample the open and closed conformations in the millisecond timescale. Ternary complexes formed in the presence of mismatched dNTPs or complementary ribonucleotides show novel FRET species, which we suggest are relevant to kinetic checkpoints that discriminate against these incorrect substrates. Our results advance the mechanistic understanding of the process of nucleotide addition by DNA polymerases and suggest ways to study conformational dynamics in other nucleic-acid polymerases.

2257-Plat

Single Molecule Studies of Eukaryotic Replisomes in Xenopus Egg Extracts

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In eukaryotes, two MCM2-7 helicases are assembled at each origin of replication in the G1 phase of the cell cycle. In S phase, the helicases are activated, leading to assembly of two sister replisomes that replicate DNA in opposite directions. At present, little is known about the spatial arrangement or molecular mechanism of MCM2-7 complexes that are engaged in DNA replication. One scenario is that the two sister MCM2-7 complexes dissociate during initiation and then travel away from one another. Alternatively, the sister helicases might remain physically coupled. To differentiate between such models, we have established a series of single-molecule visualization tools using a nucleus-free replication system of *Xenopus* egg extracts. We demonstrate that these extracts can replicate lambda phage DNA that is mechanically well-stretched and specifically tethered at both ends to a functionalized surface. Our observation of large replication bubbles from single origins on such doubly-tethered DNA argues that helicases located at sister forks can function independently during replication.

In addition, we aim to observe real-time dynamics of different replisome components on doubly-tethered DNAs. For this purpose, we have generated fluorescently tagged Cdc45, an MCM2-7 co-factor that travels with the MCM2-7 helicase. Since the high concentration of labeled Cdc45 needed to support replication causes a high fluorescence background, we tagged Cdc45 with the photoswitchable fluorescent protein mKikGR. The ability to switch on the fluorescence of only those mKikGR proteins that are bound to DNA via Cdc45 enables single-molecule imaging of active replisomes, even at high ambient concentrations of Cdc45-mKikGR. We present the results of initial experiments that prove the feasibility of these techniques as novel ways to study the activity of replication factors in a physiologically relevant environment.

2258-Plat

DNA Base Flipping: New-Found Insights into the DNA Mismatch Recognition Process in E. Coli Muts

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Detection of various base-base mismatches and small insertion/deletion loops in DNA is performed by the MutS mismatch recognition protein. This highly conserved process exists in both prokaryotes and eukaryotes and failure to recognize DNA damage can have a detrimental effect on the fidelity of the genome and, in humans, has been linked to numerous forms of cancer. Several crystal structures have emerged over the years capturing MutS bound to various mismatches. However, the events directly following mismatch recognition remain unclear. To shed light on this matter, we present nine sub-μs, all-atom molecular dynamics simulations of Escherichia coli MutS bound to a G•T mismatch with different nucleotide configurations. From these simulations, we identified significant instability in the adjacent base 5′ to the thymine mismatch. In one case, the 5′ adjacent base completely loses base stacking and flips out spontaneously via the minor groove. To the best of our knowledge, this rare event is the first ever documented case of DNA base flipping from any unrestrained protein-DNA simulation. In addition, these observations are in excellent agreement with a recent experimental study where it was suggested that the 5′ adjacent base could exist in an extrahelical state. To further understand the energetics of base flipping in MutS, we utilized the Hamiltonian replica-exchange molecular dynamics (HREMD) method simulating 44 independent replicas in an explicit water box. The free energy profile generated from HREMD shows two distinct minima, one for the stacked state and one for flipped out state, separated by a small energy barrier with the flipped out state being the more favorable of the two. Together, our results offer an unprecedented level of new insight into the mismatch recognition process and further our knowledge of this complex system.

2259-Plat

DNA Conformational Dynamics in Mismatch Recognition

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DNA mismatch recognition is done by the homodimer MutS in prokaryotes and by its homologues: heterodimers Msh2-Msh3 and Msh2-Msh6 in eukaryotes. Msh2-Msh6 binds preferentially to single insertion/deletions. Msh2-Msh3 has been shown to bind to DNA hairpins. It has been suggested that the conformational dynamics of the DNA substrate (bending and unbending) plays a fundamental role in the recognition process. Mismatch recognition allows identifying a single mismatched DNA pair among thousands of matched basepairs. The process is ATP dependent and different models for DNA discrimination have been proposed based in biochemical evidence as well as AFM studies. In this work we study the conformational dynamics of several DNA substrates and its complexes with the human MutS homologs. The DNA substrates were labeled with fluorescent dyes that constitute a fluorescence resonant energy transfer (FRET) pair. Experiments at the single molecule level allow us to follow the conformational dynamics of the substrates by determining the substrate's end to end distance. We were able to determine the binding and dissociation rates of the proteins from the substrates as well as the conformational state of the substrates under different conditions, including studies with ATP and ADP under both hydrolytic and non-hydrolytic conditions. In particular we discuss the role of the substrate's intrinsic dynamics for binding of hMsh2-hMsh3 to DNA hairpins and DNA 3-way junctions.

2260-Plat

The Dance of Chromosomes during DNA Repair

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DNA repair is an essential process for preserving genome integrity. Among the various forms of DNA damage, double-strand breaks (DSBs) are the most cytotoxic and genotoxic. To repair them, eukaryotic organisms use homologous recombination (HR): it consists of exchanging DNA strands between the broken DNA and an intact homologous DNA and it is choreographed by multi-protein complexes (1). During HR, the search for an intact homologous sequence among the whole genome is the most enigmatic stage (2). How can two homologous needles find each other in the genomic haystack? Is search the result of diffusion and chance encounters, or is there a search apparatus dedicated to bringing the homologous sequences together?

To explore the choreography of the DNA and the recombination proteins during homology search, we developed an *in vivo* 3-colors assay in diploid yeast cells where 2 homologous chromosomes are fluorescently marked at the same locus (with GFP-Lac and RFP-Tet arrays), as well as recombination factors (CFP-tagged proteins). Using deconvolution microscopy, we tracked the movement of the two chromosomes in 3-dimensions in the absence and in the presence of a unique DSB induced near one of the marked chromosome. In the absence of DSB, we found that homologous chromosomes undergo a constrain Brownian motion with a diffusion coefficient of 4.10^{-4} $\mu m^2/s$ inside a small region of 300 nm. When a DSB is induced, the two homologous DNA become highly dynamic and homologous pairing occurs within one hour. This work is the first attempt to visualize simultaneously the movement of two homologous sequences *in vivo* into and out of repair centres.

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2261-Plat

Single-Molecule Measurements of Synthesis by DNA Polymerase with Base-Pair Resolution

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