assembly with the α_1-subunit and trafficking to the plasma membrane. The β_2-subunit binds 4-5-fold more efficiently to the ER lectin chaperone, calnexin, and 2-3-fold less efficiently to the non-lectin ER chaperone, BiP, than the β_1-subunit. These results indicate that folding of the β_2- and β_1-subunits is mediated by lectin and non-lectin chaperones, respectively, consistent with the essential role of N-glycosylation for folding and trafficking of the β_2 but not of the β_1-subunit. Disruption of the α_1-β association by mutations in defined α_1-interacting regions of either β_1- or β_2-subunits results in the ER retention of unassembled mutants, indicating that α-β assembly is essential for the ER export of either β-subunit isoform. In conclusion, the ER quality control system ensures that only properly folded β-subunits assemble with the α-subunits and only assembled α-β complexes are exported to the Golgi and delivered to the plasma membrane.

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2246-Plat

Not All ABC Transporters are the Same: Correlation between Genetic, Structural, and Mechanistic Diversity

Oded Lewinson[1], Allen T. Lee[1], Kaspar P. Locher[2], Douglas C. Rees[1].
[1]California Institute of Technology, Pasadena, CA, USA, [2]ETH Zurich, Zurich, Switzerland.

ATP binding cassette (ABC) transporters constitute a ubiquitous super-family of integral membrane proteins that translocate a diverse array of substrates across cell membranes. Studies of several well-characterized systems suggested a mechanistic similarity between different members of this large family of transporters. However, more recent reports pointed out significant differences at the genetic and structural levels. We report here a functional comparison between several ABC transporters of different substrate specificities and find fundamental differences between them. Type I ABC transporters, exemplified by the arch typical maltose transporter, are characterized by an inherent instability of the transporter-receptor complex. In these systems, ATP binding promotes complex formation, and binding of substrate-loaded receptor accelerates the rate of ATP hydrolysis. In contrast, in type II ABC transporters (the metal-chelate transporters), the ''default'' complex is extremely stable. However, for productive transport to occur, the complex must dissociate, an event mediated by both substrate and ATP binding. Relative to type I transporters, high basal ATPase rates are measured with modest to negligible stimulation by substrate-loaded receptors. These and other findings presented here highlight significant mechanistic differences between ABC transport systems, indicating that considerable mechanistic diversity exists within this large superfamily of proteins.

2247-Plat

How Binding of the Signal Peptide Unlocks the Translocon

Ana Nicoleta Bondar, Douglas J. Tobias, Stephen H. White.
University of California at Irvine, Irvine, CA, USA.

In all organisms, many of the proteins newly synthesized by the ribosome are targeted to the SecY/Sec61 translocon for secretion or incorporation into the lipid membrane. Targeting of secreted proteins is generally encoded in a ~20 amino acids extension of the N-terminus of the nascent protein, denoted as the signal peptide. The translocon opens upon binding of the signal peptide and the ribosome (or the SecA motor). To understand how SecY/Sec61 opens, it is essential to know the structure and dynamics of the translocon:signal peptide complex in a hydrated lipid membrane. Molecular dynamics simulations of the SecY translocon from M. janaaschii with proOmpA signal peptide reveal that the structure and dynamics of both the translocon and the signal peptide change significantly upon formation of the complex. It appears that inside the translocon the signal peptide has a preferred location in which it interacts with water molecules and with highly conserved SecY amino acids whose mutation causes translocation defects. Binding of the signal peptide induces changes in the relative orientation of transmembrane helices of the translocon, and also affects the structure and interactions with water and the rest of the protein of the plug segment that closes the periplasmic vestibule of the translocon in the closed state. The structure and dynamics of the translocon and signal peptide are coupled: mutating the translocon induces changes in the structure and dynamics not only of the translocon, but also of the signal peptide.

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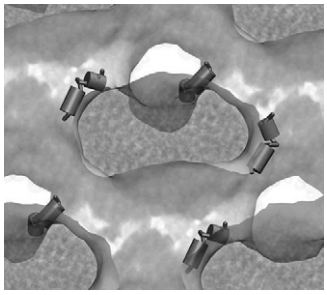
Platform AQ: Member-Organized Session: Break on through to the other side: Comparing Membrane Permeabilizers

2248-Plat

Simulation Studies of Peptide Induced Membrane Poration and Fusion

Siewert J. Marrink[1], Marc Fuhrmans[1], Durba Sengupta[1], Semen Yesylevskyy[2], Alan E. Mark[3].
[1]Univ Groningen, Groningen, Netherlands, [2]Institute of Physics of the National Academy of Science of Ukraine, Kiev, Ukraine, [3]Univ of Queensland, Brisbane, Australia.



A wide range of peptides is known to modulate the behavior of lipid membranes, in particular to be able to destabilize the normal lamellar state. Here we compare the various ways in which a lipid membrane can be distorted due to the presence of such peptides, using molecular dynamics simulations. In particular we show examples of membrane poration by antimicrobial peptides, micropinocytosis by cell penetrating peptides, and the formation of cubic phases by fusion peptides (see figure).

Snapshot of a single diamond cubic phase induced by the Influenze HA fusion peptide. The helical parts of the peptides are shown as red rods, the lipid/water interface as a green surface. Lipid tail beads are shown in gray. This particular cubic phase is special as it combines both pores (the white gaps) and stalks (filled with lipid tails) in one phase. The peptides stabilize this stalk/pore structure.

2249-Plat

Cationic Lipids: from Membrane Destabilization to Cell Signaling

Caroline Lonez, Marc F. Lensink, Bouna-Moussa Tandia, Michel Vandenbranden, **Jean-Marie Ruysschaert**.
Free University of Brussels, Brussels, Belgium.

Cationic liposomes have been used over the past decades with gene therapy or vaccination trials in mind, considering them primarily as the smart Trojan horse allowing to go trough the cell walls. Fusion-promoting lipids such as DOPE were thus added with some success to try to enhance the destabilizing properties of some cationic lipids, while others were intrinsically destabilizing. It is difficult to conceive that lipids that destabilize membranes would act innocently on the cell physiology. After all, lipids are not only the backbone of membranes, they also act as facilitators of membrane functions such as endocytosis, budding, curving; they regulate protein membrane activity and can even serve as signal transmitters (bioactive lipids). Rather than being considered as responsible for harmful side-effects that should be minimized, cationic lipids could be considered as a potential immunostimulating or pharmacological agents. We illustrate these aspects with diC14-amidine, which forms liposomes with a bilayer at the edge of instability. DiC14-amidine fuses easily with cell membranes, modifies cell signaling and activates immune responses through destabilization and/or activation of specific membrane receptors, like TLR4 [1,2]. Like it was shown for natural lipids, this demonstrates that cationic lipids are not only membrane destabilizing agents but might affect cell membrane components function.

1. Lonez C, Vandenbranden M, Ruysschaert JM. Prog Lipid Res. 2008. 47(5):340-7.
2. Tanaka T et al. Eur J Immunol. 2008. 38(5):1351-7.

2250-Plat

Cationic Lipid Vectors for Gene Delivery: Distinct Pathways of Endosomal Release

Kai K. Ewert, Alexandra Zidovska, Nathan Bouxsein, Rahau S. Shirazi, Heather Evans, **Cyrus R. Safinya**.
University of California at Santa Barbara, Santa Barbara, CA, USA.

Cationic liposomes (CLs) are used as non-viral gene vectors in worldwide human clinical trials of gene therapy. Because our understanding of the mechanisms of action of CL-DNA complexes remains poor and transfection efficiencies remain low compared to gene delivery with viral vectors, significant additional insights and discoveries will be required before the development of efficient chemical carriers suitable for long-term therapeutic applications. (For example, virtually all current human gene therapy protocols using lipofection as a vector contain cholesterol even though very little is understood about

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.
Stockholm University, Stockholm, Sweden.
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 ?
Bernard Lebleu.
Univ.Montpellier 2, Montpellier, France.
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.
McMaster University, Hamilton, ON, Canada.
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]**.
[1]UofT, Toronto, ON, Canada, [2]Univ. Saarland, Saarbruecken, Germany, [3]Uppsala Univ., Uppsala, Sweden.
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.
University of California, Santa Barbara, Santa Barbara, CA, USA.
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]**.
[1]University of Oxford, Oxford, United Kingdom, [2]Yale University, New Haven, CT, USA, [3]Yale University, New Haven, United Kingdom.
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