3160-Symp
Membrane Interactions and the Formation of Multimeric Pores by Cyclotides
David J. Craik[1], Yen-Hua Huang[1], Michelle L. Colgrave[2], Boris Martinac[3], Absed Keleshian[1], Norelle L. Daly[1].
[1]Univ Queensland, Brisbane, Australia, [2]CSIRO, Division of Livestock Industries, Brisbane, Australia, [3]Victor Chang Cardiac Research Institute, Sydney, Australia.
The cyclotides are a family of cyclic mini-proteins containing a cystine knot motif. They are produced by plants as defence-related proteins and have potent insecticidal and nematocidal activity. They also have been reported to have antimicrobial and anti-HIV activities. In this study we investigated their role in membrane interaction and disruption. Kalata B1, a prototypic cyclotide, was found to induce leakage of the self-quenching fluorophore, carboxyfluorescein, from phospholipid vesicles. Alanine-scanning mutagenesis of kalata B1 showed that residues essential for lytic activity are clustered, forming a bioactive face on the surface of the molecule. This patch of residues is not directly involved in membrane binding but we propose is involved in self assembly to facilitate the formation of pores in membranes. Patch clamp electrophysiological experiments showed that conductive pores were induced in liposome patches on incubation with kalata B1. The conductance calculated from the current-voltage relationship indicated that the diameter of the pores formed in the bilayer patches is 41-47 Å. Collectively, the findings provide a mechanistic explanation for the diversity of biological functions ascribed to this family of ultra-stable macrocyclic peptides.

3161-Symp
Role of Peptide Folding and Aggregation in Triggering Membrane Perturbation
Anne S. Ulrich[1], Parvesh Wadhwani[1], Erik Strandberg[1], Sergiy Afonin[1], Jochen Buerck[1], Johannes Reichert[1], Marina Berditsch[1], Stephan Grage[1], Christian Mink[1], Sebastian Ehni[1], Deniz Tiltak[1], Pavel Mykhailiuk[2], Igor Komarov[2].
[1]Karlsruhe Institute of Technology, Karlsruhe, Germany, [2]Taras Shevchenko University, Kyiv, Ukraine.
Many membrane-active antimicrobial peptides are cationic and fold into amphiphilic structures upon binding to the lipid bilayer of the bacterial envelope, which thereupon gets permeabilized. Peptides with an alpha-helical conformation have been thoroughly studied by solid state NMR and other biophysical techniques. A concerted re-alignment of several monomers is supposed to lead to the formation of a transient pore, i.e. a local oligomeric assembly with anionic lipids that is called toroidal wormhole. Yet, several designer-made ''helical'' peptides have been found to undergo a rapid membrane-induced concentration-dependent aggregation as beta-strands. Given that such H-bonded aggregate is thermodynamically more stable, the question arises whether membrane perturbation involves only the ''classical'' helical structures under kinetic control, or whether oligomeric beta-sheets may also contribute, as proposed e.g. for the cytotoxic Alzheimer's peptide. Even more intriguing is the same question when referring to designated beta-stranded peptides, such as the (KIGAKI)3 system designed by Blazyk et al. as a complement to the helical (KIAGKIA)3, both of which have comparable antimicrobial activity. We have studied these and other representative antimicrobial peptides with alpha-helical and beta-stranded character using solid state 19F-NMR and circular dichroism. In macroscopically oriented samples, a combination of these two methods can reveal not only the conformation and alignment of a peptide in the lipid bilayer, but also its local dynamic behavior and global aggregation kinetics. Structural results and their correlation with antimicrobial activity will be presented, in an attempt to address the mechanistic questions raised above.

3162-Symp
The Translocation Mechanism of Arginine Rich Cell Penetrating Peptides
Angel E. Garcia.
Rensselaer Polytechnic Inst, Troy, NY, USA.
The recombinant HIV-1 Tat protein contains a small region corresponding to residues **^{47}YGRKKRRQRR^{57}R** that is capable of translocating cargoes of different molecular sizes, such as proteins, DNA, RNA, or drugs, across the cell membrane in an apparently energy independent manner. The pathway that these peptides follow for entry into the cell has been the subject of strong controversy for the last decade. This peptide is highly basic and hydrophilic. Therefore, a central question that any candidate mechanism has to answer is how can this highly hydrophilic peptide be able to cross the hydrophobic barrier imposed by the cell membrane. We propose a mechanism for the spontaneous translocation of the Tat peptides across a lipid membrane. This mechanism involves strong interactions between the Tat peptides and the phosphate groups on both sides of the lipid bilayer; the insertion of charged side chains that nucleate the formation of a transient pore followed by the translocation of the Tat peptides by diffusing on the pore surface. This mechanism explains how key ingredients such as the cooperativity among the peptides, the large positive charge, and specifically the arginine amino acids contribute to the uptake. The proposed mechanism also illustrates the importance of membrane fluctuations. Indeed, mechanisms that involve large fluctuations of the membrane structure, such as transient toroidal pores and the insertion of charged amino acid side-chains, may be common and perhaps central to functions of many membrane protein functions. Consistent with this model, experiments on black lipid membranes show that Tat and Arg-9 peptides induce a current through the membrane in presence of an electric field. The current is consistent with the formations of pores. These currents are not observed in absence of the peptides.
Work collaboration with Henry Herce. Funded by NSF-NSEC.

Platform AT: Ligand-gated Channels

3163-Plat
Structural Insights into Allosteric Modulation of NMDA Receptors Through the Amino-Terminal Domain
Erkan Karakas, Noriko Simorowski, Hiro Furukawa.
Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA.
Majority of fast excitatory synaptic transmission in the mammalian brain is mediated by a class of molecules called ionotropic glutamate receptors, which include *N*-methyl-D-aspartate (NMDA) receptors. NMDA receptors are heterotetrameric ion channels that are composed of two NR1 subunits and two NR2 (A-D) subunits or NR3 subunits. NMDA receptors play key roles in numbers of important processes including synaptic plasticity and development in normal state, whereas aberrant activity of NMDA receptors is associated with ischemic brain injury and neurodegenerative diseases including Parkinson's disease and Alzheimer's disease. Activity of NMDA receptor is tightly controlled through multiple pathways. One such mechanism is allosteric modulation through binding of small molecules to the extracellular amino terminal domain (ATD) in a subtype specific manner, i.e. polyamines and protons bind NR1, Zn^{2+} binds both NR2A and NR2B, and phenylethanolamine compounds bind NR2B. To understand the molecular mechanism of the ATD-dependent allosteric modulation of NMDA receptors, we have solved the structures of NR2B ATD in the zinc-bound and -free forms. The structures reveal an overall clamshell architecture with a unique domain orientation distinct from the non-NMDA receptor ATDs and molecular determinants for the zinc binding site, ion binding sites, and the architecture of the putative phenylethanolamine binding site.

3164-Plat
Exploring the Role of Positive Allosteric Modulators in Stabilizing the GluR2 Ligand-Binding Domain Dimer
Christopher P. Ptak, Ahmed H. Ahmed, Michael K. Fenwick, Alexander S. Maltsev, Robert E. Oswald.
Cornell University, Ithaca, NY, NY, USA.
Ionotropic glutamate receptors undergo rapid desensitization after full agonist activation. By stabilizing dimers of the glutamate-bound ligand-binding domain within the tetrameric receptor scaffold, desensitization is inhibited. Physiologically, interactions between the two halves of the dimer interface are intended to be weak enough to allow for receptor desensitization. Allosteric modulators bind to a cavity at the base of this interface thereby extending the interacting surface and slowing desensitization. Recently, we solved the structures of a number of allosteric modulators in complex with the GluR2 ligand-binding domain using x-ray crystallography. The total set of new and existing modulator structures, which encompasses 4 structural classes, allows us to create a map of the preferred protein-ligand interactions along the length of the dimer cavity. The cavity is divided into five subsites (A, B, B′, C, and C′) each with a propensity for interacting with specific classes of allosteric modulators. If the modulator does not obstruct the central A subsite, the symmetrical nature of the cavity allows a second modulator to bind to the dimer. Additionally, we explore binding models in the context of dimerization for a single-cavity binding modulator using NMR spectroscopy. Our results provide guidance for the rational design of drugs that target the ligand-binding domain dimer interface and the control of desensitization.

3165-Plat
The Free Energies of Ligand-Binding to the Ionotropic Glutamate Receptor
Albert Y. Lau, Benoit Roux.
The University of Chicago, Chicago, IL, USA.
Ionotropic glutamate receptors (iGluRs) are ligand-gated ion channels activated by glutamate. The binding of glutamate and other synthetic agonist