3160-Symp
Membrane Interactions and the Formation of Multimeric Pores by Cyclotides
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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
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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
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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
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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
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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
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Ionotropic glutamate receptors (iGluRs) are ligand-gated ion channels activated by glutamate. The binding of glutamate and other synthetic agonist

molecules to the extracellular ligand-binding domains (LBDs) of these receptors drives the opening of cation-permeable transmembrane pores. Ligand-binding alters the conformational free energy landscape of LBD closure, which provides useful reversible work for opening the gate of the transmembrane ion channel. Using all-atom molecular dynamics simulations, we computed absolute LBD-ligand binding free energies for a set of different ligands to AMPA and NMDA receptor LBDs using a methodology formulated on the basis of potentials of mean force. The free energy of the full ligand-binding process is the sum of the free energy contributions from ligand-docking into an open LBD and LBD closure. Alterations in the free energy landscape of LBD closure are correlated with whether the bound ligand is a full agonist, partial agonist, or antagonist.

3166-Plat
Pore Architecture and ion Sites of Acid Sensing ion Channels and P2X Receptors
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Acid-sensing ion channels are proton-activated, sodium-selective channels composed of three subunits, and members of the degenerin/epithelial sodium channel (DEG/ENaC) superfamily. These eukaryotic channels have essential roles in sodium homeostasis, taste, and pain. Despite their roles in biology, there is little knowledge of the structural and chemical principles underlying their ion channel architecture and ion-binding sites. Here we present the crystal structure of a functional acid-sensing ion channel in a desensitized state at 3 angstrom resolution, the location of the desensitization gate, and the trigonal antiprism coordination of cesium ions bound in the extracellular vestibule. Comparison of the acid-sensing ion channel structure with the P2X receptor reveals unanticipated similarities and mechanical principles in different ligand-gated ion channels.

3167-Plat
Energetics of Allosteric ion Binding to a Ligand-Gated ion Channel
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Allosteric regulation of ligand-gated ion channels (LGICs) is ubiquitous, involving discrete transitions between resting, conducting, and desensitized states, driven by agonist binding. Small molecules that bind at specific sites have also been reported to act as allosteric modulators in many LGICs, such as glutamate, P2X, and Cys-loop receptors, but an understanding of the underlying molecular mechanisms is sparse. Of these, perhaps the best studied are kainate subtype glutamate receptors which require both extracellular Na+ and Cl- for receptor function. Studies on glutamate receptors (iGluRs) have defined the ligand binding domain (LBD) dimer assembly as the key functional unit that controls channel activation and desensitization. Using crystallographic and electrophysiological approaches, we have previously shown that for kainate, but not AMPA iGluRs, the binding of Na+ and Cl- ions to discrete, electrostatically coupled sites in the extracellular LBD dimer regulates the rate of entry into the desensitized state, which occurs when the dimer interface ruptures and the channel closes. We have now dissected the energetic effects of allosteric ions on kainate receptor dimer stability in solution using analytical ultracentrifugation. Our results show that Na+ and Cl- ions modulate dimer affinity as much as 50-fold, and that removal of either ion disrupts the dimer. We further tested the generality of this model of ion action for Ca2+ modulation of the orphan iGluR delta2 that crystallizes as a dimer which binds Ca2+. Our results indicate that ions can contribute substantial free energy to active state stabilization in both these receptors, and we postulate that in contrast to AMPARs, the dimer interface in these receptors may be intrinsically weak to serve a functional role: Allowing ion modulation. Our results provide quantitative measurements of the energetic effects of allosteric ion binding on a LGIC.

3168-Plat
Structure and Dynamics of Nicotinic Acetylcholine Receptor at the Cell Membrane
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A combination of ensemble averaging methods (confocal FRAP and FCS) and single molecule experimental techniques (single-particle tracking, high-resolution fluorescence microscopy, patch-clamp) was used to study the supramolecular organization of the acetylcholine receptor (AChR), receptor dynamics at the cell surface, and the kinetics of receptor internalization. Chol depletion produced gain-of-function of single-channel dwell time. Submicron-sized particles could be resolved into AChR ''nano-clusters'' with a peak size distribution of ~55 nm by superresolution STED and GSDIM microscopies. Chol depletion reduced the number of nanoclusters, increasing their size, and changed their supramolecular organization on larger scales (0.5-3.5 microns). FRAP, FCS and SPT experiments provided information on the dynamics of AChR nanoclusters, disclosing the dependence of their mobility on Chol content and cortical cytoskeleton. Chol content at the plasmalemma may thus modulate cell-surface organization and dynamics of receptor nanoclusters, and fine-tune receptor channel function to temporarily compensate for acute AChR loss from the cell surface.

3169-Plat
Structure Rearrangement of the Pore in P2X Receptors During Gating
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P2X receptors are cation-selective channels that open upon binding extracellular ATP. In mammals, seven P2X receptor subunits have been cloned ($P2X_{1-7}$), which can form functional homomeric as well as heteromeric channels. The recently published X-ray crystal structure of a P2X receptor confirmed that these channels are trimers and that each subunit has two transmembrane (TM) segments, a large extracellular segment containing the ligand binding site, and intracellular N and C termini. The crystallized channel appears to be in a closed state, with an extended plug of hydrophobic residues in the pore-lining TM2 helix forming a gate to prevent ion permeation. Although the structure reveals the overall molecular design of the protein, how the pore opens upon ATP binding is still unknown. Here we study the interaction of metal ions as well as MTS reagents of variable sizes with Cys residues introduced into the TM2 helices of P2X receptors. Our results suggest that the crystal structure is representative of closed P2X receptor channels in native membranes, and that the TM2 helices straighten in a translational motion that produces a modest opening of the outer pore and an accompanying constriction of the inner pore. Consideration of these constraints in light of the distinct arrangement of subunit interfaces in the ligand-binding and pore domains supports a model wherein the ligand-binding domains rotate relative to one another to straighten the TM2 helices and open the pore.

3170-Plat
Analysis of Structural Rearrangements during P2X1 Receptor Activation by Voltage Clamp Fluorometry
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P2X receptors (P2XRs) are non-selective cation channels which are activated upon binding of extracellular ATP. They are assembled as homo- or heteromers from three subunits with two transmembrane domains each and show no sequence homology to any other known ion channel or ATP-binding protein. Most recently, the first crystal structure of a P2X receptor has been resolved confirming many predictions that were based on the interpretation of mutagenesis studies. However, the conformational changes governing channel opening, desensitization and recovery remain unknown, as does the exact mode of ATP binding. The P2X1 receptor subtype is characterized by nanomolar affinity for ATP and a rapid desensitization, followed by a prolonged recovery period before reactivation is possible. Here we used voltage clamp fluorometry to identify domains undergoing conformational changes during ligand binding, activation, desensitization and recovery from desensitization of the P2X1 receptor. We have identified six residues in the extracellular domain of the P2X1 receptor that, upon substitution with cysteine, are accessible for TMRM labeling. Upon activation by ATP, five of these mutants showed significant changes in fluorescence. The kinetics of the fluorescence changes could be correlated with receptor activation or desensitization indicating that the changes were reporting discrete conformational changes. In addition, binding of the competitive antagonist NF449 produced a fluorescence change in three of these mutants. In conclusion, our observations provide insight into the conformational changes occurring during the P2X opening and desensitization and suggest that movements in a cysteine rich domain that projects over the supposed ATP binding site are involved in these processes.

Platform AU: Biotechnology & Bioengineering

3171-Plat
Parallel, Non-Contact Trapping and Translation of Yersenia Pestis Bacteria with Optoelectronic Tweezers
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Studies of host-pathogen (HP) interactions at the single cell level are critical for understanding the often elaborate, dynamical processes involved in pathogen