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.

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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