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the entire LBD or the whole protein. Hence, β-TF1 is viewed as a suppressor of $InsP_3$ binding. Additional studies suggest that β-TF1 not only helps stabilize LBD but also couples its conformational changes to the gate of the ion pore. The crystal structures of β-TF1 alone and of β-TF2 plus ARF bound with $InsP_3$ have been solved separately. The latter structure reveals how $InsP_3$ is coordinated by various side-chains in the binding sites at the ARF - β-TF2 interface, and mutation of these side-chains weakens $InsP_3$ binding. Despite this progress, the fundamental question of how $InsP_3$ biases the gating conformation of LBD remains. To address this question, we have determined the crystal structure of the entire LBD of rat $InsP_3R1$ in both $InsP_3$-bound and -unbound conformations, revealing a triangular architecture. Comparison of the $InsP_3$-bound and -unbound conformations strongly implies that β-TF1 and ARF move as a rigid unit with respect to β-TF2. While LBD without $InsP_3$-bound may spontaneously transition between gating states, binding of $InsP_3$ between β-TF2 and ARF locks it in a state that would strongly bias the gating equilibrium toward the open state of the ion pore.

3109-Plat
Using Crosslinking and Mass Spectrometry to Study Glycine Receptor Allostery
Chelsee A. Donelan, Rathna Veeramachaneni, David J. Lapinsky, Michael Cascio.
Duquesne University, Pittsburgh, PA, USA.
A wealth of high-resolution structural information regarding pentameric ligand-gated ion channels is now available, but less is known of the molecular details underlying complex allosteric mechanisms involved in channel gating and desensitization. Receptor allostery can be studied by identifying state-dependent distance constraints that may be used in molecular modeling of these receptors. Systematically generated single Cys mutations of the human α_1 glycine receptor (GlyR) expressed in insect cells were labeled with a clickable methanethiosulfonate-benzophenone crosslinker. After covalent ligation to Cys, crosslinks may then be introduced in the resting, open, or desensitized states by photoactivation. Including an alkyne tag on the crosslinker permits click chemistry addition of biotin, which allows for enrichment by avidin chromatography. Mass spectrometry (MS) fingerprinting of monomeric and higher-order GlyR bands on SDS-PAGE using ESI-QTOF MS/MS then allows for determination of the site of crosslinking. Our initial proof-of-principle studies conducted on purified GlyR have provided state-dependent information on this receptor. This approach may be broadly applicable to studies of any allosteric complex.

3110-Plat
Agonist-Induced Conformational Changes in the Ligand Binding Domain of Cyclic Nucleotide-Regulated Channels
Michael C. Puljung, William N. Zagotta.
University of Washington, Seattle, WA, USA.
Cyclic nucleotide-gated (CNG) channels and hyperpolarization-activated, cyclic nucleotide-modulated (HCN) channels are opened by the direct binding of cyclic nucleotides (e.g. adenosine 3',5'-cyclic monophosphate, cAMP). The ligand binding domain is primarily formed by an eight-stranded β-roll followed by two helices (B and C helices). Following agonist binding to residues in the β-roll, the C helix is thought to initiate the opening conformational change by moving towards the binding pocket and transitioning from a less ordered coil to a stable helix. To further investigate this hypothesis, we expressed the soluble C-terminal domain of the HCN2 channel, which contains the cyclic nucleotide binding domain, and introduced cysteines into the C helix, which were labeled with the fluorophore bimane. Into the same helix, we placed pairs of histidines in helical register. Colored transition metal ions can bind to these histidines and quench the fluorophore by a FRET mechanism. The amount of quenching was used to estimate the distance between the fluorophore and the metal binding site in the presence and absence of cAMP. For all of the constructs tested, cAMP binding induced a large increase in metal affinity at the di-histidine binding sites, indicating a stabilization of the helical structure. We also observed a change in the total amount of quenching in some constructs indicating a change in distance between the fluorophore and metal binding site. These data suggest a conformational change within the C helix induced by ligand binding. When a similar di-histidine binding site was introduced into the C helix of intact CNG channels, binding of Ni^{2+} increased the efficacy of the partial agonist inosine 3',5'-cyclic monophosphate (cIMP), consistent with the hypothesis that stabilization of the secondary structure of the C helix is part of the gating conformational change.

3111-Plat
Neuropeptide Interaction with the Extracellular Domain of the Acid Sensing Ion Channel 1A
Thomas Sherwood, Michael Ratti, Erin Frey, **Candice Askwith**.
The Ohio State University, Columbus, OH, USA.
Acidic fluctuations occur within the brain and contribute to multiple physiological and patho-physiological processes. Neurons possess specific molecules to sense and respond to these pH changes. In particular, many neurons express the acid sensing ion channels (ASICs). ASICs are ligand-gated ion channels activated by extracellular protons. ASICs are permeable to cations and depolarize neurons to mediate acid-dependent neuronal signaling. Some ASIC channels are also permeable to calcium and these channels are thought to play a particularly important role in neuronal processes. Specifically, ASIC1a subunits form channels permeable to calcium and contribute to multiple behaviors, seizure termination, pain, as well as neuronal death initiated by prolonged acidosis following inflammation and stroke. ASIC1a undergoes a process called steady-state desensitization in which slow, incremental acidification causes the channels to desensitize rather than undergo robust activation. Induction of steady-state desensitization prevents ASIC1a-mediated neuronal death. Yet, specific neuropeptides present within the brain limit steady-state desensitization and allow ASIC1a to activate following slow, incremental acidification. Two types of neuropeptides, the RFamides and dynorphin-related peptides, interact directly with the extracellular domain of ASICs to modulate channel gating. In this work, we explore the mechanism and protein domains responsible for modulation of ASC1a steady-state desensitization by endogenous neuropeptides. We determined that (1) RFamide and dynorphins alter ASIC gating in distinct, but overlapping ways; (2) dynorphins can compete with the inhibitory spider toxin venom peptide PcTx1 for interaction with the channels; and (3) the addition of RFamide and dynorphins synergizes ASIC1a modulation. We also present evidence that specific protein domains are involved in endogenous neuropeptide modulation of ASIC1a. Together, these studies further define endogenous neuropeptides as important modulators of ASIC1a activity and present data indicating that distinct protein domains control neuropeptide effects.

3112-Plat
Interdimer Contacts Paint a New Picture of Glutamate Receptor Activation
Hector P. Salazar Garcia[1], Albert Y. Lau[2], Lydia Blachowicz[3], Andrew J. Plested[1], Benoît Roux[3].
[1]Leibniz-Institut für Molekulare Pharmakologie & NeuroCure Initiative, Charité Universitätsmedizin, Berlin, Germany, [2]Johns Hopkins University School of Medicine, Dept. of Biophysics and Biophysical Chemistry, Baltimore, MD, USA, [3]The University of Chicago, Dept. of Biochemistry and Molecular Biology, Chicago, IL, USA.
The ionotropic glutamate receptors (iGluR) mediate the majority of the rapid signalling at excitatory synapses in the brain. The binding of glutamate and other agonist molecules to the ligand-binding domains (LBDs) of the iGluR provides the free energy for driving intra-LBD conformational transitions that open the gate of the ion channel. However, much less information is available about inter-LBD motions. We recently showed that disulfide crosslinks between kainate receptor LBD dimers inhibit receptor activation (Das et al, 2010, PNAS). Here, we used a combination of structural studies and electrophysiology to map the conformational transitions of the LBD dimers between different states of the GluA2 receptor. Interdimer disulfide trapping with exquisite functional sensitivity shows that the two subunit dimers must translate relative to each other during activation, with the center of the dimers moving towards the overall axis of the channel. The crosslink captures an intermediate state between resting and fully activated and has geometry (including reduced linker separation) that provides new insight to glutamate receptor activation.

3113-Plat
Computational Studies of the Molecular Mechanisms Responsible for Ca^{2+} Permeation and Mg^{2+} Block of NMDA Receptors
Lea Veras[1], Igor Kurnikov[1], Jon W. Johnson[2], Maria Kurnikova[1].
[1]Carnegie Mellon University, Pittsburgh, PA, USA, [2]University of Pittsburgh, Pittsburgh, PA, USA.
Glutamate receptors are membrane proteins activated by the neurotransmitter glutamate that mediate fast synaptic excitation in the mammalian brain. NMDA receptors constitute a glutamate receptor subfamily specifically