165-Plat
Probing the Gating of Ionotropic Glutamate Receptors with Tethered Photoswitchable Ligands
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Cellular signaling is often mediated by the binding of multiple ligands to multi-subunit receptors. In the case of ionotropic glutamate receptors (iGluRs), which are tetrameric ligand-gated ion channels that mediate excitatory transmission and synaptic plasticity in the central nervous system, the binding of glutamate both activates and desensitizes the channel, generating a fast transient current. However, the probabilistic nature inherent to the binding of diffusible ligands makes it difficult to determine how the ligand occupancy at each subunit controls these gating processes. To circumvent this problem, we use previously described photoswitchable ligands that can be covalently tethered to the ligand binding domain of iGluRs [1]. The fast cis-trans photoisomerization of the azobenzene linker, in combination with high intensity illumination, enables us to control ligand binding and unbinding with short (<100 μs) pulses of light. Here we use this approach to probe ligand-induced activation and desensitization in both homo- and heterotetrameric iGluRs.
1. Gorostiza P. et al., Proc. Natl. Acad. Sci. USA (2007) 104: 10865.

166-Plat
Occupancy of a Single Binding Site is Sufficient for AMPAR Activation
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Ionotropic glutamate receptors (iGluRs) are ligand-gated ion channels which mediate the vast majority of excitatory synaptic transmission in the vertebrate brain. The AMPAR are comprised of an N-terminal domain, a ligand binding domain (LBD, both extracellular) and the transmembrane ion channel. As tetramers, AMPAR contain four ligand binding sites and can, thus, bind up to four glutamate molecules. Upon binding, the LBD is thought to close like a clam shell, ''pulling'' the ion channel into the activated state. In order to perform voltage-clamp fluorometry studies on the AMPAR, we constructed an AMPAR mutant devoid of cysteine residues, whose functional parameters resembled those of wildtype AMPAR in the Xenopus oocytes expression system. Ectopic cysteines were then introduced and fluorescently labeled in the vicinity of the binding site to monitor ligand binding to the LBDs. We were able to record simultaneously current and fluorescence changes in response to application of glutamate. The LBDs closed upon glutamate binding and remained closed during desensitization, as probed by a non-desensitizing mutant and in the presence of CTZ. When analyzing the concentration dependence, our data is consistent with opening of the channel upon binding of a single glutamate. The result is corroborated by the fact that heteromers of AMPAR with different sensitivity always follow the concentration dependence of the single most sensitive monomer.

167-Plat
Imaged by Cryo-EM, Activated and Desensitized GluA2 Glutamate Receptors Show Extreme Flexibility
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We have reconstituted into liposomes the same truncated GluA2 protein that yielded the recent AMPA receptor crystal structure (Sobolevsky et al., Nature 2009). The reconstituted receptors show glutamate-activated and MPQX-blocked Na^+ fluxes. When the proteoliposomes are imaged in cryo-EM in the presence of MPQX or with no ligands, the receptors appear as Y-shaped particles that are indistinguishable from the X-ray structure of the MPQX-bound receptor. However, in the presence of glutamate or glutamate + cyclothiazide the extracellular ''arms'', comprising the ligand-binding domains and amino-terminal domains of the tetrameric receptor, become detached from one another. The wide range of conformations is reminiscent of previous negative-stain images of AMPA receptors from the Walz and Madden laboratories. It appears that agonist binding is accompanied by disruption of the dimer-dimer interfaces of the extracellular domains. Under conditions that favor the open and desensitized states, this AMPA-type receptor appears to be blowing in the Brownian wind.

Platform: Exocytosis and Endocytosis

168-Plat
HID-1 is a Novel Player in the Regulation of Blood Glucose
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Peptide hormones and neuropeptides are packaged and stored in specialized intracellular organelles called secretory granules (SGs, also known as dense core vesicles, DCVs). The molecular mechanisms involved in SG biogenesis from the trans-Golgi network (TGN) are largely unknown. A gene designated as hid-1 was identified during a search for mutants with a high-temperature-induced dauer formation (Hid) phenotype in C. elegans. Hid-1 is highly conserved from C. elegans to Homo sapiens. Interestingly, the Hid phenotype of hid-1 mutants is strongly suppressed in C. elegans by mutations in the daf-16 gene, which encodes for a transcription factor downstream of insulin signaling, suggesting a possible role for HID-1 in the insulin branch of the dauer pathway. Our recent studies in C. elegans have implicated an involvement of HID-1 in the early steps of SG exocytosis by controlling the correct sorting of SG cargoes. We demonstrated that HID-1 primarily localized to the medial- and trans-Golgi apparatus. We furthered our study of HID-1 functions in a pancreatic beta cell-specific HID-1 knockout mouse model. We found that HID-1 participated in the regulation of blood glucose. HID-1 deficiency in β cells leads to insufficient insulin release. The molecular mechanism of HID-1 is under investigation.

169-Plat
Molecular Dynamics Simulations of SNARE Complex Unzipping
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The SNARE proteins facilitate biological membrane fusion. The neuronal SNARE proteins, namely VAMP-2, also called synaptobrevin 2, SNAP-25, and syntaxin-1A together form a coiled-coil complex. The formation of this SNARE complex is vital for vesicle-plasma membrane fusion resulting in neurotransmitter exocytosis. VAMP-2, located on vesicular membrane, and syntaxin-1A, located on the plasma membrane, are transmembrane proteins, each with a single transmembrane domain. SNAP-25 is associated with the plasma membrane through lipid anchors and forms a binary t-SNARE complex with syntaxin-1A. For fusion to occur, the VAMP-2 zips up with the binary t-SNARE complex to form a trans SNARE complex. To better understand the mechanism and energetics of formation of the ternary SNARE complex, we carried out all-atom molecular dynamics simulations using the OPLS-AA force field to investigate the unzipping of the SNARE complex. The initial structure of the soluble part of the SNARE complex was based on the 1SFC pdb (Sutton et al., 1998 Nature 95:347-53). The missing residues R262-K265 of syntaxin-1A were modeled based on 3IPD pdb (Stein et al., 2009 Nature 460:525-28) and the two C-terminal residues S205-G206 of SNAP25 added as helical extension using Modeller. For unzipping, forces were applied between the main chain atoms of syntaxin K265 and those of VAMP-2 N92, as in unzipping experiments using optical tweezers (Gao et al., 2012 Science 337:1340-43). Application of harmonic forces between these residues resulted in unzipping of layers 8 to 6. Unexpectedly, this unzipping occurred through separation of syntaxin while the SNAP-25 SNARE domains SN1 and SN2 remained associated with VAMP-2. These results suggest that mechanical unzipping may not necessarily lead to separation of VAMP-2 from the binary t-SNARE complex and that the C-terminal association of SNAP-25 with syntaxin may be weaker than that with VAMP-2.
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170-Plat
Cholesterol Promotes Opening of the SNARE-Mediated Fusion Pore
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SNARE proteins feature in almost all known intracellular membrane fusion events, such as exocytosis, the fusion of hormone or neurotransmitter filled vesicles with the plasma membrane to release their cargo. SNAREs have been shown to be the minimal membrane fusion machinery in vitro, driving fusion on timescales from 10-100 ms. A fundamental characteristic of the initial fusion pore is its flickering between open and closed states, with live cell electrochemical and electrophysiological measurements showing flickering timescales of ~0.1 ms to 1 s during exocytosis. The flickering pore characteristics vary greatly, regulating the amount and the size of the released cargo. The molecular basis of this physiological regulation is not known. Here, we study flickering statistics of neuronal SNARE-mediated fusion pores in vitro for the first time. We used total internal reflection fluorescence microscopy (TIRFM) to measure fusion pores between reconstituted v-SNARE small unilamellar vesicles (SUVs) and cognate t-SNARE PEG-supported bilayers (SBLs). From transfer rates of fluorescently labeled vesicle lipids to the SBL, we measured the fraction of the time for which fusion pores are open on a single event basis. The accuracy was enhanced by single lipid fluorescence intensity, diffusivity and bleaching measurements. We find that increasing cholesterol content increases the openness of the fusion