

unbinding with short (<100 μ s) pulses of light and to follow channel activation and desensitization in real-time. The experiments give insight into the gating mechanism of GluK2 homotetramers and will be extended to study subunit specific activation in heteromeric GluK2/GluK5 complexes, which assemble with defined 2:2 subunit stoichiometry [2].

1. Gorostiza P. et al., Proc. Natl. Acad. Sci. USA (2007) 104: 10865.
2. Reiner A. et al., Cell. Rep. (2012) 1: 234.

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ILLUMINATING IONOTROPIC GLUTAMATE RECEPTORS: CHARACTERIZATION OF FLUORESCENT ANTAGONISTS BASED ON POLYAMINE TOXINS

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Ionotropic glutamate (iGlu) receptors, including the NMDA and AMPA subfamilies, mediate fast excitatory synaptic transmission in the mammalian central nervous system. NMDA and AMPA receptors play critical roles in learning and memory and are implicated in multiple neurological and psychiatric diseases. Argiotoxins (exemplified by the prototypical ArgTX-636) are so-called polyamine toxins isolated from the venom of the orb weaver spider *Argiope lobata*. Argiotoxins consists of an aromatic head group coupled via an amino acid linker to a polyamine moiety and act as open-channel blockers of the iGlu receptor ion channel with nanomolar affinity. Structure-activity studies of synthetic argiotoxin analogs have shown that modification of the polyamine tail can tweak NMDA versus AMPA selectivity (REF NELSON ET AL). Furthermore, the aromatic head group can tolerate substantial modification without loss of affinity. The aim of the present study is to develop NMDA- and AMPA selective argiotoxin analogs containing fluorescent moieties to enable imaging studies of native iGlu receptors and to serve as potential FRET donor or acceptor ligands. Specifically, a series ArgTX-636 analogs with fluorescein, coumarin and boron-dipyrromethene (BODIPY) derived fluorophores incorporated at or replacing the headgroup moiety were synthesized and characterized at recombinant AMPA and NMDA receptors expressed in *Xenopus Laevis* oocytes (GluA1 and GluN1/2A, respectively). Several analogs were found to possess high-affinity NMDA and/or AMPA receptor binding while maintaining desirable fluorescent properties.

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DISSECTING THE ACTIVATION OF AMPA RECEPTORS

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Ionotropic glutamate receptors are tetrameric ion channels that are activated by the neurotransmitter glutamate at excitatory synapses. It is known that after the binding of glutamate, the AMPA-subtype of glutamate receptors transits through distinct functional states to become fully activated, however, the conformations sampled by the tetramer during activation remain unknown. We have subjected plausible models of the tetramer of ligand binding domains to a panel of trapping bridges combined with electrophysiology, biochemistry and crystallography. These experiments were designed to probe the geometry of the different states sampled by the ligand binding domains during receptor activation. We show that the mutant A665C is preferentially crosslinked in the presence of the partial agonist, kainate. In addition, the same crosslink trapped a much more stable conformation in the desensitized state. We also resolved fast disulfide trapping on the millisecond time scale in the resting state of the glutamate receptor, providing insight into dynamics of the receptor complex at rest. Finally, we present engineered metal trapping bridges that trap conformations distinct from those observed in the full-length resting state crystal structure (Sobolevsky et al, 2009 Nature). Overall, our results reinforce the idea that the ligand binding domains are highly flexible and sample a surprisingly large conformational space.

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THE GLUK3 LIGAND BINDING DOMAIN HAS A ZINC BINDING SITE AT THE DIMER INTERFACE

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GluK3 receptors belong to the family of kainate subtype ligand-gated glutamate receptor ion channels. Experiments in Bordeaux revealed that zinc selectively potentiated glutamate evoked currents for GluK3 while responses for

GluK2 were inhibited. Mutagenesis and analysis of chimeric GluK2/GluK3 receptors mapped the zinc-binding site to the S2 segment of the ligand-binding domain (LBD) in a region expected to form the interface between two GluK3 subunits in an LBD dimer assembly. Multiple sequence alignments, coupled with site directed mutagenesis, revealed that a GluK3 specific aspartate residue, D759, which is exchanged for glycine in GluK1, GluK2 and in AMPA receptors, was essential for zinc potentiation. To identify additional residues contributing to the zinc binding site we attempted to solve a GluK3 LBD dimer assembly crystal structure. This was hampered by formation of zinc mediated intermolecular contacts between LBD monomers that favored other, non-biologically relevant head to tail dimer assemblies in three crystal forms solved for GluK3 glutamate and kainate complexes. However, from these structures we could generate a model dimer assembly by least squares superimposition of two copies of a GluK3 monomer on a previously solved GluK2 LBD dimer structure. This revealed that adjustment of rotamers for D730, D759 and H762 would allow formation of intersubunit contacts with appropriate bonding distances for zinc coordination. Mutagenesis experiments confirm that D730 on one subunit, together with D759 and H762 in the dimer partner, together form the binding site for zinc potentiation. The model also revealed that zinc acts as a countercharge that neutralizes the negative charges of the two coordinating aspartate residues, thereby stabilizing the dimer assembly and reducing desensitization. These results suggest that zinc may act as an endogenous positive allosteric modulator of native GluK3 containing kainate receptors.

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LIGAND-BINDING PATHWAYS IN A GLUTAMATE RECEPTOR

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Ionotropic glutamate receptors (iGluRs) are ligand-gated ion channels that play a central role in brain function. They are important in neuronal communication and are implicated in a host of neurological disorders such as schizophrenia and depression. The free energy of agonist binding to the ligand-binding domains (LBDs) of iGluRs is converted into useful work to induce conformational changes that cause receptor activation. The free energies associated with ligand docking and large-scale LBD conformational transitions have previously been computed using an approach that evaluates the two processes separately. Here, we employ a computational approach that treats ligand docking and LBD conformational transitions in a collective fashion. This enables the free energy along a physical ligand-binding pathway to be evaluated. The kinetics associated with segments of this pathway are also evaluated.

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BIOPHYSICAL STUDIES ON GLUA2 LIGAND-BINDING DOMAIN DIMERIZATION IN SOLUTION

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AMPA receptors (GluAs) are essential neuronal ligand-gated ion channels involved in learning and memory. The dimeric conformation of the GluA2 ligand-binding domain is involved in the coupling of agonist binding to channel gating. The desensitized state conformation has been shown to be disrupted along the dimer interface, however little is known about the dynamic equilibrium of the resting or activated states. A simple dimerization model is solely dependent on protein concentration, while the addition of allosteric modulator shifts the equilibrium of dimerization. By binding to the dimer interface and increasing the interface contacts, allosteric modulators enhance the stability of the dimer. Using small angle x-ray scattering (SAXS) and nuclear magnetic resonance (NMR), we screened both protein and allosteric modulator concentrations to develop an equilibrium model for modulator dependent dimerization. Depending on the specific interacting allosteric modulator, the symmetrical dimer interface can be occupied by one or two bound molecules. Both one-site per dimer (R2L) and two-site per dimer (R2L2) models are explored. In addition, dimerization led to characteristic chemical shift changes along the dimer interface as well as the ligand binding pocket. These changes have also been detected on the ligand and support that dimerization modulates the multiple conformational states involved in ligand binding. Our aim is to further understand the mechanism by which the activation process is enhanced by allosteric modulators.

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ARRANGEMENT OF THE N-TERMINAL DOMAINS IN NMDA RECEPTORS

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NMDA receptors are one of the three main types of ionotropic glutamate receptors that mediate excitatory transmission and are tetramers typically composed of glycine-binding (GluN1) subunits and glutamate-binding (GluN2) subunits. NMDA receptors are unique in that they are allosterically modulated by zinc