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Functional Insights from Glutamate Receptor Ion Channel Structures

Janesh Kumar and Mark L. Mayer

Laboratory of Cellular and Molecular Neurophysiology, Porter Neuroscience Research Center, NICHD, NIH, DHHS, Bethesda MD 20892

Abstract

X-ray crystal structures for the soluble amino terminal and ligand binding domains of glutamate receptor ion channels, combined with a 3.6 Å resolution structure of the full length AMPA receptor GluA2 homotetramer, provide unique insights into the mechanisms of iGluR assembly and function. Increasingly sophisticated biochemical, computational and electrophysiological experiments are beginning to reveal the mechanism of action of partial agonists, and yield new models for the mechanism of action of allosteric modulators. Newly identified NMDA receptor ligands acting at novel sites offer hope for development of subtype selective modulators. Many issues remain unsolved, including the role of the ATD in AMPA receptor signaling, and the mechanisms by which auxiliary proteins regulate receptor activity. The structural basis for ion permeation and ion channel block also remain areas of uncertainty, and despite substantial progress, molecular dynamics simulations have yet to reveal how binding of glutamate opens the ion channel pore.

Keywords

Synaptic Transmission; AMPA Receptors; Kainate Receptors; NMDA Receptors; Crystallography

INTRODUCTION

Glutamate receptor ion channels (iGluRs) are abundantly expressed in the postsynaptic density at the large majority of excitatory synapses in the brain of vertebrates, where they mediate chemical neurotransmission (1–4). Genetic and post-translational controls generate iGluR subtypes with diverse kinetics, calcium ion permeability, and sensitivity to voltage-dependent ion channel block by extracellular magnesium and cytoplasmic polyamines (5–7). In combination, these properties shape the ion flux and post synaptic depolarization triggered by release of glutamate from nerve terminals, generating a signaling system which can mediate rapid information transfer on the ms time scale, but in addition control excitability over the much longer time scales necessary for regulation of synaptic plasticity (8–10). There is substantial evidence for the presence of iGluRs on nerve terminals (11–13), but although their effects on neurotransmitter release are well documented, presynaptic

iGluRs are functionally less well characterized due to the difficulty of recording from these small structures.

During development of the CNS, the expression profile and extent of mRNA editing of individual iGluR genes is tightly controlled in a cell-specific manner, such that in the embryonic and adult CNS receptor subunit compositions are strikingly different. While the functional significance of this is not yet well understood, it is likely that, compared to the requirements for synaptic transmission in the adult CNS, receptors with different signaling properties and calcium permeability are required for the control of synapse formation and circuit development in the embryo. Also of note, although a neuron centric view of iGluR function is natural, given their major role in synaptic transmission, glial cells also express a variety of ligand gated ion channels (14–16). Glial iGluRs have been especially well studied in oligodendrocyte precursor cells present in the optic nerve and cerebellum (17), but much remains to be learned about the roles of iGluRs in other glial cell subtypes and brain regions.

THE THREE MAJOR GLUTAMATE RECEPTOR SUBTYPES

A major goal in the field of molecular neuroscience is to understand how the diverse functional activity of glutamate receptors and other ion channels arises from their structure. The first steps towards this began with the cloning of 18 individual mammalian iGluR cDNAs, followed by analysis of their genetic organization, leading to the discovery of subtype specific mRNA editing and alternative splicing (18). Coupled with this, amino acid sequence alignments, and analysis of ligand binding selectivity, led to the classification of iGluR subunits into three major families named AMPA, kainate and NMDA receptors, the preferred agonists for each subtype (19). In the subunit nomenclature proposed by IUPHAR in 2009 (20), AMPA receptors are formed by coassembly of GluA1-GluA4, kainate receptors by coassembly of GluK1-GluK5, and NMDA receptors by coassembly of GluN1 with GluN2A-GluN2D and GluN3A-GluN3B; with the exception of kainate receptors, for which GluK1-3 and GluK4-GluK5 are encoded by distinct gene families, for which the subunits were previously named GluR5-7 and KA1/KA2, the IUPHAR scheme nicely captures the underlying genetic substrates for iGluR assembly. A fourth iGluR gene family formed by GluD1 and GluD2 apparently does not generate glutamate or glycine activated ion channels, but is abundantly expressed in the cerebellum where its role in synaptic transmission is poorly understood.

The availability of cDNAs for iGluR genes triggered a large body of experiments using site directed mutagenesis for functional mapping. The cloning of iGluR cDNAs also made possible biochemical experiments which led to the overexpression, crystallization and X-ray diffraction analysis of an intact AMPA receptor (21), as well as structural studies on soluble domains from multiple iGluR subtypes (22–26). As more of these structures are solved it is now possible to begin to understand both the principles of iGluR gating, as well as the molecular mechanisms underlying their subtype specific properties. Central to this emerging structural biology of iGluRs, a variety of biophysical techniques in addition to crystallography are playing an increasingly important role, including analytical ultracentrifugation, calorimetry, computational chemistry, fluorescence and IR spectroscopy, NMR, and optogenetics, while functional studies on intact iGluRs using single channel

recording, rapid perfusion with outside-out membrane patches, and whole-cell recording remain indispensable.

Subunit Structure

Amino acid sequence analysis revealed that the 18 iGluR genes have a unique conserved structural profile, that distinguishes them from all other neurotransmitter receptors and ion channels. The key features which identify vertebrate iGluR subunits include (i) a 500 residue long extracellular stretch of amino acids, starting at the amino terminus and preceding the first hydrophobic membrane spanning segment; (ii) an ion channel composed of a pore-loop helix flanked by three membrane spanning hydrophobic alpha helical segments, which includes a highly conserved SYTANLAAF motif in the M3 segment; (iii) interruption of the extracellular 300 residue ligand binding domain by insertion of the ion channel pore; (iv) a cytoplasmic carboxy-terminus of variable length, which includes sites for palmitoylation and phosphorylation; (v) separate amino terminal (ATD) and ligand binding (LBD) domains which have sequence and structural homology to bacterial periplasmic binding proteins, and which are connected to each other, and to the ion channel by short polypeptide linkers (Figure 1a).

BLAST searches which capitalize on this unique profile have led to identification of iGluRs in all classes of vertebrates, as well as in insects, nematodes and primitive eukaryotic organisms. The best characterized are a family of iGluRs in the nematode *Caenorhabditis elegans* which have many functional similarities with their vertebrate counterparts (27). For other iGluR related genes the ligand binding properties are more diverse. A protein from the rotifer *Adineta vaga* named AvGluR1 encodes a functional ion channel gated by glutamate, but also by serine and glutamine (28). Surprisingly, although the GYG pore loop motif of AvGluR1 predicts high potassium selectivity, both sodium and potassium ions are permeable (P_{Na}/P_K 0.3). A large family of additional iGluR related genes named ionotropic receptors (IRs), some of which function as chemosensory receptors, has been identified in the fruit fly *Drosophila* (29). Some IRs generate ligand-gated ion channels when expressed in *Xenopus* oocytes and can reconstitute odorant responses in olfactory receptor neurons (30), but these proteins have low amino acid sequence homology in the ligand binding domain, and are gated by ligands as diverse as phenylethylamine and propionic acid (30). Although some of the IR genes have overall structural homology with vertebrate iGluRs, other lack the amino terminal domain; also, the SYTANLAAF related motif in M3 that is present even in AvGluR1 and conventional drosophila iGluRs is strikingly absent in IRs. Likewise, numerous plant genes have been identified which are structurally related to vertebrate iGluRs (31); although their ligand binding properties are not well characterized, sequence analysis suggests that plant iGluRs also will recognize a diverse spectrum of ligands. Finally, several bacterial iGluR channels have been identified (32–34); these lack both the amino terminal domain and M4 segment compared to the characteristic subunit design conserved in vertebrate, nematode, insect and *A. vaga* iGluRs. Although these bacterial proteins have significance, in that they define the minimal elements required for assembly of a functional glutamate receptor, recent work has focused on unraveling the function of more complex eukaryotic and especially vertebrate iGluRs, for which

interactions of the M4 segment with other TM domains is required for surface expression (35).

Domain Organization in Tetrameric iGluRs

After 10 years of structural studies on isolated iGluR ligand binding and amino terminal domains, the landmark full length structure of a GluA2 antagonist complex revealed how the unique sequence elements in iGluR subunits were arranged in a tetrameric assembly (21). As expected from sequence similarity with potassium channels, the ion channel has four fold rotational symmetry; consistent with work on the isolated ATDs and LBDs, the extracellular domains are arranged as a dimer of dimers (Figure 1*b*). However, unexpectedly, the GluA2 homotetramer is formed by pairs of conformationally distinct AC and BD subunits, and different subunit combinations form dimer pairs in the ATD and LBD layers, with the AB and CD subunits forming ATD dimers while the AD and BC subunits form LBD dimer pairs; as a result the tetramer has very complex geometry (Figure 1*b, c*). Each of the four ATDs has a clam shell like structure formed by upper (R1) and lower (R2) lobes, with a nearly identical conformation in each subunit; ATD dimers assemble via contacts mediated by both the upper R1 and lower R2 lobes, with a buried surface $\approx 1400 \text{ \AA}^2$ per subunit. These ATD dimer pairs form a tetramer via interactions of the lower lobes of subunits B and D, but with a much smaller buried surface $\approx 330 \text{ \AA}^2$. The ATD dimer pairs are tilted away from the overall axis of symmetry, such that subunits B and D lie proximal to the center of mass, while subunits A and C form the distal edges of the tetramer assembly. Within the LBD layer, dimer pairs like those crystallized for the isolated domains for numerous iGluRs, assemble via contacts mediated by the upper lobes, with a buried surface for each subunit of $\approx 900 \text{ \AA}^2$. Subunits A and C, which lie proximal to the center of mass, form the smaller dimer of dimers interface, buried surface $\approx 220 \text{ \AA}^2$. As a result, in contrast to previously published cartoon representations, the ATD and LBD layers in subunits B and D do not pack on top of each other in a linear arrangement, and instead there is a cross over between proximal and distal subunits in the ATD and LBD layers, generating a large cavity in the extracellular domain (Figure 1*b*). The peptide linkers which mediate these transitions between the ATD and LBD were shortened by deletion of six residues in the crystallized construct. As a result, it is possible that the ATD and LBD layers are even less closely packed with respect to each other than in the GluA2 crystal structure.

By contrast to the 2-fold symmetry of the LBD and ATD, the ion channel pore of GluA2 has 4-fold rotational symmetry, with the M4 segment of each subunit packed against M1 and M3 from an adjacent subunit, in a counterclockwise rotation when viewed from above. Lying parallel to the plane of the membrane a pre-M1 cuff helix wraps around the exterior of the ion channel assembly. A bundle crossing of the M3 helices forms a barrier to ion permeation, and above the SYTANLAAF motif the M3 helices of subunits A and C are extended by an extra turn compared to the B and D subunits. Three sets of polypeptide linkers, which have different conformations in the AC and BD subunit pairs, connect the M1, M3 and M4 transmembrane alpha helices with the LBD; these linkers mediate the 2-fold to 4-fold symmetry transition between the ion channel and ligand binding domain, and likely form entrance pathways for permeant ions and pore blockers. There are large fenestrations in the ion channel assembly which allow lipids intimate access to the pore;

these windows possibly form binding sites for iGluR auxiliary proteins, and are potential sites for the action of lipid soluble drugs. Within the ion channel, there was no electron density for the pore loop; likewise, on the cytoplasmic side of the membrane, the linkers between the TM domains, which form the turret structure in structurally related potassium channels, were also disordered. As a result the GluA2 structure provides no insight into the structure of the binding sites for permeant ions or polyamines which block Ca^{2+} permeable AMPA receptors.

While there are likely to be functionally important differences in the structure of other iGluR subtypes, cysteine mutant cross linking experiments reveal that the domain organization found in the GluA2 homotetramer is a general feature conserved across kainate and NMDA receptors (36–39), and that in the obligate heteromeric assemblies of GluK2/GluK5, and GluN1/GluN2, the ATD and LBD layers are assembled as pairs of heterodimers (37, 39, 40). Single molecule fluorescence counting techniques for NMDA receptors expressed in *Xenopus* oocytes indicate that when coexpressed GluN1, GluN2 and GluN3 assemble with a fixed stoichiometry which excludes triheteromeric assemblies, consistent with formation of GluN1:GluN2 and GluN1:GluN3 heterodimers (41). Asymmetry revealed by the GluA2 homotetramer has profound consequences for receptor activation, since the different conformations of the proximal and distal subunit pairs impacts their influence on ion channel gating. This has greatest significance in obligate heteromeric receptors, for which the GluN1 and GluK4/GluK5 subunits occupy proximal positions in the LBD layer of NMDA and kainate receptor tetramer assemblies, with GluN2, GluN3, and GluK1-GluK3 forming the distal subunits. In these receptors, due to differences in sequence, asymmetry extends to the ion channel, and although no structural data is available, homology models suggest that interactions between the TM domains of the GluN1 and GluN2 subunits underlie subtype specific Mg^{2+} block and Ca^{2+} permeation (42).

MODELS FOR ACTIVATION AND DESENSITIZATION

The availability of a full length iGluR structure provides for the first time a template that can be used to model the molecular events underlying activation and desensitization; obviously, experimental verification of such models is essential but likely to take substantial effort. Starting from the antagonist bound complex of the closed ion channel, the active and desensitized states have been modeled by two related approaches. The first was to perform rigid body manipulations, using glutamate bound crystal structures of the isolated LBD (Figure 1*d*) as a template for the activation process (21); to model desensitization a disulfide cross linked LBD structure (Figure 1*d*) was used as a surrogate for the desensitized state (43). In a related approach, similar models were used as targets for steered molecular dynamics of a GluA2 assembly in which the ATD was deleted to reduce computational overhead (44). The goal was to ask what resulting conformational changes would occur in the ion channel domain during activation and desensitization. Although both approaches have limitations, they provide preliminary structural insights, and suggest hypotheses that can be experimentally tested by site directed mutagenesis, prior to direct structural studies on other conformational states. Notably, because the distal lobes of the LBD are anchored on top of the membrane, they prevent domain closure from pulling the LBD downwards instead of opening the ion channel, and thus convert domain closure into rotational and

translational movement of M3, which expands outwards, opening the ion channel pore. The process of desensitization, in which the upper lobes of the LBD dimers separate, partially reverses glutamate triggered movement of the M3 helices. Related to this, the Arg628 side chains in the M3-S2 linker move towards the lipid bilayer during desensitization, such that it is plausible that attenuation of desensitization observed for the GluA2 R628E mutation (45) could be due to electrostatic repulsion between the mutant side chain carboxyl group and negatively charged lipid phosphate groups (44). The structural changes occurring during desensitization in NMDA receptors are much less well understood, and paradoxically, Cys mutant cross links at positions which abolish desensitization in AMPA and kainate receptors have no effect on NMDA receptor desensitization (46, 47).

Both of these modeling studies dramatically highlight the unequal role of proximal and distal subunits in the activation mechanism. At identical reference positions the M3-S2 linker moves by $\approx 7 \text{ \AA}$ following glutamate triggered domain closure for the distal subunits, but only $4 \text{ \AA}$ for the proximal subunits. It is intriguing that in heteromeric kainate and NMDA receptors, the GluK1-GluK3 and GluN2 subunits likely occupy distal positions in the LBD (21, 40). For NMDA receptors it is well known that glycine binding to the proximal GluN1 LBDs is not sufficient to activate ion channel gating, which requires in addition glutamate binding to the distal GluN2 subunits. However, for kainate receptors, selective binding of agonists to just the proximal GluK5 subunits produces partial activation, with desensitization when the distal GluK2 subunits in addition bind glutamate (48). Apparently different functional roles for kainate receptor proximal and distal subunits were reported previously (49), with selective binding of dysiherbaine to just the distal GluK1 subunit producing weakly desensitizing responses, with strong desensitization of ion channel activity when the proximal GluK5 subunits in addition bind glutamate. In combination, these experiments indicate that kainate receptor activation without substantial desensitization occurs irrespective of whether the proximal or distal subunits in diagonally apposed subunits in adjacent dimers bind agonist, while strong desensitization requires binding of agonists to both subunits in a LBD dimer assembly. Further experiments with additional LBD mutants are required to test whether separate activation of just the proximal or distal subunits in AMPA and kainate receptor LBD dimer assemblies produce equal extents of activation of ion channel gating, but clearly, for reasons that are not understood, NMDA receptors behave differently.

The most significant limitations of these computational experiments are that the templates used for modeling activation do not consider possible movements of the LBD dimers relative to each other, and that the MD study completely ignores conformational changes in the ATD tetramer assembly during desensitization. This is significant because models based on rigid body manipulation of the LBD predict that ATDs will undergo a separation during the process of desensitization (21), and that restricting this movement should limit the extent of desensitization. To date, only limited experiments have been performed to test this, and surprisingly the results showed that intermolecular ATD cross links inhibit receptor activation rather than receptor desensitization (36). In addition, the steered MD analysis of the glutamate bound active state fails to open the pore sufficiently to permit permeation of hydrated Na^+ and K^+ ions; in fact the change in radius, from $0.7 \text{ \AA}$ in the resting state, to $1.3 \text{ \AA}$ in the glutamate bound 'activated state', is much smaller than the experimentally

measured open radius of 3.8 Å (50, 51) raising major questions about the nature of additional conformational changes that must occur in an intact receptor, and why the steered MD analysis failed to open the ion channel.

THE AMINO TERMINAL DOMAIN

Role of the ATD in iGluR Assembly

The large size, layered domain organization, assembly of both the ATD and LBD as a dimer of dimers, and subunit cross over in the extracellular domains of iGluRs revealed by the full length GluA2 structure, impacts our thinking about how this complex structure is assembled during the process of receptor biogenesis. Synthesis of individual subunits, which begins with the amino terminal domain and S1 segment of the LBD, requires insertion of the M1–M3 ion channel segments through the translocon and into the lipid bilayer, before growth of the LBD polypeptide chain continues with the S2 segment, followed by M4 and the cytoplasmic carboxy terminus. At what stage individual subunits begin to interact in the process of tetramer assembly is unknown; possibly the extracellular domains begin to assemble as dimers before subunit synthesis is complete; perhaps there are membrane protein chaperones which shield the TM segments of isolated full length subunits before they assemble as oligomers. Due to subunit cross over in the ATD and LBD layers, it is very unlikely that the tetramer is zipped up in a cooperative process involving all four subunits.

Convincing evidence for assembly of iGluRs as dimer intermediates comes from synchronized induction of protein expression combined with negative stain single particle EM studies for GluA2 (52). These experiments revealed that synthesis of GluA2 dimers precedes the formation of tetramers, and that in the dimers the ATD and TM segments are closely apposed while the LBDs are too far apart to interact; by contrast, for the LBD dimer stabilizing L483Y mutant, all three segments are closely apposed, but subsequent tetramer formation is strongly inhibited. The most reasonable interpretation is that subunit cross over observed in the full length GluA2 structure cannot occur when the LBD dimer pairs are unable to separate. Relevant to this, sedimentation analysis by AUC reveals that the isolated GluA2 ATDs form dimers at sub micromolar protein concentrations (53, 54). By contrast, the isolated LBDs do not measurably interact in solution at the highest protein concentrations compatible with AUC, such that we can only set lower limits, of 5–10 mM, on the K_d for LBD dimer and tetramer assembly (46, 55). Thus, while it seems very unlikely that the LBD drives assembly in vertebrate iGluRs, subunit cross over between the ATD and LBD layers requires that the LBD plays a critical role in tetramer formation. Of note, for prokaryotic iGluRs which lack the ATD, LBD dimer assembly occurs at micromolar protein concentrations (33), suggesting that in the absence of the ATD, tighter association of the remaining extracellular domain becomes important for ion channel assembly.

More direct evidence for a role of the ATD in AMPA and kainate receptor assembly comes from AUC experiments combined with electrophysiological studies, which reveal that mutations in the ATD dimer interface alter the balance of assembly of homomeric versus heteromeric AMPA and kainate receptor assemblies (40, 54). Surprisingly, AUC experiments reveal that the GluA2 ATD forms homomeric dimer assemblies at low nM protein concentrations, and that heterodimer assembly with GluA2 is only modestly favored

over the formation of GluA2 homodimers for GluA1/GluA2, GluA3/GluA2 and GluA4/GluA2. Formation of heteromeric assemblies containing GluA2, assayed by changes in polyamine block under conditions where GluA1 is present in excess, is modestly facilitated by mutations which likely decrease the strength of GluA2 ATD homodimer assembly, suggesting that if AMPA receptors initially assemble as homodimers, tetramer assembly from pairs of heterodimers might require subunit exchange (54). These results raise important questions which remain unresolved. In native AMPA receptors are the subunits arranged as pairs of homodimers, or pairs of heterodimers; does subunit exchange occur between homodimers and heterodimers during biosynthesis; if so how is this accomplished?

By contrast the role of the ATD in the initial assembly of NMDA and kainate receptors is much easier to understand. For those iGluR subunits which are obligate heteromers, namely all NMDA receptors subunits, and the kainate receptor GluK4 and GluK5 subunits, the ATDs interact very weakly; however, the monomer-dimer K_d for heterodimer assembly is at least 1000-fold stronger for the GluN1/GluN2 and GluK2/GluK5 combinations (Figure 2a), providing a simple affinity based mechanism for assembly of heteromeric NMDA and kainate receptor assemblies (38, 40). Consistent with this, ATD Cys mutant cross linking experiments for full length NMDA and kainate receptors confirm formation of GluN1/GluN2 and GluK2/GluK5 heterodimers, while by contrast, forced GluN1 homodimer assembly abolishes formation of full length GluN1/GluN2 tetramers (37, 40, 56). Surprisingly, based on a complex series of cell biology experiments in which complementation by the isolated GluN1ATD was used to study coassembly of full length GluN2 with a GluN1ATD deletion mutant, a critical role for formation of GluN1 homodimers as a key assembly intermediate prior to formation of GluN1/GluN2 heteromers has been proposed (56). Perhaps consistent with this, single particle EM studies reveal a small population of GluN1 homodimers in solution, and two groups have reported GluN1 ATD homodimer crystal structures (38, 56). However, by contrast to AMPA and kainate receptors, for which the ATDs interact very strongly in solution, oligomerization of the GluN1 ATD was undetectable by AUC, apparently at conflict with the results of single particle EM analysis.

ATD Crystal Structures

Studies on the isolated ATDs have provided important insight into the processes of assembly and allosteric modulation, and provide much higher resolution structural information than the full length GluA2 structure, but many questions have emerged from this body of data. Crystal structures at resolutions as high as 1.4Å have been solved for ATD homodimer assemblies of GluA1 (57), GluA2 (53, 54, 58), GluA3 (59), GluK2 (60), GluK3 (61), GluK5 (61) and GluN1 (25, 56); for the Zn complex of a GluN2B ATD monomer (62); and for heterodimers of GluK2/GluK5 (40) and GluN1/GluN2B (38). For AMPA and kainate receptors the majority of these structures contain symmetrical assemblies in which the upper (R1) and lower (R2) lobes contribute nearly equally to dimer formation; for the GluA3 ATD, multiple homodimer structures were solved, and for some of these there is a modest separation of the lower lobes in the dimer assembly (59), while for the GluK5 homodimer the upper lobes have separated due to a 16° tilt, leaving only the R2 domains in contact (61). For the GluK2/GluK5 combination a dimer of heterodimers assembly that closely matches

that found in the full length GluA2 tetramer structure has been crystallized (Figure 2b); for GluA2, GluK2 and GluK3 ATD homodimers, crystallographic symmetry operations generate a similar tetramer assembly (40, 53, 58, 60, 61). Since these ATD tetramers form in the absence of the LBD and ion channel, it removes concerns that the hyper extended LBD found in the full length GluA2 complex with the competitive antagonist ZK 200775 somehow drives the ATD into an alternative conformation, as was proposed on the basis of low resolution single particle EM analysis (63).

A remarkable feature of all of these ATD structures is that the R1R2 clam shell adopts a partially closed conformation compared to structurally related periplasmic proteins, which by contrast undergo a robust 50° closure on binding ligands. In the GluK5 ATD structure a glycosylation site at the entrance to the inter lobe cleft generates a steric wedge preventing further closure (61), but no such feature is present in the other ATD structures. Limited variations in the extent of ATD domain closure across iGluR subtypes, and in different crystal forms for GluA2, GluA3 and GluK2, as well as the results of normal mode analysis and fluorescence correlation spectroscopy (59, 64), suggest only small fluctuations around this partially closed conformation in AMPA and kainate receptors compared to the robust conformation changes observed in structurally related periplasmic proteins (59, 60, 64). Whether these small amplitude changes are functionally significant, and whether non NMDA receptor ATDs can undergo larger conformational changes is an important unresolved issue relevant to allosteric modulation. Related to this, is the issue of whether AMPA and kainate receptor ATDs can even bind allosteric modulators. Small molecules and ions present in crystallization solutions have been found bound in the inter lobe clefts for some GluA2 and GluK2 ATD structures (59, 60); the most definitive structural evidence is for GluA2 crystallized in the presence of 200 mM ammonium phosphate (PDB 3HSY) for which a phosphate ion and water molecules are well resolved in the 1.75 Å resolution electron density maps (59). The bound anion forms direct and solvent mediated interdomain contacts with two Arg side chains which are conserved in AMPA receptor ATDs (Figure 2c). Functional studies to test for modulation of GluA2 by multivalent anions have not been reported, but should give insight into the potential for allosteric modulation of GluA2. However, related to this, the extent of domain closure in the phosphate bound GluA2 ATD structure is similar to that for other PDB entries for which no phosphate or sulfate was present in the crystallization solutions; for example, a GluA2 ATD dimer was refined with good statistics to a resolution of 2.3 Å (PDB 3H5V), and although 368 water molecules were modeled, difference maps, especially within the clamshell-like cleft or subunit interfaces, did not show electron density for other bound molecules or ions (53). Thus, the AMPA receptor ATD structures solved to date seem to provide no evidence for a ligand induced conformational change, and although all the available evidence argues against this possibility, it remains a tantalizing hypothesis due to the impact it could have on AMPA receptor function.

Crystal structures for the GluN1 and GluN2B ATDs differ substantially from those of AMPA and kainate receptors in two important aspects: (i) the relative orientation of the upper and lower lobes has undergone a substantial 40–50° twist (56, 62); (ii) due to this different conformation, the dimer interface is mediated exclusively by the upper R1 lobe in

the GluN1 homodimer (56), while in the GluN1/GluN2B heterodimer the interface is asymmetric and mediated by a combination of both R1:R1 and R1:R2 interactions (38). Surprisingly, the ATDs of both GluN1 and GluN2 also adopt a partially closed conformation, in which the cleft formed by apposition of the upper and lower lobes of the clamshell remains open to solvent. The GluN2B subunit was in addition crystallized as a monomer in the apo state and in a complex with Zn, which binds in the entrance to the cleft between the upper and lower lobes of the ATD clamshell without producing substantial conformation changes compared to the apo state (62). The most surprising finding from this series of ATD structures was the discovery, discussed in the following section, that GluN2B specific phenylethanolamine negative allosteric modulators of NMDA receptor activity bind at the interface between the GluN1 and GluN2B subunits in the heterodimer assembly, and not within the clam shell of the GluN2B subunit, as had been proposed on the basis of site directed mutagenesis and molecular modeling. Notably, even for this complex, the GluN2B subunit retains a stubbornly fixed partially closed conformation with only minor differences from the crystal structure of the monomeric apo state, such that it is now essential to obtain direct experimental evidence for the open state conformation.

Allosteric Modulation by the ATD

The GluN2A-GluN2D subunits, which bind glutamate, impart vastly different open probabilities, agonist potencies, deactivation time courses and single-channel conductance on NMDA receptor subtypes. It is believed that the GluN2 ATD domain plays a key role in many of these intrinsic receptor properties. Open probability is highest for GluN1/GluN2A, around 0.5, and 5-fold lower for GluN1/GluN2B (65), while GluN2C and GluN2D open with much lower open probabilities of 0.01 and 0.04 (66–68). To a large extent the GluN2 ATD, together with its short but highly divergent 16-residue ATD-LBD linker, determines these diverse channel open probabilities. Based on analysis of GluN2 chimeric receptors it was shown that exchange of the ATD, along with the ATD-LBD linker, shifts the open probability, response time course, and agonist potency in the direction of the subunit contributing the ATD, highlighting their role in regulating channel function (68, 69). NMDA receptor ATDs also mediate allosteric effects by directly binding endogenous ligands such as polyamines, Zn^{2+} and also synthetic ligands such as ifenprodil and related drugs. The first insights into this role came from studies which revealed that NMDA receptor ATDs are structurally related to periplasmic binding proteins (70, 71); that the GluN2A and GluN2B ATDs harbor nM and μM affinity binding site for Zn^{2+} respectively (71–74); and that ifenprodil binds to the GluN2B ATD (75). The high affinity and >100 fold selectivity of ifenprodil for GluN2B versus GluN2A, GluN2C or GluN2D has led to its extensive use as a lead compound for drug development and as a pharmacological tool to dissect NMDA receptor function (76). Zinc binding to the ATD also makes the channels more sensitive to proton inhibition, however the mechanism is not well understood (72, 73). In contrast to the negative allosteric action of Zn^{2+} and ifenprodil, polyamines like spermine also bind to the ATD, but act as positive allosteric modulators. This allosteric mechanism of spermine potentiation is specific to the combination of GluN2B with GluN1 subunits that lack exon 5 (77, 78). Surprisingly, recent work suggests that despite their opposite allosteric activity, both ifenprodil and spermine bind at the interface of the GluN1/GluN2 ATD assembly (38, 79).

A very appealing unified model of bidirectional regulation explains these effects (Figure 3a), and also the control of open probability by the ATDs (79, 80); this model depends in large part on elegant functional experiments that however have yet to be validated by structural studies. The need for the latter cannot be over stated given that the 1st crystal structure for an NMDA receptor ifenprodil complex (Figure 3b) revealed a totally unexpected mechanism of binding (38), while novel binding site residues identified by the crystal structure of the GluN2B Zn²⁺ complex (62) were missed by earlier studies based on ATD models derived from periplasmic proteins (74), which did not capture the characteristic twisted conformation of NMDA receptor ATDs (62, 81). In the new model of bidirectional regulation, GluN1/GluN2 NTD dimers alternate between open-cleft or 'active' and closed-cleft or 'desensitized-like' conformational states (69). The equilibrium between these states varies for individual GluN2 subunits, with the GluN2A ATD being open more frequently than GluN2C or GluN2D. The closed-cleft ATD conformation puts strain on the LBD dimer assemblies, causing them to separate, as occurs during the process of desensitization (46, 55, 80). Stabilization of the open-cleft conformation by polyamines, whereby GluN1 and GluN2B ATDs have their lower lobes extended and close to each other, would propagate little or no strain to the LBD dimers. By contrast, stabilization by zinc, ifenprodil, and its analogues, of a closed-cleft conformation, where the lower lobes have separated, introduces strain on the ATD-LBD linkers that leads to channel inhibition. Currently, it is unclear whether the GluN1 subunit ATD can alternate between open and closed cleft conformations, or whether this is limited to GluN2 subunits. Thus, although recent structures and biochemical evidence gives new insights into a central role of the ATDs in allosteric modulation of NMDA receptor function, a complete understanding of these complex mechanisms requires further exploration, and especially structures for different conformational states.

LIGAND BINDING DOMAINS

More than 120 crystal structures of AMPA, kainate and NMDA receptor ligand binding domain (LBD) complexes with various agonists, partial agonists and antagonists provide a rich substrate for analysis of receptor function (23, 24, 82). They provide a structural basis for the mechanisms underlying both ligand selectivity, and the link between ligand induced conformational changes in the LBD domain and channel opening. Recent advances include identification of glycine and D-serine as ligands for the GluD2 LBD (83, 84); the first structural studies on NMDA receptor GluN2D agonist complexes (85); and new structures for AMPA receptor GluA3 and GluA4 and kainate receptor GluK3 LBDs (86–89).

Partial Agonist Activity in AMPA and Kainate Receptors

As described above the LBD domains are arranged as back-to-back dimer assemblies mediated by D1 domain contacts; on neurotransmitter binding the two lobes of LBD close to envelope the ligand resulting in separation of the D2 domains, which in turn pulls open the ion channel. Although a strong correlation between the degree of LBD cleft closure and agonist efficacy was observed early in the structural analysis of AMPA receptors (90–92), especially for willardiines, these partial agonists activate the same series of subconductance states as glutamate, but with different probabilities, raising questions about the link between

domain closure and subconductance states that at present have no structural explanation (91, 93–95). Recently, it has been shown by NMR spectroscopy, fluorescence resonance energy transfer (FRET) measurements, and molecular dynamics (MD) simulations that the LBD domains can sample a range of conformational states on ligand binding, including but not limited to those observed in crystal structures (96–102). All-atom MD simulations using umbrella sampling to calculate the free energy landscape that governs the conformational change of the GluR2 LBD in the apo, agonist (Glu, AMPA, ACPA), partial agonist (kainate, thio-ATPA, 4-AHCP) and antagonist (ATPO, CNQX, DNQX) bound states revealed that the energy landscapes differed even within individual ligand classes, and that the trajectories traversed from apo to ligand-bound states vary (97, 101). Further calculations of LBD conformational distributions in the context of an intact GluA2 receptor revealed significant differences between the calculated thermal averages and crystal structures of the apo, AMPA and kainate bound GluA2 LBD, suggesting that crystal structures of isolated LBD complexes may not quantitatively account for the amount of conformational change transmitted to pore domain in an intact receptor. Additional evidence for a multistate equilibrium, similar to that predicted by MD simulations, has come from single-molecule FRET analysis of the GluA2 LBD bound to glutamate, which showed that it explores a range of conformations in solution, albeit on time scales much slower than required for receptor activation by glutamate (102).

Recent work has explored the idea that for channel activation the LBD adopts a closed cleft conformation that is the same for all ligands, and that the relative stability of this conformational state contributes to agonist efficacy. Measurements of LBD lobe orientation by NMR spectroscopy showed that the average solution conformations for willardiine partial agonist complexes was not strongly correlated with agonist efficacy and lies between that observed in different crystal forms (98). Using X-ray crystallography and NMR under reducing and oxidizing conditions, disulfide trapping experiments for a GluA2 LBD double cysteine A452C/S652C mutant, which locks the cleft in a closed conformation (103), reveals that the partial agonists 5-iodowillardiine, kainate, even the antagonists CNQX and DNQX, can all promote nearly full cleft closure in oxidizing conditions (Figure 4*a, b*). Crystal structures with the partial agonists kainate, 5-iodowillardiine and CNQX show that they are accommodated in the closed cleft conformation by rotation of side chains surrounding the ligand binding pocket, resulting in changes in cavity volume necessary to accommodate structurally diverse ligands (Figure 4*c*). Although the 5-iodowillardiine, kainate and CNQX bound structures are 0.9°, 3° and 1.3° more open compared to the glutamate complex, two intra lobe hydrogen bonds characteristic of a closed cleft flipped conformation are observed for the kainate and 5-iodowillardiine, but not CNQX complex. However, for the kainate complex, cleft closure is insufficient to close the cavity, which remains open to bulk solvent. A caveat of these experiments is that the crosslinks were made in the isolated LBD domain, which is free from the constraints in an intact receptor, and that functional assays of changes in efficacy for the A452C/S652C mutant were not possible. Nonetheless, these results suggest that a closed cleft conformation is sampled by LBD when bound to partial agonists. However, although a closed cleft conformation may be attained by LBD when bound to partial agonists, MD simulations for AMPA and kainate receptors suggest this is not energetically favorable (101, 104), and thus at equilibrium agonist efficacy likely reflects

both the frequency of sampling of the LBD closed state, and its stability, both of which are largest for glutamate and other full agonists. Experiments on intact receptors, for instance crystal structures of receptors in different conformational states complexed with all three classes of ligands, single molecule FRET measurements, or long duration MD simulations on intact receptors will likely be necessary to fully understand the seemingly complex mechanisms of partial agonism and ion channel substate activity.

Partial agonism in NMDA receptors

NMDA receptors are obligate heteromeric coincidence detectors requiring binding of both glycine and glutamate to activate the ion channel. Unlike non-NMDA receptors, where binding of neurotransmitter to less than four subunits can trigger channel opening, albeit to lower sub-conductance states, NMDA receptor gating requires that all four-ligand binding sites be occupied, following which the channel is opened in a concerted fashion resulting in a uniform unitary conductance level (105). It has been shown that both full and partial agonists for GluN1 and GluN2 subunits elicit single channel currents with similar amplitudes consistent with a tight functional coupling between subunits in the heterodimer assembly. However, the open probability for partial agonists is lower due to longer closures and shorter openings (106, 107). An LRET based investigation, to measure conformational changes in GluN1/GluN2A LBD domains of full-length NMDA receptors, revealed no significant differences in cleft closure of the GluN1 subunit for full and partial agonists, consistent with the single conformation observed in agonist and partial agonist crystal structures for the isolated GluN1 LBD (108). However, a graded cleft closure for the GluN2A subunit was observed by LRET for partial agonists (109). So, again the question arises as to whether full-cleft closure of GluN2 subunits can be achieved with partial agonists, as might be expected for a concerted mechanism of channel opening. Related to this, locking the GluN2A LBD in a closed cleft conformation using engineered disulfide bonds spanning the cleft increases open probability, suggesting that glutamate may not be a full agonist (110). A caveat in these experiments could be that a non-natural LBD conformation due to disulfide cross-linking mediates these effects. However, at present there are no crystal structures of either GluN2 subunits with partial agonists, or cross linked closed cleft mutants like those for AMPA receptors.

NOVEL ALLOSTERIC MODULATORS

Structural studies have given great insight into the mechanism of action and location of binding sites for iGluR allosteric modulators. In addition to the binding of ifenprodil and zinc to NMDA receptor ATD dimers, a large body of crystallographic work reveals that the LBD dimers of AMPA receptors bind allosteric drugs which either attenuate desensitization or slow deactivation, while kainate receptor LBD dimers bind modulatory ions. For AMPA receptors more than 20 crystal structures have been solved for GluA2 and GluA3 complexes with a chemically diverse range of novel allosteric modulators, including ligands which differentiate flip and flop splice isoforms (55, 111–118). All of these ligands bind to a series of five overlapping hydrophobic and hydrophilic sites at the base of the LBD dimer assembly, but the stoichiometry varies from 1:1 or 2:1 drug molecules per dimer, depending on the ligand structure and binding sites targeted. These allosteric binding sites also form the

structural determinants of deactivation, i.e. cleft closure and movement of the hinge regions, and in addition overlap with those of desensitization, i.e. subunit movements around the LBD dimer interface. Although many of these ligands bind to multiple sites in these regions, some degree of selectivity is apparent in crystallographic and functional studies (111, 117). Although this work has generated great anticipation for new classes of drug, clinical trials have met with limited success (119). No similar drugs have been reported for kainate receptors, although their disclosure likely remains only a matter of time. However, crystal structures for LBD dimer assemblies of GluK1, GluK2 and GluD2 reveal novel binding sites for Na⁺, Ca²⁺ and Cl⁻ ions (84, 120–123), which act as counter charges at polar sites on the dimer interface, thereby increasing LBD dimer stability and reducing desensitization (124).

For NMDA receptors, although structural details remain vague, recent work has identified multiple classes of novel subtype specific allosteric ligands which potentiate or inhibit activity. In addition to ifenprodil and polyamines, neurosteroids (125–127) have been identified as potential leads for GluN2A and GluN2B containing receptors. Recently, the quinazolin-4-one analogue QNZ46 and the dihydroquinolone-pyrazoline derivative DQP-1105 were identified as examples of two classes of noncompetitive inhibitors selective for GluN2C/D containing receptors (128, 129). Using QNZ46 as a representative of this class, site directed mutagenesis suggests that the putative binding site is close to the membrane proximal part of the LBD and the linkers to the TM domains (130). Related to this Cys scanning mutagenesis reveals that modification of LBD-TM linkers in NMDA receptors profoundly affects ion channel activity (131, 132). More recently, the tetrahydroisoquinoline CIQ has been identified as a subunit selective potentiator for GluN2C/D (133). However, its mechanism of action seems to be more complex, requiring both the ATD-LBD linker and residue Thr592 in the M3 TM helix for potentiation. Naphthoic and phenanthroic acid derivatives also display varied subunit selectivity and have both potentiating as well as inhibitory activity (134, 135). Although further development of these new subtype selective compounds will likely provide important pharmacological tools to analyze receptor function, and are potential lead compounds for drug development, structural investigations are required to fully elucidate their mechanism of action.

COMPUTATIONAL STUDIES

All atom molecular dynamics simulations for the isolated ligand binding domains of iGluRs are addressing increasingly fundamental questions, yielding substantial insight into the molecular mechanisms for ligand selectivity, partial agonist activity and the process of agonist triggered domain closure (101, 104, 136, 137). In a recent study, unbiased MD simulations were used to analyze cleft-closing mechanisms triggered by the spontaneous binding of glutamate to GluA2; this required 256 MD runs, each of 2 ns duration, for which six binding events were captured, only one of which lead to domain closure (138). Insights from this work include an analysis of side chain rearrangements during transitions between open, semi-open and closed-cleft LBD conformations, the existence of which had been proposed previously on the basis of hydrogen-deuterium exchange NMR experiments (100), and which were observed also in steered MD simulations with umbrella sampling (101). Cation- π interactions mediated by Tyr450, a highly conserved residue in iGluR LBDs, help to steer glutamate into the cleft during the initial docking process prior to stabilization of the

closed-cleft conformation by a series of protein-ligand and interdomain contacts triggered by the coordinated movement of Glu705, Asp728 and Lys730 (138, 139). MD studies also give insights into the mechanism of modulation of kainate receptors by allosteric ions, revealing that the binding of Na^+ ions lowers the barrier height and increases the well depth for Cl^- ions which bind in the LBD dimer interface of kainate receptors (122). Water molecules are found in the ligand binding pocket of numerous iGluR LBD crystal structures, and appear to be trapped together with glutamate in the closed cleft conformation. MD studies reveal how these water molecules contribute to crystallographically observed ligand conformations (140), and show that the trapped waters are mobile and either rearrange on the ns time scale, or are replaced by water molecules that enter the binding site through transient water channels while glutamate remains bound (141).

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Key terms/definitions and acronyms

mRNA EDITING	Enzymatic modification of mRNA, frequently adenosine to inosine, which changes the genetic code and controls Ca permeability in AMPA receptors
IUPHAR	International Union of Basic & Clinical Pharmacology maintains receptor database and issues nomenclature guidelines
Analytical ultracentrifugation (AUC)	A Powerful technique for characterizing the solution-state behavior of macromolecules by sedimentation velocity or sedimentation equilibrium
ATD and LBD	Extracellular iGluR domains which can be separately expressed as soluble proteins and crystallized
BLAST searches	Basic Local Alignment Search Tool used to query DNA and protein sequence data bases for bioinformatic analysis
Buried surface	Surface area not accessible to solvent. An important tool for structural analysis of intermolecular contacts in protein assemblies
Desensitization	A decrease in receptor response on prolonged exposure to agonist. In iGluRs mediated by separation of the upper lobes of LBD dimer
Translocon	A large protein assembly which mediates translocation of polypeptide chains across membranes during biosynthesis of nascent membrane proteins

Negative stain single particle EM	Proteins are embedded in an electron dense medium to generate a three-dimensional outline by combining two-dimensional images
Normal mode analysis	Analysis of large-scale macromolecule motions carried out by studying harmonic oscillations around a local energy minimum
Allosteric modulation	A mechanism for receptor regulation by the binding of ligands to regulatory sites distinct from the active site
Conductance and subconductance	Refers to the measure of current flowing through ion channels in their fully and partially open states
Open probability (Po)	The amount of time an ion channel is in the open state
Partial agonist	Compounds that elicit less than maximal responses at full occupancy relative to full agonists
Agonist efficacy	Refers to the magnitude of a receptor response following ligand binding. See also partial agonist
LRET	Luminescence resonance energy transfer is a modification of conventional FRET using a luminescent chelate as donor

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SUMMARY POINTS

1. Glutamate receptor ion channels are tetrameric assemblies with a unique architecture distinct from other ion channels. The amino terminal and ligand binding domains can be expressed as soluble proteins, yielding high resolution crystal structures, while a 3.6 Å resolution full length AMPA receptor structure reveals the overall architecture.
2. Biochemical techniques, including analytical ultracentrifugation, NMR, and numerous other forms of spectroscopy, together with increasingly sophisticated molecular dynamics calculations, are providing complimentary insights to X-ray crystal structures.
3. The information obtained by combining these results is beginning to give insight into the mechanisms for receptor activation, partial agonists, desensitization, and allosteric regulation.

FUTURE ISSUES

1. There has been essentially no progress in understanding how an expanding family of glutamate receptor auxiliary membrane proteins modulates receptor function; given that most native iGluRs are likely to exist as complexes with auxiliary membrane proteins overcoming this limitation is essential for further progress.
2. Additional high resolution structures of novel ligand complexes and allosteric modulators, as well as lower resolution structures of full length AMPA, kainate and NMDA receptors in different conformational states, remain essential goals.
3. Sustained efforts in medicinal chemistry, which played a key role early in the discovery of iGluR receptor subtypes, will likely remain very important in the immediate future, as judged by the recent development using classical high throughput screening techniques of ligands which likely target novel structural motifs.
4. Microsecond and longer duration MD simulations will eventually give insight into the mechanisms of activation and desensitization, but the large size, and conformational freedom of iGluRs, which result from their unique architecture, will make these challenging computational targets.

iGluR Auxiliary subunits

Ionotropic glutamate receptors *in vivo* interact with several auxiliary proteins that regulate their trafficking, turnover, subcellular localization, synaptic stabilization, and kinetics. Diverse families of auxiliary subunits have been identified for each receptor subtype including transmembrane AMPA receptor regulatory proteins (TARPs), Cystine-knot AMPAR modulating protein (CKAMP44), Cornichon homologs-2 and -3 (CNIH-2 and CNIH-3), Synapse differentially induced gene 1 (SynDIG1); neuropilin tolloid-like 1 (NETO1) and neuropilin tolloid-like 2 (NETO2) for kainate receptors; and NETO1 for NMDA receptors, respectively. Accumulating experimental evidence indicates that native iGluRs function as supra-molecular complexes with these various auxiliary subunits, which fine tunes receptor function; for instance while TARPs & Cornichons enhance AMPA receptor surface expression and increase receptor responses, CKAMP44 on the other hand has an opposite effect. The presence of auxiliary subunits has also helped to reconcile differences in channel kinetics observed between native and recombinant AMPA and kainate receptors, e.g. TARPs and NETO2 slow down both the deactivation and desensitization of AMPA and kainate receptors respectively. However, understanding the mechanisms of modulation of receptor function by these auxiliary subunits is still in its nascent stages and is likely to improve only with structural and biochemical information.

Figure 1.

Domain organization and mechanism of iGluR activation and desensitization. (a) An iGluR subunit, with extracellular amino terminal (ATD) R1 R2 and ligand binding (LBD) S1 S2 domains interrupted by insertion of the ion channel pore; the highly conserved “SYTAN” motif present on the M3 TM helix is shaded in red. (b) Crystal structure of the full length GluA2 tetramer (3KG2) with each subunit colored differently. The extracellular and ion channel domains are arranged as layers, with subunit crossover taking place at the LBD layer for the B and D subunits. (c) Least squares superpositions of the AC and BD subunit pairs; distinct A/C and B/D subunit conformers result from subunit crossover. (d) Crystal structures of the GluA2 LBD dimer with a cartoon representation of the ion channel in resting (1FTO), glutamate activated (1FTJ) and desensitized (2I3V) states. A 21° rotation of the lower lobe on ligand binding increases separation of the S2 domains by ~5 Å compared to the apo structure, leading to ion channel opening, as shown by the position of glycine residue Cα atoms in the GT linker (red spheres). In the desensitized state, the dimer interface is broken due to a 14° rotation of domain S1, resulting in ~13 Å separation of the upper lobes, depicted by blue spheres for the Ser741 Cα atoms on the top of helix J, which decouples LBD domain closure from channel opening.

Figure 2.

ATD structure and function. (a) Superimposed size exclusion chromatography profiles for the isolated GluR6 and KA2 ATD domains injected separately (dashed lines) or as a mixture of the two proteins at equal concentrations (solid lines); the increase in dimer peak and corresponding decrease in monomer peak indicates formation of GluR6/KA2 ATD heterodimers; red data points show mass values of the eluted peaks estimated by multi angle light scattering. (b) Crystal structure of GluR6/KA2 ATD heterotetramer assembly; two GluR6/KA2 heterodimer pairs are arranged such that the dimer of dimers interface is mediated by the GluR6 subunit (green), while KA2 subunits (red) occupy the lateral edges of the tetramer. (c) Stereoview of the cleft between the R1 and R2 domains of the GluA2 ATD (3HSY) showing an omit map contoured at 7σ revealing a characteristic tetrahedral density in which a phosphate ion present in crystallization condition could be easily modeled along with a well ordered water molecule.

Figure 3.

Models for allosteric modulation by NMDA receptor ATD domains. (a) Schematic representation of a model for bidirectional allosteric regulation of NMDA receptors; spermine binding to the lower lobes of the ATD heterodimer interface stabilizes the open channel state, potentiating receptor function, whereas ifenprodil binding to the upper lobe dimer interface leads to channel inhibition. (b) Crystal structure of the GluN1b (exon5 variant) and GluN2B heterodimer complexed with ifenprodil (3QEL); a composite model generated after least-squares superpositions of GluN1 subunit homodimer crystal structures (3QEK) shows the position of Arg and Lys residues inserted due to exon 5 splicing (blue sticks), preceded by Glu and Asp residues which form the putative polyamine binding site.

Figure 4.

Partial agonists induce nearly full LBD closure. (a) Plot of mean domain closure for the GluA2 LBD for either WT or disulfide trapping mutants in complex with glutamate, kainate, 5-iodowillardine and CNQX, measured with respect to the apo LBD crystal structure (1FTO); the “error” bars indicate minimum and maximum domain closure values in individual subunits. (b) Least squared superimposition of GluA2 LBD disulfide trapping mutant in complex with CNQX, crystallized in either oxidizing (yellow 3T9U) or reducing conditions (dark brown 3T9V), showing an average cleft closure of $\sim 17^\circ$ in the oxidizing condition. (c) Stereoview of the oxidized state ligand binding cavities for the glutamate (green 3T93), kainate (cyan 3T9H), 5-iodowillardine (red 3T96) and CNQX (yellow 3T9U) complexes; note that despite similar domain closure the cavity sizes differ to accommodate diverse ligand molecules, and that in the kainate complex the cavity is continuous with bulk solvent; sticks show the location of the A452C and S652C mutant disulfide bond that traps the closed cleft conformation.