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## Glutamate receptor ion channels

Mark L Mayer

Glutamate receptor ion channels mediate excitatory responses at the majority of CNS synapses. They are the only ligand gated ion channels for which multiple high resolution crystal structures have been solved. Highlights of information gained from mechanistic studies based on crystal structures of their ligand binding domains include explanations for strikingly diverse phenomena. These include the basis for subtype specific agonist selectivity; mechanisms for desensitization and allosteric modulation; and mechanisms for partial agonist activity. In addition, multiple lines of evidence, including low resolution electron microscopic studies, suggest that native AMPA receptors combine with an auxiliary subunit which regulates activity and trafficking. Functional studies suggest that glutamate receptor gating is distinct from that of structurally related voltage gated ion channels.

### Addresses

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### Abbreviations

<b>ACBC</b>	1-aminocyclobutanecarboxylic acid
<b>ACPC</b>	1-aminocyclopropanecarboxylic acid
<b>AMPA</b>	$\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
<b>ATPO</b>	(S)-2-amino-3-[5-tert-butyl-3-(phosphonomethoxy)-4-isoxazolyl]propionic acid
<b>iGluR</b>	glutamate receptor ion channel
<b>DNQX</b>	6,7-Dinitro-2,3-quinoxalinedione
<b>NMDA</b>	N-methyl-D-aspartic acid
<b>TARP</b>	transmembrane AMPA receptor regulatory protein

### Introduction

Ligand gated ion channels are membrane proteins which mediate information transfer at synapses. Their function relies on the ability to respond very rapidly to the transient release of a neurotransmitter to produce a change in membrane potential of the postsynaptic cell. Genome and cDNA sequencing analysis reveals that there are three major subtypes of neurotransmitter activated ligand-gated ion channel, each with a unique architecture. These are the glutamate receptors, tetrameric cation selective channels which are activated by glutamate and a number of environmentally significant plant neurotoxins; the so called cys-loop receptors, which form pentameric ion channels gated by acetylcholine, GABA, glycine and serotonin; and the P2X receptors which are believed to be trimeric ion channels gated by ATP.

The glutamate receptor ion channels (iGluRs) are abundantly expressed in the brain and spinal cord and mediate responses at the vast majority of excitatory synapses.

Mammalian iGluRs are encoded by 18 genes which assemble to form four major families, the AMPA, kainate, NMDA and delta receptors. There are four AMPA receptor genes (GluR1-4); five kainate receptor genes (GluR5-7, plus KA1 and KA2); seven NMDA receptor genes (NR1, NR2A-D, NR3A and NR3B); and two delta subunits. Coassembly of iGluRs within but not between families, generates a large number of receptor subtypes *in vivo*. Unique amongst ligand gated ion channels, high resolution crystal structures have been obtained for multiple subtypes of iGluRs, and such structures provide the means to gain unprecedented insight into their mechanism of action and modulation. The unique architecture of iGluRs is a key factor which has facilitated their structural analysis (Figure 1). Unlike the cys-loop receptors, in which ligand binding sites are formed at the interface between subunits, in iGluRs the ligand binding cores are discrete domains, one copy of which is present in each subunit. The ligand binding domains can be genetically excised, expressed as water soluble proteins, and crystallized. To date structural analysis has been successfully applied to the ligand binding cores of AMPA [1], kainate [2] and NMDA [3] receptors; work is ongoing in several labs on other iGluR domains but the experience gained indicates that these are more challenging targets. Due to this difficulty in solving structures for the amino terminal, and ion channel domains, significant new information continues to emerge from more traditional cell biological and genetic based approaches. The material covered in this review focuses on experiments designed to resolve the molecular mechanisms of iGluR gating and does not address the topics of targeting and synaptic function.

### **Agonists antagonists and ligand binding domains**

Prior structural work on AMPA receptors established that the ligand binding cores assemble as pairs of dimers, and suggested that agonist stabilized domain closure of the individual subunits in a dimeric assembly leads to channel opening. Graded closure of the ligand binding cores produced partial agonist responses. Desensitization resulted from a reorganization of the dimer assembly which allowed the channel to close even though the individual ligand binding cores remained in their active agonist bound conformation. A substantial body of new experimental work reinforces this picture. Crystallization of the ligand binding domains of the kainate receptor GluR5 [2,4] and GluR6 [2,5] subunits, in conjunction with previously solved structures for the glycine binding NMDA receptor NR1 subunit [3] and the AMPA receptor GluR2 subunit [6], permits for the 1<sup>st</sup> time an understanding of the molecular mechanisms underlying the subtype specific ligand selectivity characteristics of individual iGluR subunits. The overall fold of the iGluR ligand binding domains for AMPA, kainate and NMDA receptors is nearly identical. For GluR6 expression in insect cells provides for the 1<sup>st</sup> time information about the structure of glycosylation sites [2,5]. Key amino acid side chains which interact with the ligand  $\alpha$ -NH<sub>2</sub> and  $\alpha$ -COOH groups are the same in all four iGluR families. What differs are the amino acids which interact with the glutamate  $\gamma$ -COOH group or in the case of NR1 subunits prevent the binding of glutamate. In the agonist bound complex of all iGluRs the ligand is buried in the interior of the protein, but the volume of the ligand binding cavity varies substantially, from 56 Å<sup>3</sup> for NR1 to 218, 305 and 255 Å<sup>3</sup> for GluR2, GluR5 and GluR6, respectively (Figure 2). One amino acid in the NR1 subunit glycine binding pocket, a Trp side chain at position 731, plays a key role in selectivity by occupying space required for the glutamate  $\gamma$ -COOH group; as a result glutamate cannot bind to the

NR1 subunit [3]. In GluR5 and GluR6 steric occlusion mediated by Ser/Asn and Leu/Phe exchanges play key roles in subtype selectivity [2,5]. Strikingly, in the NR1 binding pocket there is no ordered solvent, while in the GluR2, GluR5 and GluR6 glutamate complexes there are 4, 6 and 5 bound water molecules. The displacement of solvent permits the binding of large heterocyclic agonists, such as quisqualate, ATPA and AMPA, with relatively little or in some cases no change in the extent of domain closure [2,6,7]. Notably the larger volumes of the kainate receptor ligand binding cores do not result from differences in the extent of domain closure, since for both GluR5 and GluR6 the ligand binding cores are more closed than for GluR2. NMR measurements for AMPA receptor binding cores are in their early stages but reveal subtle differences in conformational flexibility for individual agonist complexes [8] and line broadening for residues in the dimer interface [9].

Substantially less work has been done with iGluR antagonist complexes. The structure of a GluR2 complex with ATPO reveals the same open cleft conformation but a different set of ligand protein interactions than observed previously for the quinoxaline DNQX; of note the phosphonate group in ATPO acts as a substitute for a  $[\text{SO}_4^{2-}]$  ion observed in the DNQX complex [10]. For kainate receptors no antagonist structures have been solved but a molecular model has been developed for a novel GluR5 selective antagonist [11]. For the NR1 subunit of NMDA receptors structures of complexes with 5,7,-dichlorokynurenic acid and cycloleucine also reveal the now familiar open cleft conformation for competitive antagonists [3,12]. Of potential significance for functional studies on receptor turnover and trafficking *in vivo* is development of a photoactive irreversible AMPA receptor antagonist [13]. Also of note was the development of thiol reactive NR1 antagonists used for modeling the structure of the NR1 ligand binding core [14]. To date structures for NMDA receptor NR2 subunit glutamate binding domains have not been reported but mutagenesis and molecular modeling suggests plausible models [15,16].

### **Mechanisms for partial agonist action**

Partial agonists, compounds which produce less than the maximum response of full agonists, are powerful tools with which to probe receptor gating mechanisms. Historically, two distinct models have been proposed to explain such behavior. The MWC concerted transition model, in which receptors exist in an equilibrium between resting and active conformations, explains difference in agonist efficacy as resulting from different relative affinities of agonists for the resting and active states. The induced fit model suggests instead that the receptor can exist in multiple conformational states and that the ligands which best fit the receptor binding pocket produce the largest extent of activation. Crystal structures for the ligand binding cores of wild type and mutant AMPA, kainate and NMDA receptor complexes indicate that both of these strikingly different mechanisms underlie partial agonist activity. In the most extensive analysis performed a series of 5-substituted willardiines was shown to promote graded closure of the AMPA receptor ligand binding core consistent with the induced fit model [17]. Variation in the extent of domain closure due to steric occlusion, which was correlated with the efficacy for activation of channel gating, and inversely with the extent of desensitization, strongly suggests an induced fit model. Similar observations on individual compounds including

kainate, a very weak partial agonist [18], (S)-2-amino-3-(4-bromo-3-hydroxy-isoxazol-5-yl)propionic acid [19] and CPW399 [20] stronger partial agonists, reinforce this model. Surprisingly, a similar analysis of the AMPA receptor L650T mutant, reveals a second mechanism for partial agonist action at AMPA receptors, reduced stability of the fully closed agonist bound conformation as required by the MWC model [18]; notably this occurred only for AMPA which produced 38% of the maximum response, with glutamate and quisqualate continuing to act as full agonists. Spectroscopic studies on the related GluR4 L651V mutant provide another biophysical approach to measure ligand induced conformation changes [21]. For the NR1 subunit of NMDA receptors the partial agonists D-cycloserine, ACPC and ACBC all produce the same extent of domain closure as the full agonists glycine and D-serine again consistent with the MWC model [3,12]. For kainate receptors less work has been done, and notably the structure of the Apo state or an antagonist bound complex has yet to be solved to provide a reference point for comparison with other iGluR subtypes. For GluR6 the kainate and domoate complexes are 3 and 14 degrees more open than the glutamate complex, consistent with the action of kainate as a partial agonist of much higher efficacy for GluR6 versus GluR2 and an induced fit mechanism for partial agonist action based on domain closure [2,5,22].

### **Ligand binding core dimer assemblies**

Different from the cys-loop receptors the ligand binding cores of iGluRs are believed to assemble as a dimer of dimers. The dimeric assembly of iGluRs was first described for AMPA receptors and is generated by apposition of the backside of the domain 1 surfaces of each subunit, burying a solvent accessible surface of about 2400 Å<sup>2</sup> [6]. Stabilization of dimer contacts at the domain 1 interface is known to attenuate AMPA receptor desensitization [24]. Similar dimer crystal structures have now been observed for the ligand binding cores of the NR1 [3] and GluR6 subunits [5] and at low resolution in single particle electron microscopic images of intact AMPA receptors [23]. Although the 3.1 Å resolution of the GluR6 crystal structure is too low to reveal details of the dimer interface, it appears that many of the contacts present in AMPA receptors are conserved. Surprisingly, a strikingly different dimer structure was observed in the GluR5 glutamate complex [5]. In the GluR5 dimer the pair of subunits have separated at their domain 1 interface; because this is accompanied by a rotation of each subunit the separation of the linkers leading to the ion channel segments remains unchanged from that in the AMPA like dimer observed for GluR6. As a result the novel packing observed in the GluR5 dimer is unlikely to correspond to the desensitized state and is more likely a consequence of crystal packing forces.

### **Desensitization**

The rapid and profound desensitization which follows activation of iGluRs has provided a powerful experimental tool for analysis of the underlying allosteric mechanism. Prior crystallographic, biochemical and functional studies for GluR2 revealed that domain 1 ligand binding core dimer contacts must be broken for desensitization to proceed [24]. Extensive tests using site specific mutagenesis to disrupt domain 1 intermolecular contacts observed in AMPA receptor dimers strongly reinforce the conclusion that the dimeric assembly observed in crystal structures of the isolated ligand binding cores is present in full length membrane bound AMPA receptor ion channels [25]. Single particle

electron microscopic analysis of intact AMPA receptors purified from rat brain reveals that this mechanism now has to be extended to include movement of the associated N terminal domains [26]. Surprisingly, despite 88% amino acid sequence identity in the ligand binding cores of AMPA and kainate receptors, mutations in the GluR6 dimer interface which would be expected to perturb intersubunit contacts and accelerate desensitization have the opposite effect [22]. However, only a limited set of mutants was studied, and glycine instead of alanine was used to replace GluR6 side chains, potentially allowing the main chain to adopt conformations inaccessible to other amino acids due to removal of steric constraints. Therefore, additional experiments are required to evaluate potential differences in AMPA and kainate receptor gating. A notable feature of the established model for AMPA receptor desensitization is that it defines the active state in great detail but reveals nothing about the structure of the desensitized state itself the crystal structure of which has not been determined. The report that mutations in the linker between the 2<sup>nd</sup> transmembrane segment and the ligand binding core disrupt desensitization of both AMPA and kainate receptors, albeit to different extents, provides the 1<sup>st</sup> clues about conformational states that are occupied during desensitization [27]. Analysis of the action of novel allosteric modulators of AMPA receptor desensitization confirms a key role for the dimer interface and extends the binding site beyond residues known to interact with the drug cyclothiazide [28,29].

### **Auxiliary subunits**

iGluRs coassemble with a vast horde of cytoplasmic and cytoskeletal proteins via PDZ binding motifs and numerous other signals [30]. A growing body of evidence indicates that these proteins modulate iGluR function. For example, coexpression of GluR6 and PSD 95 accelerates the rate of recovery from desensitization 2-fold [31]. However, none of these proteins can be considered true accessory subunits as found for example in voltage gated ion channels. This situation has now changed as a result of recent studies which show convincingly that a subset of membrane proteins, that are members of a gene family which includes calcium channel  $\gamma$  subunits, interact specifically with AMPA receptors to modify both their transport to the plasma membrane and their gating properties [32,33,48]. These are named transmembrane AMPA receptor regulatory proteins (TARPs). Native PAGE, single particle electron microscopy, mass spectral analysis of affinity purified native AMPA receptors from rat brain, which were decorated with Fab fragments, independently established the coassembly of AMPA receptors with TARPs, and revealed substantial density in the transmembrane region that was attributed to TARPs [26,34]. Amongst many notable aspects in these studies is the observation that the kinetics of AMPA receptor and synaptic responses are modified by TARPs; this is noteworthy because it raises the prospect that there may be accessory proteins for other iGluR subtypes, especially kainate receptors, for which there is a striking mismatch between the kinetics of synaptic responses and those of recombinant kainate receptors in heterologous expression systems.

### **Modulation assembly and gating**

NMDA receptors are notable for many characteristic properties, including complex kinetics, allosteric modulation by ligands acting at the amino terminal domain, and the fact that they are obligate heteromers. The description of modal gating adds to this

complexity [35]. Detailed kinetic analysis permits for the 1<sup>st</sup> time a physical model of proton inhibition [36] and a description of NMDA receptor gating during repetitive synaptic activity [37]. Analysis of NMDA receptors formed by coexpression of the NR1, NR2A and either NR2B or NR2C subunits reveals that the inhibitory action of nM Zn<sup>2+</sup> at the NR2A subunit persists in triheteromeric assemblies which likely form NMDA receptor complexes *in vivo*, but that the inhibition saturates at about 15% [38]. The much lower affinity inhibition of the NR2B subunit by Zn<sup>2+</sup> has the consequence that in native NMDA receptor assemblies containing both NR2A and NR2B subunits Zn<sup>2+</sup> modulation occurs over a broad concentration range and may have both tonic components mediated by nM Zn<sup>2+</sup> as well as an activity dependent component mediated by vesicular release of Zn<sup>2+</sup> [39]. Similar to NMDA receptors it has now been established that kainate receptors show modulation by extracellular protons and polyamines [40] while on the other hand cytoplasmic polyamines, long established as modulators of AMPA and kainate receptors also modulate NMDA receptors [41]. The mechanisms underlying modulation of GluR5 subtype kainate receptors by endogenous ligands is less well understood than for NMDA receptors, in part due to difficulties in achieving sufficient levels of expression for routine functional analysis. The discovery that the GluR5b splice variant has uniform kinetic properties compared to the 5a splice variant, and that mutations in the C-terminus greatly increase the cell surface expression of GluR5b, opens up the hope for more detailed functional studies [42].

Regulation of the assembly and trafficking of iGluRs is beyond the scope of this review but a couple of interesting themes are relevant to iGluR function. Surprisingly it is possible to express functional NMDA receptor tandem constructs in which NR1 and NR2 subunits truncated after the 2<sup>nd</sup> transmembrane segment were fused to wild type subunits and coexpressed with the missing final TM and intracellular C terminus [43]. Another unexpected result was the demonstration that agonist binding is a critical determinant for plasma membrane expression of kainate receptors [44,45]. The underlying mechanisms have yet to be determined as has the ligand occupancy of iGluRs in the endoplasmic reticulum, golgi apparatus, and other intracellular membrane bound environments. Also of note is the observation that the assembly of AMPA receptor tetramers is regulated by RNA editing at the Q/R site in the pore loop [46]. Relevant to this is a growing realization that the pore structure of iGluRs is not 4-fold symmetric [47●].

## Conclusions

Structural work on iGluRs has undergone an explosive burst of activity such that it is now possible to explain at the atomic level the ligand binding properties of key individual subtypes. It is anticipated that similar work on the NR2 glutamate binding NMDA receptor subunits as well as other less well characterized iGluRs will complete this picture in the near future. This should provide the groundwork for enormous advances in medicinal chemistry. As illustrated in Figure 2 the ligand binding cores account for a mere 25% of the mass of an AMPA receptor subunit, and substantially less in the case of NMDA receptor NR2 subunits which have 50-70 kDa cytoplasmic C terminal domains. Structural information is almost entirely lacking for the remaining 75% of an iGluR subunit and as a result the molecular mechanisms underlying ion permeation, gating, assembly, trafficking, and activity dependent modulation are much less well understood.

This is unlikely to change until the technical challenges of working with eukaryotic membrane proteins are solved.

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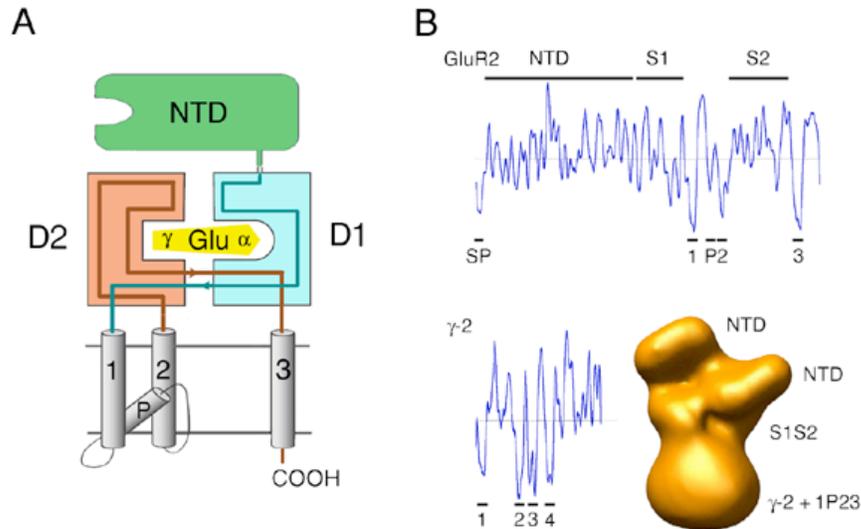
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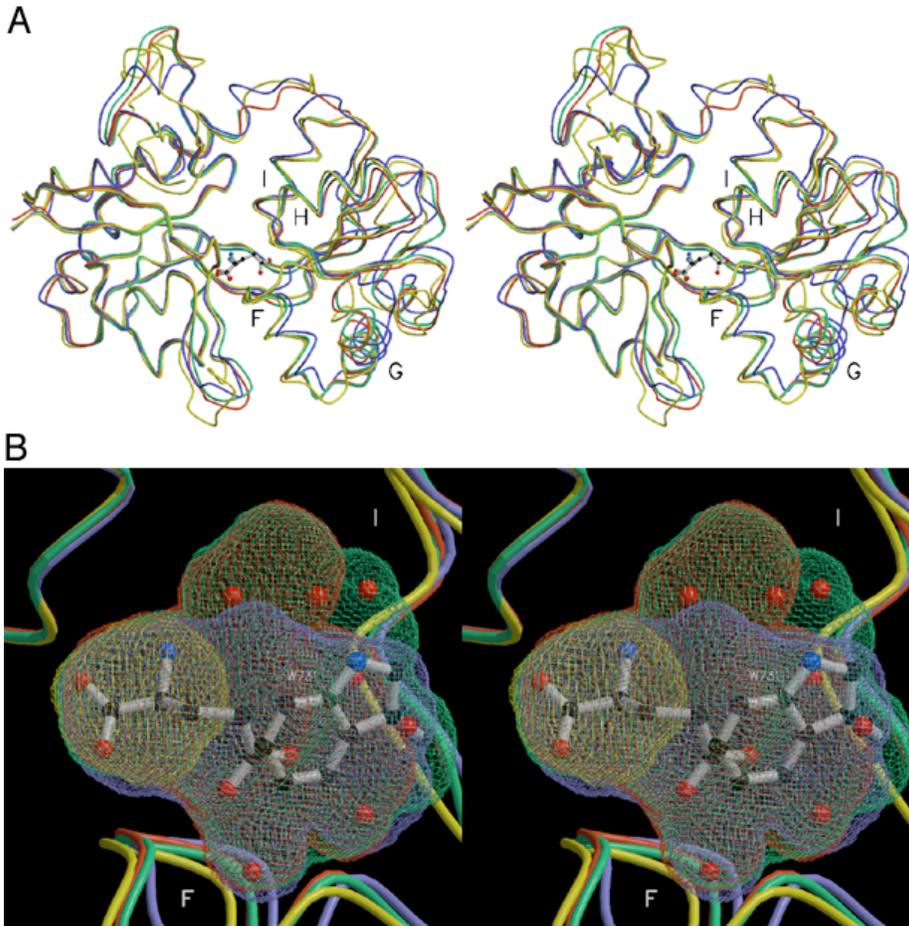
Elegant biochemical studies reveal how RNA editing of the GluR2 AMPA receptor subunit affects assembly prior to transport to the plasma membrane

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● 48. Priel A, Kolleker A, Ayalon G, Gillor M, Osten P, Stern-Bach Y. **Stargazin reduces desensitization and slows deactivation of the AMPA-type glutamate receptors** *J Neurosci* 2005 **25**:2682-2686. This study is complementary to [● ● 32] and focuses on the novel result that TARPs modulate AMPA receptor gating kinetics.



**Figure 1** Domain structure in glutamate receptor ion channels. (A) Each subunit consists of a bilobed amino terminal domain (NTD), the two domain ligand binding core (D1 D2), an ion channel with 3 membrane spanning segments (1-3) and a pore loop (P), and a cytoplasmic domain of variable length. (B) Low resolution structures of iGluRs and accessory subunits illustrated by hydropathy plots for GluR2 (top) and the  $\gamma$ -2 subunit of the TARP family (lower left), and by a single particle image of an AMPA receptor complex with TARPs [26].



**Figure 2** Crystal structures of the agonist bound complexes for the ligand binding domains of iGluRs. **(A)** Stereoview of NR1 (yellow), GluR2 (blue), GluR5 (green) and GluR6 (red) superimposed using domain 1 coordinates [2,3,6]; the positions of helices F, G H and I in domain 2 are indicated by labels. Ball and stick representations show the glycine ligand from NR1 and the glutamate ligand from GluR6 for which the  $\alpha$ -carbon functional groups almost completely overlap. **(B)** Stereoview of solvent accessible surfaces of the ligand binding pocket interiors; the surfaces and coils are colored using the same scheme as used above to illustrate secondary structure surrounding the binding site; helices F and I in domain 2 are labeled for orientation. Ball and stick representations show the glutamate ligand and six water molecules trapped in the GluR5 ligand binding pocket and the W731 side chain of NR1. All of the water molecules lie outside the NR1 cavity, which is just big enough to accommodate glycine; the upper 3 water molecules in the GluR5 structure also lie outside the envelope of the GluR2 cavity; the top right water molecule in addition lies outside the envelope of the GluR6 cavity. W731 projects into the cavity of the AMPA and kainate receptors preventing the binding of glutamate to NR1.