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TOPICAL REVIEW

The multifaceted subunit interfaces of ionotropic glutamate receptors

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Abstract The past fifteen years has seen a revolution in our understanding of ionotropic glutamate receptor (iGluR) structure, starting with the first view of the ligand binding domain (LBD) published in 1998, and in many ways culminating in the publication of the full-length structure of GluA2 in 2009. These reports have revealed not only the central role played by subunit interfaces in iGluR function, but also myriad binding sites within interfaces for endogenous and exogenous factors. Changes in the conformation of inter-subunit interfaces are central to transmission of ligand gating into pore opening (itself a rearrangement of interfaces), and subsequent closure through desensitization. With the exception of the agonist binding site, which is located entirely within individual subunits, almost all modulatory factors affecting iGluRs appear to bind to sites in subunit interfaces. This review seeks to summarize what we currently understand about the diverse roles interfaces play in iGluR function, and to highlight questions for future research.

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Abbreviations iGluR, ionotropic glutamate receptor; LBD, ligand binding domain; NTD, N-terminal domain; TMD, transmembrane domain; WT, wild type.

Introduction

The three main subfamilies of iGluRs (AMPA, kainate and NMDA) all respond to the binding of glutamate by opening an integral cation-selective pore. This leads to both neuronal excitation and a range of subtype-specific physiological effects (Traynelis *et al.* 2010). While structural studies have focused on iGluR subunits, these do not function in isolation. As obligate tetramers, each subunit is in direct contact with at least two neighbours (Fig. 1*A*; Sobolevsky *et al.* 2009). In addition, they interact with the lipid bilayer, and associate with accessory proteins such as TARPs and cornicons for AMPA receptors (Milstein & Nicoll, 2008; Schwenk *et al.* 2009; Straub & Tomita, 2012) and NETOs for kainate receptors (Copits & Swanson, 2012). In terms of contacts within the tetramer, both the N-terminal domain (NTD) and the LBD in the extracellular part of the receptor form dimers with adjacent subunits, while in the transmembrane domain (TMD), subunits interact with their two neighbours. These interfaces are central to receptor assembly and function, helping to control subunit composition and mediate the transduction of glutamate binding into the transient opening of the pore. Advances in our knowledge of iGluR structure have helped us understand some of the conformational changes defining both assembly and function (see recent reviews by Sukumaran *et al.* 2012;

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Kumar & Mayer, 2013; Sobolevsky, 2013). This review will focus specifically on the functional role of iGluR interfaces and the molecules that bind to them.

Structure overview of iGluR interfaces

Since the cloning of the first iGluR subunit nearly 25 years ago, our knowledge of their structure has progressed in zigzags, with unexpected twists coupled with the discovery of unique features. Initial topology models assumed homology with the pentameric nACh superfamily, but iGluRs were revealed to be tetramers with a distinct fold; the ligand binding domain is formed from two non-contiguous segments flanking the first three membrane domains (Hollmann *et al.* 1994; Stern-Bach *et al.* 1994). Further surprises have come from the first structure of a full-length iGluR (Sobolevsky *et al.* 2009). This not only confirmed that these receptors have a unique switch in symmetry (from 4-fold to 2-fold), but revealed an entirely unpredicted cross-over in the subunit pairings between the NTD and LBD (Fig. 1*B*).

Of the interfaces evident in the $GluA2_{cryst}$ structure, we have the most information on the contacts between neighbouring pairs of LBD and NTD domains. This knowledge has mostly come from X-ray crystallographic studies, first for the LBD (Armstrong & Gouaux, 2000) and more recently for the NTD (Jin *et al.* 2009; Kumar *et al.* 2009; Furukawa, 2012). Including the delta (GluD) subfamily, which is structurally but not functionally homologous, there are four (iGluR) subfamilies (Traynelis *et al.* 2010). Structural information is available for all four. To date a total of 253 LBD structures have been determined for subunits from all the subfamilies, along with 25 NTD structures representing all iGluR subfamilies except delta (Table 1). Of these, the majority have contained the two distinct 2-fold symmetric dimers (hereafter simply 'dimers') present in the $GluA2_{cryst}$ structure. The LBD has a bi-lobate fold, with the ligand bound in the cleft between lobes. The dimer is formed 'back-to-back', through interactions between the upper ('D1') lobes only. This results in an interface area averaging 860, 960 and 1100 $Å^2$ for wild-type AMPA, kainate and NMDA receptor LBDs respectively (average interface-areas determined from WT structures using PISA web interface (www.ebi.ac.uk).

The NTD also has a cleft between upper and lower lobes, although it is less pronounced. While zinc ions

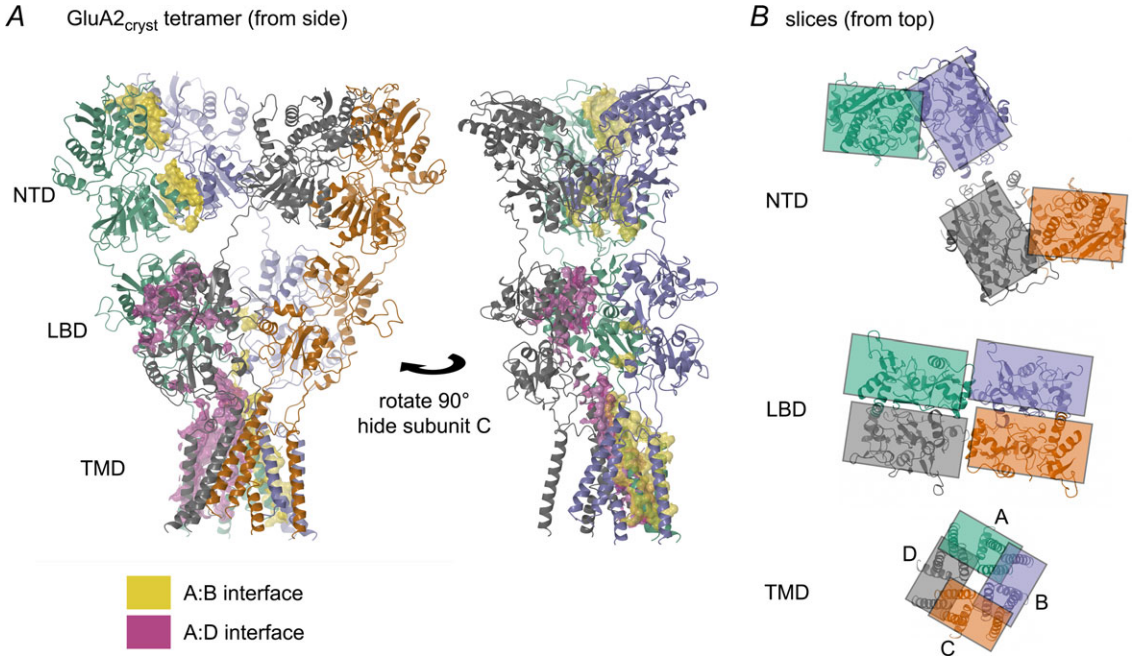

Figure 1. Domain organization in $GluA2_{cryst}$
A, the $GluA2_{cryst}$ tetramer (PDB (protein data bank) code 3KG2; Sobolevsky *et al.* 2009), showing two side-views, rotated 90 deg. Subunits are coloured green (A), blue (B), orange (C) and grey (D). Interfaces between subunit A and neighbouring subunits are shown as magenta (the A:D interface) and yellow (the A:B interface) surfaces. The A:D interface is most visible in the left panel and consists of contacts in the LBD and TMD. The A:B interface is most visible in the right panel and mainly consists of contacts in the NTD and TMD. *B*, sectional views from above the tetramer, centred on the three main domains. Rectangles indicate the approximate extent of the subunits to show the switch from A:B and C:D contacts in the NTD to A:D and B:C contacts in the LBD. These views also highlight the change from (pseudo) 4-fold to 2-fold symmetry between the TMD and extracellular domains, as well as the right-handed subunit 'twist' (moving up from TMD to NTD), which is greatest for the B and D subunits. Structure cartoons in this and other figures were generated using CCP4mg (McNicholas *et al.* 2011). Interface surfaces were calculated by the PISA service (www.ebi.ac.uk; Krissinel & Henrick, 2007) called from within CCP4mg.

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Table 1. iGluR domain structures in protein data bank (PDB)

	AMPA (GluA*n*)				Kainate (GluK*n*)				NMDA (GluN*n*)						Delta (GluD*n*)
Subunit[a]	1	2	3	4	1	2	3	5	1	2A	2B	2D	3A	3B	2
#NTD	1	7	2	1	0	5*	1	4*	4*	0	4*	0	0	0	0
(w. dimers)	(1)	(7)	(2)	(1)	—	(5)	(1)	(4)	(4)	—	(4)	—	—	—	—
#LBD	0	130	17	6	34	29	8	0	14*	7*	0	5	4	2	2
(w. dimers)	—	(127)	(17)	(6)	(27)	(23)	(1)	—	(7)	(7)	—	(0)	(0)	(0)	(1)
[w. allosteric]	—	[45]	[3]	[0]	[0]	[0]	[0]	—	[0]	[0]	—	[0]	[0]	[0]	[0]

#Total number of structures in the PDB (at end of April 2014), with the number containing dimeric complexes in parentheses. A dash means not applicable. *Includes heteromeric complexes between NTDs from GluK2/GluK5 (2 structures) and GluN1/GluN2B (2) and LBDs from GluN1/GluN2A (5). [a]No structures had been deposited for GluK4, GluN2C or GluD1 at time of writing.

have been shown to bind at the cleft entrance in the GluN2B subunit (Karakas *et al.* 2009), no ligand has yet been identified that binds within the cleft. In AMPA and kainate receptor NTD structures the domains are arranged as dimers in a 'side-to-side' orientation, with close contacts between the adjacent upper and lower lobes (Fig. 2*A*). Structures for NMDA receptor NTDs exhibit a range of dimer conformations with contacts mainly between the upper lobes (Fig. 2*B*; Furukawa, 2012). The NTD dimer interfaces average 1290 and 1410 Å^2 for AMPA and kainate receptor NTDs, but only 1130 Å^2 for NMDA receptor NTDs (calculated as for LBD interface), reflecting the less extensive lower-lobe contacts. The functional roles of these interfaces are considered in more detail later.

The switch in dimer pairings between the NTD and LBD was one of the key findings from the GluA2$_{cryst}$ structure (Fig. 1*B*). This arrangement has been confirmed for kainate receptors (Das *et al.* 2010), as well as an

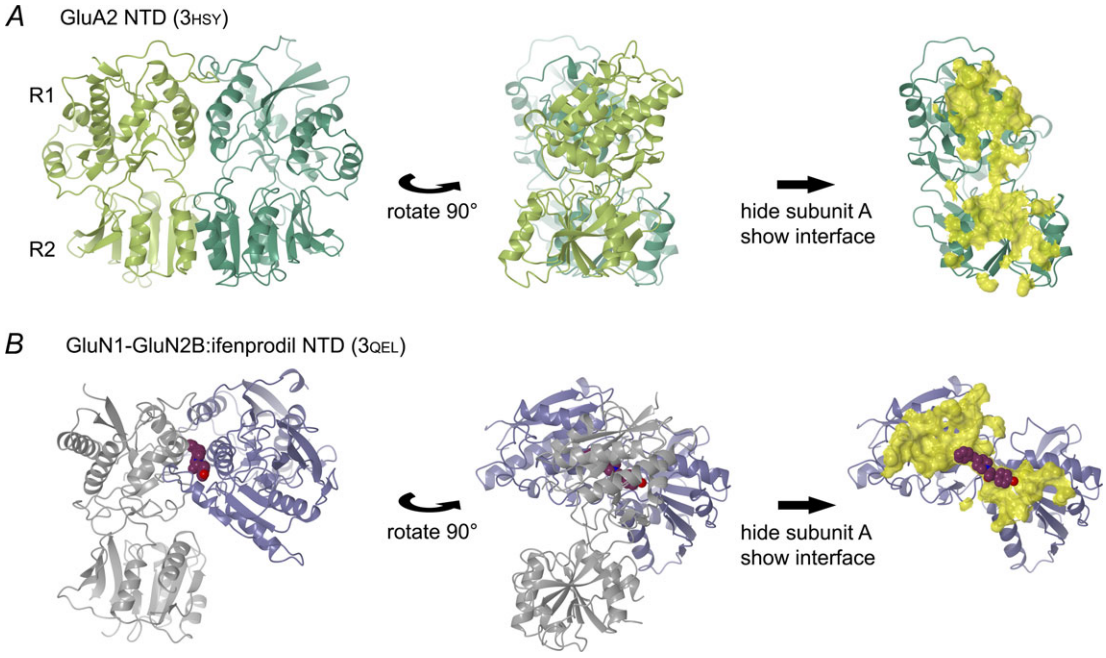

Figure 2. NTD dimer interfaces
A, views of the GluA2 NTD dimer (PDB code 3HSY; Rossmann *et al.* 2011). Domains are shown in cartoon representation, with N-terminal residues at the top. The left and central panels show both protomers (light and dark green), with the view rotated 90 deg. The top and bottom lobes of the NTD are commonly designated as R1 and R2, so the interfaces form as R1:R1 and R2:R2. In the right panel only protomer B is shown, along with the interface between A and B (yellow surface) as calculated by PISA from within CCP4mg. *B*, views of the GluN1–GluN2B NTD dimer in complex with ifenprodil (3QEL; Karakas *et al.* 2011). The *Xenopus* GluN1 and rat GluN2B protomers (light grey and blue, respectively) and the interface surface are shown as in *A*. The interface largely consists of R1:R1 interactions. Ifenprodil is shown in purple space-fill. The initial orientation was fixed by aligning protomer A (GluN1) with the equivalent GluA2 protomer, leaving protomer B oriented with R2 pointing towards the viewer in the left panel.

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AMPA subunit without the linker deletions present in $GluA2_{cryst}$ (Midgett *et al.* 2012). It is reasonable to assume NMDA receptors assemble in the same way, although this has yet to be demonstrated. One question that the $GluA2_{cryst}$ structure does not answer, however, is how subunits are arranged in a heteromeric receptor. Subunits could be arranged A/B/A/B or A/A/B/B around the pore. This affects which dimer contacts are formed: in the former arrangement both LBD and NTD dimers must be heteromeric, while in the latter arrangement only one or the other would be. An alternating subunit arrangement has been determined for AMPA receptors (Mansour *et al.* 2001), but for NMDA receptors there is evidence in support of both alternating (Rambhadran *et al.* 2010; Salussolia *et al.* 2011; Riou *et al.* 2012) and paired arrangements (Balasuriya *et al.* 2013).

From a purely structural perspective, evidence on this aspect of receptor stoichiometry is incomplete; NTD dimers almost certainly preferentially assemble as heteromers, but the evidence is less clear-cut for LBD dimers. Hetero-dimerization of NTDs has been directly demonstrated both for kainate (Kumar *et al.* 2011) and NMDA receptors (Karakas *et al.* 2011; Lee & Gouaux, 2011), while heteromerization in AMPA receptors has been shown to be driven by interactions in the NTD (Shanks *et al.* 2010; Rossmann *et al.* 2011). Although homodimeric NTD structures have been observed for subunits which form obligate heteromers (i.e. GluK5, GluN1 and GluN2B), it is reasonable to ascribe these to the combined effects of crystal packing and high protein concentration. There is less information for LBD dimers, however. Heteromeric LBD structures have only been determined for GluN1 in complex with GluN2A, and there is biochemical evidence that this is the native form (Furukawa *et al.* 2005). However, both of these subunits have also been observed in crystal structures as homodimers, so although the alternating arrangement seems most likely for all receptor subtypes, definitive structural proof is still required.

Interface movements during receptor gating

The first AMPA LBD structures led to the development of a gating model for non-NMDA (i.e. AMPA and kainate) receptors in which the LBD dimer plays a literally pivotal role in channel activation and desensitization (Sun *et al.* 2002). In this model the dimer interface enables ligand binding to be transmitted into channel opening, while desensitization results from dissociation of the dimer interface, relieving strain on the pore and allowing closure. Consistent with this, dimer stability is inversely correlated with the rate of desensitization in both AMPA (Sun *et al.* 2002) and kainate receptors (Zhang *et al.* 2006; Chaudhry *et al.* 2009*b*). At the extreme, macroscopic desensitization can be blocked either with stabilizing point mutations (Stern-Bach *et al.* 1998; Nayeem *et al.* 2009) or introduced disulphides (Priel *et al.* 2006; Weston *et al.* 2006). Conversely, a disulphide link at the base of the dimer interface blocks GluA2 function, resulting in a conformation with a dissociated D1:D1 interface that has been ascribed to the desensitized state (Armstrong *et al.* 2006).

One consequence of this model is that the 'normal' dimer conformation is generally considered both the

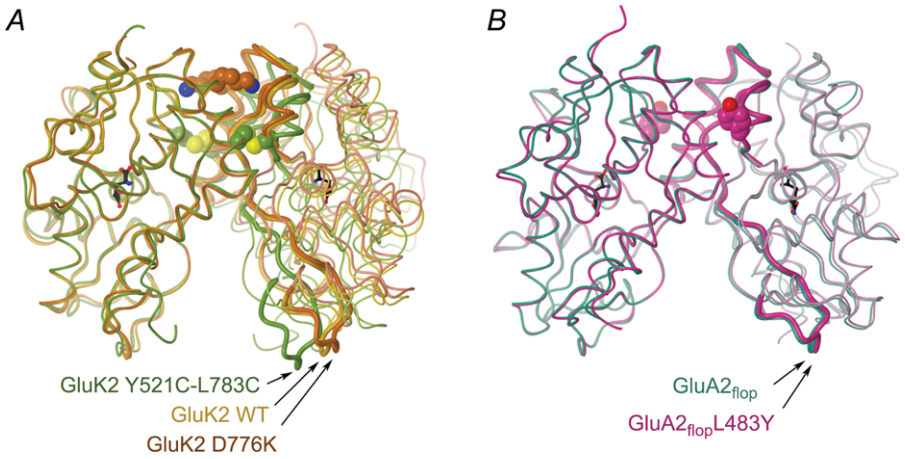

Figure 3. Dimer conformations of LBDs containing non-desensitizing iGluR mutants
A, comparison of LBD dimer conformations for GluK2 WT, D776K (PDB codes 2XXR and 2XXX; Nayeem *et al.* 2011) and Y521C–L783C (2I0C; Weston *et al.* 2006). Polypeptide is shown as a tube, with mutations shown as spheres and ligand (glutamate) as black sticks. Dimers are aligned on the left protomer. Key residue stretches at the top (P769–W798) and bottom (A518–S670) of the right protomer are displayed as thicker tubes to highlight relative movements. *B*, LBD dimers for $GluA2_{flop}$ WT (1FTM; Armstrong & Gouaux, 2000) and L483Y (1LB8; Sun *et al.* 2002) in complex with AMPA, displayed as in panel *A* (highlighted residue stretches in the right protomer are T480–S635 and P737–G757). Here and elsewhere residue numbering follows the general convention; full-length except for GluA2, where it is for the mature polypeptide.

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resting and active conformation. This fits the observations of several electron microscopy studies (Midgett *et al.* 2012; Schauder *et al.* 2013). These show symmetry-related density thought to correspond to NTD and LBD dimers. In other studies, however, LBD dimers appear not to be present in the resting (i.e. apo) state (Shanks *et al.* 2010). Experiments using luminescence resonance energy transfer to measure LBD separation in AMPA subunits lacking the NTD came to a similar conclusion (Gonzalez *et al.* 2010): dimers were only observed when antagonist was added, or with a non-desensitizing construct (GluA4 L505Y). If confirmed, these results would represent a significant challenge to the accepted gating model, which depends on an intact LBD dimer to enable channel opening. While it is tempting to look for possible technical factors, such as residual glutamate causing desensitization in the 'apo' forms (and with antagonist allowing recovery to a true resting state), another twist in the iGluR story cannot be ruled out.

When considering what the LBD dimer interface looks like in the active state there are variations in the LBD structures, both from mutations and ligand-specific conformational shifts (Nayeem *et al.* 2011). Recent single-channel recordings of non-desensitizing kainate receptor mutants show a close link between the dimer conformation and function. Although the cross-linked GluK2 Y521C–L783C mutant blocks macroscopic desensitization, it does not open either continuously or to the main conductance level (Daniels *et al.* 2013). This is actually consistent with the associated LBD structure (Weston *et al.* 2006), which was described as 'relaxed' compared to wild type (WT; Fig. 3*A*). In contrast, a second non-desensitizing mutant, GluK2 D776K, does open continuously to the main conductance level (Dawe *et al.* 2013). This mutant exhibits a tighter association at the top of the dimer interface than GluK2 WT (Fig. 3*A*; Nayeem *et al.* 2011), probably representing the active state of the receptor. Equivalent single-channel experiments have not been reported for non-desensitizing AMPA mutations, although for GluA2 L483Y the LBD dimer conformation is also similar to WT (Fig. 3*B*).

Of interfaces in the other domains, the NTD is not thought to play a significant role in non-NMDA receptor function, but does have an effect in NMDA receptors. Receptor properties such as single channel open probabilities and ligand potency are defined by the GluN2 subunit, and it has been shown that the NTD dominates this effect (reviewed in Hansen *et al.* 2010; Paoletti, 2011). This appears to be related to the greater conformational flexibility of the NTD in NMDA subunits (Karakas *et al.* 2011), although how conformational changes in the NTD are transmitted to the TMD is not known. Unsurprisingly given the functional role of the NMDA NTD, a number of endogenous and exogenous factors have been found to bind there. These are discussed in the following sections.

Structural information on interfaces between subunit TMDs is currently limited to the closed conformation observed for $GluA2_{cryst}$, but for obvious reasons they are central to iGluR function. In addition, accessory proteins interacting with non-NMDA subunits appear to do so via the TMD (Shanks *et al.* 2010) but also modulate the function of the LBD (Straub & Tomita, 2012). Further structural information on this region is therefore an essential prerequisite for a full understanding of iGluR function.

Physiological binding sites within domain interfaces

A number of endogenous factors interact with iGluRs in addition to glutamate (and glycine for NMDA receptors). These include polyamines and a wide range of ions. Polyamines affect the responses of all three receptor subtypes by binding to the TMD, while Mg^{2+} ions selectively block the NMDA receptor (Traynelis *et al.* 2010). In terms of the domains described here, both the NMDA receptor NTDs and kainate receptor LBDs have binding sites for

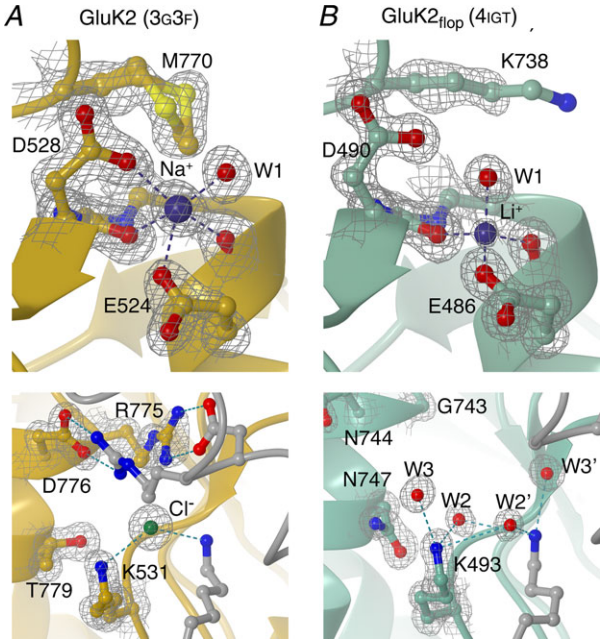

Figure 4. Ion binding to the LBD dimer interface in iGluR subunits
A, cation and anion binding sites in GluK2 (PDB code 3G3F; Chaudhry *et al.* 2009*b*) shown in the upper and lower panels respectively. In both cases residues and solvent in contact with the ion are indicated, along with the associated electron density (grey mesh; contoured at 1.5σ). For clarity in the lower panel density is only shown for ions, solvent and residues in the further protomer. *B*, equivalent views for $GluA2_{flop}$ WT (4IGT; Assaf *et al.* 2013), in which lithium binding is observed at the 'cation' site, but no anion is present. Symmetry-related waters are indicated with a prime ('). Structure factors for electron density maps were downloaded from the Electron Density Server (eds.bmc.uu.se/eds/; Kleywegt *et al.* 2004) from within CCP4mg.

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endogenous modulators. Polyamines, H^+ and Zn^{2+} modulate NMDA receptor responses by binding to the NTD (Paoletti & Neyton, 2007), and monovalent cations and anions affect GluK1 and GluK2 responses through sites in the LBD (Paternain *et al.* 2003; Wong *et al.* 2007). In the other receptor families, Ca^{2+} binds GluD2 at a site equivalent to the cation site in kainate subunits (Naur *et al.* 2007; Hansen *et al.* 2009) while Zn^{2+} binds to GluK3 at a site lower down the dimer interface (Veran *et al.* 2012). Binding sites for many of these factors have been identified in X-ray structures; these are described below.

Polyamines and zinc both bind to the NTD of NMDA receptors. The binding site for polyamines has been identified as the NTD dimer interface, where they act by increasing conformational freedom (Mony *et al.* 2011). Zinc binds with high affinity to GluN2A-containing NMDA receptors, and with lower affinity to receptors containing the GluN2B subunit (Paoletti & Neyton, 2007). Karakas *et al.* (2009) crystallized the GluN2B NTD in the presence of Zn^{2+}, revealing five binding sites per protomer. A Zn^{2+} ion bound at the mouth of the cleft between lobes was identified as the physiologically relevant site. However, this ion is chelated by only two sidechains and is 50% occupied. A structure of the GluN1–GluN2A heteromeric NTD complex with zinc bound will be more convincing, and may even reveal an alternative binding mode. Indeed, a modulatory binding site outside of the various subunit interfaces would be the exception for iGluRs.

The other main binding sites for endogenous factors are those for monovalent ions in kainate receptors (Plested & Mayer, 2007; Plested *et al.* 2008; Bowie, 2010). In both GluK1 and GluK2 subunits, monovalent cations bind to two sites on either side of the LBD dimer 2-fold symmetry axis, while a single anion binds to a site on the axis (Fig. 4*A*). The cation sites are contained within individual protomers, while the anion site spans the two protomers. Binding of Na^+ and Cl^- ions have been shown to stabilize the dimer, and thereby slow desensitization (Chaudhry *et al.* 2009*a*). However, mutations to residues forming these sites give a more mixed picture. While elimination of Na^+ binding invariably disrupts the dimer, as expected (Plested *et al.* 2008; Nayeem *et al.* 2011), mutations to residues forming the anion binding site have varied functional effects. Most mutations do accelerate the rate of desensitization (Plested & Mayer, 2007; Wong *et al.* 2007), but three mutants have been identified where the rate of desensitization is slowed (Nayeem *et al.* 2013). For two of these, sidechain rearrangements can explain the phenotype, but for one mutant (GluK2 R775A), the loss of chloride binding was the only apparent structural change. One possible explanation is that the charge balance in the WT receptor tends to destabilize the interface, and the loss of two basic sidechains counteracts this (Nayeem *et al.* 2013).

AMPA receptors do not respond to external monovalent ions in the same way as kainate receptors, although many of the residues in the two binding sites are conserved. Two recent structures show Li^+ bound at the equivalent of the cation site (Fig. 4*B*; Assaf *et al.* 2013; Harms *et al.* 2013). The significance of this, if any, is unclear. Identification of lithium requires a high resolution structure, so it might have been missed before (33 AMPA LBD structures have been crystallized in the presence of lithium). On the other hand, there has been no indication from other structures that Na^+ or another physiological cation binds at this site. This highlights one of the difficulties of using X-ray

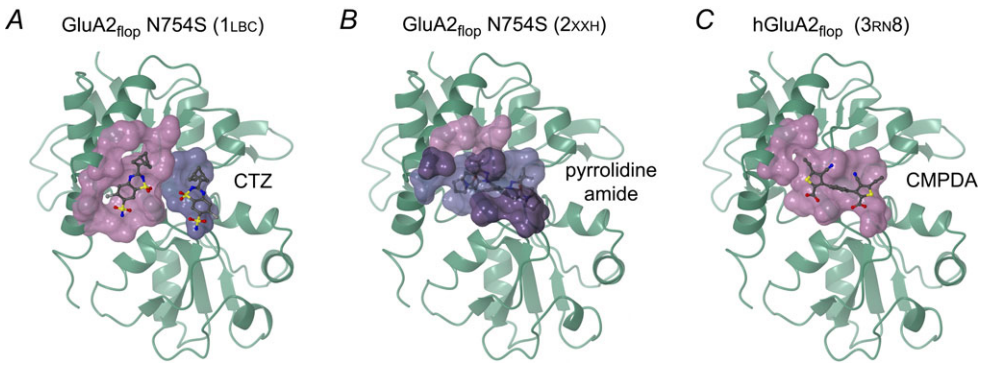

Figure 5. Allosteric potentiator binding to AMPA subunits
A, two molecules of cyclothiazide (CTZ) bind within the $GluA2_{flop}$ N754S LBD dimer interface (PDB code 1LBC; Sun *et al.* 2002). The main interface is shown as a pink surface and the minor interface as a blue surface. As the two binding sites are related by 2-fold symmetry, equivalent binding surfaces are present on the other protomer (not shown). The N745S mutation results in a 'flip-like' binding profile for allosteric modulators. *B*, several AMPA modulators bind to a single site on the dimer 2-fold axis, but in two orientations. In the example shown (2XXH; Ward *et al.* 2011), an amide designated 10a is bound in overlapping conformations, with an occupancy of 0.5 in each. Surfaces are shown as in *A* for the two binding modes, with areas of overlap in purple. *C*, allosteric potentiators with 2-fold symmetry have been identified that bind in a single orientation (Timm *et al.* 2011). The structure shown (3RN8) is of human $GluA2_{flop}$ in complex with phenyl-1,4-bis-alkylsulfonamide (CMPDA). Breaking such ligands' symmetry could potentially lead to compounds with selectivity for specific heteromeric receptor complexes.

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structures to identify binding sites. Not counting those sites described earlier, over 100 of the reported iGluR domain structures have a potentially physiological ion bound at one or more sites (e.g. 77 structures with Zn^{2+} bound; 8 with Na^{+}; 35 with Cl^{-}). Crystallization conditions are often far from physiological; in the reported iGluR structures pH varies between 4.0 and 9.5, for example. Ions such as sulphate or lithium can be present in the crystallization buffer at levels of several molar, while ions found in the brain can be present at concentrations very different from their normal physiological levels. In addition to those ions present as presumed artefacts of crystallization, real sites may be missed if they are not being explicitly looked for, particularly at lower resolutions. The discovery of further modulatory sites cannot therefore be ruled out.

Drug binding to domain interfaces

While the number of therapeutically useful drugs targeting iGluRs is currently limited (e.g. memantine for Alzheimer's disease and perampanel for epilepsy), there is no shortage of potential therapeutic targets (Bowie, 2008). In addition to compounds acting competitively at the agonist binding sites, there are non-competitive antagonists acting at all three receptor subtypes, and allosteric potentiators of AMPA receptors. Of these, several classes are thought to bind to the region between the LBD and the pore. This includes non-competitive antagonists of non-NMDA receptors such as GYKI53655 and LY 300164 (Balannik *et al.* 2005), as well as more recently identified NMDA antagonists (Acker *et al.* 2011; Hansen & Traynelis, 2011). Given the close apposition of subunit polypeptides in this region (Fig. 1*A*), it is a reasonable assumption that these compounds will bind to more than one subunit. However, the extent to which subunit interfaces are directly involved in their functional effects will have to await more detailed structural information for the TMD.

Two other classes of iGluR modulators have been definitively shown to bind to domain interfaces. Phenylethanolamines such as ifenprodil, a GluN2B-selective non-competitive antagonist, bind at the GluN1–GluN2 NTD dimer interface (Karakas *et al.* 2011), while AMPA potentiators typified by cyclothiazide bind in the LBD dimer interface (Sun *et al.* 2002). In the former case, NMDA receptor function is apparently inhibited through restrictions in NTD conformational freedom (Karakas *et al.* 2011). It seems unlikely that similar inhibition can occur in non-NMDA receptors, where the NTD has no reported effect on function. AMPA potentiators, in contrast, act by stabilizing the LBD dimer interface (Sun *et al.* 2002), binding to the lower half of the D1:D1 interface. Increased dimer stability, perhaps coupled with steric block, slows the rate of macroscopic desensitization. While equivalent compounds acting at kainate receptors have not been described, there appears no intrinsic reason a similar effect cannot be achieved.

Three binding modes have so far been identified for AMPA potentiators using X-ray crystallography (Fig. 5). Some compounds, including cyclothiazide itself, bind to two sites on either side of the dimer 2-fold symmetry axis (Fig. 5*A*). Others bind in two overlapping conformations to a more central site (Fig. 5*B*), while chemically symmetric compounds have now been identified that bind to a central site with a single conformation (Fig. 5*C*; Timm *et al.* 2011). In all cases the modulators interact with equivalent residues on both subunits, and will therefore bind to homomeric receptors preferentially. This is necessarily the case for any compound which binds to multiple sites related by 2-fold symmetry. However, by breaking the symmetry of compounds in the third class, it should be possible to develop AMPA receptor modulators which are selective for heteromeric receptors. Ultimately this level of selectivity may prove essential in developing safe and effective therapies targeting these ubiquitous receptor classes.

Conclusion

Structural studies on isolated subdomains have brought us a surprising way towards understanding how glutamate binding to iGluRs is translated into channel opening. They have led directly to models of receptor activation explaining properties ranging from ligand affinity and efficacy to desensitization and the effects of endogenous ions. In all of these, domain interfaces, and an understanding of how their conformation varies, have proved to be of central importance. The full-length $GluA2_{cryst}$ structure has served to confirm the utility of the isolated LBD and NTD structures, showing that the same dimeric associations exist in the intact receptor. It has also highlighted their limitations, revealing an unpredicted cross-over between the two extracellular domains. Despite this progress, significant gaps remain, not least how the conformation of the TMD changes when the receptor is activated, and the mechanisms by which accessory proteins modify this process. Future protein structures will undoubtedly play a role in answering these questions, although it remains to be seen whether structures of isolated domains still have more to reveal, or additional full-length iGluR structures will instead be required.

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Additional information

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