SYMPOSIUM REVIEW

Structure and function of glutamate receptor amino terminal domains

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Abstract The amino terminal domain (ATD) of ionotropic glutamate receptor (iGluR) subunits resides at the extracellular region distal to the membrane. The ATD is structurally and functionally the most divergent region of the iGluR subunits. Structural studies on full-length GluA2 and the ATDs from three iGluR subfamilies have shed light on how the ATD facilitates subunit assembly, accommodates allosteric modulator compounds, and controls gating properties. Here recent developments in structural and functional studies on iGluR ATDs are reviewed.

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Abbreviations ATD, amino terminal domain; CTD, carboxyl terminal domain; LBD, ligand-binding domain; TMD, transmembrane domain.

Ionotropic glutamate receptors

The majority of excitatory transmission in the mammalian brain is mediated by L-glutamate. This excitatory transmission is critical in brain development, as well as in basic functions including learning and memory formation (Kandel *et al.* 1995). The glutamate-mediated excitatory transmission is elicited by actions of metabotropic glutamate receptors (mGluRs) and ionotropic glutamate receptors (iGluRs), which are classified as G protein-coupled receptors and ligand-gated ion channels, respectively. There are four subfamilies of iGluRs, including α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors (GluA1–GluA4), kainate receptors (GluK1–GluK5), *N*-methyl-D-aspartate (NMDA) receptors (GluN1, GluN2A–D, GluN3A-B) and delta receptors (GluD1 and GluD2) (Traynelis *et al.* 2010). The combination of AMPA, kainate and NMDA receptors at the synaptic and extrasynaptic sites determines the amplitude and kinetics of excitatory postsynaptic currents (Lester *et al.* 1990), and thus the overall property of excitatory synaptic transmission. Therefore, understanding the regulatory mechanisms of these receptors is crucial in dissecting the rather complex pharmacology of the neuronal synapses.

All of the iGluR subunits are composed of four distinct domains: the ATD, ligand-binding domain (LBD), transmembrane domain (TMD), and C-terminal domain (CTD) (Traynelis *et al.* 2010) (Fig. 1*A*). Of all the domains,

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the ATD has the most divergent primary sequences among the iGluR subunits. While AMPA, kainate and delta receptor ATDs have approximately 20–25% sequence identity, there is little or no sequence identity between the non-NMDA receptors and NMDA receptors. The sequence identities are higher within the subfamilies: AMPA receptor subunits (∼55% among GluA1–4); kainate receptor subunits (∼75% among GluK1–3, 65% among GluK4–5 and ∼30% between GluK1–3 and GluK4–5); NMDA receptors (35–55% among GluN2A–D and ∼15% between GluN1 and GluN2A–D); and delta receptors (60% between GluD1 and GluD2). Compared

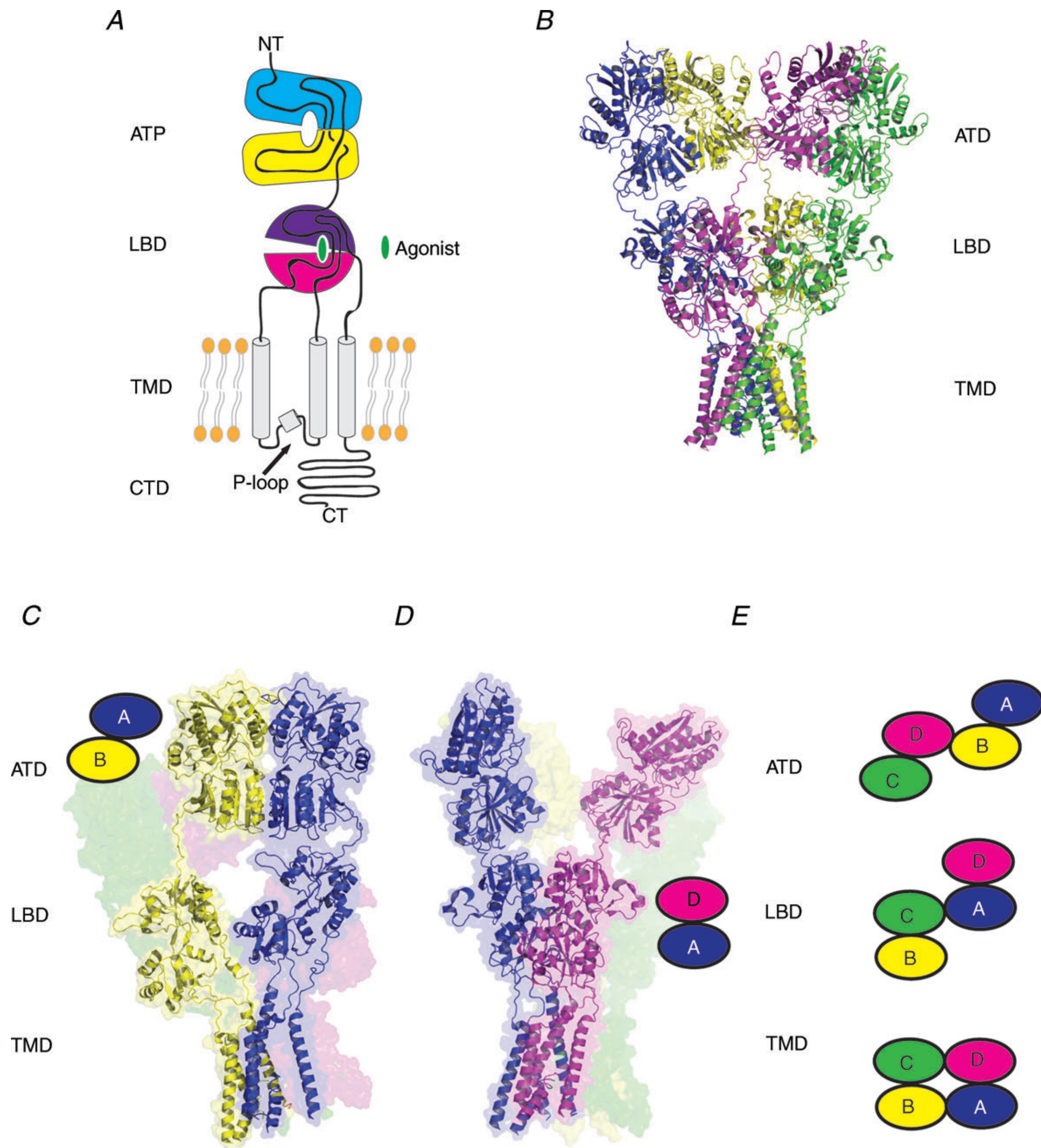

Figure 1. Organization of domains and subunits in iGluRs
A, iGluR subunits are composed of distinct domains including the amino terminal domain (ATD), ligand-binding domain (LBD), transmembrane domain (TMD), and carboxyl terminal domain (CTD). *B*, crystal structure of the homotetrameric full-length GluA2 receptors (PDB code: 3KG2) showing the pattern of subunit arrangement and domain organization in the tetrameric assembly (Sobolevsky *et al.* 2009). The four subunits (A–D) are coloured as blue (A), yellow (B), green (C) and magenta (D). *C*–*E*, ATD dimers (panel *C*) and LBD dimers (panel *D*) are formed by an A–B (shown as a cartoon) or C–D pair and A–D (shown as a cartoon) or B–C pair, respectively. This results in a crossover of the dimer pairs in the ATD and LBD sections. Panels *C*, *D*, and *E* are modified from Hansen *et al.* (2010) with permission from the American Society for Pharmacology and Experimental Therapeutics.

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to ATD, sequence identities are significantly higher within LBDs (80–90% within similar groups) or TMDs (80–95% within similar groups).

History of structural studies on iGluRs

The structural study of iGluRs started in the late 1990s with the isolated LBD of GluA2 AMPA receptor (Armstrong *et al.* 1998). A series of GluA2 LBD structures in complex with different ligands and allosteric modulators or structures of mutant GluA2 LBDs has provided insights into receptor activation, deactivation and desensitization (Armstrong & Gouaux, 2000; Sun *et al.* 2002; Jin *et al.* 2005; Armstrong *et al.* 2006). Extensive studies have also been conducted on kainate receptor and NMDA receptor LBDs (Furukawa & Gouaux, 2003; Furukawa *et al.* 2005; Inanobe *et al.* 2005; Mayer, 2005; Yao *et al.* 2008; Vance *et al.* 2011). The common findings in those studies are as follows: (1) iGluR LBDs have bi-lobed clamshell-like architecture; (2) opening and closing of the LBD clamshell structures are coupled to gating activities; (3) non-NMDA receptor and NMDA receptor LBDs form homodimers in crystals while a GluN1–GluN2 heterodimer has also been observed; and (4) the dimer interface regulates speed of deactivation and the extent of desensitization. It is worth mentioning that some non-NMDA receptors can function as homotetramers in heterologous expression systems; however, they exist mostly as heterotetramers in the mammalian brain. Although there have not been any reports of heterodimeric structures of non-NMDA receptor LBDs to date, such studies may provide some important insights. It has been difficult to conduct studies of heterodimeric assembly in LBDs due to weak association between subunits. Assessment of oligomerization has relied on crystal packing since the wild-type LBD proteins for all of the iGluR families exist as monomers in solution, which is in contrast to ATD proteins that form dimers in solution. Nevertheless, the dimeric arrangement of LBDs observed in those crystallographic studies has proven to be physiological as the recent full-length GluA2 structure contains the same LBD dimers.

Meanwhile, an image of an intact AMPA receptor was revealed by a single particle electron microscopy analysis, providing the insight into how native AMPA receptors adopt various conformations and exist with membrane proteins belonging to the Stargazin/TARP family (Nakagawa *et al.* 2005). After an enormous number of crystallographic studies on iGluR LBDs, the structures of ATDs from all of the subfamilies became available in 2009 and later, as discussed in the following section (Hansen *et al.* 2010). Finally, completion of the full-length GluA2 AMPA receptor crystal structure in late 2009 marked a historical end to the mystery involving the subunit stoichiometry and the domain organization, and started a new era in the structural and functional studies of iGluRs (Sobolevsky *et al.* 2009). Perhaps the most surprising aspect of the structure is the presence of two conformers (A/C and B/D types) of four subunits with a crossover at the ATD and LBD sections, which results in staggering of ATD and LBD dimers (A–B and C–D dimers at ATD and A–D and B–C dimers at LBD; Fig. 1*B–D*). Importantly, with this full-length structure, one can now predict how the conformational movement of each modular domain in the iGluR subunits may couple to function in a much more precise manner than before. AMPA, kainate and NMDA receptors have similar architectures in the LBD and probably TMD and thus, have a similar LBD–TMD inter-domain orientation. However, the recent crystallographic studies showed that NMDA receptor ATDs are clearly different from non-NMDA receptor ATDs, not only in basic architecture (Karakas *et al.* 2009, 2011; Farina *et al.* 2011), but also in the pattern of subunit arrangement in the ATD dimers (Karakas *et al.* 2011). Thus, ATD is structurally the most diverse region among the iGluR subunits and the tetrameric arrangement in ATD is also expected to differ significantly between non-NMDA receptors and NMDA receptors. This is understandable considering the low sequence identity between non-NMDA receptor ATDs and NMDA receptor ATDs (<10%).

Comparison of amino terminal domain structures from different subfamilies

A series of iGluR ATD structures have emerged in the last two years including the ones for GluA2 and 3 AMPA receptors (Clayton *et al.* 2009; Jin *et al.* 2009; Sukumaran *et al.* 2011), GluK2, 3 and 5 kainate receptors (Kumar *et al.* 2009; Kumar & Mayer, 2010; Kumar *et al.* 2011) and GluN1 and GluN2B NMDA receptors (Karakas *et al.* 2009, 2011; Farina *et al.* 2011). Both AMPA and kainate receptor ATDs have bi-lobed clamshell-like architectures that are composed of R1 (upper lobe) and R2 (lower lobe) domains and are similar to leucine/isoleucine/valine-binding protein (LIVBP) and mGluR LBDs (Fig. 2*A*). While there are robust conformational changes in LIVBP (Quiocho & Ledvina, 1996) and mGluR LBDs (Kunishima *et al.* 2000; Tsuchiya *et al.* 2002) featuring opening and closing of clamshell-like structures upon ligand binding and unbinding, there appears to be no such conformational variability in non-NMDA receptor ATDs. That is, all of the non-NMDA receptor ATD structures obtained to date adopt similar intermediate conformations that reside between the open-cleft and closed-cleft of LIVBP or mGluR LBDs. The non-NMDA receptor ATDs are organized as homodimers in crystals as well as in solution, indicating that dimers are basic units in ATDs (Fig. 2*C*) (Clayton *et al.* 2009; Jin *et al.* 2009; Kumar *et al.* 2009; Kumar & Mayer, 2010; Sukumaran *et al.* 2011)

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except for the recently reported heterodimeric structure of GluK2 and GluK5 (Kumar *et al.* 2011). In general, the non-NMDA receptor subunits are symmetrically arranged within the ATD dimers in a side-by-side orientation mediated by strong R1–R1 and R2–R2 interactions with the inter-R1–R2 clefts facing the front and back side of the dimers (Fig. 2*C*). The recent structure of the GluK2–GluK5 ATD heterodimer shows a pattern of

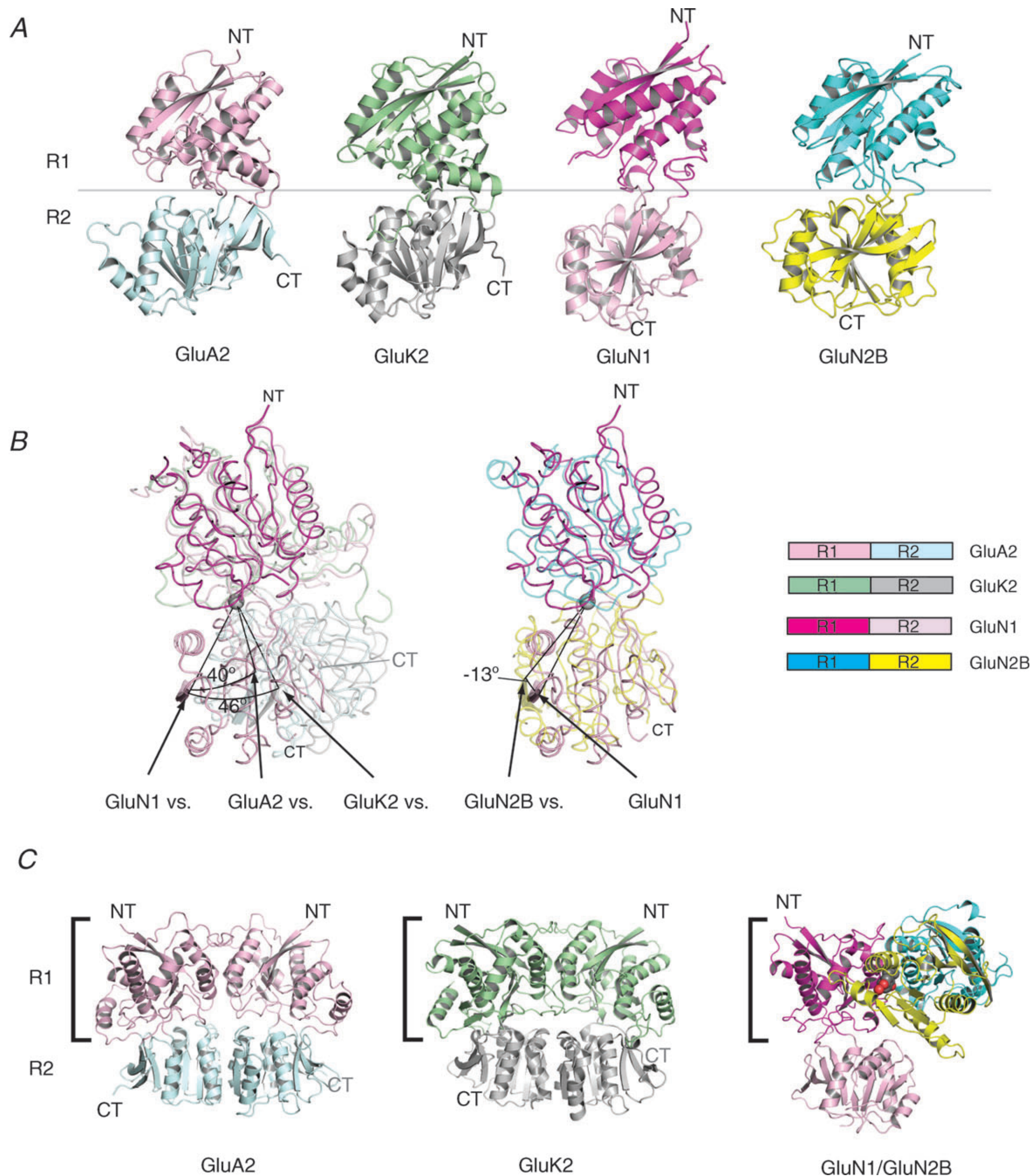

Figure 2. Structures of iGluR ATDs
A, structures of ATD monomers from the AMPA, kainate and NMDA receptor subfamilies. The overall architecture of iGluR ATDs is shaped like a bi-lobed clamshell composed of the upper lobe (R1) and the lower lobe (R2), coloured differently. The structures (PDB codes are 3H5V, 3H6G, 3QEK and 3JPY*B* for GluA2, GluK2, GluN1 and GluN2B, respectively) are aligned with the similar R1 orientation. *B*, distinct R1–R2 orientation in NMDA receptors. Superposition of the R1 domains shows that the ATD clamshells from NMDA receptor subunits (both GluN1 and GluN2B) are substantially 'twisted' compared to those from non-NMDA receptors. The pivotal points for the R1–R2 twist are displayed as grey spheres. *C*, comparison of GluA2 ATD homodimer, GluK2 ATD homodimer, and GluN1–GluN2B ATD heterodimer. The R1s of ATDs on the left (square bracket; GluN1 R1 in GluN1–GluN2B ATD heterodimer) are similarly oriented. Note a substantial difference in the subunit orientation of GluN1–GluN2B ATDs compared to those of GluA2 or GluK2 ATDs. Panels *B* and *C* are modified from Karakas *et al.* (2011).

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dimeric subunit arrangement similar to that observed in GluK2 or GluK3 ATD homodimers with strong R1–R1 and R1–R2 interactions (Kumar *et al.* 2011). In contrast, substantially weaker R1–R1 interactions are observed for GluK5 ATD homodimers due to a 16 deg tilt in the R1 (Kumar & Mayer, 2010; Kumar *et al.* 2011). GluK5 subunits are obligate heteromers that form functional ion channels when combined with GluK1–3. Thus, it is understandable that heteromeric assembly of GluK2–GluK5 ATDs is considerably favoured over homomeric assembly of GluK5 ATDs. Furthermore, the tetrameric assembly of GluK2 kainate receptors or GluK2–GluK5 at the ATD in the crystals is shown to be similar to the one observed in the crystal structure of the full-length GluA2 AMPA receptor by extensive disulfide based cross-linking experiments (Das *et al.* 2010; Kumar *et al.* 2011).

In contrast to non-NMDA receptor ATDs, the GluN2B NMDA receptor ATD exists as monomers in crystals and in solution (Karakas *et al.* 2009). The GluN2B ATD also has an overall clamshell-like structure, but with a strikingly different R1–R2 orientation that involves twisting by ~50 deg (Fig. 2*A* and *B*) (Karakas *et al.* 2011). A similar twist is also observed in recent structures of GluN1 ATD (Farina *et al.* 2011; Karakas *et al.* 2011) and is suggested to exist in the GluN2A ATD based on functional experiments (Stroebel *et al.* 2011) indicating that the twist in R1–R2 orientation is a specific structural feature of NMDA receptor ATDs.

It is now known that GluN1 and GluN2 ATDs form heterodimers in mature NMDA receptors (Fig. 2*C*). This has been concluded based on the following observations: (1) GluN1 and GluN2B ATD proteins form dimers in solution only when they are mixed together (Karakas *et al.* 2011); (2) GluN1 and GluN2B can be cross-linked by disulfide bonds (Karakas *et al.* 2011; Lee & Gouaux, 2011); and (3) GluN1 and GluN2B ATDs form heterodimers in crystals (Karakas *et al.* 2011). Furthermore, an allosteric modulator, ifenprodil, significantly strengthens the heteromeric interaction in solution (Karakas *et al.* 2011). This observation is consonant with the crystallographic study that identified an ifenprodil binding site at the GluN1–GluN2B heterodimer interface (Karakas *et al.* 2011). The GluN1–GluN2B ATD heterodimer has a subunit arrangement that is highly distinct from those observed in non-NMDA receptors. While, non-NMDA receptor ATDs associate with each other symmetrically through R1–R1 and R2–R2 interactions, GluN1 and GluN2B ATDs do so through asymmetrical interactions involving R1–R1 and R1(GluN1)–R2(GluN2B) (Karakas *et al.* 2011) (Fig. 2*C*). This mode of subunit association results in the complete lack of R2–R2 interactions in NMDA receptors in contrast to non-NMDA receptors with strong R2–R2 interactions. The lack of R2–R2 interaction in NMDA receptor ATDs and the resulting freedom in the motion of R2 is perhaps an important structural feature that facilitates ATD-mediated allosteric regulation. Indeed, the recent study of Karakas *et al.* (2011) shows that trapping the movement of GluN2B R2 by disulfide cross linking prohibits allosteric inhibition by ifenprodil. Thus, although multiple conformations of clamshells have not yet been captured by crystallography, it is plausible that the ATD clamshells undergo conformational changes.

It is also suggested that the GluN1 subunit forms homodimers through ATD in the initial stage of NMDA receptor assembly before being replaced by GluN2 subunits to form mature tetrameric NMDA receptors (Atlason *et al.* 2007; Farina *et al.* 2011). In the recent study, a small portion (~5%) of the purified GluN1a ATD protein sample was shown to form dimers by negative staining and single particle electron microscope analyses (Farina *et al.* 2011). Crystallographic study of GluN1a ATD identified a dimer in an asymmetric unit, which may represent the premature form of NMDA receptors (Farina *et al.* 2011).

The strikingly different subunit arrangement in the GluN1–GluN2B ATD dimers places the C-terminal ends rather far apart (by ~79 Å) compared to the equivalent distance in non-NMDA receptors (Fig. 3*A*). With an assumption that the GluN1 and GluN2 subunits are arranged in the GluN1–GluN2–GluN1–GluN2 orientation, and that the domain swap between ATD and LBD observed in the GluA2 AMPA receptor structure occurs in NMDA receptors, the distance at the N-terminal ends of non-dimer forming LBDs (A/B or C/D in Fig. 1*D*) is ~68 Å on the average (Fig. 3*B*). While there are 11 and 9 linker residues between the end of ATD structures and the beginning of LBD structures of GluN1 and GluN2B, respectively, how they fill this ~11 Å distance gap is an unanswered question. One possible explanation may be that the recent crystal structure of GluN1–GluN2B ATDs is in the phenylethanolamine-bound form, and thus may represent a state similar to a desensitized state (Kew *et al.* 1996). It is plausible that the tetrameric arrangement of LBDs in the ifenprodil-bound GluN1–GluN2B NMDA receptors may be different from the antagonist-bound state that is represented by the recent full-length GluA2 AMPA receptor structure. Nevertheless, the highly distinct heterodimeric arrangement of the GluN1–GluN2B ATDs implies that the inter-domain organization between ATD and LBD may be significantly different between non-NMDA receptors and NMDA receptors, and that the pattern of the inter-subunit and inter-domain organization in NMDA receptors may not be precisely predicted by simply extrapolating the structural information obtained from the crystallographic study of the full-length GluA2 homotetramer. More structural or equivalent work capturing different functional states may be necessary to further understand the mode of inter-domain arrangement and cross talk between ATD and LBD in NMDA receptors.

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Role of amino terminal domain in iGluRs

Despite numerous studies, the exact role of non-NMDA receptor ATDs on ion channel activities remains unknown to date. The lack of allosteric regulation in non-NMDA receptors is suggested to stem from inflexibility of the lower lobe of the ATD clamshell (R2) through tight R2–R2 interaction within ATD dimers of both GluA2 and GluK2 subunits (Clayton *et al.* 2009; Jin *et al.* 2009; Kumar *et al.* 2009). In contrast, three different dimeric arrangements with significantly weaker R2–R2 interaction are observed within the GluA3 ATD crystal structures (Sukumaran *et al.* 2011). This, along with results of dynamic studies suggests that opening and closing as well as the rearrangement of the subunit interface similar to that observed in mGluR1 LBD can occur in the AMPA receptor ATD (Sukumaran *et al.* 2011). Additionally, unidentified electron density is observed at the clamshell cleft of the GluA2 ATD structure in a crystallization condition indicating the possible existence of an allosteric modulator that may bind AMPA receptor ATDs (Sukumaran *et al.* 2011). The deposited coordinate of the crystal structure accounts for this electron density by a sulfate ion. Furthermore, a phosphate ion in the crystallization condition along with water molecules can perhaps explain this density. Thus, whether or not an authentic ligand for non-NMDA receptors exists is an issue that remains to be resolved. Further work needs to be carefully conducted to assess a regulatory role of the non-NMDA receptor ATD on the ion channel activities.

In contrast to non-NMDA receptors, the functional roles of NMDA receptor ATDs are widely known (Hansen *et al.* 2010; Paoletti, 2011). There are two basic roles of NMDA receptor ATDs: (1) regulation of open probability

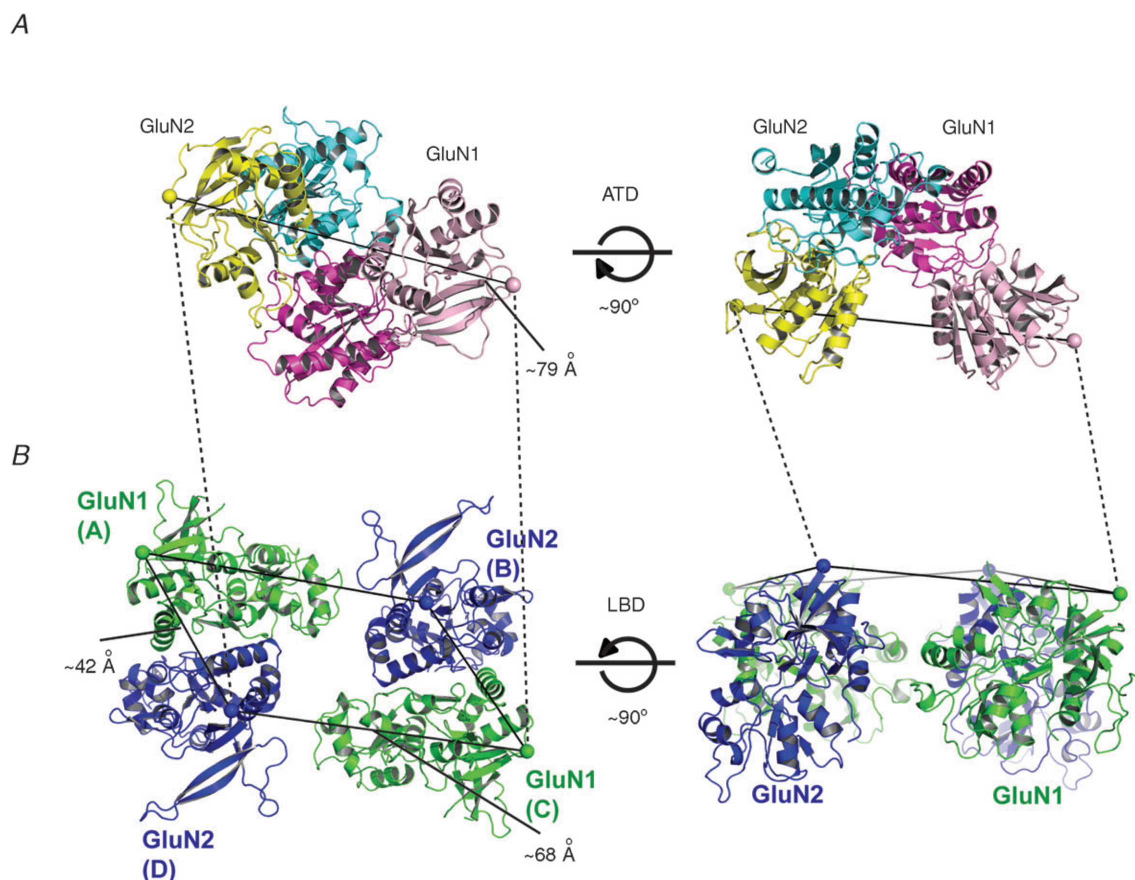

Figure 3. Possible subunit arrangement at the extracellular region of NMDA receptors
A, GluN1–GluN2B ATD dimer viewed from the C-terminal ends (spheres; left panel) or the side of the C-termini. The colour code of GluN1 and GluN2B is as in Fig. 2. *B*, tetrameric model of NMDA receptor LBDs assuming the GluN1–GluN2–GluN1–GluN2 orientation viewed from the sides of the N-terminal ends (spheres; left panel) and the side of N-termini. This model is built by superposing the top portion (domain 1) of the GluN1 and GluN2A LBD bi-lobed structures (PDB code: 2A5T) (Furukawa *et al.* 2005) onto the equivalent portion of the full length GluA2 receptor structure. Specifically, two GluN1 LBDs are superposed to the A and C subunits of GluA2 AMPA receptor structure in Fig. 1 whereas two GluN2 LBDs are superposed to the B and D subunits. The distance between the C-terminal ends of GluN1 and GluN2B ATDs is ∼79 Å whereas the GluN1–GluN2 distance between the N-termini of non-dimer forming LBDs is ∼68 Å.

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and deactivation; and (2) allosteric regulation of the ion channel activity by binding to modulator compounds. An important feature of NMDA receptors is their functional diversity, including different open probability and deactivation kinetics, which relies upon which of the four GluN2 subunits (A–D) is present in the tetrameric receptors (Paoletti, 2011). Recent studies using chimeric receptors revealed that the ATD and the short linker between the ATD and LBD are at least in part responsible for controlling both open probability and deactivation rates (Gielen *et al.* 2009; Yuan *et al.* 2009). For example, substitution of ATDs between GluN2A and GluN2B or GluN2D shifts the open probability and deactivation rates toward those of the subunit providing the ATD (Gielen *et al.* 2009; Yuan *et al.* 2009). Perhaps the most distinct feature of NMDA receptor ATDs is that they bind allosteric modulators and regulate the ion channel activities. One of the allosteric modulators, zinc, binds both GluN2A (Paoletti *et al.* 1997) and GluN2B (Rachline *et al.* 2005) ATDs and allosterically inhibits the ion channel activity in a voltage-independent manner with IC_{50} values at low nanomolar and micromolar, respectively. Within the GluN2B ATD, zinc was shown to bind to the clamshell cleft and stabilize a 'closed' conformation in a recent crystallographic study (Karakas *et al.* 2009). Based on numerous mutagenesis studies, it is known that the high-affinity zinc binding site is also located at the similar cleft of the GluN2A ATD (Fayyazuddin *et al.* 2000; Stroebel

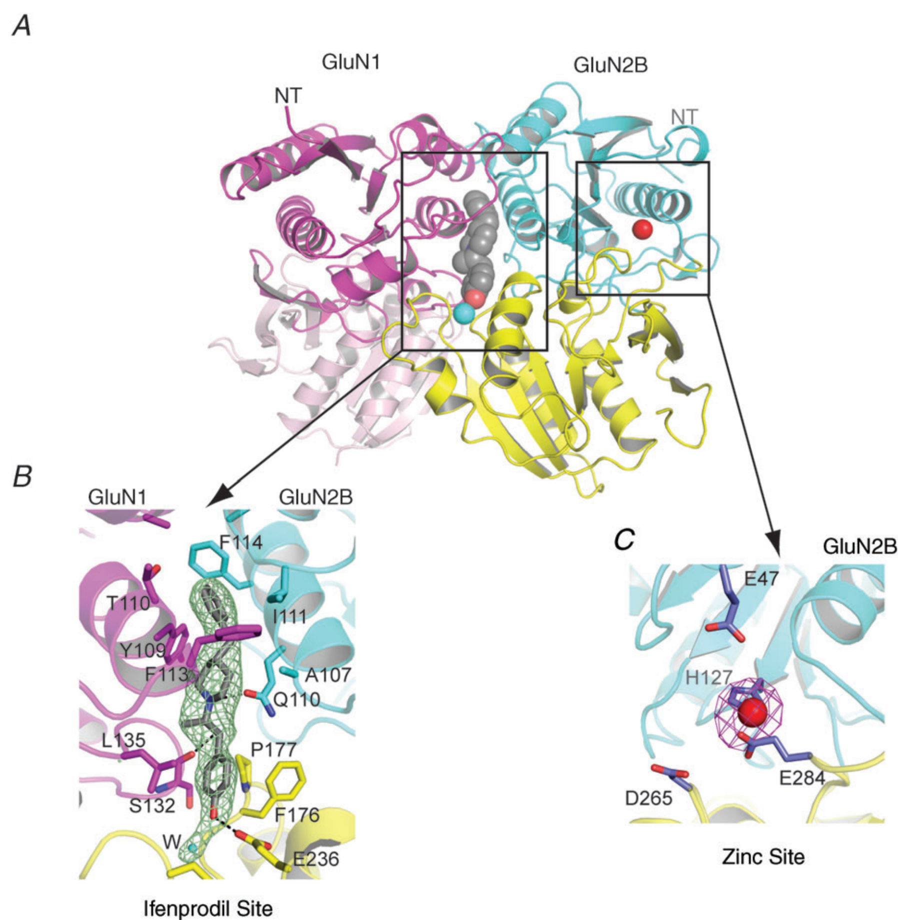

Figure 4. Binding sites for allosteric modulators in GluN1–GluN2B ATDs
A, GluN1–GluN2B ATDs with the binding site for ifenprodil (grey sphere) at the subunit interface and for zinc (red sphere) within the GluN2B cleft. The cartoon represents the composite structure of GluN1–GluN2B ATDs in complex with ifenprodil (PDB code: 3QEL) and GluN2B ATD in complex with zinc (PDB code: 3JPY). Cyan sphere represents a water molecule at the ifenprodil binding site. *B*, blow-up view of the ifenprodil binding site. Binding of ifenprodil involves residues from both GluN1 and GluN2B subunits, which form hydrophobic and polar interactions. Shown in mesh is the *Fo-Fc* omit electron density map contoured at 3σ. A cyan sphere represents a water molecule. *C*, zinc binding site at the clamshell cleft of GluN2B ATD. His127 and Glu284 coordinate directly to zinc. Glu47 and Asp265 are proximal to the zinc binding site and have been previously shown to affect zinc sensitivity (Rachline *et al.* 2005). Water molecules that are likely to be present but not visible in this crystal structure due to limited resolution of the crystallographic data may play an important role in zinc coordination along with Glu47 and Asp265. Shown in magenta mesh is the anomalous difference Fourier map at 6σ.

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et al. 2011). Binding of zinc to the ATD increases sensitivity to protons that inhibit NMDA receptor activities (Choi & Lipton, 1999; Low *et al.* 2000). Although several regions of the receptors, including the LBD dimer interface (Gielen *et al.* 2008) and the region adjacent to the gate (Low *et al.* 2003), have been proposed to serve as proton sensors, there is currently no clear view on how zinc binding at the ATD can affect those proton sensors.

Phenylethanolamines are di-aryl compounds that show neuroprotective effects by specifically targeting and allosterically inhibiting GluN2B containing NMDA receptors (>100-folds over GluN2A), and thus there has been substantial enthusiasm for applying them for treatment of a number of neurological diseases (Mony *et al.* 2009; Koller & Urwyler, 2010). The recent crystallographic study showed that the binding site for phenylethanolamine compounds, ifenprodil and Ro 25-6981, is located at the GluN1–GluN2B ATD subunit interface rather than the previously predicted site within the clamshell cleft (Karakas *et al.* 2011). The phenylethanolamine binding site has no positional overlap with the zinc binding site, which is located within the clamshell cleft of GluN2B ATD (Fig. 4). While, this crystallographic study revealed the precise architecture of the correct phenylethanolamine binding site, the mechanism underlying specific binding to GluN2B over GluN2A remains unresolved. Surprisingly, GluN2A and GluN2B differ only by one residue at the phenylethanolamine binding site (Ile111 in GluN2B is Met112 in GluN2A) and interchanging this residue between GluN2A and GluN2B does not abolish or confer ifenprodil sensitivity (Karakas *et al.* 2011). One possible explanation is that the patterns of subunit interaction between the GluN1–GluN2A heterodimer and the GluN1–GluN2B ATD heterodimer may be different from each other. The correlation between the subtype specific patterns of GluN1–GluN2 subunit interactions and binding of allosteric modulators needs to be clarified by further crystallographic studies on other NMDA receptor subtypes. Nevertheless, the recent structural identification of the phenylethanolamine binding site should facilitate the development of ATD-targeting compounds in the right direction. A further challenge in the development of phenylethanolamine-based compounds for therapeutic usage includes minimizing off-target effects towards the human ether-*a-go-go* channel and α_1-adrenergic receptors.

Another role of the iGluR ATD is to serve as sites for interaction with extracellular proteins and *cis*- or *trans*-synaptic proteins. For example, the AMPA receptor ATD interacts with N-cadherin either *cis*- or *trans*-synaptically and promotes formation of dendritic spines (Saglietti *et al.* 2007). In addition, binding of EphrinB to EphB receptor tyrosine kinase facilitates interaction with NMDA receptors through the extracellular domain of the EphB receptor and ATD (Dalva *et al.* 2000; Takasu *et al.* 2002). This interaction stabilizes the surface expression of NMDA receptors (Nolt *et al.* 2011). Recent work reports that binding of β-amyloid to the EphB receptor reduces the number of NMDA receptors on the cell surface, perhaps by interfering with the EphB receptor–NMDA receptor interaction thereby reducing the synaptic function (Cisse *et al.* 2011). Thus, these findings suggest a potentially important role of EphB receptor–NMDA receptor interaction in Alzheimer's disease (Cisse *et al.* 2011).

Conclusions

Recent structural studies on iGluR ATDs provide important insights into how this domain assembles and functions. However, several fundamental questions remain unanswered: (1) can non-NMDA receptors be allosterically regulated by binding of small compounds or proteins to the ATD? (2) How does the ATD mediate allosteric regulation in GluN2A and GluN2B containing NMDA receptor ion channels? (3) Are there small compounds that allosterically regulate GluN2A, GluN2C and GluN2D NMDA receptors in a subtype-specific manner? Finding answer to these questions will not only deepen our understanding of iGluR functions, but will also pave the way to developing iGluR-based compounds with therapeutic potentials for neurological disorders and diseases.

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