Review

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Emerging structural insights into the function of ionotropic glutamate receptors

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Ionotropic glutamate receptors (iGluRs) are ligand-gated ion channels that mediate excitatory neurotransmission crucial for brain development and function, including learning and memory formation. Recently a wealth of structural studies on iGluRs including AMPA receptors (AMPARs), kainate receptors, and NMDA receptors (NMDARs) became available. These studies showed structures of non-NMDARs including AMPAR and kainate receptor in various functional states, thereby providing the first visual sense of how non-NMDAR iGluRs may function in the context of homotetramers. Furthermore, they provided the first view of heterotetrameric NMDAR ion channels, and this illuminated the similarities with and differences from non-NMDARs, thus raising a mechanistic distinction between the two groups of iGluRs. We review mechanistic insights into iGluR functions gained through structural studies of multiple groups.

A brief history of structural studies on iGluRs

Glutamate, a simple amino acid, is the major excitatory neurotransmitter in mammalian brains [1]. The presynaptically released glutamate binds different subclasses of ionotropic glutamate receptors (iGluRs) including α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (AMPARs), kainate receptors, and *N*-methyl-D-aspartate (NMDA) receptors (NMDARs). Glutamate binding mediates opening of their cationic ion channels to generate synaptic current pivotal to brain function. The iGluR subunits contain modular domains including the amino-terminal domain (ATD), ligand-binding domain (LBD), transmembrane domain (TMD), and carboxy-terminal domain (CTD), and assemble into tetramers within respective subclasses to form ligand-gated ion channels (Figure 1). While NMDARs form functional ion channels only as heterotetramers of at least two distinct subunits, non-NMDARs do so by forming either homo or heterotetramers. Enthusiasm to understand the structure and function of iGluRs has been enormous over several decades owing to the involvement of iGluRs in various aspects of neuronal function as well as in neurological diseases and disorders such as depression, schizophrenia, Alzheimer's and Parkinson's diseases, autism, seizure, and stroke [2].

The structural biology of iGluRs started in the late 1990s after the molecular cloning of various iGluR genes [3–5]. Taking advantage of the modular nature of iGluR subunit proteins, structural determination of individual water-soluble domains such as the ATD and LBD was pursued first (primarily by X-ray crystallography) because this was substantially less technically demanding than solving the entire tetrameric transmembrane ion channels [6]. As of 2015, over 268 crystal structures of LBDs of various subunits from all of the major iGluR subclasses in complex with agonists, antagonists, partial agonists, and allosteric modulators are available. Despite low sequence identity between NMDAR and non-NMDAR subunits (~20%), the LBD structures have similar bi-lobed clamshell-like architectures that undergo opening and closing upon unbinding and binding of ligands, respectively [7–14]. The structural mobility of LBD was also extensively tested and elaborated by nuclear magnetic resonance [15], molecular dynamics free energy simulation [16], and single molecule fluorescence resonance energy transfer [17]. Meanwhile, ATD structures for non-NMDARs [18–24] and NMDARs [25–27] became available but in limited numbers compared to LBD structures. ATDs from both NMDARs and non-NMDARs have bi-lobed structures but are completely unrelated in primary sequence and overall architecture to LBDs. However, consistent with low sequence identity (<10%), conformations of bi-lobed architectures are highly distinct between NMDARs and non-NMDARs [28,29]. In NMDARs, allosteric sites, not present in non-NMDARs, were observed at the heterodimeric subunit interface between GluN1 and GluN2B for ifenprodil [27], and within GluN2B ATDs for zinc [26]. In both studies of ATD and LBD, homodimeric and heterodimeric assemblies were observed in non-NMDARs and NMDARs, respectively. This led to the speculation that the basic assembly pattern of an iGluR subunit is a dimer of dimers, instead of a *bona fide* fourfold symmetrical tetramer as in other tetrameric ion channels such as potassium and sodium channels [30–32]. However, integration of fragments of knowledge about the structure and function of individual extracellular domains required an overall view of the intact iGluR ion channels. This was an enormous challenge in X-ray crystallography because iGluR channels are tetramers of large transmembrane proteins

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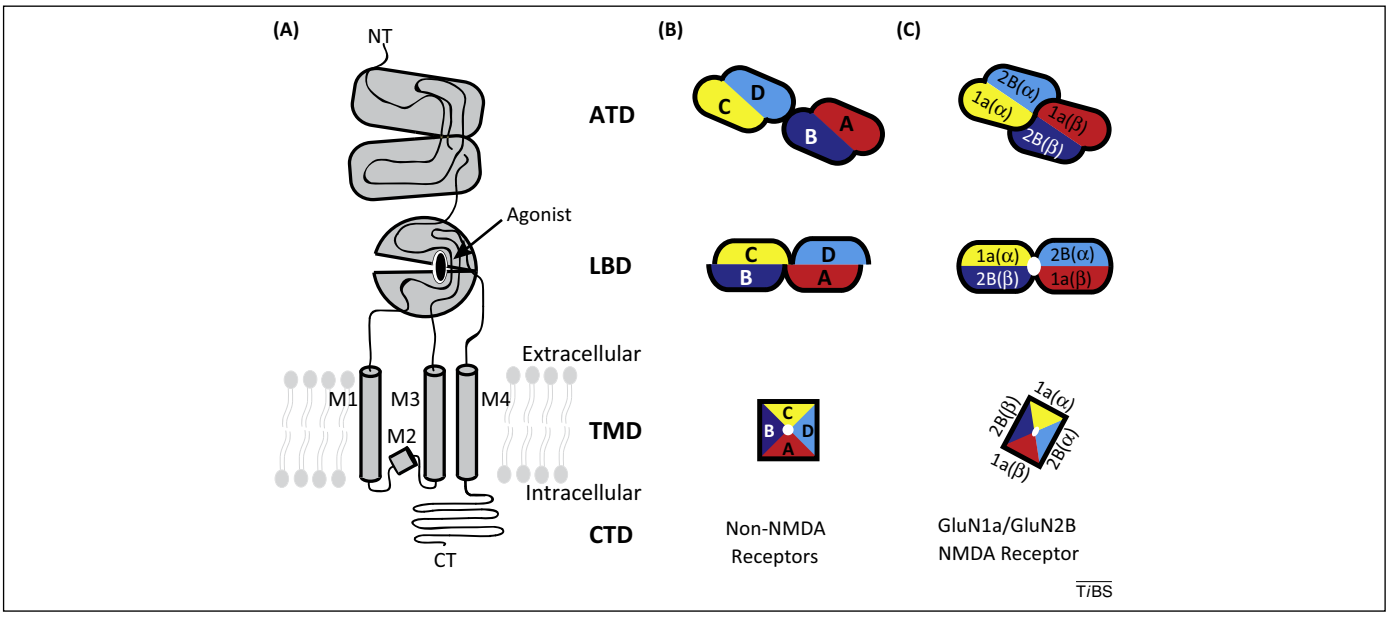

Figure 1. Domain organization and subunit arrangement of iGluRs. **(A)** The ionotropic glutamate receptor subunits are composed of distinct domains including the amino-terminal domain (ATD), ligand-binding domain (LBD), transmembrane domain (TMD), and the carboxy-terminal domain (CTD). The TMD of one subunit is composed of M1–M4 helices. Schematic representation of tetrameric subunit organization of non-NMDARs **(B)** and NMDARs **(C)** at ATD, LBD, and TMD layers are shown. Dimer pairs at ATD and LBD are indicated by lack of black lines at the interface. The four subunits in non-NMDARs are noted as (A–D).

that are heavily glycosylated. Attempts were made to observe intact AMPAR structures in the early 2000s by cryo-electron microscopy (cryo-EM); however, the overall resolution of the structure, and thus the structural interpretation, were limited by the technical restriction of the time [33–36]. The first clear-cut structure of the intact iGluR channel became available when Gouaux and colleagues solved the structure of homotetrameric GluA2 AMPARs in complex with an antagonist by X-ray crystallography, thereby revealing the pattern of subunit and domain arrangement and opening the new era of structural studies for intact iGluRs [37]. Today, the iGluR field has multiple structures of the intact GluA2 AMPAR representing different functional states, the GluK2 kainate receptor, and even intact heterotetrameric NMDARs, which were once considered overwhelmingly challenging.

Structural biology of intact non-NMDARs – a giant step toward mechanistic understanding

Recently several intact non-NMDAR structures have been published by multiple groups, providing solid molecular templates that permit a mechanistic understanding of their activation and desensitization. Specifically, in addition to the original GluA2 AMPAR structure in the antagonist-bound form, GluA2 AMPAR structures in the pre-activated (or pre-open) state, and structures of the desensitized state of GluA2 AMPAR and GluK2 kainate receptor (obtained by both X-ray crystallography and cryo-EM), have become available. These studies provide clear 'snap-shots' for making valid predictions of how domains and subunits move upon entry into different functional states (Figure 2).

GluA2 AMPARs in apo, antagonist-bound, and agonist-bound states have an overall Y-shaped architecture formed by staggered layers of ATD, LBD, and TMD (Figures 1 and 2) [37]. The structure has a symmetry mismatch between the extracellular domains (ATDs and LBDs) and TMDs, which are arranged as a dimer of dimers with twofold symmetry and a tetramer with fourfold rotational symmetry, respectively. The subunit and domain assembly is complex because the extracellular ATD and LBD have swapping of dimer pairs (i.e., homotetrameric subunits assigned with letters A through D form AB and CD dimers at ATD, and AD and BC dimers at LBD; Figures 1 and 2).

How ligand-gating occurs in non-NMDARs is a fundamental question that began to be addressed by extensive observation of conformational changes in isolated LBDs, for which binding of an agonist or antagonist stabilized the closed or open conformations, respectively [6,7,13,14]. This major conformational change in the LBD has been suggested to create tensions in the LBD–TMD linker leading to the opening of the channel [38,39]; however, discerning what this actually means in the context of intact tetrameric iGluRs has been a challenging task that required intensive structural studies of full-length receptors. Recently, three independent studies attempted to trap the presumably active state of the GluA2 AMPAR by binding an allosteric modulator or a *Conus striatus* cone snail toxin, con-ikot-ikot, both of which block entry of AMPAR into the desensitized state [40]. These studies solved structures of trapped receptors by X-ray crystallography and cryo-EM [41–43] (Table 1). In the agonist/modulator-bound form, the receptor retains its Y-shaped architecture, but the vertical dimension of the receptor is shortened by 7–10 Å, bringing the ATD and LBD tetramers closer together compared to apo and antagonist-bound forms. Conversely, in the toxin-bound structure, the ATD and LBD are further separated by 10 Å because the toxin intercalates into the space between these domains and interacts with both ATDs and LBDs (Figure 2C). Consistent with the studies on isolated LBDs, the LBD in the intact AMPAR bound to agonist/modulator had a closed cleft conformation,