CellPress

Ionotropic glutamate receptors made crystal clear

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Two recent crystallographic studies of the full-length GluA2 AMPA receptor provide our first insights into how the modular domains of the tetrameric complex coordinate the process of activation. These findings herald a new era in the structure–function analyses of neurotransmitter receptors, a fitting achievement for the 'International Year of Crystallography'.

Advances in our understanding of membrane-bound proteins have been unprecedented in the last decade with the publication of many full-length ion-channel and transporter structures that fulfill key signaling roles in the vertebrate brain [1]. Leading the way has been the study of ionotropic glutamate receptors (iGluRs), which began with the structural dissection of the isolated ligand-binding domains (LBDs) of AMPA-[2], NMDA-[3], kainate-[4] and orphan-type [5] subfamilies. In 2009, the first full-length iGluR structure was reported in a *tour de force* treatise of the GluA2 AMPAR [6]. As anticipated from earlier work, the iGluR tetramer complex had a modular design of an amino-terminal domain (NTD) responsible for subunit assembly, a LBD providing the clamshell agonist-binding pocket and the transmembrane helices (or TMD) forming the permeation pathway for cations (Figure 1). The most unexpected finding was that the iGluR tetramer contained 2 conformationally distinct subunits, the A/C and B/D subunit pairs, which was due to symmetry mismatch between the extracellular (i.e., LBD and NTD) and pore domains of each subunit [6]. Whether this structural distinction plays a key role in channel gating is a point of continual debate and thus has spurred on structural biologists to consider the iGluR as a whole. In keeping with this, recent months have witnessed a flood of high-impact papers that begin to address how each modular domain contributes to bringing about channel activation [7–11]. Two of these studies highlighted here report new insights into iGluR activation that will undoubtedly motivate the field in the coming years.

Yelshanskaya and colleagues examined the structural basis of AMPAR activation by looking for differences in the full-length GluA2 structure when in complex with the partial agonist, 5-nitrowillardiine ($GluA2_{NOW}$), and competitive antagonist, ZK 200775 ($GluA2_{ZK}$) [11]. Appreciating that receptor agonists elicit a greater degree of closure in the agonist-binding pocket than competitive antagonists, the authors planned to exploit this distinction by examining how other domains of the AMPAR, such as the NTD or pore helices, re-organize their structure in response to ligand binding. Surprisingly however, the gross architecture of $GluA2_{NOW}$ and $GluA2_{ZK}$ were rather similar in both the NTD and pore forming helices. The ion-channel pore of $GluA2_{NOW}$ was in the closed conformation like that of $GluA2_{ZK}$ though the authors did observe small differences in the cross-pore dimensions between adjacent subunits. Given that 5-nitrowillardiine is a fairly weak partial agonist, the structural similarities are not entirely unexpected since the AMPAR would be expected to spend much of its time in a non-conducting state sojourning for only milliseconds into the open/activated state(s). Greater differences were observed by comparing LBD clamshell structures which showed that the degree of closure in $GluA2_{NOW}$ was more pronounced by approximately 11°. As a result, the back-to-back interface formed between two adjacent LBD pairs was wider and shorter with $GluA2_{NOW}$ than $GluA2_{ZK}$. Interestingly, the architecture of the LBD in complex with 5-nitrowillardiine was different between the full-length (i.e., $GluA2_{NOW}$) and isolated domain [12] structures suggesting perhaps that other domains (i.e., NTD, pore helices) and their linkers constrain the LBD architecture of the tetramer. Consistent with this idea, the altered conformation of the LBD dimers in $GluA2_{NOW}$ increased tension on the ATD-LBD and LBD-TMD linkers which was observed as a 1–2° tilt of the ATD away from its twofold axis of symmetry, an overall shortening of the $GluA2_{NOW}$ structure with a tighter and more expansive LBD dimer interface. This arrangement suggested an unappreciated heterogeneity in the distance between contact points along the LBD dimer interface which was probed by introducing single cysteine residues on either side of it and testing for disulphide bond formation. When experiments placing cysteines in the upper (or D1) and lower (or D2) lobes of the clamshell structure were compared, a pattern emerged suggesting that strengthening of the D1-D1 interface promoted AMPAR activation whereas crosslinking residues at the D2-D2 interface interfered with it; an idea that is emerging for all iGluR families [13]. The authors offer two possible models to explain their observations that form a useful framework for testing new aspects of AMPAR structure–function relationships.

Chen and colleagues [7] took a different approach in examining the structural basis of activation by crystallizing the full-length GluA2 AMPAR in complex with two positive allosteric modulators, a snail toxin from *Conus striatus* [14] and a high-affinity ligand, (R,R)-2b [15]. Positive allosteric modulators promote channel openings and

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0166-2236/
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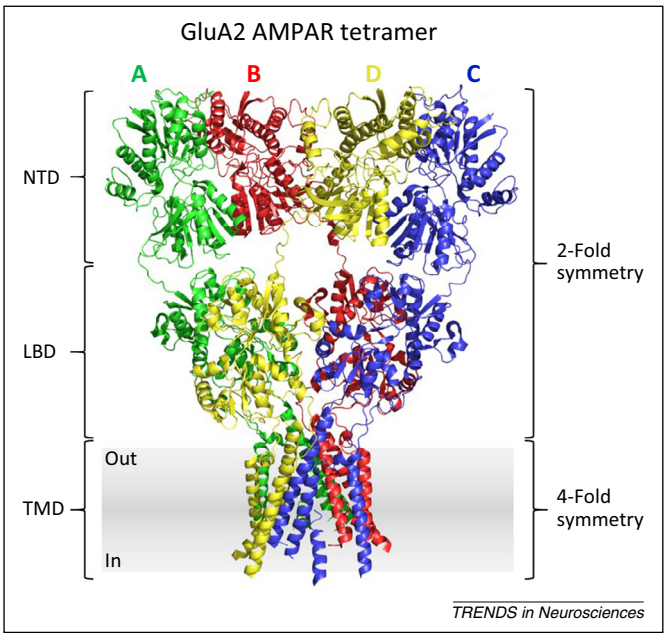

Figure 1. Full-length X-ray crystal structure of the GluA2 AMPAR tetramer. GluA2 receptor complex consists of 3 distinct modular domains that are referred to as the N-terminal domain (NTD), ligand-binding domain (LBD), and transmembrane domain (TMD). The NTD and LBD have a two-fold symmetry whereas the TMD has a four-fold symmetry. Individual subunits are colored to highlight the A–C and B–D subunit pairs. Adapted, with permission, from [6].

attenuate receptor desensitization and therefore it was reasoned that the GluA2 AMPAR structure would be more likely to adopt an activated conformation. The snail toxin is wedged in a solvent-filled cavity between the ATD and LBD layers forming a rigid structure that extends the overall height of the GluA2 AMPAR by almost 10 Å. The binding of the modulator, (R,R)-2b, improved diffraction quality of GluA2 crystals, possibly by stabilizing a homogenous population of receptor conformations, and, as a result, it was included to aid crystallization. Although the toxin makes few contact points with the ATD, it establishes a meshwork of electrostatic and polar interactions across the LBD 'gating ring' strengthening both intra- and inter-dimer interfaces. Specifically, because the toxin binds to both the D1 and D2 lobes of the LBD A/C subunits, it acts like a 'straightjacket' trapping the GluA2 AMPAR in a ligand-bound conformation. In this arrangement, greater closure in the clamshell structure is observed with partial agonists, kainate and fluorowillardiine, compared to the ZK antagonist and thus more separation of the D2 lobes which pulls on the M3 pore helices to bring about channel activation. Importantly, there is a greater D2 lobe separation in the B/D subunit pairs compared with the A/C pair suggesting that channel gating is brought about by an asymmetric pulling force on the LBD-TMD linkers with the B/D pairs contributing more. Curiously, the channel pore of the agonist-toxin-GluA2 AMPAR complex is in the closed conformation which the authors propose represents a 'pre-open' state.

Taken together, these studies provide our first insight into how the modular design of iGluRs is coordinated during activation emphasizing the value of studying full-length structures in the future.

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