**Cryo-EM structures of the ionotropic glutamate receptor GluD1 reveal a non-swapped architecture**

Ananth Prasad Burada*, Rajesh Vinnakota and Janesh Kumar*

Ionotropic orphan delta (GluD) receptors are not gated by glutamate or any other endogenous ligand but are grouped with ionotropic glutamate receptors (iGluRs) based on sequence similarity. GluD receptors play critical roles in synaptogenesis and synapse maintenance and have been implicated in neuronal disorders, including schizophrenia, cognitive deficits, and cerebral ataxia. Here we report cryo-EM structures of the rat GluD1 receptor complexed with calcium and the ligand 7-chlorokynurenic acid (7-CKA), elucidating molecular architecture and principles of receptor assembly. The structures reveal a non-swapped architecture at the interface of the extracellular amino-terminal domain (ATD) and the ligand-binding domain (LBD). This finding is in contrast with structures of other families of iGluRs, where the dimer partners between the ATD and LBD layers are swapped. Our results demonstrate that principles of architecture and symmetry are not conserved between delta receptors and other iGluRs and provide a molecular blueprint for understanding the functions of the ‘orphan’ class of iGluRs.

Delta receptors belong to the iGluR family, along with the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), kainate and N-methyl-D-aspartate (NMDA) receptors. This enigmatic class of iGluRs is referred to as ‘orphan’, because they are not activated by endogenous ligands. The family consists of two members, GluD1 and GluD2, expressed in multiple regions of the brain, with GluD1 being predominantly expressed in the inner ear and GluD2 in cerebellar Purkinje cells. The two subtypes share ~50% sequence similarity with each other and ~20–30% with other iGluRs. Although multiple structures of the intact iGluRs have been reported for AMPA, kainate and NMDA receptors, structural insight into a full-length delta receptor is still lacking. Unlike other iGluRs, delta receptors do not generate ligand-gated currents and are believed to exert their physiological function lacking. Unlike other iGluRs, delta receptors do not generate ligand-gated currents and are believed to exert their physiological functions via a noncanonical (metabolic) pathway. Dysfunction of GluD receptors is associated with social and cognitive deficits, cerebellar long-term depression and cerebellar ataxia and atrophy, and is also linked to retinal dystrophy, schizophrenia, and cocaine addiction. The primary function of GluD receptors is believed to be their ability to act as a bidirectional synaptic organizer via trans-synaptic interactions at presynaptic termini, mediated through neurexin and cerebellin, and at postsynaptic sites via direct interactions with Shank scaffold proteins. However, the ionotropic roles of GluD receptors are enigmatic, because they possess a functional ion channel, as demonstrated by electrophysiological assays on both recombinantly expressed GluD receptors harboring the Lurcher point mutation and on chimeric receptors in which the LBDs were swapped with those from AMPA or kainate receptors. Furthermore, the ionotropic properties of Lurcher mutant receptors could be modulated by ligands, like d-serine, 7-CKA and Ca2+, that bind to the LBD. Moreover, recent reports have suggested that GluD receptors not only interact directly with metabolotropic glutamate receptors but are also gated by their activation.

Although crystal structures of isolated ATDs, LBDs and the intact extracellular region (ATD–LBD) have been reported for GluD receptors, the full-length structure of either member of this family remains elusive. In order to address this and to gain structural insight into the function of these orphan receptors, we have determined the structure of homotetrameric rat GluD1 receptors using single-particle cryo-EM. The structure reveals a distinct architecture compared with that of other iGluRs. We validated the observed receptor assembly via cysteine cross-linking experiments and whole-cell patch-clamp electrophysiology. Our results give insight into architecture and assembly of orphan delta receptors and provide molecular blueprints for understanding their functions.

**Results**

Screening of C-terminally truncated rat GluD1 via fluorescence-detection size-exclusion chromatography identified GluD1 Δ851 as a promising construct for large-scale expression and purification from HEK293 GnTI− cells in suspension (using established protocols). Purified receptor (GluD1 Δ851) was complexed with 1 mM 7-CKA to trap the LBD in an open-cleft conformation and 1 mM Ca2+ to stabilize the LBD dimer assembly and subjected to cryo-EM analysis. Two-dimensional and three-dimensional (2D and 3D) classification of the cleaned-up particle stack resulted in seven distinct classes showing heterogeneity in the extracellular domains (Extended Data Fig. 1c,d), primarily due to the movement of the two extracellular arms (Extended Data Fig. 2). Out of the seven 3D classes, we focused on classes 5 and 7, representing a ‘compact’ and a ‘splayed’ conformation (Extended Data Fig. 2 and Supplementary Fig. 1) for 3D refinement, resulting in final maps at 8.1-Å and 7.6-Å resolution, respectively, as estimated by the gold-standard Fourier shell correlation (FSC) 0.143 criteria (Extended Data Fig. 3), into which protein coordinates were modeled and refined (Table 1, Supplementary Figs. 2 and 3 and Supplementary Notes).

An unprecedented architecture of the GluD1 receptor. Our cryo-EM analysis revealed a Y-shaped GluD1 receptor tetramer with a three-layered arrangement of the ATD, LBD, and transmembrane (TM) domains. The ATD and LBD are arranged in a two-fold symmetric dimer-of-dimers configuration, as observed for other
could also be superimposed, with an r.m.s. deviation of ~2.6 Å, but the TM domains did not superimpose, such that the angle formed between the M4 helices of the two subunits is ~65° (BC) and 63° (AD) (Fig. 1c). This assembly is distinct from the reported structures of AMPA, kainate and NMDA receptors, where the ATD dimer pairs are formed by AB and CD subunit pairs. Due to domain swapping, the LBD dimers are formed between subunits AD and BC. This arrangement results in the formation of receptor tetramer by pairs of conformationally distinct AC and BD subunits (Extended Data Fig. 7 and Supplementary Fig. 4). The non-swapped arrangement of GluD1 receptors seemingly allows more conformational freedom for the movements of the two dimer arms (Fig. 1a,d and Extended Data Fig. 4a,d), leading to the heterogeneity observed in our cryo-EM analysis.

### Assembly of the extracellular domains in GluD1
Each of the four ATDs has a clamshell-like structure formed by the upper (R1) and lower (R2) lobes, with a nearly identical conformation in each subunit. ATD dimers assemble via contacts mediated by both the R1 and the R2 lobes, with a buried surface of ~2,120 Å² per subunit. Furthermore, due to the movement of the two extracellular arms, the GluD1 ATD dimers form tetrameric (dimer-of-dimers) contacts only in the compact conformation, with a small buried surface of ~28 Å² (Extended Data Fig. 8), unlike that in AMPA and kainate receptors, where the buried surface is much larger, at ~300 Å². However, consistent with the nanomolar affinity for ATD homodimer formation observed for GluD2 receptors\(^5\), the ATD dimer interface is intact in both the compact and the extended conformations (Fig. 2).

Furthermore, due to the absence of subunit crossover, the two ATD–LBD arms of the receptor tetramer are in the same plane (Extended Data Figs. 5 and 6). In AMPA and kainate receptors, domain swapping and the dimer-of-dimers interface interactions lead to tilting of the ATD dimer pairs away from the overall axis of symmetry, such that subunits B and D lie proximal to the center of mass (COM), and subunits A and C form the distal edges of the tetramer assembly (Supplementary Fig. 4). Owing to this, the COMs of the R2 lobes of ATD-proximal subunits B and D in the compact conformation are at a distance of ~45 Å, and an angle of ~162° is formed between COMs of subunits A, B and D, indicating that the two dimers are almost in the same plane. This finding is in contrast with what was observed with the AMPA receptor GluA2, in which the BD COMs are a distance of ~35 Å, and the COMs of subunits A, B and D make an angle of ~119° (Fig. 1d and Extended Data Fig. 4d).

Within the LBD layer, dimer pairs like those crystallized for the isolated domains for AMPA, kainate and delta receptors assemble via contacts mediated by the upper lobes, with a buried surface for each subunit of ~1,184 Å² (Extended Data Fig. 8b). The dimer-of-dimers interface in the compact conformation for the LBD is ~510 Å², which is similar to that observed in AMPA and kainate receptors (Fig. 2e). Furthermore, due to the non-crossover architecture in GluD1, the ATD and LBD layers in subunits AB and CD pack on top of each other in an almost-linear arrangement (Fig. 2a). We also observed that the ATD and LBD layers pack closer to each other than in AMPA and kainate receptors. Whether this assembly is due to the non-crossover architecture and the shorter ATD–LBD linker in GluD1 needs further exploration (Fig. 2b).

Our imaging conditions had 1 mM 7-CKA and Ca\(^2+\), which helped in trapping the LBD in the dimeric state. The LBD adopts an open cleft conformation, similar to that of antagonist-bound AMPA and kainate receptor LBDs, with a cleft opening of ~25° compared to the d-serine-bound GluD2 LBD\(^6\) (Supplementary Fig. 5). Thus, the LBD layer arrangement is similar to that observed in antagonist-bound GluA2 or GluK2 receptors trapped in a closed state, with a classical two-fold symmetric dimer and dimer-of-dimers assembly.
Transmembrane domain arrangement. In contrast to the two-fold symmetry of the LBD and the ATD, the ion channel pore of GluD1 has four-fold rotational symmetry, such that the M4 segment of each subunit is packed against M1 and M3 from an adjacent subunit in a counterclockwise rotation, ABCD, when viewed from above (Fig. 1f,g). However, due to domain swapping, the TM arrangement is clockwise ABCD in the AMPA and kainate receptors (Extended Data Fig. 7c). Similarly to that in the AMPA and kainate receptors, the pre-M1 cuff helix in GluD1 that lies parallel to the plane of the membrane wraps around the exterior of the ion channel assembly. As observed in other iGluRs, the M3 helix bundle forms a barrier to ion permeation. We also observed that although the TMs adopt a closed pore, their assembly is more splayed, similar to that observed in NMDA receptors and unlike AMPA or kainate receptors, in which the assembly is more compact. Consistent with this, the top of the M3 helix is more constricted and immediately separates below to form an expanded vestibule. Thus, the distances between residues R632 (top), L622 (middle) and L611 (bottom) of the M3 helices from two subunits, D and B, are ~13, 22 and 35 Å, respectively (Fig. 2f). In contrast, the TM packing is more compact in the case of GluA2 receptors, where the corresponding residues on M3 helix L620 (top), L610 (middle) and R599 (bottom) are separated by ~15, 16 and 30 Å, respectively (Supplementary Fig. 4b). Similarly, the distance between M4 helix residues A812 (top), L823 (middle) and A836 (bottom) between subunits C and D are separated by ~33, 38 and 49 Å, while those in GluA2 for corresponding residues are at a distance of ~29, 36 and 38 Å, respectively (Supplementary Figs. 4c and 6).

Validating receptor interfaces and assembly. In order to validate that the unprecedented subunit arrangement and molecular symmetry of the GluD1 receptor is physiological, we performed cysteine-mutant cross-linking experiments. We introduced cysteine residues into the ATD dimer-of-dimers (F385C), ATD dimer (I155C) and LBD dimer (K514C) interfaces. These are the sites that should result in spontaneous disulfide bond formation due to the two-fold symmetric assembly at these layers (Fig. 3a and Supplementary Fig. 7). Our results showed bands corresponding to GluD1 dimers in non-reducing conditions for I155C and K514C, indicating that the ATD and LBD indeed exist in a two-fold symmetric dimeric arrangement (Fig. 3a). Furthermore, the F385C mutant also formed dimers, suggesting that the two extracellular arms of the receptor tetramer can interact at the ATD dimer-of-dimers interface, reminiscent of that observed in AMPA and kainate receptors.

We also made the double cysteine mutant I155C K514C, which would lock the receptor at both the ATD and LBD layers, resulting in bands corresponding to tetramers on non-reducing SDS–PAGE gels in the case of domain-swapped architecture and dimers.
for non-swapped assemblies. Consistent with our GluD1 structure, bands corresponding to dimers and not tetramers were observed, indicating non-swapped architecture of GluD1 receptors in vivo; however, we did observe higher oligomers (bigger than tetramers), probably due to nonspecific linkages (Fig. 3a).

In order to validate this assembly further, we carried out glutaraldehyde cross-linking of purified GluD1 receptors, with GluA2 as a control. Due to its non-swapped architecture, the probability for getting dimers would be higher in GluD1 receptors compared to GluA2 receptors, in which, due to subunit crossover, the probability for all protomers becoming cross-linked would be higher, leading to tetramers in SDS–PAGE gels. Consistent with this notion, cross-linking with 3 mM glutaraldehyde for 2 and 5 min yielded dimers primarily on SDS–PAGE for GluD1 receptors, whereas tetramers were observed for GluA2 receptors (Supplementary Fig. 8).

C-terminal truncation does not affect the assembly of GluD1 receptors. We strived to use a minimally modified construct of GluD1 for protein expression and purification. The only modification we made was C-terminal truncation at residue 851. In order to demonstrate that this C-terminal deletion does not affect the receptor assembly and its functionality, we utilized a chimeric receptor approach, because native GluD1 receptors do not evoke ligand-gated currents. For this, we generated chimeric receptors in which the LBD of GluD1 is swapped with that of GluK2, resulting in glutamate- and kainate-sensitive receptors32 (Supplementary Notes).
are in Extended Data Fig. 9 and Supplementary Notes. Data for graphs in b, c in HEK-293T cells on the application of 10 mM glutamate is shown in the normalized traces for GluD1Δ851 and GluD1(K2LBD)Δ shown. The error bars represent standard error of the mean. The number of cells used for the recordings is 3 and GluD1(K2LBD)Δ for GluD1(K2LBD)Δ851 receptors. Although we got no measurable response on 10 mM glutamate application from GluD1Δ851 receptors, robust currents were blocked when Na+ in extracellular solution was replaced with the large cation N-methyl-d-glucamine (NMDG), demonstrating that these constitutive currents are mediated by GluD1 receptors (Extended Data Fig. 9c–e). Thus, our electrophysiology experiments demonstrate that the C-terminal deletion that is necessary for overexpression and purification of GluD1 does not affect receptors assembly.

Discussion

Using a minimally modified construct, we have purified and determined the structure of detergent-solubilized GluD1 receptors in solution. Owing to the conformational heterogeneity of GluD1, the resolution of our EM maps is limited to ~8 Å. However, by fitting crystal structures and models of various domains into the constraints of an EM density map, we provide the first insight into the subunit arrangement for a homotetrameric GluD1 receptor. While all the previous models for GluD receptors depict domain swapping at the ATD−LBD interface, our study shows a unique non-swapped architecture for this enigmatic class of receptors. However, whether this non-crossover architecture is the sole cause for the inactive ion channel needs to be addressed in the future.

Due to the non-crossover architecture, the two extracellular arms of the receptor seem to have a broad range of movement, resulting in conformations in which the receptor could adopt a splayed conformation. Recent data show that other iGluRs, which, unlike delta receptors, have swapped architecture and show agonist-gated ion channel activation, can also adopt splayed ATD conformations in detergent-solubilized states (Extended Data Fig. 7), albeit to a limited extent. It is to be noted that, the splayed conformations may not be physiological due to the higher concentration of proteins in membranes and interactions with other synaptic proteins[35,49], auxiliary subunits[63] that would likely limit movements.

Another important feature of the GluD1 receptor is the close packing of ATD and LBD domains. Although it is not currently clear whether this is driven by the shorter ATD−LBD linker compared with other iGluRs, it has been reported that mutation of the linkers leads to loss of function in GluD2 receptors. Pertaining to this notion, d-serine application induced parallel fiber (PF) long-term depression in cerebellar slices in Purkinje cells (PC) expressing wild-type receptors, but it failed to do so in Purkinje cells expressing GluD2, in which a glycosylated linker was inserted between the two domains[21]. This finding could result from the uncoupling of the ATD−LBD interactions, which might lead to reduced transduction of forces generated by ligand binding to the TM domains. However, the GluA2 receptor with a shorter ATD−LBD linker still crystallized in a domain-swapped configuration and was gated by glutamate[42]. This finding, along with other recent pieces of evidence, suggests that not only the short linkers but the ATD−LBD interface interactions and the contribution of the hinge region of the GluD2 LBD to the weak ligand affinity[38, among other things, might also contribute to the inactivity of the GluD receptors. However, this needs to be established by future studies.

Our study also raises the important questions of what drives the subunit crossover in AMPA and kainate receptors, and why is this not observed in GluD1, the answers to which are still unclear. For

![Figure 3](https://example.com/figure3.png)
AMPA and kainate receptors, evidence from EM of intermediates of the biosynthesis process shows that GluA2 dimer synthesis precedes tetramer formation and that in the dimers, the ATD and TM segments are closely apposed, whereas the LBDs are too far apart to interact. This arrangement is suitable for domain swapping on the assembly of a second dimer pair to form tetramers. By contrast, for the LBD-dimer-stabilizing L483Y mutant, all three segments are closely apposed, similarly to that observed in our GluD1 structure. However, subsequent tetramer formation in GluA2 L483Y was observed to be strongly inhibited, because the subunit crossover observed in the full-length GluA2 structure cannot occur when the LBD dimer pairs cannot separate. This assembly mechanism is supported by nanomolar affinities of the ATD dimerization and low micromolar to millimolar affinity for LBD dimerization. The GluD1 structure with closely apposed ATD−LBD domains and no subunit crossover, however, points toward a different mode of assembly. It is speculated that due to subunit crossover in the ATD and LBD layers, the tetramers in AMPA and kainate are unlikely to be assembled in a cooperative process involving all four subunits. In the case of GluD1, the assembly is likely to be straightforward, with subunits assembling as two dimers and the tetramer being formed by a simple assembly of the two dimers, with no subunit crossover at the ATD−LBD layer. This is also supported by the nanomolar affinities of ATD dimerization in GluD receptors and the fact that isolated GluD2 LBDs in the apo state have been shown to crystallize as dimers in the presence of Ca²⁺ ions, which can modulate the strength of dimer formation. Furthermore, the extracellular domains (ATD−LBD) of GluD2 also crystallized as dimers but with interactions only at the ATD layer, and the apo LBDs adopted an unusual 'swing-out' conformation in the absence of Ca²⁺ ions and antagonists. This GluD2 ATD−LBD dimer could not be modeled into a receptor tetramer without significant reorganization and reorientation of the LBD domains. We believe that in the absence of the constraints exerted by linkers and the TM domain, and due to crystal lattice contacts, the LBDs likely adopt a non-physiological conformation in this study.

Furthermore, AMPA, kainate and NMDA are able to assemble into functional receptors even after ATD deletion. However, a recent study on ATD-deleted NMDA receptors revealed that a fraction of the receptor population adopted LBD packing analogous to what we observe in the GluD1 structure, thus highlighting the importance of ATDs in guiding the subunit arrangement of the LBD layer. The well-resolved LBD-TM linkers in this ΔATD NMDA receptor adopted a relaxed conformation, likely rendering the receptor inactive. Thus, aspects of our structures are seen in other iGluRs, but the lack of subunit crossover is unique to GluD1.

Multiple studies have shown that ATDs, which are the most distal domains of GluD receptors, directly interact with cerebelins, which are the most distal domains of GluD receptors, directly interact with cerebelins,

**Fig. 4 | Orientation and arrangement of the ATD and LBD domains.** a, b, Arrangement of the planes of ATD and LBD dimers in GluA2 and GluD1 in side (a) and top (b) views. The ATD planes are shown in red, and the LBD planes are in blue. The angles formed between the ATD−LBD planes are measured and shown in a, c, Model of the GluD1−Cbln complex generated via superimposition of the GluD2 ATD−Cbln complex onto the ATD dimers of GluD1 (compact conformation). d, Top view of the GluD1−Cbln binary complex is shown with the distances between the COMs of the Cbln1 trimers. e, Schematic representation of the tripartite neurexin, Cbln and GluD receptor trans-synaptic complex. The non-swapped architecture may allow movements of the GluD receptor arms to accommodate the entire trans-synaptic complex in the 20- to 25-nm synaptic cleft.
which in turn couple with neurexins, the trans-synaptic complex. The arrangement and orientation of ATDs in GluD1 (Fig. 4a,b) does not occlude or restrict the cerebellin interaction surface. Upon superimposing the ATD-cerebellin complex onto GluD1 ATD, the receptor ectodomain (ATD-LBD) and cerebellin (Cbln) has a length of ~16 nm, similar to the ~17-nm distance reported earlier11 (Fig. 4c,d). Based on this finding, we propose a model (Fig. 4e) for the tripartite complex formed between GluD receptors, cerebellin and neurexin that mediate trans-synaptic interactions and is essential for maintaining the synaptic integrity of PF-PC synapses. Our model is similar to that proposed earlier20,21, except for the non-crossover architecture observed in the GluD1 receptor. We also postulate that this anchoring of GluD receptors to the β-NRX1(+)–Cbln1 complex will limit or prevent large-scale motions of the two extracellular arms.

In summary our results provide a molecular framework to design future studies directed toward resolving the long-standing questions concerning this family of receptors. These results suggest that orphan delta receptors of the iGluR family are likely to have a different mode of assembly and provide a foundation for future studies directed toward understanding the functions of these receptors in light of this structural information.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41594-019-0359-y.

Received: 14 August 2019; Accepted: 27 November 2019; Published online: 10 January 2020

References

1. Gao, J. et al. Orphan glutamate receptor 61 subunit required for high-frequency hearing. Mol. Cell. Biol. 27, 4500–4512 (2007).

2. Yuzaki, M. The δ2 glutamate receptor: a key molecule controlling synaptic plasticity and structure in Purkinje cells. Cerebellum 3, 89–93 (2004).

3. Yuzaki, M. & Aricescu, A. R. A GluD coming-of-age story. Trends Neurosci. 40, 138–150 (2017).

4. Twomey, E. C. & Sobolevsky, A. I. Structural mechanisms of gating in ionotropic glutamate receptors. Biochemistry 57, 267–276 (2018).

5. Greger, J. H. & Mayer, M. L. Structural biology of glutamate receptor ion channels: towards an understanding of mechanism. Curr. Opin. Struct. Biol. 57, 185–195 (2019).

6. Yamazaki, M., Araki, K., Shibata, A. & Mishina, M. Molecular cloning of a cDNA encoding a novel member of the mouse glutamate receptor channel family. Biochem. Biophys. Res. Commun. 183, 886–892 (1992).

7. Lomelí, H. et al. The δ1 delta-1 and δ2 subunits extend the excitatory amino acid receptor family. FEBS Lett. 315, 318–322 (1993).

8. Schmid, S. M. & Hollmann, M. To gate or not to gate: are the δ subunits in the glutamate receptor family functional ion channels? Mol. Neurobiol. 37, 126–141 (2008).

9. Hirai, H. et al. Rescue of abnormal phenotypes of the δ2 glutamate receptor-null mice by mutant δ2 transgenes. EMBO Rep. 6, 90–95 (2005).

10. Yuzaki, M. New insights into the structure and function of glutamate receptors: the orphan receptor δ2 reveals its family’s secrets. Kinet. J. Med. 52, 92–99 (2003).

11. Kakegawa, W., Kohda, K. & Yuzaki, M. The δ2 ‘ionotropic’ glutamate receptor functions as a non-ionotropic receptor to control cerebellar synaptic plasticity. J. Physiol. 584, 89–96 (2007).

12. Kohda, K. et al. The δ2 glutamate receptor gates long-term depression by coordinating interactions between two AMPA receptor phosphorylation sites. Proc. Natl Acad. Sci. USA 110, E948–E957 (2013).

13. Yadav, R. et al. Deletion of glutamate delta-1 receptor in mouse leads to aberrant emotional and social behaviors. PLoS One 9, e93269 (2012).

14. Kondo, T., Kakegawa, W. & Yuzaki, M. Induction of long-term depression and phosphorylation of the δ2 glutamate receptor by protein kinase C in cerebellar slices. Eur. J. Neurosci. 22, 1817–1820 (2005).

15. Utine, G. E. et al. A homozygous deletion in GRID2 causes a human phenotype with cerebellar ataxia and atrophy. J. Child Neurol. 28, 926–932 (2013).

16. Miyoshi, Y. et al. A new mouse allele of glutamate receptor delta 2 with cerebellar atrophy and progressive ataxia. PLoS One 9, e107867 (2014).

17. Van Schd, K. et al. Early-onset autosomal recessive cerebellar ataxia associated with retinal dystrophy: new human hotfoot phenotype caused by homozygous GRID2 deletion. Genet. Med. 17, 291–299 (2015).

18. Guo, S.-Z. et al. A case-control association study between the GRID1 gene and schizophrenia in the Chinese Northern Han population. Schizophr. Res. 93, 385–390 (2007).

19. Benamer, N. et al. Gluδ1, linked to schizophrenia, controls the burst firing of dopamine neurons. Mol. Psychiatry 23, 691–700 (2018).

20. Liu, J., Gandhi, P. J., Pavuluri, R., Shelkar, G. P. & Dravid, S. M. Glutamate delta-1 receptor regulates cocaine-induced plasticity in the nucleus accumbens. Transl Psychiatry 8, 219 (2018).

21. Uemura, T. et al. Trans-synaptic interaction of GluRδ2 and neurexin through Cbln1 mediates synaptic formation in the cerebellum. Cell 141, 1068–1079 (2010).

22. Lee, S.-J., Uemura, T., Yoshida, T. & Mishina, M. GluRδ2 assembles four neurexins into trans-synaptic triad to trigger synapse formation. J. Neurosci. 32, 4688–4701 (2012).

23. Tao, W., Díaz-Alonso, J., Sheng, N. & Nicoll, R. A. Postsynaptic δ1 glutamate receptor assembles and maintains hippocampal synapses via Cbln2 and neurexin. Proc. Natl Acad. Sci. USA 115, E3573–E3581 (2018).

24. Yasumura, M. et al. Glutamate receptor δ1 induces preferentially inhibitory presynaptic differentiation of cortical neurons by interacting with neurexins through cerebellin precursor protein subtypes. J. Neurochem. 121, 705–716 (2012).

25. Elegehej, J. et al. Structural basis for integration of Gluδ receptors within synaptic organizer complexes. Science 353, 295–299 (2016).

26. Uemura, T., Mori, H. & Mishina, M. Direct interaction of GluRδ2 with Shank scaffold proteins in cerebellar Purkinje cells. Mol. Cell. Neurosci. 26, 330–341 (2004).

27. Takeuchi, T. et al. Control of synaptic connection by glutamate receptor δ2 in the adult cerebellum. J. Neurosci. 25, 2146–2156 (2005).

28. Wollmuth, L. P. et al. The Lurcher mutation identifies δ2 as an AMPA/kainate receptor-like channel that is potentiated by Ca2+. J. Neurosci. 20, 5973–5980 (2000).

29. Ikeno, K., Yamakura, T., Yamazaki, M. & Sakimura, K. The Lurcher mutation reveals Ca2+ permeability and PKC modification of the GluRδ channels. Neurosci. Res. 41, 193–200 (2001).

30. Yadav, R., Rimmerman, R., Scofield, M. A. & Dravid, S. M. Mutations in the transmembrane domain M3 generate spontaneously open orphan glutamate δ1 receptor. Brain Res. 1382, 1–8 (2011).

31. Schmid, S. M., Kott, S., Sager, C., Huelsken, T. & Hollmann, M. The glutamate receptor subunit δ3αβ is capable of gating its intrinsic ionic channel as revealed by ligand binding domain transplantation. Proc. Natl Acad. Sci. USA 106, 10320–10325 (2009).

32. Orth, A., Tapken, D. & Hollmann, M. The δ subfamily of glutamate receptors: characterization of receptor chimeras and mutants. Eur. J. Neurosci. 37, 1620–1630 (2013).

33. Naur, P. et al. Ionotropic glutamate-like receptor δ2 binds D-serine and glycine. Proc. Natl Acad. Sci. USA 104, 4116–4121 (2007).

34. Hansen, K. B. et al. Modulation of the dimer interface at ionotropic glutamate-like receptor δ2 by D-serine and extracellular calcium. J. Biol. Chem. 283, 253–262 (2016).

35. Perroy, J. et al. Direct interaction enables cross-talk between ionotropic and group I metabotropic glutamate receptors. J. Biol. Chem. 283, 6799–6805 (2008).

36. Suryawanshi, P. S. et al. Glutamate delta-1 receptor regulates metabotropic glutamate receptor 5 signaling in the hippocampus. Mol. Pharmacol. 89, 253–262 (2016).

37. Scheres, S. H. W. & Chen, S. Prevention of overfitting in cryo-EM structure determination. Nat. Methods 9, 853–854 (2012).

38. Tao, W. et al. Mechanisms underlying the synaptic trafficking of the glutamate delta receptor GluD1. Mol. Psychiatry 24, 1451–1466 (2019).

39. Nakagawa, E., Cheng, R. S., Ramon, E., Sheng, M. & Walz, T. Structure and different conformational states of native AMPA receptor complexes. Nature 433, 545–549 (2005).
45. Dürr, K. L. et al. Structure and dynamics of AMPA receptor GluA2 in resting, pre-open, and desensitized states. *Cell* **158**, 778–792 (2014).
46. Meyerson, J. R. et al. Structural mechanism of glutamate receptor activation and desensitization. *Nature* **514**, 328–334 (2014).
47. Jalali-Yazdi, F., Chowdhury, S., Yoshioka, C. & Gouaux, E. Mechanisms for zinc and proton inhibition of the GluN1/GluN2A NMDA receptor. *Cell* **175**, 1520–1532.e15 (2018).
48. Zhao, Y., Chen, S., Swensen, A. C., Qian, W.-I. & Gouaux, E. Architecture and subunit arrangement of native AMPA receptors elucidated by cryo-EM. *Science* **364**, 355–362 (2019).
49. Matsuda, K. et al. Transsynaptic modulation of kainate receptor functions by C1q-like proteins. *Neuron* **90**, 752–767 (2016).
50. Chen, S. et al. Activation and desensitization mechanism of AMPA receptor-TARP complex by cryo-EM. *Cell* **170**, 1234–1246.e14 (2017).
51. Twomey, E. C., Yelshanskaya, M. V., Grassucci, R. A., Frank, J. & Sobolevsky, A. I. Structural bases of desensitization in AMPA receptor- auxiliary subunit complexes. *Neuron* **94**, 569–580.e5 (2017).
52. Sobolevsky, A. I., Rosconi, M. P. & Gouaux, E. X-ray structure, symmetry and mechanism of an AMPA-subtype glutamate receptor. *Nature* **462**, 745–756 (2009).
53. Tapken, D. et al. The low binding affinity of D-serine at the ionotropic glutamate receptor GluD2 can be attributed to the hinge region. *Sci. Reports* **7**, 46145 (2017).
54. Shanks, N. F., Maruo, T., Farina, A. N., Ellisman, M. H. & Nakagawa, T. Contribution of the global subunit structure and stargazin on the maturation of AMPA receptors. *J. Neurosci.* **30**, 2728–2740 (2010).
55. Zhao, H. et al. Preferential assembly of heteromeric kainate and AMPA receptor amino-terminal domains. *eLife* **6**, e32056 (2017).
56. Zhao, H. et al. Analysis of high-affinity assembly for AMPA receptor amino-terminal domains. *J. Gen. Physiol.* **141**, 747–749 (2013).
57. Chaudhry, C., Plested, A. J., Schuck, P. & Mayer, M. L. Energetics of glutamate receptor ligand binding domain dimer assembly are modulated by allosteric ions. *Proc. Natl Acad. Sci. USA* **106**, 12329–12334 (2009).
58. Cheng, S., Seven, A. B., Wang, J., Skiniotis, G. & Özkan, E. Conformational plasticity in the transsynaptic neurexin-cerebellin-glutamate receptor adhesion complex. *Structure* **24**, 2163–2173 (2016).
59. Song, X. et al. Mechanism of NMDA receptor channel block by MK-801 and memantine. *Nature* **556**, 515–519 (2018).
60. Matsuda, K. et al. Cbln1 is a ligand for an orphan glutamate receptor δ2, a bidirectional synapse organizer. *Science* **328**, 363–368 (2010).

**Publisher’s note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations. © The Author(s), under exclusive licence to Springer Nature America, Inc. 2020
Methods
Cell lines. The HEK293 GnT1 (ATCC CRL-3022) were obtained and authenticated at ATCC, and no further authentication or mycoplasma testing was performed.

Construct design. Rat GluD1 was cloned into the pEGBacMam vector in frame with a C-terminal thrombin-recognition site (GLV/PRGSAAAA) and EGFP (A207K mutant) with a C-terminal octa-histidine (His8) tag. Full-length GluD1 had weak expression and stability, as determined via FSEC. Further screening of constructs identified GluD1 ΔS51 as a promising candidate for overexpression and purification.

For cysteine cross-linking experiments, mutants were generated by using site-directed mutagenesis. The cysteine-knockout constructs C625A and C839A were first generated on the GluD1 ΔS51 background (GluD1-2x) and then used for incorporating mutations H155C (ATD), F385C (ATD dimer-of-dimers interface) and K514C (LBD). Additionally, combination mutant GluD1-2x H155C K514C was also generated.

For electrophysiology experiments, wild-type rat GluD1 and GluD1 ΔS51 was cloned into a pPK5 expression vector. GluK2 LBD chimeras were generated by exchanging GluD1 ΔS51, T148–P528, S2, P664–D792) with that of GluK2: S1, S398–N515; S2, P636–E775 to generate constructs GluD1(K2LBD) and GluD1(K2LBD) ΔS51. The constitutively active receptors were generated by site-directed mutagenesis of alanine 634 in the SYTANLA motif to cysteine (numbering as per mature polypeptide) in GluD1 wild type and the GluD1 ΔS51 construct. All the constructs were verified by sequencing of the entire coding region.

Expression and purification. HEK293GnT1 cells were adapted to grow in suspension cultures in freestyle 293 expression media supplemented with 2% FBS (Gibco), 2 mM glutamine (Gibco) and 1% Pen-Strep (Gibco) and were infected at a multiplicity of infection 1.5 to 3.5 with P2 baculovirus at a multiplicity of infection 1. To boost the expression of the protein, 10 mM sodium butyrate (Sigma) was added 20 h post-infection, and cultures were incubated at 30 °C. The cells were harvested ~48–52 h later by centrifugation at 6,000 rpm for 20 min. The cell pellet was collected and stored at −80 °C for further processing. The frozen cell pellet was thawed at room temperature for 10 min and was resuspended in a buffer (20 mM L-1 cell culture) containing 150 mM NaCl, 20 mM Tris, pH 8.0, along with protease inhibitor cocktail (Roche). The resuspended cells were disrupted by ultrasonication (Qsonica sonicator, four cycles of 90 s (15 s on/15 s off) with power level 7, using a medium-sized probe) with constant stirring. Care was taken to keep the temperature below 12 °C throughout the sonication. The lysate was first clarified by low-speed centrifugation, and membranes were collected by ultracentrifugation at 40,000 rpm for 1 h. Membrane pellets were homogenized and solubilized for 45 min in buffer containing 150 mM NaCl, 20 mM Tris, pH 8.0, 40 mM n-dodecyl-β-D-maltopyranoside, and 6.7 mM cholesterol hexanicuminate at 4 °C. The detergent-solubilized fraction was collected by centrifugation at 40,000 rpm for 1 h, and cobalt-charged TALON metal affinity resin (~4 ml bed volume) was added to the supernatant, together with 10 mM imidazole to allow batch binding for 3 h at 4 °C. The resin was packed in a column, washed with 40 mM imidazole-containing buffer (20 mM Tris, 150 mM NaCl, 0.75 mM n-dodecyl-β-D-maltopyranoside, 0.01 mM cholesterol hexanicuminate) until the baseline reached zero. Bound GluD1 receptors were eluted by 250 mM imidazole-containing buffer. The fractions containing the protein of interest were pooled and kept at −80 °C. The purified protein was incubated in 150 mM NaCl, 851 mM potassium chloride, 10 mM HEPES, 5 mM EGTA, 2 mM Na2ATP and 0.5 mM CaCl2, pH 7.2 (osmolarity 295–305 mOsmol L−1). Viral particles were then incubated for 15 min with monoclonal His, antibodies (Sigma) raised in rabbit. After four 15-min washes with TBST (TBS + Tween-20 0.05%), the membranes were incubated for 1 h at room temperature with anti-rabbit goat antibodies conjugated to horsedarhid peroxidase. Then the membranes were washed twice for 15 min with TBST with 2% Tween and 2% with TBS, and immunoreactivity was visualized using the ECL detection kit (Invitrogen).

Cysteine cross-linking and western blots. For GluD1 cysteine cross-linking experiments, plasmid DNA encoding GluD1-2x, GluD1-1x with mutants H155C (ATD), F385C (ATD dimer-of-dimers interface), K514C (LBD) and GluD1-2x– H155C K514C were transiently transfected for expression in HEK293T cells. Cells were harvested 24–48 h after transfection and resuspended in TBS buffer (20 mM Tris, pH 8.0, 150 mM NaCl) supplemented with protease inhibitor cocktail.

Cells were sonicated, and membrane fractions were harvested following low-speed and ultralow centrifugation. Membranes were solubilized in TBS buffer supplemented with 40 mM DDM and 2 mM CHS for 1 h at 4 °C, clarified by ultracentrifugation and then run on a 6% SDS–PAGE gel either in the absence (non-reducing condition) or in the presence (reducing condition) of 100 mM DTT. Protein bands were electroblotted onto PVDF membranes (Amersham Biosciences) and were blocked for 1 h at room temperature in TBST (150 mM NaCl, 10 mM Tris–HCl, pH 7.6, 0.1% Tween-20) containing 5% non-fat milk and then incubated for 1 h with monoclonal His, antibodies (Sigma) raised in rabbit. After four 15-min washes with TBST (TBS + Tween-20 0.05%), the membranes were incubated for 1 h at room temperature with anti-rabbit goat antibodies conjugated to horseradish peroxidase. Then the membranes were exposed twice for 15 min with TBST with 2% Tween and 2% with TBS, and immunoreactivity was visualized using the ECL detection kit (Invitrogen).

Glutaryldeacyl hydrolase cross-linking. Purified and detergent-solubilized rat GluD1 ΔS51 and GluA2cryst receptors in 20 mM HEPES, 150 mM NaCl and 1 mM DDM were incubated with 3 mM of glutaryldeacyl for 2, 5 and 10 min at 37 °C to allow cross-linking. The cross-linking reaction was stopped by quenching with 50 mM Tris, after which, the protein was resolved on SDS–PAGE and stained with comassie blue for band visualization.

Electrophysiology. Rat GluD1 wild type, GluD1 ΔS51, GluD1(K2LBD), and GluD1(K2LBD) ΔS51 receptors were tested for activity by whole-cell patch-clamp recordings. Assays were carried out 36–48 h post-transfection in HEK293T cells. Pipettes were pulled (Sutter, P-1000) from borosilicate glass capillaries (1.05 OD x 1.17 x 100 mm, Harvard Apparatus) and polished to 2–3 MΩ resistance, filled with internal solution containing 30 mM CsCl, 100 mM CsA, 4 mM NaCl, 10 mM HEPES, 5 mM EGTA, 2 mM Na2ATP and 0.5 mM CaCl2, pH 7.2 (osmolarity ranging from 290–300 mOsmol L−1). External solution contained 110 mM KCl, 10 mM HEPES, and 0.5 mM CaCl2, pH 7.3 and osmolarity ranging between 295–305 mOsmol L−1. 10 μM of glutamate or 1 mM kainate dissolved in external solution was applied for 100 ms to measure the whole-cell desensitization kinetics. The whole-cell recordings were carried out using Patchmaster V2X902 (Heka Elektronik) 3 min after the establishment of the whole-cell configuration. Raw data files were exported into Igor pro (TIX) and converted into abf files, compatible for pClamp by using ABF Utility. The macroscopic rate of desensitization (∝) was measured by the exponential fit to the decay of current from ~90% of its peak amplitude (∝) to baseline. The desensitization kinetics were fitted by using the single exponential, two-term fitting (Levenberg–Marquardt). The weighted ∝ fitted from two-term fitting using Eq. (4) and ∝ fitted from two-term fitting using Eq. (5) at T1 = A1 x T1 + A2 x T2) (A1 + A2), where weight = 1, weight = 2, A1 = tau 1, τ2 = tau 2. Ratios of glutamate and kainate-evoked currents were determined in 3 or 4 independent experiments and subjected to statistical analysis using Prism 8.0.

For recording constitutively active currents, mutation A634C (numbering as per mature polypeptide) was introduced by site-directed mutagenesis in both full-
length GluD1 and GluD1 Δ851. Recordings were performed between 16 and 28 h post-transfection from HEK293T cells. Pipettes were pulled as described above and were filled with internal solution containing 140 mM CsCl, 10 mM HEPES, 1 mM Ba2+ and 2 mM Na3ATP with pH adjusted to 7.2 with CsOH (osmolarity ranging 290–300 mOsmol L−1). The extracellular solution consisted of 135 mM NaCl, 5.4 mM KCl, 0.5 MgCl2, 5 mM HEPES, pH 7.2, adjusted by NaOH as described previously28. For 7-CKA, α-serine or calcium application, solutions were prepared by addition of either 2 mM CaCl2 or 1 mM 7-CKA, 10 mM α-serine in the extracellular solution. The NMDG solution consisted of 140 mM NMDG, 0.5 mM MgCl2, and 5 mM HEPES. The seal resistance before entering into the whole-cell configuration was always at least 1 GΩ. Currents were recorded at room temperature, using HEKA EPC10 with Patchmaster as described previously.

Statistics. No statistical methods were used to determine the sample size. The experiments were not randomized, and the investigators were not blinded to allocation during experiments and outcome assessment.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
The cryo-EM density reconstruction and final models were deposited in the Electron Microscopy DataBase (accession code EMD-0744 for the compact conformation and EMD-0773 for the splayed conformation) and in the Protein Data Bank (accession code PDB 6KSS for the compact conformation and EMD-0773 for the splayed conformation). The raw movie data have been submitted to the EMPIAR database. All other relevant data supporting the key findings of this study are available within the article and its Supplementary Information files or from the corresponding author upon reasonable request. Source data for Fig. 3a–c and Extended Data Fig. 9e are available with the paper online.

References
61. Kandiah, E. et al. CM01: a facility for cryo-electron microscopy at the European Synchrotron. Acta Crystallogr. D Struct. Biol. 75, 528–535 (2019).
62. Zheng, S. Q. et al. MotionCor2: anisotropic correction of beam-induced motion for improved cryo-electron microscopy. Nat. Methods 14, 311–332 (2017).
63. Zhang, K. Geif: Real-time CTF determination and correction. J. Struct. Biol. 193, 1–12 (2016).
64. Punjani, A., Rubinstein, J. L., Fleet, D. J. & Brubaker, M. A. cryoSPARC: algorithms for rapid unsupervised cryo-EM structure determination. Nat. Methods 14, 290–296 (2017).
65. Pettersen, E. F. et al. UCSF Chimera—a visualization system for exploratory research and analysis. J. Comput. Chem. 25, 1605–1612 (2004).
66. Kidmose, R. T. et al. Naminator—automatic molecular dynamics flexible fitting of structural models into cryo-EM and crystallography experimental maps. IUCrJ 6, 526–531 (2019).
67. Afonine, P. V. et al. Real-space refinement in PHENIX for cryo-EM and crystallography. Acta Crystallogr. D Struct. Biol. 74, 531–544 (2018).
68. Davis, I. W., Murray, L. W., Richardson, J. S. & Richardson, D. C. MOLPROBITY: structure validation and all-atom contact analysis for nucleic acids and their complexes. Nucleic Acids Res. 32, W615–W619 (2004).

Acknowledgements
This work was supported by the Wellcome Trust DBT India Alliance fellowship (grant number IA/I/13/2/501023) awarded to J.K. A.P.B. thanks ICMR (3/1/3/JRF-2014/ HRD-86(60237), India for a senior research fellowship. R.V. thanks SERB for an N-PDF fellowship (N-PDF/2016/002621). M. L. Mayer, NIH, Bethesda kindly gifted the various iGluR constructs that were subcloned and used for construct optimization and mutational studies. E. Gouaux (OHSU, Portland) kindly provided the pEG BacMam vector. We acknowledge the European Synchrotron Radiation Facility for provision of microscope time on CM01, and we thank M. Hons for assistance in EM data collection. We thankfully acknowledge the kind help of M. Karuppusamy, EMBL in grid vitrification. Help with initial screening of conditions for grid vitrification from P. I. Peters, R. Raveli at Måå, Maastricht University, Maastricht, the Netherlands and V. K. Ragunath, National Electron Cryo-Microscopy facility at the Bangalore Life Sciences Cluster (DBT/PR12422/MED/31/287/2014), NCBS, Bangalore is gratefully acknowledged. We are also thankful to A. Kembhavi and K. Vaghmare, The Inter-University Centre for Astronomy and Astrophysics, Pune for helping with transfer and storage of raw EM data.

Author contributions
A.P.B. optimized the construct and purified protein, did all of the molecular biology and biochemical experiments, and processed EM data with assistance from J.K. Electrophysiology experiments were done by R.V. J.K. supervised the overall project design and its execution. All authors contributed to the analysis and preparation of the manuscript and approved the final draft.

Competing interests
The authors declare no competing interests.

Additional information
Extended data is available for this paper at https://doi.org/10.1038/s41594-019-0359-y. Supplementary information is available for this paper at https://doi.org/10.1038/s41594-019-0359-y.

Correspondence and requests for materials should be addressed to J.K.

Peer review information Katarzyna Marcinkiewicz was the primary editor on this article and managed its editorial process and peer review in collaboration with the rest of the editorial team.

Reprints and permissions information is available at www.nature.com/reprints.
Extended Data Fig. 1 | GluD1 purification and cryo-EM data processing. **a**, Schematic representation of the optimized GluD1 construct showing the C-terminal truncation at residue 851 and C-terminal thrombin cleavage site, along with GFP and octa-histidine tag (Supplementary Notes). **b**, Size-exclusion profile of the final purified protein showing receptor stability in optimized buffer conditions. **c**, Selected 2D class averages from reference-free 2D classification of GluD1 in complex with 1 mM 7-CKa and 1 mM Ca²⁺. The white arrows mark a few classes that show conformational heterogeneity of the extracellular receptor domains. **d**, 3D classification of GluD1 into seven classes reveals heterogeneity due to the movement of the two extracellular arms. More details are in Extended Data Fig. 2 and Supplementary Fig. 1.
Extended Data Fig. 2 | Cryo-EM data processing work flow. A total of 72,149 good particles were obtained by several cycles of 2D class averaging of particles from 4,120 micrographs. The 3D map generated by ab initio 3D reconstruction was further refined heterogeneously into seven conformationally distinct 3D classes. The 3D classes showed heterogeneity due to movement of the two extracellular arms. For the purpose of model building and analysis, a compact (class 5) and a splayed (class 7) conformation map were further refined to a resolution of 8.1 Å and 7.6 Å, respectively.
Extended Data Fig. 3 | Local resolution estimates of the cryo-EM maps. a, d, The sharpened cryo-EM densities of GluD1 Δ851 in 7-CKA and calcium bound form, colored based on local resolution. b, e, Euler angle distribution of particles for the two models is shown. c, f, FSC curves for the cryo-EM maps with mask (red) without mask (blue). The resolution of map corresponding to FSC 0.5 and 0.143 is indicated.
Extended Data Fig. 4 | Splayed conformation of GluD1 receptor. a–f, Architecture of splayed conformation of GluD1 receptors in complex with 7-CKA and calcium. a, Side view highlighting the broadest face of the Y-shaped receptor and 90° rotated views of the sharpened 3D density map. Each subunit is depicted in a different color. The EM reconstructions clearly show the non-swapped arrangement of the ATD and LBD layers. The distances between the centroids (R1-R1 of ATD domains) for AB and CD dimer pairs are shown above the model. The vertical separation between the COMs of ATD dimers and LBD dimers are also shown. Panel b shows the segmented density map for subunits A and B fitted with protein coordinates. c, Superimposition of subunits B/D, B/C, A/D and A/C are shown highlighting similar AB and BC conformations. Helices and sheets are represented as pipes and planks, respectively. Top views of ATD (d), LBD (e) and TM domains (f) are shown. The distances and the angles subtended between the COM of various subunits were measured and are indicated below the top views. More details are in Extended Data Figs. 5 and 6.
Extended Data Fig. 5 | Architecture and domain arrangement in compact GluD1 model. 

a, Cryo-EM density map of compact GluD1 model is shown in a view parallel to the membrane. The four subunits A, B, C, D are colored in orange, green, yellow and cyan, respectively. The colored spheres represent the COM of the ATD and LBD domains. 

b, Top view of ATD with segmented EM density map fitted with atomic models is shown. The distances between the COMs of ATDs shown with dashed lines below the EM density map, depicting the arrangement of ATDs in the plane. 

c, Densities corresponding to LBDs fitted with atomic models. 

d, The distances from COMs of ATD and LBD are shown. The LBD plane is depicted as a circular disk, and the ATD plane is shown as a dashed ellipse. 

e, f, Side and top views of angles subtended by COMs of ATD with the COM of the LBD layer. COM plane of the LBD layer is indicated by metallic disk.
Extended Data Fig. 6 | Architecture and domain arrangement in splayed GluD1 model. 
a, Cryo-EM density map of splayed GluD1 model is shown in a view parallel to the membrane. The four subunits A, B, C, D are colored in orange, green, yellow and cyan, respectively. The colored spheres represent the COM of ATD and LBD domains. 
b, Top view of ATD with segmented EM density map fitted with atomic models is shown. The distances between the COMs of ATDs are shown with dashed lines below the EM density map, depicting the arrangement of ATDs in the plane. 
c, Densities corresponding to LBDs fitted with atomic models is shown. 
d, The distances from COMs of ATD and LBD are shown. The LBD plane is depicted as a circular disk, and the ATD plane is shown as a dashed ellipse. 
e, f, Side and top views of angles subtended by COMs of ATD with the COM of the LBD layer. COM plane of the LBD layer is indicated by metallic disk.
Extended Data Fig. 7 | Domain arrangement in GluD1, GluA2, GluK2, GluN1/GluN2A and GluN1/GluN2B receptors. a. Top views of the ATD (a), LBD (b) and TM domains (c) are shown for GluD1, GluA2, GluK2, GluN1/GluN2A and GluN1/GluN2B receptors highlighting the subunit arrangement. Each chain is uniquely colored, and domain arrangement is also depicted in cartoon below each layer. Comparisons for compact and super-splayed conformations of NMDA receptors with that of GluD1 are shown highlighting the fact that in all the conformations of AMPA, kainate and NMDA receptors, the domain swapping between the ATD and LBD layers exists unlike that in GluD1.
Extended Data Fig. 8 | Buried surface area between the subdomains. Surface illustration of the isolated subdomains in gray with buried surface represented in green. The calculated buried surface area for the various domains is also shown. a–d, Analysis for ATD dimer, LBD dimer, ATD dimer-of-dimer and LBD dimer-of-dimer interface for the compact GluD1 model.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | C-terminal truncation does not affect assembly of GluD1 receptors. a, Representative traces for the whole-cell recording of the GluD1, GluD1 Δ851, GluD1(K2LBD) and GluD1(K2LBD) Δ851 expressed in HEK-293T cells are shown in response to 10 mM glutamate application. b–e, Whole-cell patch-clamp recordings (holding potential = −60 mV) from constitutively active GluD1 A634C point mutant receptors. The seal resistance before entering into the whole-cell configuration was always at least 1 GΩ. b, No spontaneous currents were observed for wild-type GluD1 or GluD1 Δ851 receptors, and no effect was observed on 2 mM Ca2+ application. c, d, Overlay of representative traces showing application of either NMDG solution or 1 mM 7-CKa (red), 10 mM d-serine (green) or 2 mM CaCl2 (blue). Dashed line indicates zero current level achieved by application of impermeant NMDG, which blocks the constitutive inward currents for both GluD1 A634C (c) and GluD1 Δ851 A634C receptors (d). The constitutive currents are also modestly inhibited by D-ser or 7-CKa application and potentiated by Ca2+ (c, d) for both the full-length and CT truncated GluD1 receptors. e, Percent inhibition of spontaneous currents by 7-CKa and d-serine calculated with respect to NMDG inhibition. Data for graphs are available as Source data. The number of cells used for the recordings is shown. The error bars represent standard error from the mean.
Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- n/a
- Confirmed
- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
  - Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
  - Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on Statistics for Biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection
Cryo-EM data was collected using EPU. Electrophysiology data were acquired with HEKA Patchmaster.

Data analysis
Cryo-EM data were analyzed with MotionCor2 v1.0.5, gctf v3.0.6, and cryoSPARC v2.9.0. Structures were built, refined, and analyzed in cocot version 0.9., phenoix version 1.16, pymol 2.1.0, and chimera 1.12. Electrophysiology data were analyzed with Clampfit 8.2.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The cryo-EM density reconstruction and final model were deposited with the Electron Microscopy Data Base (accession code EMD-0773 & EMD-0774) and with the Protein Data Bank (PDB ID: 6KSP & PDB ID: 6K5S). All other relevant data supporting the key findings of this study are available within the article and its Supplementary information files or from the corresponding author upon request.
Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
- Behavioural & social sciences
- Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-list.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

- **Sample size**
  For electrophysiology, sample sizes represent the number of cells used for recordings and analysis. The sizes were based on consistency and quality of data across conditions and multiple experiments.

- **Data exclusions**
  For patch clamp recording, uniform sized cells were selected for recordings and no data was excluded unless the data quality was poor due to noise of patch stability.

- **Replication**
  Patch clamp recordings were replicated as per sample size indicated in figure/text.

- **Randomization**
  For whole-cell recordings, samples were grouped based on mutants/receptor chimeras tested.

- **Blinding**
  The investigators were not blinded to group allocation.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
|----------------------------------|---------|
| n/a | n/a |
| ☒ Antibodies
  ☒ Eukaryotic cell lines
  ☒ Palaeontology
  ☒ Animals and other organisms
  ☒ Human research participants
  ☒ Clinical data | ☒ ChiP-seq
  ☒ Flow cytometry
  ☒ MRI-based neuroimaging |

**Antibodies**

- **Antibodies used**
  Primary antibody for Western analysis was purchased from Cell signaling Technology [His-Tag(D3110)XP Rabbit mAb. Secondary was purchased from Santa Cruz Biotechnology, Inc. (goat anti-rabbit IgG-HRP [sc-2004]).

- **Validation**
  Characterization data for both the antibodies is available with the vendor. The HEK293S GnTI - ATCC ® CRL-3022 were obtained and authenticated by ATCC and no further authentication was performed.

**Eukaryotic cell lines**

Policy information about [cell lines](#)

- **Cell line source(s)**
  The HEK293S GnTI - ATCC ® CRL-3022 were purchased from ATCC.

- **Authentication**
  The HEK293S GnTI - ATCC ® CRL-3022 were obtained and authenticated by ATCC and no further authentication was performed.

- **Mycoplasma contamination**
  HEK293S GnTI - ATCC ® CRL-3022 cell line was not tested for mycoplasma contamination.

- **Commonly misidentified lines (See ICLAC register)**
  NA