Channel opening and gating mechanism in AMPA-subtype glutamate receptors

Edward C. Twomey1,2, Maria V. Yelshanskaya1, Robert A. Grassucci1,3, Joachim Frank1,3,4 & Alexander I. Sobolevsky1

AMPA (\(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid)–subtype ionotropic glutamate receptors mediate fast excitatory neurotransmission throughout the central nervous system. Gated by the neurotransmitter glutamate, AMPA receptors are critical for synaptic strength, and dysregulation of AMPA receptor–mediated signalling is linked to numerous neurological diseases. Here we use cryo–electron microscopy to solve the structures of AMPA receptor–auxiliary subunit complexes in the apo, antagonist– and agonist–bound states and determine the iris–like mechanism of ion channel opening. The ion channel selectivity filter is formed by the extended portions of the re–entrant M2 loops, while the helical portions of M2 contribute to extensive hydrophobic interfaces between AMPA receptor subunits in the ion channel. We show how the permeation pathway changes upon channel opening and identify conformational changes throughout the entire AMPA receptor that accompany activation and desensitization. Our findings provide a framework for understanding gating across the family of ionotropic glutamate receptors and the role of AMPA receptors in excitatory neurotransmission.

Excitatory neurotransmission is tightly governed by the AMPA subtype of the ionotropic glutamate receptors (iGluRs)1–2. Gated by the neurotransmitter glutamate, AMPA receptors (AMPArs) activate on a sub-millisecond timescale and rapidly depolarize the post-synaptic membrane. AMPAR-mediated neuronal signalling has profound effects on synaptic strength, and many neurological diseases have been directly linked to changes in AMPAR synaptic signalling3–5.

AMPArs are tetrameric, two-fold symmetric membrane proteins with a three-layer, ‘Y’-shaped architecture6,7. Extracellularly, the amino-terminal domains (ATDs) form the top of the Y. Below the ATDs are the ligand-binding domains (LBDs) that bind the neurotransmitter glutamate. Two polypeptide stretches (S1 and S2) make up each of the four AMPAR subunit LBDs, which are tethered to the transmembrane domain (TMD) at the base of the Y by flexible linkers. The nearly four-fold symmetric TMD is made up of three membrane-spanning helices (M1, M3 and M4) and a re-entrant pore loop (M2) between helices M1 and M3. The cation-selective ion channel is lined by the M3 helices with their bundle crossing forming a tight gate to regulate permeation.

Since the first, closed-state intact AMPAR structure8, many studies have pursued different receptor conformations using both X-ray crystallography9–12 and cryo-electron microscopy (cryo-EM)13–17. However, the structure of the ion channel is yet to be resolved to high resolution, and the open-state of the receptor has yet to be seen. This is a fundamental barrier to understanding AMPAR biology and structurally informed design of therapeutic agents that target AMPARs in neuropathological conditions. Here, we use cryo-EM to solve the structure of an AMPAR ion channel at high resolution and resolve the opening of the AMPAR ion channel in response to binding of glutamate.

Ion channel structure

We used cryo-EM to analyse the structure of the homomeric GluA2 flip splice variant AMPAR in complex with the auxiliary subunit germline-specific gene 1-like (GSG1L), which we surmised would stabilize the closed-state structure of the ion channel as it lowers the ion channel open probability compared to the transmembrane AMPAR regulatory protein (TARP)–2 or stargazin (STZ)15,16. Indeed, using a covalent fusion construct (Methods) between truncated forms of GluA2 and GSG1L, we solved structures of the digitonin–solubilized receptor complex bound to the antagonist ZK200775 in two states, termed GluA2–GSG1LZK-1 (4.6 Å) and GluA2–GSG1LZK-2 (4.4 Å) (Extended Data Table 1; Extended Data Fig. 1), with local qualities of the maps showing higher resolution details in the ion channel pore (Extended Data Fig. 2).

The GluA2–GSG1LZK complex has a typical Y-shaped GluA2 tetramer in the centre (Fig. 1a), with two GSG1L subunits assembling between protomers A/B and C/D on the periphery of the GluA2 TMD (Fig. 1b). The GluA2 TMD is completely resolved and allows building of the entire ion channel pore–forming region (Fig. 1c, d). The M3 bundle crossing, formed by T617, A621, T625 and M629, occludes cation permeation, as previously observed in the original GluA2 crystal structure8. Below T617, the M3 bundle crossing opens up into a hydrophobic cavity in the middle of the channel pore. Below this cavity is a second constriction formed by the extended regions of the M2 re–entrant loops. Similar to other tetrameric ion channels, this constriction might serve as a lower gate of the channel as well as a selectivity filter18–20. At the re–entrant loop tip, the glutamine/arginine (Q/R)–site glutamine residues (Gln586) protrude towards the centre of the ion channel pore, occluding the permeation pathway and forming a lower gate. Their location is consistent with the observation that channels composed of edited GluA2 subunits (Gln586Arg) have reduced Ca\(^{2+}\) permeation and polyamine block, probably due to electrostatic repulsion21,22. The pore loop, under the Q/R-site, appears to be more flexible in our closed-state structures, compared to the rest of the channel, but we expect it to become more ordered upon channel opening to form a selectivity filter along the permeation pathway.

The \(\alpha\)-helical portion of M2 is largely hydrophilic (Fig. 1c) and contributes to cross-subunit interfaces within the TMD (Extended Data...
Fig. 1a, b. Model of GluA2–GSG1LZK1 viewed parallel to (a) or from the intracellular side of (b) the membrane with GluA2 subunits A and C coloured purple, B and D in green and GSG1L in red. The competitive antagonists ZK200775 (ZK) are shown as space-filling models. c, Close-up view of the pore-lining domains M2 and M3 in subunits A and C with cryo-EM density shown as blue mesh. d, Ion conduction pathway (violet) with pore-lining residues in the M2 and M3 segments of subunits A and C shown as sticks.

Fig. 3b). These hydrophobic interactions involve Leu540 and Tyr533 from M1, as well as Trp605 and Phe608 from the neighbouring M3, each from the same subunit as the M2, and Trp606 and Ile600 from M3 of the adjacent subunit. The M2 helix contributes to these hydrophobic interfaces through Leu577, Trp578, Leu581 and Phe584. For a helix that has often evaded structural studies, the M2 helix has quite an extensive network of hydrophobic interactions.

The second closed state structure, GluA2–GSG1LZK2, has the same ion channel architecture as in GluA2–GSG1LZK1, although with higher resolution details throughout the TMD (Extended Data Fig. 2). While the TMD structure is the same, the ECD in GluA2–GSG1LZK2 is markedly different, being shorter by 6 Å (Extended Data Fig. 4). Of symmetry (Extended Data Fig. 4c, d), and the LBD dimers rotate by 90° as a rigid body around the overall two-fold axis of symmetry (Extended Data Fig. 4e, f), and the LBD dimers rotate by 4°, creating a separation between the proximal subunits A and C (Extended Data Fig. 4e, f). An elongated density at the ATD–LBD interfaces inside subunits A and C perfectly matches a digitonin molecule (Extended Data Fig. 4b, inset). Thus, digitonin binding might help to trap GluA2 in a distinct closed state conformation. We hypothesize that the ATD–LBD interface represents a new binding site for hydrophobic molecules with a possible therapeutic importance and may bind to fatty acids that have recently been identified as non-competitive AMPAR inhibitors23,24.

Cryo-EM of activated GluA2–STZ complex
To understand how the native neurotransmitter glutamate (Glu) activates and opens the AMPAR ion channel, we used a fusion construct between GluA2 and STZ (Methods) that promotes GluA2–STZ complex formation and expression while maintaining its functional properties. In contrast to GSG1L, which stabilizes the AMPAR inactive states, STZ favours the open state25–27, as signified by increased steady-state current in response to Glu application in whole-cell patch-clamp recordings (Fig. 2a). Additional stabilization of the open state is provided by the positive allosteric modulator cyclothiazide (CTZ)28, which makes AMPAR responses non-desensitizing (Fig. 2a).

We solved a cryo-EM structure of the GluA2–STZ complex in the presence of Glu and CTZ to 4.2 Å resolution (Extended Data Fig. 1), with two-dimensional class averages showing the secondary structure features and diverse orientations (Fig. 2b). The reconstruction (Fig. 2c) shows the entire GluA2–STZ complex in the agonist-bound state, with high-resolution features in the GluA2 TMD core (Extended Data Fig. 2n, o) but lower local resolution for STZ. To achieve unambiguous side chain definition in the TMD, we performed directed refinement (Fig. 2c) that resulted in a 4.0 Å cryo-EM reconstruction (Fig. 2d) and distinct side-chain densities (Extended Data Fig. 5e). The TMD cryo-EM map shows clear differences in the central pore (Fig. 2c), compared to the closed-state GluA2–GSG1L complex (Fig. 1b) and the STZ molecules bound. Also, glutamate and CTZ are easily discernible in the cryo-EM density (Extended Data Fig. 5a, d), suggesting that we trapped the complex in an activated state. The original, closed-state cryo-EM structures of the GluA2–STZ complex suggested that acidic residues in the STZ 34–TM2 loop mediate electrostatic interactions with positively charged residues in the lower membrane.
Glutamic acid 2 (GluA2) LBD and S1-pre-M1 linker to enhance receptor activation\(^{17,29}\). In our active state cryo-EM reconstruction, this region of STZ seems to be disordered. However, based on well-defined adjacent regions of STZ (Extended Data Fig. 5e), the same acidic residues are juxtaposed to the GluA2 basic patch. Perhaps rather than directly interacting in the activated state, the \(\Delta 4\)-TM2 loop has an important role in the structural transitions necessary for receptor activation.

Our density, and the corresponding GluA2–STZ\(_{\text{Glu}+\text{CTZ}}\) model (Fig. 3, Methods) show four STZ molecules bound around the AMPAR core (Fig. 2e, Extended Data Fig. 6c). We used the same fusion design to solve the first structure of the GluA2–STZ complex in the closed state\(^{15}\) but observed only one or two STZ molecules bound to the AMPAR core. Here we solubilized the complex in digitonin instead of dodecyl maltoside (DDM) detergent and observed maximum occupancy of STZ binding sites around the AMPAR core, similar to GluA2–STZ co-expression\(^{17}\). Accordingly, instead of the four-layer particle architecture in DDM\(^{15}\), we see a clearly discernible three-layer architecture in the two-dimensional (2D) class averages (Extended Data Fig. 6a). Interestingly, the GluA2–GSGL1 complex in digitonin, which showed a four-layer architecture when solubilized in DDM\(^{16}\), also showed the three-layer particle architecture (Extended Data Fig. 6b) but with only two, not four, molecules of GSGL1 bound to GluA2 core (Fig. 1a, Extended Data Fig. 6d). As the nature of the fusion construct design does not preclude full occupancy of the auxiliary subunit binding sites, we hypothesize that STZ and GSGL1 use different stoichiometries to assemble with AMPARs.

Open ion channel pore

As indicated by the markedly different architecture of the AMPAR core in GluA2–STZ\(_{\text{Glu}+\text{CTZ}}\) compared to the closed state, the width of the TMD increases by 10 Å from 55 Å in the closed state structures to 65 Å in GluA2–STZ\(_{\text{Glu}+\text{CTZ}}\) and the ion channel pore undergoes widening (compare Figs 1b and 3b). We probed the ion conduction pathway in GluA2–STZ\(_{\text{Glu}+\text{CTZ}}\) (Fig. 4a) and found it much wider than in the closed state GluA2–GSGL1 structures (Fig. 1d). Measurements of the pore radius (Fig. 4b) confirmed that in contrast to the pore-occluded conformations in the closed GluA2–GSGL1\(_{\text{ZK}1}\), GluA2–GSGL1\(_{\text{ZK}2}\) and desensitized GluA2–GSGL1\(_{\text{Quis}}\)\(^{16}\) states, the GluA2–STZ\(_{\text{Glu}+\text{CTZ}}\) pore is wide open for ion conductance. The distances between the Co\(_{\alpha}\) atoms of diagonal Gln586, Thr617 and Ala621 pairs increased almost uniformly by about 3 Å in GluA2–STZ\(_{\text{Glu}+\text{CTZ}}\) (Fig. 4b, c), indicating nearly four-fold symmetric, iris-type opening of the main portion of the pore (Fig. 4d), reminiscent of other tetrameric ion channels\(^{19,30,31}\). In stark contrast, the distance between diagonal Thr625 pairs, which is similar for the A/C and B/D subunit pairs in the closed state (9.5 and 11 Å), increases to 16 Å for the A/C pair and to 32 Å for the B/D pair in GluA2–STZ\(_{\text{Glu}+\text{CTZ}}\). This 6.5 Å versus 21 Å separation upon channel opening indicates that at the level of Thr625, the AMPAR pore loses its pseudo four-fold symmetry and becomes two-fold symmetric, consistent with previous functional experiments\(^{32}\). We also compared our recent molecular dynamics model of the open state\(^{33}\) and the GluA2–STZ\(_{\text{Glu}+\text{CTZ}}\) structure. The narrowest portions of the pore in the molecular dynamics model, estimated as Co\(_{\alpha}\) distances between the diagonal Gln586 (9.6 Å) and Thr617 (12.6 Å) pairs, are narrower than the corresponding portions of the GluA2–STZ\(_{\text{Glu}+\text{CTZ}}\) pore (10.2 Å and 14.1 Å, respectively). Yet, we clearly observed ion conducting events through our molecular dynamics model\(^{33}\), an observation strongly supporting the conducting conformation of the GluA2–STZ\(_{\text{Glu}+\text{CTZ}}\) pore. In addition, a clear density in the centre of the pore (Extended Data Fig. 5b, c), probably representing a sodium ion, also suggests that the selectivity filter of the channel is indeed in a conducting state.

The selectivity filter located at and below the Q/R site (Fig. 4a, b), which is not visible in the closed state, is clearly resolved in GluA2–STZ\(_{\text{Glu}+\text{CTZ}}\) and is approximately four-fold symmetric, similar to K\(^+\) channels\(^{30,31}\). Just below the Q/R site, the backbone carbonyls of Gln587, Gly588 and Cys589 line the selectivity filter, while the side chains of Gln587 and Cys589 point away from the central pore axis. At the bottom of the selectivity filter is Asp590 that creates a negative entry point into the cytoplasm from the channel pore, perhaps having a role in cation versus anion selectivity of AMPAR channels.
Gating mechanism
To understand the conformational changes that are associated with AMPAR gating, we compared the open-state GluA2–STZ + CTZ with the closed-state GluA2–GSG1LZK-1 and the desensitized-state GluA2–2×GSG1LQuis structures (Fig. 5, Supplementary Video 1). Since gating starts with binding of glutamate to an unliganded AMPAR, we first wanted to see whether GluA2–GSG1LZK-1 represents the resting, apo state of the receptor. We solved cryo-EM structures of the digitonin-solubilized GluA2–GSG1L complex in the absence of ligands, which, similar to the ZK200775-bound structures, are represented by two different states. The apo state structures, GluA2–GSG1Lapo,1 and GluA2–GSG1Lapo,2, turned out to be very similar to GluA2–GSG1LZK-1 and GluA2–GSG1LZK-2 (Extended Data Figs 3, 4), superposing on each other with root mean square deviation (r.m.s.d.) values of 0.526 Å and 0.701 Å, respectively. We therefore used the higher resolution GluA2–GSG1L ZK-1 structure that is nearly identical to GluA2–GSG1Lapo-1 as an accurate representation of the closed resting state.

In the first approximation, activation, which includes ion channel opening, is represented by the GluA2–GSG1L ZK-1 to GluA2–STZGlu + CTZ transition. At the level of a single LBD, glutamate binding...
The structures of AMPAR complexes allow better understanding of the AMPAR function and may provide insights into the mechanisms of synaptic plasticity.

**Discussion**

The structures of AMPAR complexes allow better understanding of the iGluR gating mechanism (Extended Data Fig. 8). Channel opening in GluA2–STZGlu4+CTZ is accompanied by the outward flipping of Gln586, which occludes the channel in the closed state, away from the central pore axis. Therefore, the Q/R site at the tip of M2 seems to function as a lower gate. We propose that individual AMPAR subunit contributions to the lower gate result in the sub-conductance states observed in single-channel AMPAR studies17,44. AMPARs occupy their highest conductance state in the presence of glutamate49,50 and STZ46. We expect that the highest conductance state results from all subunits being engaged by glutamate51,52 when channel occlusion is relieved at both gates. STZ also promotes the higher conductance states by stabilizing the open conformation of the lower gate through interaction with the M2 helices. We therefore believe that our GluA2–STZGlu4+CTZ structure represents the maximum–conductance state of the channel.

In addition, the prominent placement of Gln586 of the iGluR family, as it is part of the highly conserved STYANLAAF motif52. Also in this motif is the Lurcher mutation Ala622Thr, which notably changes function across iGluR subtypes, resulting in constitutively activated receptors and markedly different gating properties54. The proximity of this site to Ala618 suggests that the Lurcher mutation may change iGluR gating through altering the properties of M3 kinking upon channel opening. Better understanding of AMPAR gating and availability of the high-resolution open-state structure will help to overcome barriers in the design of therapeutic agents and the treatment of neurodegenerative diseases.
16. 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 54, 569–580.e5 (2007).

17. Zhao, Y., Chen, S., Yoshioka, C., Barondes, S. & Gouaux, E. Architecture of fully occupied GluA2 AMPA receptor–TARPs complex elucidated by cryo-EM. Nature 536, 108–111 (2016).

18. Doyle, D. A. et al. The structure of the potassium channel: molecular basis of K+ conduction and selectivity. Science 280, 69–77 (1998).

19. Cao, E., Liao, M., Cheng, Y. & Julius, D. TRPV1 structures in distinct conformations reveal activation mechanisms. Nature 504, 113–118 (2013).

20. Huettner, J. E. Glutamate receptor pores. J. Physiol. (Lond.) 593, 49–59 (2015).

21. Priel, A. & Tomita, S. Stargazin modulates AMPA receptor gating and trafficking by distinct domains. Nature 435, 1052–1058 (2005).

22. MacLean, D. M., Ramaswamy, S. S., Du, M., Howe, J. R. & Jayaraman, V. Structural bases of desensitization in AMPA receptor–auxiliary subunit complexes elucidated by cryo-EM. Nature 536, 108–111 (2016).

23. Chang, P. et al. Seizure control by decanic acid through direct AMPA receptor modulation. Brain 139, 431–443 (2016).

24. Zhang, W. et al. Seizure control by derivatives of medium chain fatty acids associated with the ketogenic diet show novel branching-point structure for enhanced potency. J. Pharmacol. Exp. Ther. 352, 43–52 (2015).

25. Prieto, M. L. & Wollmuth, L. P. Gating modes in AMPA receptors. Neuron 897–903 (2015).

26. Chang, P. et al. Stargazin reduces desensitization and slows deactivation of the AMPA-type glutamate receptors. J. Neurosci. 25, 2682–2686 (2005).

27. Yelshanskaya, M. V., Saotome, K., Singh, A. K. & Sobolevsky, A. I. Probing GluR2 AMPA receptor–TARP complex elucidated by cryo-EM. Nature 536, 108–111 (2016).

28. Patneau, D. K., Vyklicky, L. Jr & Mayer, M. L. Hippocampal neurons exhibit cyclohexidine-sensitive rapidly desensitizing responses to kainate. J. Neurosci. 14, 503–512 (2014).

29. Patneau, D. K., Vyklicky, L. Jr & Mayer, M. L. Hippocampal neurons exhibit cyclohexidine-sensitive rapidly desensitizing responses to kainate. Neuron 14, 503–512 (2014).

30. Jiang, Y. et al. The open pore conformation of potassium channels. Nature 417, 523–526 (2002).

31. Long, S. B., Campbell, E. B. & Mackinnon, R. Crystal structure of a mammalian voltage-dependent Shaker K+ channel. Science 309, 1690–1693 (2005).

32. Sobolevsky, A. I., Yelshanskaya, M. V. & Wollmuth, L. P. The outer pore of the GluK2 kainate receptor channels revealed by thermodynamic mutant cycles. J. Gen. Physiol. 142, 225–239 (2013).

33. Ayalon, G. & Stern-Bach, Y. Functional assembly of AMPA and kainate glutamate receptors. Cell Reports 17, 139–149 (2016).

34. Ayalon, G. & Stern-Bach, Y. Functional assembly of AMPA and kainate receptors is mediated by several discrete protein-protein interactions. Neuron 31, 103–113 (2001).

35. Ayalon, G. & Stern-Bach, Y. The tetrameric structure of a glutamate receptor channel. Science 280, 1996–1999 (1998).

36. Smith, T. C. & Howe, J. R. Concentration-dependent substrate behavior of native AMPA receptors. Nat. Neurosci. 3, 992–997 (2000).

37. Poon, K., Ahmed, A. H., Nowak, L. M. & Oswald, R. E. Mechanisms of modal activation of GluA3 receptors. Mol. Pharmacol. 80, 49–59 (2011).

38. Prieto, M. L. & Wollmuth, L. P. Glutamate receptor substrates required for tetramerization in AMPA receptors. Neuron 30, 4449–4459 (2010).

39. Swanson, G. T. & Kamojo, S. K. & Cull-Candy, S. G. Single-channel properties of recombinant AMPA receptors depend on RNA editing, splice variation, and subunit composition. J. Neurosci. 17, 58–69 (1997).

40. Kohda, K., Wang, Y. & Yuzaki, M. Mutation of a glutamate receptor motif reveals its role in gating and i2 receptor channel properties. Nat. Neurosci. 3, 315–320 (2000).

Supplementary Information is available in the online version of the paper.

Acknowledgements We thank H. Kao for computational support, A. des Georges, I. S. Fernandez, M. Fislaie and A. K. Singh for processing advice. E.C.T. is supported by National Institutes of Health (NIH) F31 NS093838. A.I.S. is supported by the NIH (R01 NS083660, R01 CA206573), the Pew Scholar Award in Biomedical Sciences, and the Irma T. Hirschl Career Scientist Award. J.F. is supported by the Howard Hughes Medical Institute and the NIH (R01 GM029169). Cryo-EM data were collected at the Columbia University Medical Center cryo-EM facility and at the Simons Electron Microscopy Center and National Resource for Automated Molecular Microscopy located at the New York Structural Biology Center, supported by grants from the Simons Foundation (492427), NYSTAR, and the NIH National Institute of General Medical Sciences (GM103310).

Author Contributions E.C.T. designed the constructs, prepared the protein samples, carried out cryo-EM data collection and processing, built models, analysed data and wrote the manuscript. M.V.Y. carried out electrophysiology experiments, assisted in protein production and edited the manuscript. R.A.G. assisted in cryo-EM data collection. J.F. advised on the cryo-EM workflow and provided funding. A.I.S. supervised the project, built models, analysed data, wrote the manuscript and provided funding. E.C.T and A.I.S. designed the project.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Reviewer Information Nature thanks M. Mayer, S. Traynelis and the other anonymous reviewer(s) for their contribution to the peer review of this work.
Cells were passed twice a week and were used until the 30th passage. (GIBCO) supplemented with 2% heat inactivated fetal bovine serum (Crystalgen). Cells were obtained from ATCC and were not further authenticated. Cells of thrombin to eluted protein) for 2 h at 22 °C. The sample was then loaded onto 0.1% digitonin (0.05% digitonin was used in the GluA2–STZ purification). Elution homogenized, then solubilized for 2 h in 150 mM NaCl, 20 mM Tris-HCl pH 8.0, 1% trifugation (186,000 g) was collected and mechanically clustered control by a piezoelectric translator. Typical 10–90% rise times were 200–300 μs, as measured from junction potentials at the open tip of the patch pipette after recordings. Data analysis was performed using the computer program Origin 9.1 (OriginLab Corp.).

Expression and purification. The GluA2–GSG1L and GluA2–STZ bacmid and baculovirus were made using standard methods55, and the following methods were applied to the expression and purification of both constructs. P2 virus, produced from S09 cells (GIBCO 12659017), was added to HEK293S GnTI− cells (ATCC CRL-3022) at 37 °C and 5% CO2. At 12 h after infection, 10 mM sodium butyrate, 50 μl of this was quickly added and pipetted up-and-down in a mixture of 0.5 μl 700 mM glutamate (final concentration 100 mM). 3 μl of this mixture was added to the grid, blotted as described above.

The GluA2–GSG1L data (apo/ZK) were collected on a Tecnai F30 Polara (FEI) microscope operating at 300 kV, using Leginon® with a Gatan K2 Summit detection (DED) camera (Gatan) in counting mode with a pixel size of 0.98 Å. Data were collected across 40 frames (0.2 ms per frame), with a dose rate of 8 e− pixel−1 s−1 (total dose of approximately 67 e− Å−2), with defocus range −1.5 μm to −3.5 μm, Cc 2.26 mm. The GluA2–STZ data were collected on a Titan Krios microscope (FEI) operating at 300 kV, using Leginon with a post-GIF quantum energy filter (20 eV slit) and Gatan K2 Summit direct camera DED camera in counting mode operating at a pixel size of 1.0 Å pixel−1. A dose rate of 8 e− pixel−1 s−1 (total dose of approximately 55 e− Å−2) was used across 40 frames (0.2 ms per frame), within the defocus range −1.0 μm to −3.0 μm.

Image processing. All frame alignment and dose-weighting was carried out in MotionCor2. CTF correction, with gctf60, was performed on non-dose-weighted micrographs, with all further processing on dose-weighted micrographs using RELION 2.061. From 2,938 micrographs for GluA2–GSG1L bound to ZK200775, 541,261 particles were picked. The data were binned to 3.92 Å and subject to 2D classification, which resulted in cleaning the particle pool to 531,406 particles. These particles were then subject to 3D classification without symmetry into ten classes and the GluA2–2×GSG1Lapo model16–low-pass filtered to 40 Å as a reference. Three classes, with two showing different ECD conformations (that is, state 1 and state 2) and two auxiliary subunits bound were chosen for additional 3D sub-classification. This resulted in a group of 234,426 particles, which were unbinned to a pixel size of 1.98, and subject to classification with the GluA2−2×GSG1Lapo model (40 Å filter) as the reference. This resulted in two well-structured groups of particles: GluA2−GSG1Lapo (26,971 particles) and GluA2−GSG1Lapo (41,926 particles). The particles were unbinned to the original 0.98 Å pixel−1, and refined with C2-symmetry. Of note is that we did not observe any stoichiometric heterogeneity, as was observed in cryo-EM performed on the GluA2−GSG1L complex solubilized in DDM14.

For the GluA2–GSG1L collection in the absence of ligand, 242,078 particles were automatically picked from 2,593 micrographs using six 2D classes as references (generated from manual picking as described above). The particle images were binned to 3.92 Å pixel−1, and 235,543 particles remained following 2D classification. Next, 3D classification was carried out without symmetry into ten classes with the 40 Å low-pass filtered GluA2–GSG1Lapo map. Six classes, numerating 115,120 particles showed structural details that warranted further image processing. The particles were unbinned to 0.98 Å pixel−1 and classified in 3D into 10 classes with the GluA2–GSG1Lapo model. Resulting were two prominent classes, GluA2−GSG1Lapo (20,392 particles) and GluA2−GSG1Lapo (18,926 particles), which were refined with C2-symmetry, and resemble the two states observed for GluA2–GSG1Lapo in the presence of ZK200775 (Extended Data Figs 3, 4).

A total of 4,116 micrographs were collected for GluA2–STZ, and 595,889 particles were automatically picked, with manually picked particles used to generate reference classes as described above. The particles were binned twice to 2.1 Å pixel−1 and subject to 2D classification, which resulted in 581,495 particles being subject to further image processing. The original GluA2−2×STZ antagonist-bound map15, low-pass filtered to 40 Å, was used as a reference model for 3D classification into ten classes without symmetry imposed. 3D classification resulted in 278,454 particles, from four classes, being chosen for further image processing, without observation of stoichiometric heterogeneity as in the DDM-solubilized complex15, and the particles were unbinned to 1.08 Å pixel−1 and classified in 3D to ten classes. Particles from three classes, numerating 69,207
particles and showing structurally similar features, were chosen for refinement. Initial refinement, with C2-symmetry, resulted in a 4.2 Å map, which showed side-chain features in the GluA2 core but was lacking details for STZ. To align particles better according to the GluA2 TMD and STZ, we generated a soft spherical mask around the detergent micelle (which encompassed all of the GluA2 and STZ TMDs and STZ extracellular head). Resulting in an overall 4.0 Å map, with distinct side-chain features throughout the TMD for model building of the GluA2 and STZ TMDs. We did not observe a digitonin-bound state of GluA2–STZ Glu + CTZ. As we did for GluA2–GSG1L in the resting states (GluA2N,GSG1L,ZK–2, GluA2–GSG1L apo–2), the resolution for all reconstructions was estimated using the Fourier shell correlation (FSC) = 0.143 criterion between independent half maps on corrected FSC curves in which the influences of the mask were removed (Extended Data Fig. 1). All maps were post-processed using a softmask in RELION, and B-factors for map sharpening were automatically estimated (Extended Data Table 1). All visualization of electron microscopy densities was done in UCSF Chimera. Local resolution for each map (Extended Data Fig. 2) was calculated with unfiltered half maps using ResMap. Model building. To build the state 1 ZK200775-bound and GluA2–GSG1L models, we individually isolated the ATD and LBD dimers, as well as the TMD, from the GluA2–2 × GSG1L,ZK complex structure using rigid-body fitting in COOT. Side chains in the TMD were defined based on local resolution, as was the structure of the M2 helix and pore loop. The resulting model was refined against an unfiltered half map (work) in real space with constraints using PHENIX. The refined model was tested for overfitting (Extended Data Fig. 2) by shifting the coordinates with a half map (work) in real space with constraints using PHENIX. The refined model was also real space refined in PHENIX and tested for overfitting (Extended Data Fig. 2), of which there are no signs of over-fit models. Structures were visualized and figures were prepared in Pymol.

Data availability. Cryo-EM density maps have been deposited in the Electron Microscopy Data Bank (EMDB) under accession numbers EMD-8819 (GluA2–GSG1L,ZK–1), EMD-8820 (GluA2–GSG1L,ZK–2), EMD-8821 (GluA2–GSG1L apo–1), EMD-8822 (GluA2–GSG1L apo–2), and EMD-8823 (GluA2–STZ Glu + CTZ, including directed TMD map). Model coordinates have been deposited in the Protein Data Bank (PDB) under accession numbers 5WIE (GluA2–GSG1L,ZK–2), 5WEL (GluA2–GSG1L,ZK–1), 5WEM (GluA2–GSG1L apo–1), 5WEN (GluA2–GSG1L apo–2), and 5WEO (GluA2–STZ Glu + CTZ). All other data are available from the corresponding author upon reasonable request.

50. Goehring, A. et al. Screening and large-scale expression of membrane proteins in mammalian cells for structural studies. Nat. Protocols 9, 2574–2585 (2014).
51. Kawate, T. & Gouaux, E. Fluorescence-detection size-exclusion chromatography for precrystallization screening of integral membrane proteins. Structure 14, 673–681 (2006).
52. Russo, C. J. & Passmore, L. A. Electron microscopy: ultrastable gold substrates for electron cryomicroscopy. Science 346, 1377–1380 (2014).
53. Suloway, C. et al. Automated molecular microscopy: the new Legion system. J. Struct. Biol. 151, 41–60 (2005).
54. Zheng, S. Q. et al. MotionCor2: anisotropic correction of beam-induced motion for improved cryo-electron microscopy. Nat. Methods 14, 331–332 (2017).
55. Zhang, K. Gct: Real-time CTF determination and correction. J. Struct. Biol. 193, 1–12 (2016).
56. Kimanius, D., Forsberg, B. O., Scheres, S. H. & Lindahl, E. Accelerated cryo-EM structure determination with parallelisation using GPUs in RELION-2. eLife 5, e18722 (2016).
57. Scheres, S. H. & Chen, S. Prevention of overfitting in cryo-EM structure determination. Nat. Methods 9, 853–854 (2012).
58. Chen, S. et al. High-resolution noise substitution to measure overfitting and validate resolution in 3D structure determination by single particle electron cryo-microscopy. Ultramicroscopy 135, 24–35 (2013).
59. Pettersen, E. F. et al. UCSF Chimera—a visualization system for exploratory research and analysis. J. Comput. Chem. 25, 1650–1654 (2004).
60. Kucukelbir, A., Sigworth, F. J. & Tagare, H. D. Quantifying the local resolution of cryo-EM density maps. Nat. Methods 11, 63–65 (2014).
61. Emsley, P., Lohkamp, B., Scott, W. G. & Cowtan, K. Features and development of Coot. Acta Crystallogr. D 66, 486–501 (2010).
62. Afonine, P. V. et al. Towards automated crystallographic structure refinement with phenix.refine. Acta Crystallogr. D 68, 352–367 (2012).
63. Tang, G. et al. EMAN2: an extensible image processing suite for electron microscopy. J. Struct. Biol. 157, 38–46 (2007).
Extended Data Figure 1 | FSC curves for cryo-EM reconstructions. FSC curves calculated between half-maps for GluA2–GSG1L_{ZK-1}, GluA2–GSG1L_{ZK-2}, GluA2–GSG1L_{apo-1}, GluA2–GSG1L_{apo-2} and GluA2–STZ\textsubscript{Glu+iCTZ} cryo-EM reconstructions, as well as for the GluA2–STZ\textsubscript{Glu+iCTZ} TMD reconstruction from directed refinement. The dashed line indicates FSC = 0.143.
Extended Data Figure 2 | Local resolution and fitting of cryo-EM maps. a–p. Local resolution calculated using Resmap and two unfiltered halves of the reconstruction for GluA2–GSG1L_{apo-1}, GluA2–GSG1L_{apo-2}, GluA2–GSG1L_{apo-1}, GluA2–GSG1L_{apo-2} and GluA2–STZGlu+CTZ structures viewed parallel to the membrane as a surface (a, d, h, k, n) and slice through the centre of the receptor (b, e, i, l, o), with the cross-validation FSC curves for the refined model versus unfiltered half maps (one used in the refinement, work, and another one not, free) and the unfiltered summed maps shown on the right (c, f, j, m, p).
Extended Data Figure 3 | Closed state 1 cryo-EM density and comparison of ZK200775-bound and apo states. a–d, Fragments of GluA2–GSG1LZK-1 and GluA2–GSG1Lapo-1 with the corresponding cryo-EM density. a, d, ATD and LBD of subunit A in GluA2–GSG1LZK-1 (a) and GluA2–GSG1Lapo-1 (d) with density for ZK200775 indicated in the GluA2–2×GSG1LZK-1 structure. b, c, M2 helix (b) and selectivity filter (c) with the Q/R-site Gln586 side chains pointing towards the centre of the pore in GluA2–GSG1LZK-1. e, Superposition of GluA2–GSG1LZK-1 (blue) and GluA2–GSG1Lapo-1 (red) viewed parallel to the membrane. Note that the structures are almost indistinguishable (r.m.s.d. = 0.526 Å). Densities are shown at 6σ.
Extended Data Figure 4 | Closed state 2 structure and digitonin-binding pocket.  

**a, b.** Structures of GluA2–GSG1L ZK-1 (a) and GluA2–GSG1L ZK-2 (b) viewed parallel to the membrane. The GluA2 subunits A and C are coloured purple, B and D in green and GSG1L in red. The competitive antagonist ZK200775 and digitonin are shown as space-filling models. In b, inset shows expanded view of the boxed region, demonstrating cryo-EM density for digitonin (blue mesh, 4σ). Digitonin and the surrounding residues in the inset are shown in stick representation.  

**c–h.** Top down views along the axis of the overall two-fold rotational symmetry on the ATD (c, d), LBD (e, f) and TMD (g, h) layers. Rigid-body rotation of the ATD tetramer in d and rotation of LBD dimers in f are indicated by red arrows.  

**i, j.** Superposition of GluA2–GSG1L ZK-2 (blue) and GluA2–GSG1L apo-2 (red) viewed parallel to the membrane. Note that the structures are almost indistinguishable (r.m.s.d. = 0.701 Å).
Extended Data Figure 5  |  Cryo-EM density for the open state.

**a–e,** Fragments of GluA2–STZ<sub>glu+CTZ</sub> with the corresponding cryo-EM density. **a,** Zoomed view of the glutamate-binding pocket. **b, c,** The ion channel pore with a central density at the selectivity filter, probably for a sodium ion that is hydrated based on the pore diameter, viewed from the top of the selectivity filter looking down into the cytoplasm (b) or parallel to the membrane with two (front and back) GluA2 subunits removed (c). **d,** Density for CTZ. **e,** Transmembrane domain segments for GluA2 (top row) and STZ (bottom row).
Extended Data Figure 6 | Overview of single-particle cryo-EM and stoichiometry for GluA2–STZ and GluA2–GSG1L solubilized in digitonin. a, b, Two-dimensional class averages for GluA2–STZ (a) and GluA2–GSG1L (b) indicating three-layer architecture of the particles. c, d, Final densities for GluA2–STZ (c) and GluA2–GSG1L (d) with the GluA2 subunits A and C coloured purple, B and D in green, STZ in cyan and GSG1L in red. Insets show 2D slices made parallel to the membrane through the refined, nonfiltered map. Note, although four STZ molecules bind one receptor, only two copies of GSG1L can bind per GluA2 tetramer.
Extended Data Figure 7 | Conformational differences between the closed, open and desensitized states. a–c, Structures of GluA2–GSG1LZK-1 in the closed state (a), GluA2–STZGlu+CTZ in the open state (b) and GluA2–2×GSG1LQuis in the desensitized state (c), viewed parallel to the membrane. The GluA2 subunits A and C are coloured purple, B and D in green, GSG1L in red and STZ in cyan. The competitive antagonist ZK200775, agonists Glu and Quis and positive allosteric modulator CTZ are shown as space-filling models. d–l, Top down views along the axis of the overall two-fold rotational symmetry on the layers of ATD (d–f), LBD (g–i) and TMD (j–l). Rigid-body rotation of the ATD tetramers in e and f, broadening of LBD layer in h and rotation of subunit A/C LBDs in i are indicated by red arrows. Note the dramatic opening in the middle of the LBD layer (h) and pore dilation (k) in the open state.
Extended Data Figure 8 | iGluR gating mechanism. Two out of four iGluR subunits are shown with the ATDs omitted. Four basic states of iGluR gating are illustrated: resting, represented by apo (GluA2–GSG1L_{apo,1}) or antagonist-bound closed state (GluA2–GSG1L_{ZK,1}) structures; closed, agonist-bound (pre-active state crystal structures\textsuperscript{10,11}); open (GluA2–STZ\textsubscript{GluA}+CTZ\textsubscript{L}); and desensitized (GluA2–2\times GSG1L\textsubscript{Quis}, complex\textsuperscript{16}). Transitions between the states are indicated by black arrows, conformational rearrangements by blue arrows, and ionic current through the open channel by an orange arrow. Upper and lower gates are indicated by one and two red asterisks, respectively, with red sticks at the upper gate representing channel occluding residues at the bundle crossing and the Q/R site at the lower gate. Glutamate molecules are illustrated by orange wedges. The receptor sits in a resting, closed state, with its LBD clamshells in the maximally open conformations, unoccupied by the neurotransmitter glutamate. Upon glutamate binding, the LBD clamshells close, as described in the pre-activated crystal structures, to an intermediate state that does not put enough strain on the LBD–TMD linkers to open the channel. The LBDs then transition to their maximally closed state, which strains the LBD–TMD linkers, causing the channel pore to open and conduct ions. Most AMPARs, however, quickly desensitize, transitioning to the desensitized state from the open state via the agonist-bound, closed state. Desensitization is accompanied by the rupture of the upper LBD interfaces, with the LBDs adopting their maximally closed clamshell conformations, as described in the desensitized-state GluA2–GSG1L complex.
## Extended Data Table 1 | Cryo-EM data collection, refinement and validation statistics

| Data collection and processing | GluA2-GSG1L	extsubscript{2EK1} (EMDB-8819) (PDB 5WEK) | GluA2-GSG1L	extsubscript{3RK1} (EMDB-8820) (PDB 5WEL) | GluA2-GSG1L	extsubscript{ARG1} (EMDB-8821) (PDB 5WEM) | GluA2-GSG1L	extsubscript{ARG2} (EMDB-8822) (PDB 5WEN) | GluA2-STZ	extsubscript{ICER-CTE} (EMDB-8823) (PDB 5WEO) |
|------------------------------|------------------------------------------------|------------------------------------------------|------------------------------------------------|------------------------------------------------|------------------------------------------------|
| Magnification                | 39,000x                                        | 39,000x                                        | 130,000x                                       | 39,000x                                        | 130,000x                                       |
| Voltage (kV)                 | 300                                            | 300                                            | 300                                            | 300                                            | 300                                            |
| Electron exposure (e⁻/Å\(^2\)) | 67                                             | 67                                             | 55                                             | 67                                             | 55                                             |
| Defocus range (μm)           | -1.5 to -3.5                                   | -1.5 to -3.5                                   | -1.0 to -3.0                                   | -1.0 to -3.0                                   | -1.0 to -3.0                                   |
| Pixel size (Å)               | 0.98                                           | 0.98                                           | 1.08                                           | 0.98                                           | 0.98                                           |
| Symmetry imposed             | C2                                             | C2                                             | C2                                             | C2                                             | C2                                             |
| Initial particle images (no.)| 541,261                                        | 419,261                                        | 20,392                                         | 18,926                                         | 595,889                                        |
| Final particle images (no.)  | 26,971                                         | 41,926                                         | 20,392                                         | 18,926                                         | 69,207                                         |
| Map resolution (Å)           | 4.6                                            | 4.4                                            | 6.1                                            | 6.8                                            | 4.2                                            |
| Map resolution range (Å)     | 3 to 9                                         | 3 to 9                                         | 4 to 9                                         | 4 to 9                                         | 3 to 9                                         |
| Refinement                   |                                                |                                                |                                                |                                                |                                                |
| Initial model used (PDB code)| 5VHY                                           | 5VHY                                           | 5VHY                                           | 5VHY                                           | 5KBU                                           |
| FSC-0.143                    |                                                |                                                |                                                |                                                |                                                |
| Model resolution (Å)         | 4.6                                            | 4.4                                            | 6.1                                            | 6.8                                            | 4.2                                            |
| Model resolution range (Å)   | 3 to 9                                         | 3 to 9                                         | 4 to 9                                         | 4 to 9                                         | 3 to 9                                         |
| Map sharpening B factor (Å\(^2\)) | -172                                           | -196                                           | -225                                           | -653                                           | -160                                           |
| Model composition            |                                                |                                                |                                                |                                                |                                                |
| Non-hydrogen atoms           | 27,468                                         | 27,468                                         | 27,360                                         | 27,360                                         | 30,940                                         |
| Protein residues             | 27,360                                         | 27,360                                         | 27,360                                         | 27,360                                         | 30,804                                         |
| Ligands                      | 108                                            | 108                                            | N/A                                            | N/A                                            | 136                                            |
| B factors (Å\(^2\))          |                                                |                                                |                                                |                                                |                                                |
| Protein                      | 263.37                                         | 248.89                                         | 337.97                                         | 409.74                                         | 240.10                                         |
| Ligand                       | 217.60                                         | 88.03                                          | N/A                                            | N/A                                            | 113.71                                         |
| R.m.s. deviations            |                                                |                                                |                                                |                                                |                                                |
| Bond lengths (Å)             | 0.01                                           | 0.01                                           | 0.01                                           | 0.01                                           | 0.01                                           |
| Bond angles (°)              | 1.18                                           | 1.25                                           | 1.37                                           | 1.25                                           | 1.16                                           |
| Validation                   |                                                |                                                |                                                |                                                |                                                |
| MolProbity score             | 1.69                                           | 1.81                                           | 1.85                                           | 1.86                                           | 2.00                                           |
| Clashscore                   | 6.12                                           | 6.79                                           | 7.83                                           | 7.84                                           | 9.25                                           |
| Poor rotamers (%)            | 0.23                                           | 0.34                                           | 0.14                                           | 0.37                                           | 0.59                                           |
| Ramachandran plot            |                                                |                                                |                                                |                                                |                                                |
| Favored (%)                  | 93.11                                          | 93.22                                          | 93.48                                          | 93.34                                          | 91.10                                          |
| Allowed (%)                  | 5.48                                           | 6.63                                           | 6.34                                           | 6.55                                           | 8.20                                           |
| Outliers (%)                 | 1.42                                           | 0.14                                           | 0.17                                           | 0.12                                           | 0.70                                           |

© 2017 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.
# Experimental design

1. **Sample size**
   - Describe how sample size was determined.
   - **Amount of cryo-EM data collected was limited by time allocation at the microscopes**

2. **Data exclusions**
   - Describe any data exclusions.
   - **No data have been excluded**

3. **Replication**
   - Describe whether the experimental findings were reliably reproduced.
   - **No replication attempts have failed. All cryo-EM data collections were consistent from the beginning to the end.**

4. **Randomization**
   - Describe how samples/organisms/participants were allocated into experimental groups.
   - **N/A**

5. **Blinding**
   - Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   - **N/A**

   Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. **Statistical parameters**
   - For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

   - **n/a**
   - **Confirmed**

   - □ √ The **exact sample size** (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
   - □ □ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
   - □ □ A statement indicating how many times each experiment was replicated
   - □ □ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
   - □ □ □ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
   - □ □ □ The test results (e.g. P values) given as exact values whenever possible and with confidence intervals noted
   - □ □ □ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
   - □ □ □ Clearly defined error bars

   *See the web collection on [statistics for biologists](https://www.nature.com/natureresearch) for further resources and guidance.*
7. Software

Describe the software used to analyze the data in this study.

Origin 9.1.0, Leginon, MotionCor2, gCTF, RELION 2.0, UCSF Chimera, ResMap, COOT, PHENIX, EMAN2, Pymol

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

None

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

N/A

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

HEK293S GnTi-, ATCC, Cat#CRL-3022
HEK293, ATCC, Cat#CRL-1573
Sf9, Gibco, Cat#12659017

b. Describe the method of cell line authentication used.

None of the cell lines used have been authenticated

c. Report whether the cell lines were tested for mycoplasma contamination.

The cell lines used have been tested for mycoplasma contamination by the providers (negative results) but have not been retested in the lab

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

N/A

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

N/A

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

N/A