# THE STRUCTURE AND FUNCTION OF GLUTAMATE RECEPTOR ION CHANNELS

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As in the case of many ligand-gated ion channels, the biochemical and electrophysiological properties of the ionotropic glutamate receptors have been studied extensively. Nevertheless, we still do not understand the molecular mechanisms that harness the free energy of agonist binding, first to drive channel opening, and then to allow the channel to close (desensitize) even though agonist remains bound. Recent crystallographic analyses of the ligand-binding domains of these receptors have identified conformational changes associated with agonist binding, yielding a working hypothesis of channel function. This opens the way to determining how the domains and subunits are assembled into an oligomeric channel, how the domains are connected, how the channel is formed, and where it is located relative to the ligand-binding domains, all of which govern the processes of channel activation and desensitization.

ION CHANNEL STRUCTURE

Ion gradients are widely used in transmembrane signalling. These gradients are established by the active transport of ions from low to high electrochemical potentials across the membrane, driven by coupling transport to an energetically favourable process, such as ATP hydrolysis or respiration. Once established, such a gradient is relatively stable, as the lipid bilayer is essentially impermeant to ions. Furthermore, the partitioning of ions is typically not electroneutral, so that in addition to the concentration gradient for each ionic species, an overall electrostatic potential is built up across the membrane. Electrochemical gradients represent sources of potential energy that can be tapped for a variety of purposes. In neurons, the encoding, transmission and integration of information relies primarily on the carefully choreographed control of transmembrane electrostatic potential by a host of ion channels. Fast synaptic communication between nerve cells is one such process, mediated by ion channels that are located in the postsynaptic membrane and activated by neurotransmitters that are released from the presynaptic cell.

The development of patch-clamp techniques that are sensitive to the opening and closing of even a single ion channel[1] has permitted the detailed characterization of the electrophysiological behaviour of many ligand-gated ion channels, including those responsible for neurotransmission[2]. Typically, ligand-gated ion channels are closed in the resting state, but open in response to the binding of agonist, allowing selected ions to flow down their electrochemical gradients through an internal pore. After activation, the channels undergo spontaneous desensitization, closing even in the continuing presence of agonist. In a synapse, such channels mediate local depolarization or hyperpolarization of the plasma membrane, depending on the channel's ion selectivity and the nature of the corresponding transmembrane gradients. The change in membrane potential represents a signal that can be further processed by the receiving cell, and the magnitude and duration of the signal depends on several factors, including the time course of neurotransmitter concentration in the cleft, and the kinetics and extent of channel activation and desensitization.

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DOI: 10.1038/nrn725

Box 1 | **Cloned mammalian glutamate receptors**

Ionotropic glutamate receptors (iGluRs) with high affinity for the agonist AMPA (α-amino-5-methyl-3-hydroxy-4-isoxazole propionic acid) are homo- or hetero-oligomers composed of subunits known as GluRA–GluRD (or GluR1–GluR4)[114–116]. They are responsible for fast excitatory synaptic signalling in the brain. In addition, recent evidence indicates that they are involved in the activity-dependent modulation of synaptic strength[117], a key component of the synaptic plasticity that is thought to underpin learning and memory.

Kainate receptors are homo- or hetero-oligomers of the subunits GluR5–GluR7, KA1 and KA2 (REFS 118–122). Like the AMPA receptors, they can mediate excitatory synaptic signals, but they are also involved in modulating the presynaptic release of neurotransmitter, and therefore in regulating the strength of synaptic connections. The kainate receptors are also potential therapeutic targets in the treatment of epilepsy and pain[123].

A third family of glutamate receptors is activated by NMDA (*N*-methyl-D-aspartate). These receptors are formed as heteromers that contain both NR1 (REF. 124) and NR2 (NR2A–NR2D)[125–128] subunits, and in some cases NR3 subunits (NR3A and NR3B)[129–131]. NMDA receptors require co-agonism by glycine[132] or D-serine[133], which bind to the NR1 subunits, and glutamate, which binds to the NR2 subunits. Another unique feature of NMDA receptors is that extracellular $Mg^{2+}$ blocks these channels at resting membrane potentials; this block is relieved by depolarization of the membrane, allowing NMDA receptors to act as integrators of synaptic activity[134,135].

So, all three families of glutamate receptor contain multiple subunits that can co-assemble, but only within families[136–138], to produce many receptor combinations. In addition, alternative splicing and RNA editing generate further variability[11,139]. As these modifications can affect both the extent and kinetics of the channel's electrophysiological response, the iGluR family shows an enormous molecular and functional diversity that is likely to be physiologically important.

In addition to these iGluR subunits, two orphan mammalian subunits have been cloned (δ1 and δ2)[140]; although no ion channel functionality has yet been shown for these subunits, a mutation in one of them is responsible for the neurological defects associated with the lurcher phenotype in mice[75,141]. Finally, iGluR homologues have also been cloned from a prokaryote[44] and from various invertebrates[142,143] and plants[144]. The GluR family is therefore likely to be evolutionarily old[145].

ALLOSTERIC
A term originally used to describe enzymes that have two or more receptor sites, one of which (the active site) binds the principal substrate, whereas the other(s) bind(s) effector molecules that can influence its biological activity. More generally, it is used to describe the indirect coupling of distinct sites within a protein, mediated by conformational changes.

P-LOOP SEQUENCE
A conserved structural motif found in many different ion channels that constitutes part of the channel pore.

PDZ DOMAIN
A peptide-binding domain that is important for the organization of membrane proteins, particularly at cell–cell junctions, including synapses. They can bind to the carboxyl termini of proteins or can form dimers with other PDZ domains. PDZ domains are named after the proteins in which these sequence motifs were originally identified (PSD95, Discs large, zona occludens 1).

How agonist binding triggers the temporally regulated, multistep sequence of functional transitions in ligand-gated ion channels is one of the fundamental questions in ion channel molecular biology. This question can be broken down according to the functional steps that are associated with agonist binding, channel activation, ion permeation and channel desensitization. As the site of agonist binding is usually distant from the ion pore, activation of the channel must be mediated by ALLOSTERIC coupling of conformational changes at the binding site to corresponding changes at the channel's gate.

Recent experimental breakthroughs have provided new information on several of these processes. For glutamate receptor ion channels, crystallographic analyses of the ligand-binding domain in various functional conformations have revealed several activation-dependent conformational states and a dimerization interface that is implicated in receptor desensitization[3–5]. In parallel, crystallographic and electron-microscopic data have been obtained for a prokaryotic $K^+$ channel[6,7], the *Torpedo* acetylcholine receptor (nAChR)[8] and an acetylcholine-binding protein[9], with general and specific implications for the working of ion channels. This review aims to integrate structural insights with new functional and biochemical data, and to describe advances in our understanding of the mechanism of glutamate receptor ion channel function that are emerging as a result.

### iGluR: a modular receptor

Ligand-gated ion channels are generally formed as homo- or hetero-oligomeric assemblies of integral membrane protein subunits. On the basis of pharmacological and sequence-homology criteria, they can be divided into several families; one such family comprises glutamate-gated channels, which are found in the pre- and postsynaptic cell membranes of the central nervous system. These glutamate receptor ion channels (iGluRs) are responsible for most fast excitatory signalling in the brain, and are thought to contribute to the synaptic plasticity that has been implicated in our ability to learn and form memories. In keeping with the physiological importance of iGluRs, their dysfunction is implicated in a range of neuropathologies, including epilepsy, stroke damage and the perception of pain[10]. Pharmacologically distinct subfamilies of iGluR have been identified, characterized by their affinities for the synthetic agonists AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid), NMDA (*N*-methyl-D-aspartate) and kainate[11] (BOX 1).

An important advantage of the iGluRs for studies of ion channel function is their modular construction. This allows a reductionist approach to the questions outlined above. As illustrated in FIG. 1 (side view), a typical iGluR subunit consists of an amino-terminal domain (NTD), a ligand-binding domain (S1S2), three transmembrane domains and a re-entrant pore loop, and a carboxy-terminal domain[12]. The NTD shows sequence homology to the leucine/isoleucine/valine-binding protein LIVBP, one of the bacterial periplasmic binding proteins (PBPs)[13]. In the NR2 subunits (BOX 1), it forms or contributes to the binding site of many NMDA receptor modulators[14–25], and influences receptor desensitization[26,27]. For the non-NMDA receptors, it contributes to receptor assembly[28–30]. This domain is absent from the related kainate-binding proteins of frog, chick and goldfish brains[31–33]. The S1S2 ligand-binding domain is formed by two sequences that share sequence and structural homology with the glutamine-binding protein QBP[3,34,35]. The re-entrant pore loop is homologous to the P-LOOP SEQUENCES of other channels[36,37], and is located between the first and second transmembrane domains, forming a pore domain similar to those found in $K^+$ channels. In iGluRs, as in $K^+$ channels, the P-loop sequence lines the channel and determines many of its electrophysiological properties[38–41]. In addition, neuronal iGluRs contain a third transmembrane domain and a cytoplasmic carboxy-terminal domain that interacts with PDZ-DOMAIN proteins of the postsynaptic density, which control the targeting and localization of these receptors[42].

The modular design indicates that the iGluR might have been assembled from components, in line with the 'genes-in-pieces' hypothesis[43]. Support for this idea came from the discovery of a prokaryotic glutamate receptor ion channel (GluR0) — a potential evolutionary 'missing link'[44]. As GluR0 lacks an NTD and has only two transmembrane domains, it could have been formed by the fusion of a PBP with a primitive $K^+$ channel[44]. Like the $K^+$ channel KcsA, the structure of

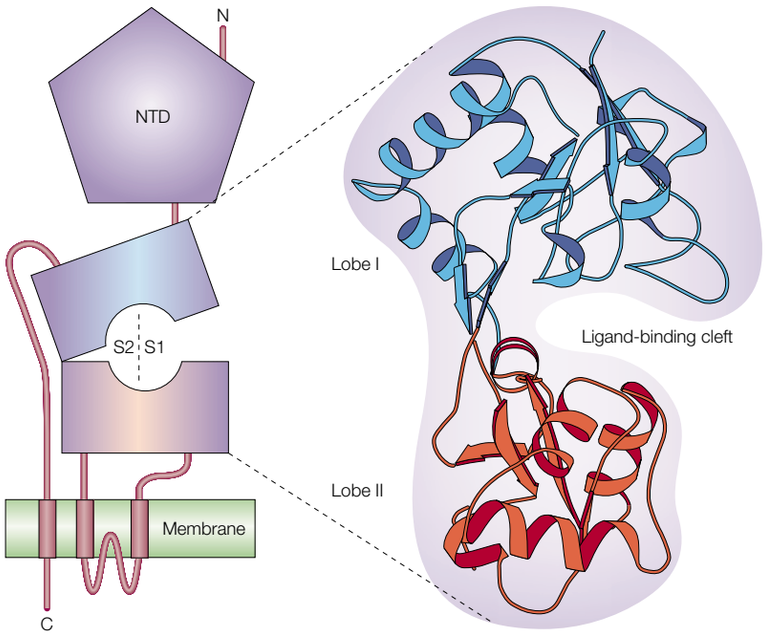


Figure 1 | **The modular nature of iGluR subunits.** The amino terminus of ionotropic glutamate receptor (iGluR) subunits is extracellular. An amino-terminal domain (NTD) is followed by the S1 half-domain, two transmembrane domains with an intervening re-entrant P loop, the S2 half-domain and a third transmembrane domain. The carboxyl terminus is located in the cytoplasm, where it can interact with proteins of the postsynaptic density. The S1 and S2 half-domains form the iGluR ligand-binding domain, which is homologous to the bacterial glutamine-binding protein QBP. The structure of this domain has been determined crystallographically, and is shown in enlarged form as a ribbon diagram. It consists of two lobes (lobe I, blue; lobe II, red), separated by a ligand-binding cleft[4].

which has been determined by X-ray crystallography (see Protein Data Bank (PDB) entry 1BL8)[6], GluR0 forms a glutamate-gated channel that is selective for potassium ions[44]. As a result of this structural and functional homology of the pore-forming domain, the extensive structural and biophysical studies of $K^+$ channel ion permeation can be mined for insights into iGluR function.

### S1S2: a molecular piston

A fortunate consequence of the modularity of the iGluR construction is that its ligand-binding domain can be expressed in isolation from the rest of the subunit[45–49]; isolated constructs from AMPA receptors have been shown to bind ligand with both high affinity and high specific activity[45,46,50]. It is this characteristic of the protein that has provided the greatest insights into potential mechanisms of iGluR channel gating, as the conversion of the free energy of agonist binding into mechanical work takes place entirely within the context of a self-contained domain that can be studied in isolation. This is consistent with the observation that gating of non-NMDA glutamate receptors seems to involve the independent association of four molecules of agonist, one with each of four receptor subunits[51]. By contrast, the agonist-binding site of nAChR is located at the interfaces between adjacent subunits in the oligomer[9,52].

The existence of the S1S2 domain was first postulated on the basis of the above-mentioned homology to a bacterial PBP that is specific for glutamine[13,34]. X-ray crystallography has shown the PBPs to be bilobate molecules that bind their ligands using a 'Venus-flytrap' mechanism[53]: in the absence of ligand, the cleft between the lobes is open, but in the presence of ligand, the cleft is closed, trapping the ligand within it[54]. The cleft closure can be described as a rigid-body motion of one lobe relative to the other, typically involving a rotation of ~35–55° about an axis through the hinge[54–56]. However, various PBP-homologous proteins have been identified, some of which show much smaller domain closures in response to ligand binding. These include the purine and Lac repressor proteins, which close by 17–24° and 6°, respectively[57–59]. The smaller cleft closure seems to be dictated by the repressors' need to oligomerize[60], a constraint that would also be expected to apply to the iGluR[61].

Since 1998, crystallographic insights have been obtained into the structure of the S1S2 domain core of the AMPA receptor GluR2. The overall structure of the domain core closely resembles that of QBP[3], with two lobes packed together to form a ligand-binding cleft on one side of the molecule (FIG. 1). The transmembrane domains of the receptor are tethered to the core domain by peptides that are attached to the carboxyl terminus of S1, and the amino and carboxyl termini of S2. These attachment sites are clustered on the back and at the bottom end of the molecule, as shown in FIG. 1. Parallel small-angle X-ray scattering measurements of the molecular envelope of a larger S1S2 construct showed that the peptide tethers extend beyond the end of the core domain, forming an even more elongated particle[61]. These peptides are therefore distant from both the agonist-binding cleft and the hinge domain on the back of the molecule.

Further insights have now been provided by the determination of crystallographic structures of the S1S2 core, alone and in complexes with a number of antagonists and agonists[4]. One key finding is that the extent of cleft closure more or less tracks the extent of channel activation and desensitization (FIG. 2). In response to binding of the agonists glutamate (see PDB entry 1FTJ) and AMPA (PDB 1FTM), the S1S2 core undergoes a cleft closure of ~20° relative to its ligand-free (apo) conformation (PDB 1FTO)[4] (FIG. 2). This closure is smaller than that seen for the bacterial PBP, and more in keeping with the conformational changes that are seen in oligomeric repressor proteins[61]. Most interestingly, the cleft closure in the presence of the agonist kainate (PDB 1FTK and 1FW0) was only ~12°, even smaller than that seen with glutamate or AMPA (FIG. 2). Kainate is a 'partial agonist' of AMPA receptors, inducing both a smaller extent of activation and a smaller degree of desensitization than AMPA or glutamate[62]. Finally, the antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX; PDB 1FTL) stabilizes the open conformation of the cleft. In addition to the extent of cleft closure, the direction of rotation varies between the different S1S2–agonist complexes[4]. Such behaviour has been observed previously in examining other PBP homologues[60], and might provide a second mechanism by which different agonists can induce different channel responses.

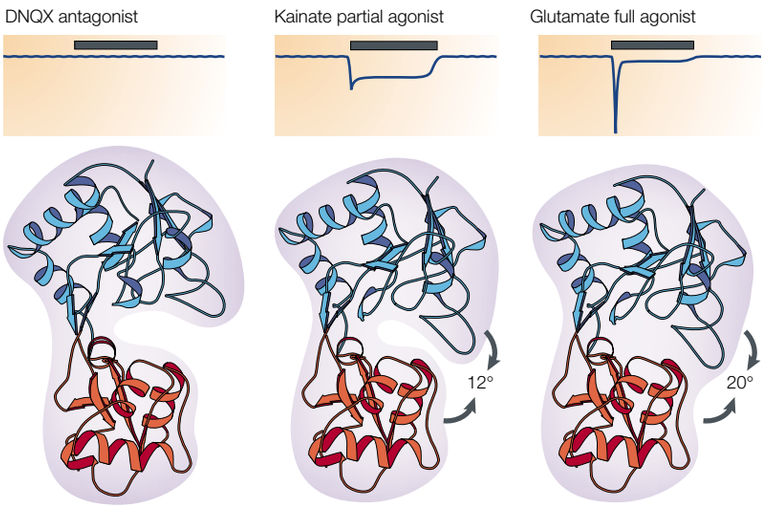


Figure 2 | **Cleft closure mirrors extent of activation.** Upper panel: schematic current traces indicating the response of non-NMDA (*N*-methyl-D-aspartate) ionotropic glutamate receptors to the antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX; left), the partial agonist kainate (middle) and the full agonist glutamate (right)[62]. Lower panel: corresponding structures of the S1S2 domain. DNQX binding to the S1S2 domain stabilizes the open, apo conformation of the domain, which is associated with the closed state of the channel (left). Binding of kainate (middle) induces a ~12° cleft closure in the S1S2 domain, and leads to smaller peak currents and lower levels of channel desensitization than does glutamate binding (right), which causes a ~20° cleft closure in the S1S2 domain[4].

## The closing of the cleft

The structures of the S1S2 core also provide a framework for understanding the stepwise interaction of agonist with the iGluR binding site (FIG. 3). STOPPED-FLOW FLUORESCENCE STUDIES of wild-type and mutant S1S2 domains show that agonists first dock to one side of the binding cleft, interacting with two side chains (Tyr451 and Glu403 in GluR4)[63] that form a ridge-like structure at the edge of the open binding site[4]. This docking interaction is modelled in FIG. 3 (middle) on the basis of the structure of the ligand-free domain and the interactions of glutamate with lobe I in the co-crystal structure. In a subsequent, slower step, S1S2 undergoes a conformational change that locks the agonist into the closed cleft (FIG. 3, right). This locking step presumably corresponds to the cleft closure detected crystallographically, and involves a glutamate side chain (Glu706 in GluR4) that forms a hydrogen bond with the agonist α-amino group[3,63]. This side chain repositions itself within the cleft as a result of ligand binding[4]. The hydrogen bond is part of a three-way network that links side chains on each lobe of the protein (Glu706 and Thr481) together with the agonist, and it might be responsible for the high stability of the resulting complex: the backward isomerization rate constant is only ~10 $s^{-1}$ (REF. 63).

What is the mechanism of cleft closure? It seems to involve a pre-existing open⇔closed equilibrium for the ligand-binding domain. In the absence of agonist, the balance of the equilibrium favours the open state; the balance is shifted in favour of the closed state in the presence of agonist, which interacts more strongly with this conformation (FIG. 4). Consistent with such an equilibrium, the structure of the ligand-free form of the GluR2 S1S2 core has an open binding cleft (FIG. 4, upper left)[4], whereas that of the GluR0 S1S2 domain has a closed conformation (FIG. 4, upper right; see PDB 1IIW)[5]. All agonist–iGluR complexes studied so far have been closed (FIG. 4, lower right). However, crystallographic studies of the related ligand-binding domain of the metabotropic glutamate receptor mGluR1 have revealed a mixture of open and closed conformations in the presence of glutamate (FIG. 4, lower left and lower right; see PDB 1EWK)[64].

For the PBP, the existence of the equilibria shown in FIG. 4 is well established by biochemical analyses[65–67]. In addition to the expected open, empty, and closed, ligand-bound structures, crystallographic studies have shown PBP in both the closed, empty state[68] and the open, ligand-bound state[69,70]. For the PBP, the open conformation is not universally favoured in the apo state. Although both the phosphate- and arabinose-binding proteins adopt a predominantly open conformation in the absence of ligand[71,72], the cleft of the dicarboxylate-binding protein DctP is predominantly closed in the absence of ligand, opens to allow ligand to enter, and then recloses[73]. The kinetics of GluR4 S1S2 ligand binding are qualitatively similar to those of the phosphate-binding protein[63]. As iGluR agonists do not have access to the binding site in the closed state[4,5], this indicates that the eukaryotic iGluR domains preferentially adopt an open conformation in the absence of ligand (FIG. 4, upper left).

Assuming a simple allosteric coupling between the states of the ligand-binding domain and the channel gate, observations of spontaneous cleft closure correlate well with evidence of spontaneous activation of prokaryotic, NMDA and certain mutant non-NMDA glutamate receptors. In particular, although spontaneous activation is not observed for GluR2, it is observed for the prokaryotic GluR0 channel and for NMDA receptors, presumably reflecting the conformational equilibrium observed for the isolated binding cleft[5,74]. Furthermore, on introducing the LURCHER MUTATION[75] into both AMPA and kainate receptor subunits, spontaneous activation can be detected in the form of membrane current leakage in the absence of agonist[76–78]. The closed-channel state of the wild-type eukaryotic receptors seems to be sufficiently stable to prevent spontaneous opening (see below); however, when destabilized by mutations, the conformational equilibrium becomes apparent. Spontaneous activation has also been reported for nAChR mutants[79]. So, it is quite possible that entire receptor subunits exist in a conformational equilibrium that is differentially stabilized in the absence or presence of agonist (simple allostery), as has been proposed for iGluRs and other ion channels[80,81]. However, even though there seems to be an allosteric switch between the well-defined end states, the linear free-energy relationships of transition-state energies in nAChR mutants indicate that the switching process might involve a 'conformational wave' that propagates from the binding site to the channel gate[82].

Whereas simple allostery seems to operate in iGluRs and other ligand-gated ion channel subunits, the question

STOPPED-FLOW FLUORESCENCE STUDIES
The properties of a fluorophore are dependent on its chemical environment, and can therefore be influenced by molecular interactions or conformational changes. In stopped-flow experiments, interacting molecules are rapidly mixed in a flow cell, and the temporal evolution of their fluorescence signals is monitored to determine the kinetics of the interaction.

LURCHER MUTATION
A spontaneous gain-of-function mutation in the δ2 glutamate receptor gene of mice that causes neuronal cell death, leading to loss of motor control in heterozygotes and to early postnatal mortality in homozygotes.

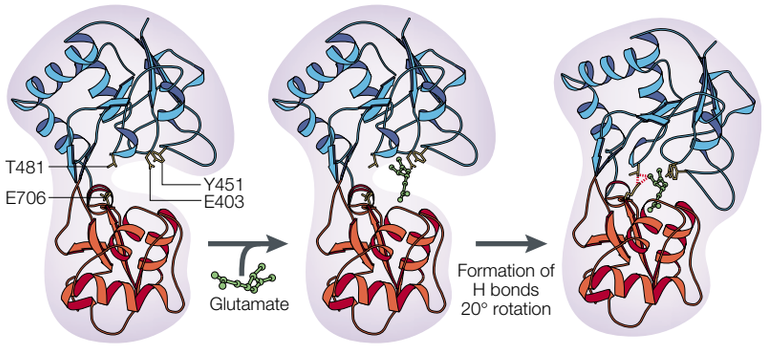


Figure 3 | **Ligand binding to the S1S2 domain.** From left to right, glutamate docks in the open ligand-binding cleft on lobe I (blue), near to side chains Tyr451 and Glu403, shown as modelled (middle). A subsequent, kinetically slower cleft closure allows the formation of a three-way hydrogen-bond network (right) between the glutamate α-amino moiety and side chains Thr481 and Glu706 from both sides of the cleft[3,63].

remains as to whether these channels are gated by a concerted Monod–Wyman–Changeux (MWC) mechanism, such as that used to describe haemoglobin cooperativity[83]. For the nAChR, several lines of evidence indicate a concerted mechanism[79]. The ability of an iGluR point mutation to convert an antagonist into an agonist[84] indicates that these receptors might also have a concerted gating mechanism[79]. However, reports that binding of agonist to individual iGluR subunits can lead to incremental gating of the channel[51] would argue that concerted transitions are not always required. Using non-desensitizing GluR3/GluR6 chimaeras or cyclothiazide to block desensitization, and antagonist presaturation to slow the kinetics of agonist association, a three-step opening of the iGluR pore could be detected. The dwell times in the different states could be well described if it was assumed that the small-conductance state required the binding of two agonist molecules, and the medium- and large-conductance states required one additional agonist molecule each, with independent binding to each site[51]. Conductance sublevels have also been observed for various $K^+$ channels; although the resulting models differ in detail from each other and from that proposed for the iGluR, these observations generally support the functional homology between iGluR and $K^+$ channels[85,86]. The altered ion selectivity of the $K^+$ channel substates supports the hypothesis that they represent partially gated channels (rather than showing rapid switching between fully open and closed states)[87].

## Pharmacological specificity

One of the curious observations provided by the first iGluR S1S2 structure was that the ligand-binding site showed an overall negative charge, despite the fact that its physiological agonist and many high-affinity synthetic ligands are themselves negatively charged[3]. A similar coulombic mismatch has been observed between the phosphate-binding protein and its ligand, and is apparently resolved in that case by the precise distribution of both hydrogen-bonding partners and dipolar interactions within the binding site[72,88].

Our understanding of the pharmacological specificity of the glutamate receptors has been greatly advanced by high-resolution structural analysis of the ligand-binding domain of GluR2 in a complex with several different ligands[4]. On the basis of these studies, it seems that complementarity of the agonist-binding pocket for various ligands is mediated by a series of 'subsites', not all of which need to be occupied for high-affinity binding of a given agonist; water molecules are sometimes found in unused subsites[4]. This strategy presumably accounts for the ability of the iGluR binding site to bind various pharmacological agents[89]. Unlike those of the PBP, the subsites responsible for interaction with the agonist side-chain moieties are located such that the ligand lies across, rather than along, the domain's hinge[63]. In keeping with its status as a primordial glutamate receptor, the orientation of agonist in the GluR0 binding site corresponds to that found in the PBP[5]. Ultimately, a full understanding of the differential selectivity of the AMPA, NR1, NR2 and kainate receptor subunits will probably require comparative structural analysis of the corresponding binding-site complexes.

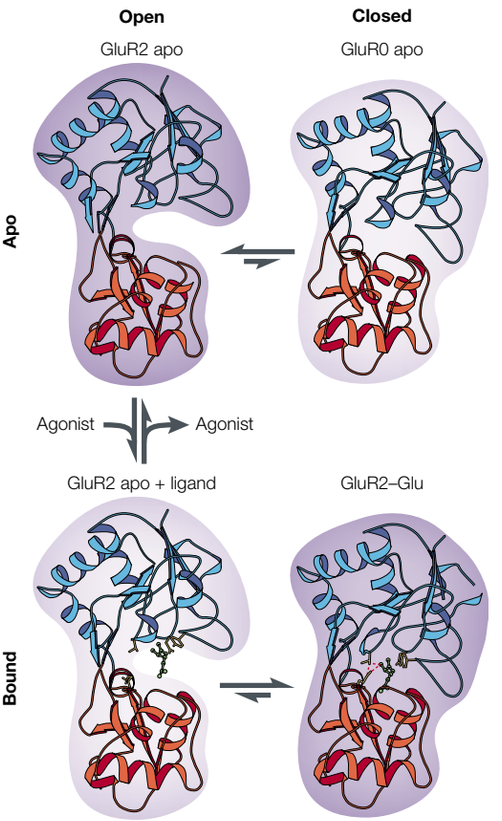


Figure 4 | **Conformational transitions in the S1S2 domain.** In the absence of ligand (top), the S1S2 domain of the glutamate receptor (GluR) probably exists in an open⇔closed equilibrium that is skewed towards the open form. Ligand can bind only to the open cleft (apo⇔bound equilibrium; left-hand side). In the presence of agonist (bottom), the closed conformation is stabilized relative to the open conformation, shifting the open⇔closed equilibrium towards the right. Glu, glutamate.

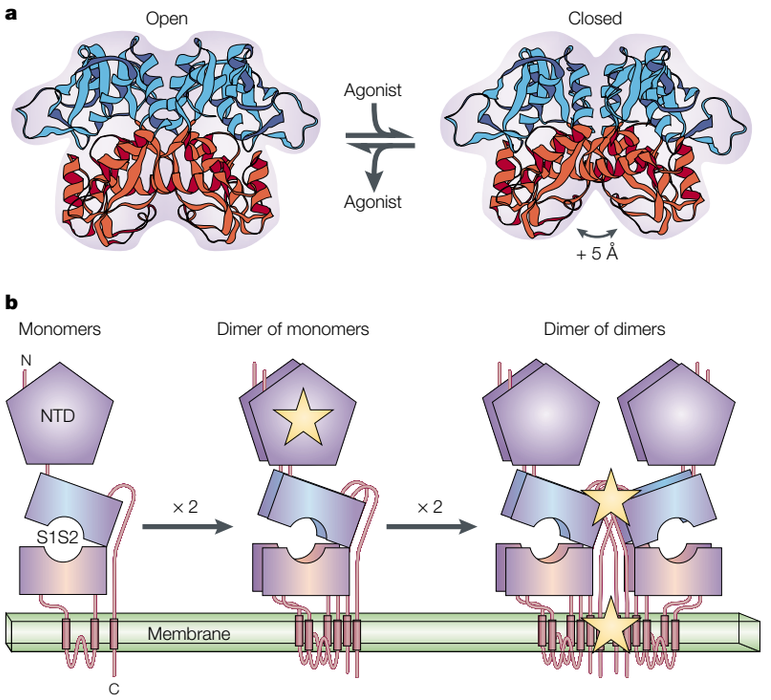


Figure 5 | **Assembly of the iGluR. a** | In most crystal structures of ionotropic glutamate receptors (iGluRs), a dimer is formed between two S1S2 domains[4]. Dimerization is mediated by interactions on the back of lobe I (blue), and involves side chains that have been implicated in channel desensitization. The cleft closure associated with agonist binding moves the ends of lobes II (red) ~5 Å apart. **b** | The 'dimer-of-dimers' model of iGluR assembly[30]. Monomers associate most strongly through interactions between their amino-terminal domains (NTDs) (star in middle figure). Dimers undergo a secondary dimerization, mediated by interactions in the S2 and/or transmembrane domains (stars in right-hand figure). The crystallographically observed S1S2 dimer probably corresponds to this secondary dimerization interaction.

In addition to structural studies, INFRARED SPECTROSCOPY offers an alternative method for detailed analysis of protein–ligand interactions. In combination with crystallographic structure, the vibrational spectra also allow the characterization of the ligand electronic bonding configurations stabilized by interaction with the binding site — data that is inaccessible to structural analysis alone. In the case of the iGluR, they show that the ligand charge distribution adapts to optimize binding; nevertheless, electronegative ligand moieties interact with electronegative functional groups in the binding site, and therefore may not be bound with maximum affinity[90,91]. Extended to include binding-site mutants, this approach offers the prospect of detailed structure–activity information that might prove useful in the design of new agonists and antagonists.

INFRARED SPECTROSCOPY
Many interatomic molecular vibrations show energy transitions that lead to the absorption of photons at characteristic frequencies in the infrared range. The energy transitions, and therefore the associated infrared absorption spectra, are highly sensitive to the stereochemical environment of the functional group(s) involved.

### Putting the pieces together

Despite our schematic understanding of the sequence of building blocks that constitutes a glutamate receptor subunit (FIG. 1), we know neither the spatial disposition of these domains within a subunit nor the packing of the subunits required to form a functional ion channel. However, it is this molecular architecture that provides the context within which conformational changes induced in S1S2 by agonist binding can be communicated to the channel gate.

To understand how the conformational changes in the ligand-binding domain are harnessed to produce physiological signals, it will be necessary to determine the relative orientations of the domains within an oligomeric channel. On the basis of electrophysiological, biochemical and hydrodynamic analyses, iGluRs are thought to be tetramers[28,30,51,92–94]. One set of subunit–subunit interactions seems to be mediated by the NTD[29,30]. In cross-linking experiments, these domains have been shown to dimerize when expressed as soluble constructs[28].

The GluR4 S1S2 domain cannot be cross-linked in dimeric form[28]. Moreover, dimers are not observed in solution-scattering studies at protein concentrations as high as 0.75 mM (REF. 61). However, the GluR2 S1S2 domain forms crystallographic or non-crystallographic dimers in many different crystal forms[4] (FIG. 5a). The crystal environment provides access to far higher concentrations than can be achieved in solution: the effective concentration of a ~30-kD protein, such as S1S2, exceeds 20 mM, assuming standard values for solvent content (50%) and protein density (1.33 g $ml^{-1}$). The most straightforward interpretation of these results is that the dimerization of eukaryotic S1S2 domains is much weaker than that of the NTD. The physiological relevance of the weak S1S2 dimerization is supported by the fact that the dimer is observed in many different crystal forms, that the interface contains numerous amino-acid residues that are implicated in the modulation of iGluR desensitization[95], and that it provides a plausible model for iGluR activation by the S1S2 cleft closure[4,5] (see below). Interestingly, in the case of the GluR0 S1S2 domain, dimers are observed not only crystallographically, but also in solution by analytical centrifugation, with an equilibrium dissociation constant of 0.8 μM (REF. 5).

Recently, using epitope-tagged AMPA receptor chimaeras, Ayalon and Stern-Bach[30] provided evidence of a sequential assembly of tetrameric iGluR channels as dimers of dimers (FIG. 5). In this model, an initial subunit dimerization is mediated primarily by interactions between compatible NTDs (FIG. 5b, step 1: 'dimer of monomers'). However, assembly of functional receptors (FIG. 5b, step 2: 'dimer of dimers') requires that these dimers undergo a second dimerization, this time requiring compatibility between the S2 and transmembrane domains of the subunits. This secondary dimerization is not observed in the absence of NTD compatibility[30], indicating that it might be weaker than the primary dimerization. Given the domains involved, the crystallographically observed dimerization presumably corresponds to part of the secondary interaction. A dimer-of-dimers model of iGluR assembly is consistent with images of GluR2 homomers determined by electron microscopy, with dimensions of 11 × 14 × 17 nm: a fourfold symmetric assembly would have been expected to have at least two approximately equal dimensions[94]. A dimer-of-dimers assembly is not unprecedented for ion channels: it has also been proposed for the cyclic-nucleotide-gated channels[96].

ELECTRON PARAMAGNETIC RESONANCE SPECTROSCOPY
When an atom with an unpaired electron is placed in a magnetic field, the spin of the unpaired electron can align, either in the same direction as the field or in the opposite direction. Electron paramagnetic resonance (EPR) spectroscopy is used to measure the absorption of microwave radiation that accompanies the transition between these two states.

$EC_{50}$
The concentration of agonist that evokes a half-maximal response.

INHIBITION CONSTANT
A measure of affinity, determined by the displacement of a labelled reporter ligand.

## Pull to open?

Models of iGluR activation and desensitization have been developed that account for the observations described above[4,5]. In these models (FIG. 6), cleft closure in a given subunit pulls the transmembrane domains of that subunit away from the pore axis (FIG. 6, side view, middle panel), opening the channel gate. Subsequent slippage between subunits allows the gate to close again, while the subunits remain in the agonist-bound conformation (FIG. 6, side view, right panel).

This model derives from the following considerations[4,5]. To a first approximation, the crystallographically observed S1S2 dimers are formed 'back to back', with the agonist-binding clefts of each monomer facing out from the twofold symmetry axis (FIG. 5a). Furthermore, the dimerization interaction is mediated exclusively by residues that are located in the upper lobe (FIG. 1, lobe I). As a result, cleft closure in the two domains would increase the separation between the two bottom lobes, which are connected to the transmembrane domains, by ~5 Å (FIG. 1 and FIG. 6). As the domains are positioned such that cleft closure can also have a horizontal component (FIG. 5a), a model can be constructed in which the transmembrane domains are also twisted in a manner analogous to the rigid-body motions detected in the KcsA transmembrane domain by ELECTRON PARAMAGNETIC RESONANCE SPECTROSCOPY[97]. Finally, side chains implicated by mutagenesis in the kinetics and/or extent of desensitization are found at the dimer interface. As it is known that agonist remains bound to iGluRs in the desensitized state[98,99], it is likely that the domain remains in the closed-cleft conformation, indicating that interdomain rather than intradomain rearrangements are required.

However, the mechanism of desensitization must also account for the fact that the desensitized state shows much higher affinity for agonist than does the resting state, as indicated by the difference between $EC_{50}$ and INHIBITION CONSTANT values[11], and by the characteristics of receptor desensitization in the presence of AMPA

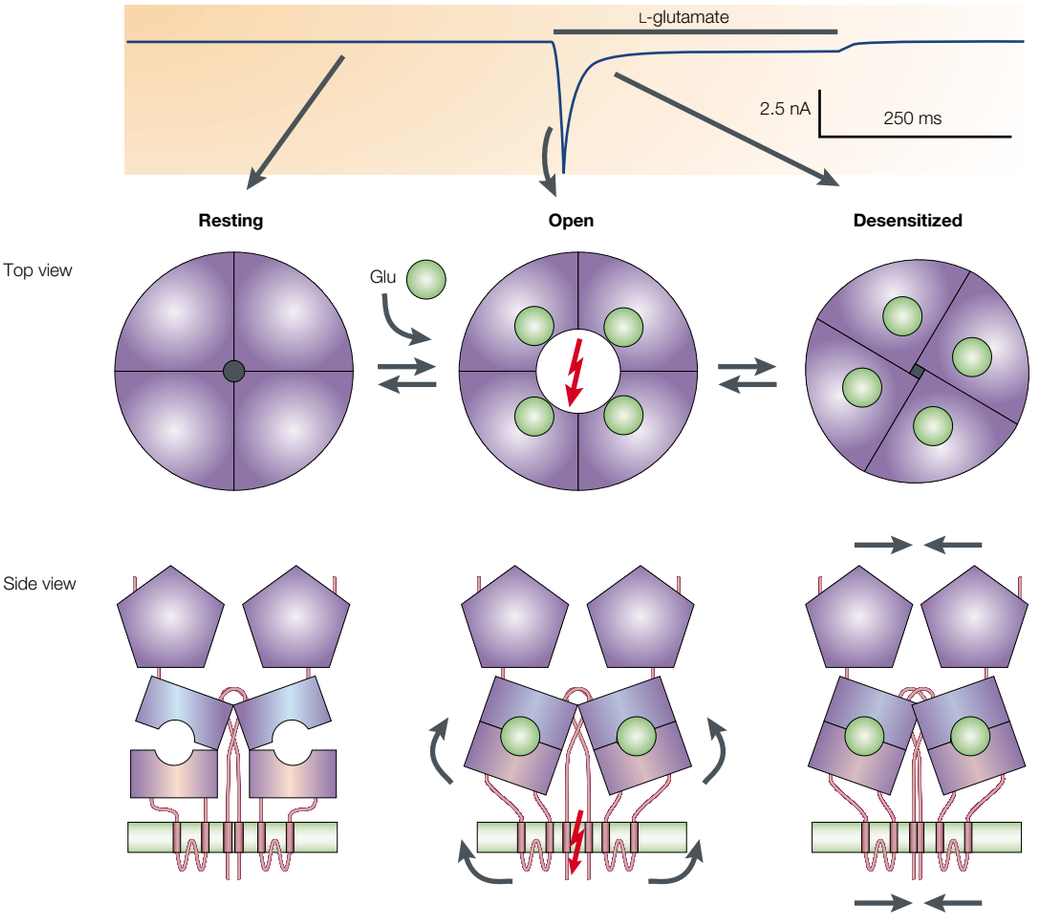


Figure 6 | **A model for iGluR activation and desensitization[4,5].** Upper row: ionotropic glutamate receptor (iGluR) channel physiology. In response to the application of glutamate to mouse embryonic hippocampal neurons, non-NMDA (*N*-methyl-D-aspartate) iGluRs open and then rapidly desensitize to a steady-state level[146]. Middle row: schematic top view of channels in the resting, open and desensitized states. Binding of glutamate (Glu) triggers a conformational change that opens the ion pore (red lightning bolt). Although agonist remains bound, a further, most likely interdomain conformational change allows the channel to close again. Lower row: schematic side view of two of the four iGluR subunits in a channel. S1S2 cleft closure is coupled to a conformational change in the transmembrane domains that pulls open the ion pore (arrows, middle figure). Repacking of the subunits (arrows, right-hand figure) recloses the pore, even though S1S2 and the transmembrane domains remain in the closed-cleft conformation.

and kainate[98]. Furthermore, the high ligand-binding affinity of S1S2 constructs more closely matches that of the desensitized than of the resting state[4,45,100]. How can these observations be reconciled with the proposal that the crystallographically observed agonist-bound state of S1S2 reflects its activated rather than its desensitized state? The low affinity of the resting state for agonist presumably reflects the conformational coupling of S1S2 cleft closure to the energetically unfavourable process of opening the ion channel — allostery in its original sense of interaction between remote binding sites[81]. If channel opening were sufficiently unfavourable, this could effectively eliminate the problem of spontaneous activation described above; mutations such as lurcher would act by destabilizing the closed channel relative to the open channel. Given such a situation, it is possible that the transition from the activated to the desensitized state might allow slight conformational changes within S1S2, leading to the formation of further energetically favourable interactions[4]. Alternatively, additional conformational changes might not be required within S1S2. Instead, desensitization might simply involve effective decoupling of cleft closure from the energetically unfavourable open state of the channel. This could occur either by intersubunit rearrangements or by rearrangements between the domains within a subunit; for example, as mediated by the connecting peptides. In any case, decoupling of S1S2 from the channel is unlikely to be absolute, as agonist remains trapped in the binding site during open-channel block[99].

### Pass the salt

An important question associated with the model shown in FIG. 6 is how conformational changes in the transmembrane domains can lead to ion permeation in the iGluR. Despite their apparent evolutionary and overall structural relationship, the iGluR and $K^+$ channel ion pores differ in their selectivity profiles.

For the prototypical bacterial $K^+$ channel, KcsA, crystallographic and electrophysiological analyses have provided a very detailed understanding of the mechanism of ion conduction[6,7,101]. Potassium ions are dehydrated at the entrance of the channel by protein functional groups that are arranged to mimic the hydration shell, and the precision of the geometry required to achieve dehydration accounts for the observed high selectivity for potassium ions[6,7]. Dehydrated ions are hastened through the channel by repulsive coulombic interactions between multiple ions bound in the permeation pathway[6,101].

This mechanism seems to be exploited by voltage-gated eukaryotic $K^+$ channels[102], and might also apply to the prokaryotic GluR0 channel, which shares the ion selectivity characteristics of the $K^+$ channels[44]. Nevertheless, it is unlikely to apply in detail to the eukaryotic glutamate receptors, which are permeable not only to both $K^+$ and $Na^+$, but, depending on subunit composition, to divalent cations as well[10]. Many other ligand-gated ion channels also show broader ion selectivity, including the nAChR.

What channel opening mechanism is likely to account for the broader ion selectivity and incremental gating of the iGluR? The 4.6-Å-resolution structure of the closed form of the *Torpedo* nAChR channel reveals a hole with a radius of ~3.5 Å along the channel axis at the position of the gate, which is only slightly smaller than the radius of hydrated sodium and potassium ions; this constriction seems to be surrounded by hydrophobic leucine side chains[8]. Furthermore, electron-microscopic analysis of nAChR freeze-trapped in the open state revealed rotation of the kinked helices lining the pore, leading to a widening of the narrowest part of the permeation pathway[103]. It therefore seems likely that, in this case, the channel opens wide enough to allow ions to pass through together with their hydration shells. Consistent with this idea, the open-channel dimensions of the nAChR pore have been estimated as 6.4–8.4 Å (REFS 104–106).

Corresponding measurements give values in excess of 7 Å for recombinant non-NMDA receptors[107], and ~5.5 Å for NMDA receptors[108]. So, it is likely that the broad cation selectivity of the iGluRs results from their ability to pass hydrated or partially hydrated ions through a pore that is significantly wider than that of the KcsA channel. Despite these differences, analysis of the iGluR pore-loop structure by scanning cysteine mutagenesis confirms its similarity to the $K^+$ channel pore loop[40,109]. Indeed, $Ca^{2+}$ channels, which are also homologous to the $K^+$ channels, seem to have large pores (~6 Å in diameter) that are highly selective for $Ca^{2+}$, but also conduct monovalent and divalent cations in its absence[110]. Furthermore, $K^+$ channel mutants have been identified that reduce or eliminate selectivity among monovalent cations (although not divalent cations), indicating that relatively minor sequence changes are sufficient to relax the highly selective interactions in the permeation pathway[111,112]. Given the underlying structural similarities, it will be interesting to determine the mechanisms by which homologous P loops mediate the selective and geometrically precise dehydration of ions in some channels, but act as relatively non-selective molecular sluice gates in other channels.

### Peptides leading to the channel

Another interesting structural question concerns the nature of the symmetry of the iGluR transmembrane domains. Most P-loop-containing channels are fourfold or pseudo-fourfold symmetrical assemblies. However, as described above, the iGluRs are thought to be assembled as dimers of dimers[30]. If the transmembrane domains preserve a true or approximate fourfold symmetry, then the peptides connecting them to the extracellular domains will be required to mediate a transition from two- to fourfold symmetry. Alternatively, it might be that the transmembrane domains show a breakdown of fourfold symmetry, and that this explains the ion selectivity differences described above. In any case, the dimer-of-dimers assembly also raises questions about the independence and equivalence of agonist binding to each of the four iGluR subunits, suggested by the analysis of conductance substates[51].

A further question concerns the role of the peptides that connect the PBP-homologous S1S2 core to the

transmembrane domains. Ranging in length from about 5 to 20 amino acids, these peptides would be responsible for coupling the motions between the ends of lobe II to the channel gate, and they have been implicated in determining the desensitization characteristics of the iGluR[26,27,113]. What is not clear is whether the peptides act as passive 'tie rods', transmitting the bulk motion directly to all three transmembrane domains of a subunit, or whether they undergo independent conformational changes, in which case differential forces would be applied to the three transmembrane domains of a given subunit.

## Concluding remarks

The modular design of the iGluR has made possible the most extensive structural analysis yet of the interaction of ligands with an ion channel binding site. Together with detailed biophysical analyses of the ligand-binding interaction, and the homology of the S1S2 domain core to the well-characterized PBP family, these data provide unparalleled insights into the conformational changes that convert the free energy of agonist binding into mechanical work that can be harnessed to drive channel activation.

In parallel, our understanding of glutamate receptor function has benefited both from new information on the assembly of the channels, and from the explosion of structural information on the homologous bacterial $K^+$ channel KcsA. This has led to the first schematic models of the steps that lead from agonist binding to channel activation and subsequent desensitization.

However, as outlined above, several intriguing questions remain. Given the likely functional differences in ion selectivity, it will be interesting to establish in molecular detail how the P-loop sequence mediates two fundamentally different gating mechanisms; that is, how the relatively rigid and highly selective ion pore of KcsA becomes a less selective, mechanically gated pore in the iGluR. Another important issue concerns the orientations of all the domains in an assembled tetrameric glutamate receptor channel relative to one another in various functional states. These molecular-packing interactions will determine how the closure of the four S1S2 domains is translated into an activation signal, and the nature of the postulated slippage required for desensitization. The details of these structural contacts might also reveal how the NTD and connecting peptides modulate the kinetics of the channel response. Except for the S1S2 dimerization, none of these interactions has been characterized in molecular detail. As structural information on larger domains or intact iGluRs begins to emerge, we can look forward to the development of a framework for understanding the biophysical basis of these versatile molecular machines.


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Acknowledgement

This review is dedicated to the memory of Don C. Wiley, a great scientist, mentor and friend.


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**Protein Data Bank:** http://www.rcsb.org/pdb/
1BL8: potassium channel (KcsA) | 1EWK: mGluR subtype 1, complex with glutamate | 1FTJ: GluR2 S1S2J, complex with glutamate | 1FTK: GluR2 S1S2I, complex with kainate | 1FTL: GluR2 S1S2J, complex with DNQX | 1FTM: GluR2 S1S2J, complex with AMPA | 1FTO: GluR2 S1S2J, apo state | 1FW0: GluR2 S1S2J, complex with kainate | 1IIW: GluR0 ligand-binding core, apo state

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