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Review article

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The AMPA receptor life cycle: assembly, regulation and synaptic diversity

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Abstract

AMPA receptors (AMPARs) mediate the majority of fast excitatory neurotransmission in the mammalian brain. Recent structural, functional and proteomic advances have reshaped our understanding of how these receptors assemble, gate and diversify within distinct synaptic environments. Notably, AMPAR function is governed by an interlocking set of regulatory layers that include alternative splicing and RNA editing within regions of the receptor subunits responsible for gating and permeation, and direct allosteric coupling with different families of auxiliary proteins. The assembly of AMPARs through a dedicated endoplasmic reticulum biogenesis pathway ensures accurate tetramer formation and provides a regulatory checkpoint for synaptic receptor abundance. Brain development introduces additional layers of control as editing, splicing and auxiliary-subunit expression shift from embryonic to adult states, whereas interactions with extracellular proteins contribute to the organization and diversification of synaptic architecture across circuits. Thus, AMPARs are dynamic macromolecular assemblies whose diversity underlies the breadth of glutamatergic signalling in health and disease. This Review synthesizes these emerging principles, highlighting how AMPARs transition from their molecular 'birth' to their deployment at functionally specialized synapses.

Sections

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Introduction

Fast excitatory neurotransmission in the vertebrate brain relies almost entirely on AMPA-type ionotropic glutamate receptors (AMPARs)[1]. For decades, these receptors were viewed primarily through the lens of their pore-forming subunits, GluA1–GluA4, and their core biophysical properties[2]. However, recent advances in genetics, cryo-electron microscopy (cryo-EM), proteomics and developmental neurobiology have led to a fundamental re-evaluation of how AMPARs are built, how they operate and how their molecular diversity contributes to the organization and plasticity of neural circuits[3–7]. These discoveries now situate AMPARs not merely as ligand-gated channels but as modular, dynamically regulated signalling assemblies whose composition and function are tightly choreographed from transcription through to synaptic deployment.

A unifying theme emerging from this body of work is that AMPARs adhere to strict rules of construction that govern how distinct layers of regulation are integrated within the receptor life cycle. At the structural level, allosteric sites within the ligand-binding domain (LBD) of the pore-forming subunits coordinate with evolutionarily conserved auxiliary subunits to shape gating kinetics, desensitization and ion permeability[8–10]. In addition, AMPARs undergo assembly within a dedicated endoplasmic reticulum (ER) biogenesis pathway that enforces the ordered progression from monomer to functional tetramer[11–13]. This early processing step establishes a major checkpoint for the number and type of receptors that ultimately populate the synapse[13].

Complementing these architectural constraints are the post-transcriptional mechanisms that modulate AMPAR function. RNA editing at two key sites (the Q/R site within the receptor pore[14,15] and the R/G site in the LBD[16]), together with alternative splicing of another LBD region known as the flip/flop cassette[17], tunes the receptors' gating and permeation properties, as well as their sensitivity to auxiliary-subunit modulation[8–10]. These features are not static; rather, they change across development in a coordinated manner that results in a highly diversified adult landscape[16,18]. Auxiliary subunits, including transmembrane AMPA receptor regulatory proteins (TARPs), cornichon homologues (CNIHs), cystine-knot AMPAR-modulating proteins (CKAMPs) and the more sparsely expressed germ cell-specific gene 1-like protein (GSG1L), are likewise developmentally and regionally regulated[4,19,20], enabling synapses to fine-tune AMPAR behaviour according to circuit demands.

Emerging evidence suggests that this molecular diversity extends beyond individual receptors to influence how AMPARs are matched with specific synaptic environments. Classical synapse-organizing complexes formed by neurexins, neuroligins and leucine-rich repeat transmembrane proteins, as well as LGI1–ADAM22–membrane-associated guanylate kinase (MAGUK) assemblies, have long been recognized as determinants of synaptic identity[21–23]. Recent proteomic and functional work has identified secreted noelins as extracellular AMPAR-interacting proteins that may influence receptor positioning or stabilization at synapses in a cell-type-specific manner[24]. Thus, AMPAR subunit and auxiliary-subunit composition may constitute a molecular 'barcode' that helps synapses to establish distinct modes of transmission and plasticity.

Together, these findings reveal an AMPAR life cycle that is far more intricate than previously appreciated. From their transcriptional origin and ER assembly through to their deployment and stabilization at specific synapses, AMPARs are influenced by multiple layers of regulation that together define the breadth of excitatory signalling in the brain. In this Review, we synthesize this rapidly evolving field by outlining the structural, developmental and trans-synaptic rules that shape AMPAR diversity.

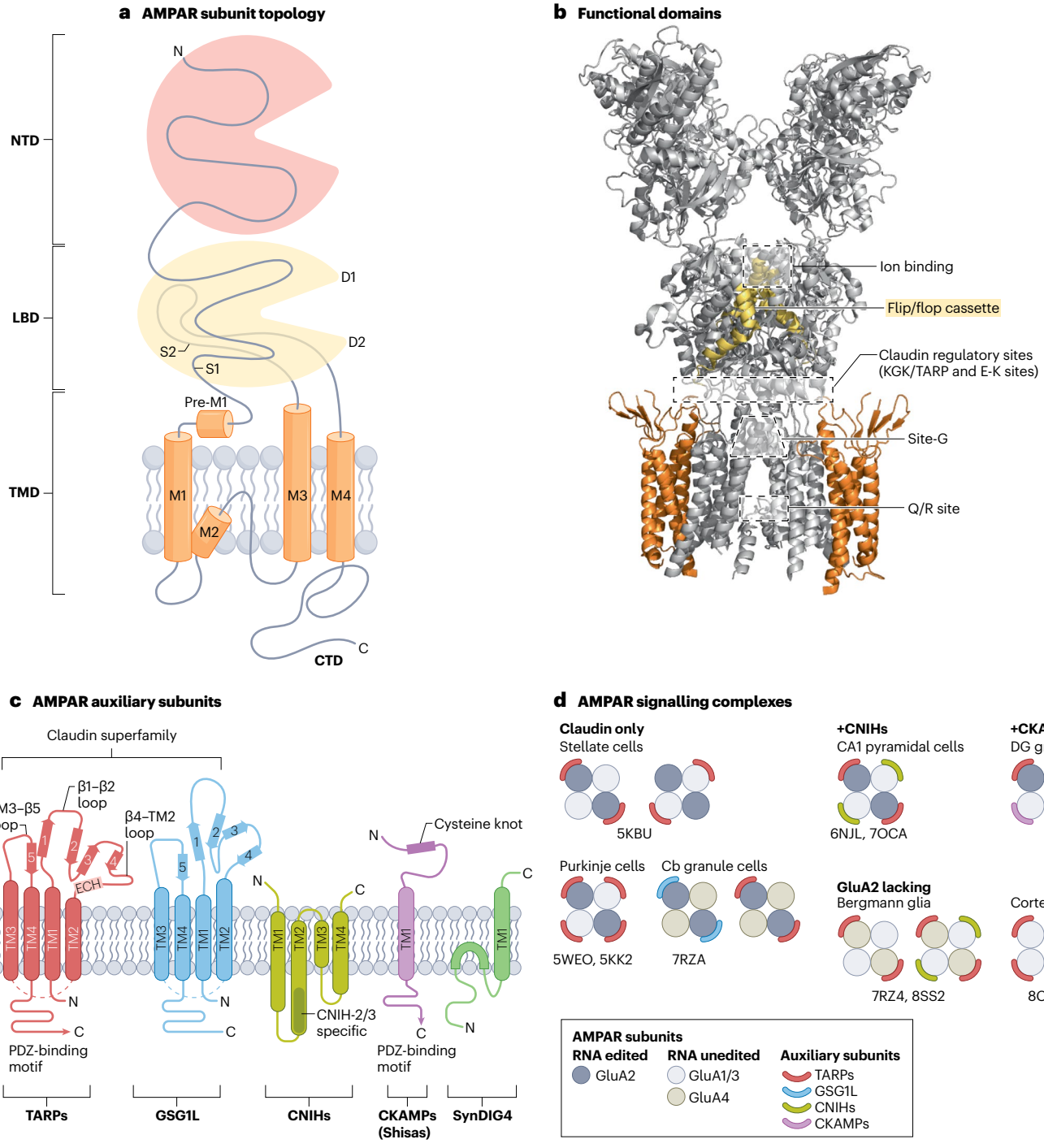
Structural features of AMPARs

In AMPARs, glutamate binding is transduced into pore opening through coordinated movements of distinct structural domains[25,26] (Fig. 1). Each AMPAR subunit comprises an extracellular amino-terminal domain (NTD), a bi-lobed LBD that binds glutamate and initiates channel gating, a transmembrane domain that forms the ion-conducting pore, and a cytoplasmic carboxy-terminal domain involved in intracellular signalling and receptor regulation (Fig. 1a). The LBD is formed by two discontinuous segments, S1 and S2, and adopts a clamshell-like architecture whose closure drives channel activation[27]. The transmembrane domain contains three membrane-spanning helices (M1, M3 and M4) and a re-entrant M2 pore loop that lines the selectivity filter and contributes to ion permeation and channel block[28]. The alternatively spliced flip/flop cassette laying at the lower (D2) lobe of the LBD (Fig. 1a) modulates desensitization and gating kinetics[10,17,29,30].

Despite their conserved modular architecture, GluA1–4 subunits differ in several regulatory features that influence receptor function and assembly. GluA2 subunits undergo RNA editing at the Q/R site

Fig. 1 | Structural organization and molecular diversity of native AMPAR complexes. a, AMPA receptor (AMPAR) subunits (GluA1–GluA4) share a conserved modular architecture composed of an extracellular amino-terminal domain (NTD), a bi-lobed ligand-binding domain (LBD), a transmembrane domain (TMD) that forms the ion channel, and a cytoplasmic carboxy-terminal domain (CTD)[1]. The S1–S2 segments form the clamshell-like LBD, whereas the TMD contains three membrane-spanning helices (M1, M3 and M4) and the re-entrant M2 pore loop[1]. The position of the alternatively spliced flip/flop cassette is indicated in yellow. **b**, A schematic representation of the cryo-electron microscopy structure of the GluA2-transmembrane AMPA receptor regulatory protein (TARP) γ2 (RCSB Protein Data Bank (PDB) identifier: 5KBU[52]). Functional hot spots within the LBD and TMD that govern AMPAR behaviour are shown, including ion-binding sites at the LBD apex[98], the flip/flop cassette at the LBD dimer interface[10], claudin regulatory sites that couple auxiliary-subunit loops to gating[20,73], the 'Site-G' motif near the channel gate that influences Ca^{2+} permeability[9,75] and the Q/R site that regulates both monovalent and divalent-ion permeation[14,40]. **c**, In neurons, AMPARs assemble with a diverse set of auxiliary subunits — including TARPs, germ cell-specific gene 1-like protein (GSG1L), cornichon homologues (CNIHs), cystine-knot AMPAR-modulating proteins (CKAMPs) and synapse differentiation-induced gene 1 (SynDIG4, also known as proline-rich transmembrane protein 1, PRRT1) — that adopt either claudin-like folds or distinct single-pass architectures[1]. TARPs, GSG1L and CNIHs share a four-transmembrane (4-TM) claudin-like scaffold with extracellular loops that interact with the receptor ligand-binding domain and linkers. By contrast, CKAMPs and SynDIG4 are single-pass (1-TM) proteins with large extracellular regions; CKAMPs contain a characteristic cystine-knot motif stabilized by disulfide bonds, whereas SynDIG4 features extended extracellular loops and a relatively short cytoplasmic tail[4]. **d**, Native AMPAR signalling complexes vary across the brain. GluA2-containing receptors predominate[7,10,63], but there are notable exceptions in specialized cell types such as Bergmann glia[14,58,59]. The stoichiometry of these complexes can differ: many receptors incorporate two or more TARPs[8], whereas some inhibitory-type complexes contain GSG1L alone[10]. PDB identifiers are shown beneath each schematic: these refer to representative, experimentally determined structures of recombinant AMPAR assemblies with similar subunit and auxiliary subunit compositions. These structures are provided as structural exemplars to guide the reader and do not represent native, cell-type-specific receptor configurations found in situ. DG, dentate gyrus. Part **b** is adapted with permission from ref. 52, AAAS.

within the pore-forming M2 loop, rendering receptors containing GluA2 impermeable to Ca^{2+}, whereas GluA1, GluA3 and GluA4 remain unedited at this position[2,14,15,28]. Subunits also differ in their C-terminal domains, which mediate interactions with intracellular scaffolding proteins and regulate receptor trafficking and synaptic targeting[2,31]. Additional regulatory mechanisms affect all subunits, including alternative splicing at the flip/flop cassette, which generates isoforms with distinct gating and desensitization kinetics[2,17,32]. All AMPAR subunits are expressed early in the rat embryonic brain and display overlapping but regionally distinct expression patterns[18]. During development, flip variants predominate at early stages, whereas flop isoforms increase postnatally and become prominent in mature neurons[18]. In addition to splicing, RNA editing at the R/G site, located in the LBD just upstream of the flip/flop cassette, converts an arginine to glycine and primarily regulates receptor gating by accelerating recovery from desensitization rather than altering ion permeation[16]. Although less well characterized



than the Q/R site, R/G editing is thought to fine-tune synaptic response kinetics. This modification occurs in GluA2–4 but not GluA1 subunits and is developmentally regulated, being largely absent early in the rodent brain and increasing with maturation[16,32]. Disruption of these developmental regulatory processes has been linked to neurological disease. For example, impaired editing of the GluA2 Q/R site has been associated with motor neuron degeneration in amyotrophic lateral sclerosis[33], while alterations in AMPAR subunit expression and splice isoforms have been reported in temporal lobe epilepsy[34] and in a rat model following pilocarpine-induced status epilepticus[35].

AMPAR subunits assemble as tetrameric receptors and can, in principle, form either homomeric or heteromeric assemblies. However, expression studies indicate that most neurons co-express multiple AMPAR subunits, leading to the formation of heteromeric receptor populations, most of which include GluA2. In rodent hippocampal and cortical neurons, the predominant native receptors are thought to be GluA1/GluA2 or GluA2/GluA3 heteromers[36–38]. Other combinations occur in specific neuronal populations, such as GluA2/GluA4 heteromers in cerebellar granule cells[18,39] and, in some cases, homomeric assemblies (including GluA2 homomers)[40,41]. Recent studies have also demonstrated the existence of predominant triheteromeric GluA2-containing AMPAR assemblies[42]. Nevertheless, certain neurons and glial cells, such as Bergmann glia, express little or no GluA2, resulting in receptors assembled from other subunits, such as GluA1 and GluA4 (refs. 43–45). Consequently, AMPAR subunit composition varies across brain regions and cell types, contributing to the functional diversity of glutamatergic synapses.

All native AMPARs co-assemble with one of two claudin-like proteins: TARPs or GSG1L (Fig. 1c,d). TARP isoforms are members of either the type I class (subdivided into Ia (γ2 and γ3) and Ib (γ4 and γ8) TARPs) or the type II class (γ5 and γ7)[6,7]. These isoforms show distinct regional expression patterns in the mammalian brain. The type I TARPs γ2 (also known as stargazin) and γ3 are broadly expressed in neurons across the brain, with γ2 being particularly abundant in the cerebellum and γ3 enriched in cortical and other telencephalic regions[19]. By contrast, γ4 and γ8 display more restricted distributions: γ4 is enriched in subcortical regions (including the striatum and olfactory bulb), whereas γ8 is highly expressed in hippocampal neurons[19,46]. The Type II TARPs γ5 and γ7 are also regionally specialized, with prominent expression in cerebellar circuits and selected forebrain regions[19,46–50]. Together these complementary expression patterns suggest that different neuronal populations assemble AMPARs with distinct TARP isoforms. A more detailed summary of the regional and cellular expression of each TARP isoform is provided in Supplementary Table 1. Interestingly, GSG1L-containing AMPARs make up only around 5% of all AMPARs and, unlike those associated with TARPs, are expressed late in CNS development[20]. TARPs typically associate with AMPARs in either a 2:4 or 4:4 stoichiometry[8,42,51–55], whereas GSG1L-containing AMPARs generally assemble in a strict 2:4 stoichiometry[20,56,57] (Fig. 1d). Structural and functional studies suggest that the 2:4 TARP:AMPAR stoichiometry can be found in both neuronal and glial cells whereas the 4:4 stoichiometry is apparently neuronal specific[8,58,59].

AMPAR–TARP complexes can co-assemble with other auxiliary subunits, most notably members of the CNIH family, which are present in approximately 80% of AMPAR assemblies[6,7]. Additional, less abundant auxiliary subunits include members of the CKAMP (also known as Shisa) family[6,7,60] (Fig. 1c) and the recently identified claudin auxiliary proteins, claudin-22 and claudin-24, which are prominently expressed in rodent cerebellar granule cells[61]. Of these proteins, CNIH2 and CNIH3 are widely distributed across the rodent brain, with particularly strong enrichment in forebrain regions such as the cortex and hippocampus, relative to the cerebellum[6,7,62,63]. Structural and biochemical studies indicate that CNIHs associate with AMPARs in positions within the receptor complex that are distinct from those that bind TARPs[60,64], where they contribute to receptor assembly and influence AMPAR subunit composition as well as trafficking to the cell surface[10,65–68]. Although synapse differentiation-induced gene 4 (SynDIG4, also known as PRRT1) (Fig. 1c) has been identified as a possible auxiliary protein[69], its precise integration into AMPAR complexes remains unclear[70,71]. The expression of many of these AMPAR-auxiliary subunit complexes appears region specific: the cerebellum primarily expresses claudin-only AMPARs[8,20], whereas CKAMP-containing, CNIH-containing and SynDIG4-containing AMPARs predominate in the hippocampus and cortex[63,68,71,72] (Fig. 1d).

From a functional perspective, several allosteric binding sites, shared by all AMPAR subunits, have been identified and found to control distinct aspects of channel gating and ion permeation (Fig. 1a,b). This includes a network of electrostatic interactions atop the LBD that coordinates with a separate and distant binding site for TARPs, called the KGK/TARP site, to determine the time course of channel gating[73,74] (Fig. 1b). Allosteric coordination between these sites is further modified by alternative splicing of the flip/flop cassette[8,20] (Fig. 1a,b). Finally, a recent study has uncovered a novel divalent binding pocket, Site-G, in the vestibule of the pore that acts as a sieve to funnel Ca^{2+} into the selectivity filter[75] (Fig. 1b).

Structural control of AMPAR gating

In the sections that follow, we will examine the structural determinants that regulate AMPAR gating in detail. However, it first is useful to outline the basic sequence of conformational transitions (Fig. 2) through which glutamate binding to the extracellular LBDs of AMPAR subunits is mechanically coupled to opening of the ion channel pore[3,25,76,77].

In the resting state, the bi-lobed LBD clamshells of AMPAR subunits are open and the channel pore is closed (Fig. 2a). Binding of glutamate promotes progressive closure of the LBD clamshells, generating mechanical tension in the LBD–transmembrane domain linkers that connect the extracellular domains to the pore-forming helices[76,78]. Transmission of this force to the M3 helices, which form the bundle crossing that constitutes the upper activation gate, results in separation of the helices and opening of the channel pore to permit ion conduction (primarily Na^+ and K^+, and in some subunit compositions Ca^{2+})[25,53,79] (Fig. 2a,b). Single-channel recordings and recent structural studies indicate that AMPARs can exist in multiple open conformations associated with distinct subconductance levels, reflecting the progressive activation of the pore as additional subunits transition towards the active state[39,80]. The pore also contains a second constriction near the Q/R site, the lower activation gate, that contributes to ion permeation and selectivity[28,53,75,81] (Fig. 2a).

A defining feature of AMPAR architecture is that the four subunits occupy functionally asymmetric positions within the tetramer: these are referred to as the A/C and B/D subunits, according to their position[25] (Fig. 2b). A similar architectural arrangement of subunits is also found in kainate receptors[82–84] (Fig. 2b). Structural studies of native and recombinant AMPAR heteromers indicate that the extracellular domains of A/C subunits, which are often GluA1 or GluA3 subunits, are positioned closer to the pore axis, whereas those of the B/D subunits, usually GluA2 subunits but can be GluA4[58,59], are located further from the pore[42,85] (Fig. 2b). Consistent with this arrangement, the linkers

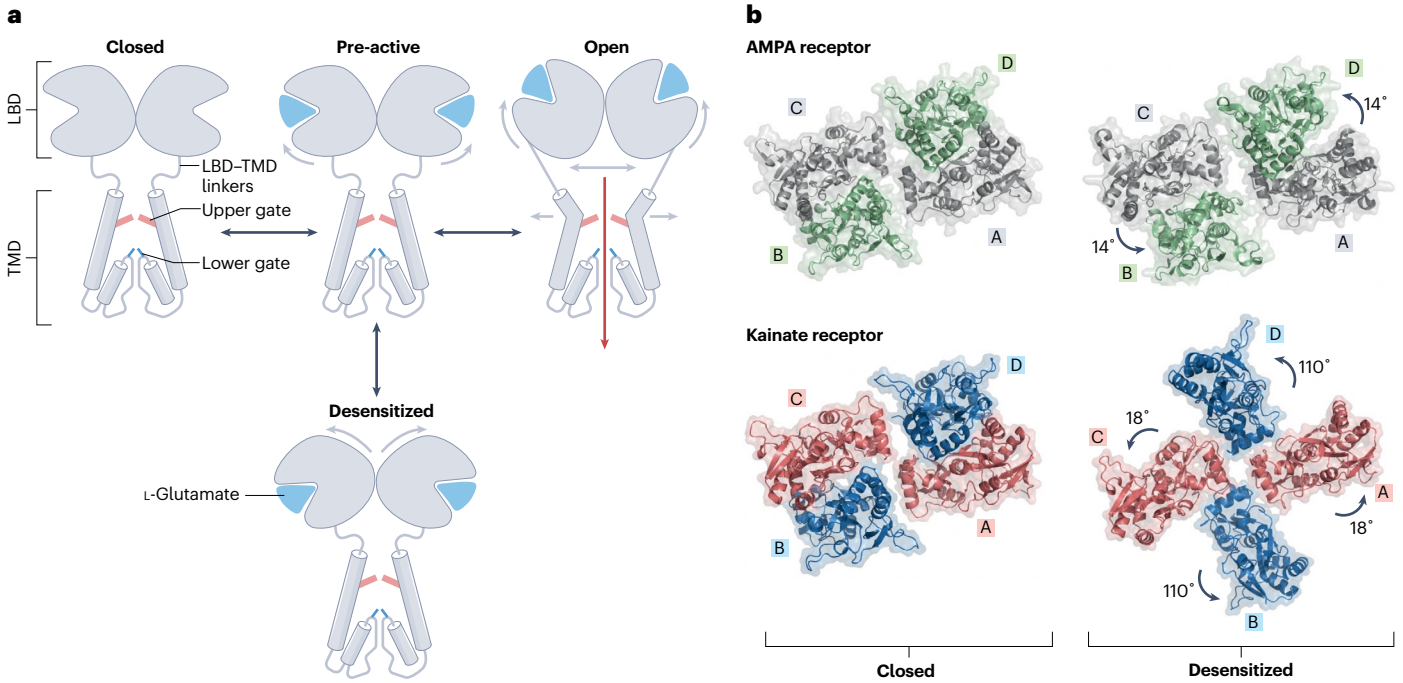


Fig. 2 | The AMPAR gating cycle. a, A schematic representation of the conformational transitions underlying AMPA receptor (AMPAR) gating. In the resting (closed) state, the ligand-binding domains (LBDs) adopt an open clamshell conformation and the ion channel pore is closed. Agonist binding promotes progressive closure of the LBD clamshells, generating mechanical tension in the LBD–transmembrane domain (TMD) linkers that is transmitted to the M3 helices, which form the upper activation gate. This transition produces a pre-active intermediate and ultimately an open, conducting state in which the M3 bundle crossing is dilated. The pore also contains a lower gate near the selectivity filter. In the continued presence of agonist, receptors enter a desensitized state in which rearrangements within the LBD layer uncouple ligand binding from channel opening, yielding a non-conducting conformation despite maintained agonist occupancy. **b**, Structural basis of subunit rearrangements during gating and desensitization in AMPARs compared with kainate receptors. The upper panels show AMPAR structures in the closed state (RCSB PDB identifier: 5VHY[56]) and desensitized state (PDB identifier: 5VHZ[56]). The lower panels show kainate receptor structures in the closed state (PDB identifier: 7KS0[82]) and desensitized state (PDB identifier: 7KS3[82]). AMPARs are tetramers organized as A/C and B/D subunit pairs that occupy distinct positions within the receptor. During activation, conformational changes are transmitted asymmetrically, with larger movements in B/D subunits. Transition to the desensitized state is associated with a rotation of the LBD dimers relative to one another around the central axis, with A/C and B/D subunits moving in opposite directions. In AMPARs, this rearrangement is relatively modest (typically ~10–20° rotation, depending on subunit composition and auxiliary proteins)[56,57,176,177], preserving much of the dimer interface. By contrast, kainate receptors undergo substantially larger rotational and translational movements (~90–120° rotation, with additional ~10–20° local subunit rearrangements)[82,90,178–180], resulting in pronounced disruption of the LBD dimer interface and a more extensive reorganization of the extracellular domain. These differences highlight distinct structural mechanisms of desensitization across ionotropic glutamate receptor subtypes. The upper panels in part **b** are adapted with permission from ref. 56, Elsevier. The lower panels in part **b** are adapted from ref. 82, CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

that couple the LBD to the M3 gate undergo larger conformational changes in the B/D subunits during activation, suggesting that these subunits exert a stronger mechanical pull on the channel gate[53,79,86]. This asymmetric architecture contributes to differential gating behaviour within the tetramer and to the modulation of receptor function by auxiliary subunits[8,20].

In the continued presence of glutamate, AMPARs rapidly enter a desensitized state, in which rupture of the interface between the upper (D1) lobes of the LBD dimer, formed by the association of the LBDs from two neighbouring subunits, uncouples ligand binding from pore opening, producing a non-conducting conformation despite continued agonist occupancy[87–89]. This transition is accompanied by a reorganization of the LBD layer in which the two LBD dimers rotate relative to one another around the central axis of the receptor, with A/C and B/D subunits moving in opposite directions to adopt a more four-fold-symmetric arrangement. In AMPARs, this rotational movement is relatively modest, resulting in limited separation and reorientation of subunits within the tetramer[3–5] (Fig. 2b). By contrast, kainate receptors undergo a markedly larger rotational rearrangement during desensitization, with substantial twisting and spatial separation of subunits that produces a more pronounced disruption of the dimer interface and a more extensively reorganized LBD layer[82–84,90] (Fig. 2b). Stabilization of this dimer interface in AMPARs markedly reduces desensitization, as demonstrated by the action of positive allosteric modulators such as cyclothiazide, which bind at the LBD dimer interface and prolong receptor activation[91,92].

Thus, AMPAR gating arises from coordinated conformational changes across multiple structural layers of the receptor, including the LBD dimers, the LBD–transmembrane domain linkers and the M3 activation gate, as well as rotational rearrangements of subunits within the tetramer that differ in magnitude between receptor families. In native receptors, these transitions are further shaped by auxiliary

subunits and other regulatory elements. In the sections that follow, we examine how specific structural features modulate these transitions within the AMPAR gating cycle.

Electrostatic control of AMPAR activation

The topology of all ionotropic glutamate receptor (iGluR) subunits is highly conserved, especially at the LBD (Fig. 1a and Supplementary Fig. 1). In both AMPARs and kainate-type iGluRs (KARs), there is an extensive network of electrostatic and hydrophobic interactions at the interface between the D1 lobes of neighbouring subunits, forming the LBD dimer interface that stabilizes coupling between ligand binding and channel gating, is important for channel gating[73,74,93,94]. Interestingly, in KARs, the electrostatic residues form discrete binding pockets for both Na^+ and Cl^- at the apex of this interface[95,96], which are essential for channel gating[73,94,97,98] (Supplementary Fig. 1). In GluK2 KARs, occupancy of the Na^+-binding pocket is an absolute requirement for activation[94], with the time course of channel activity regulated by the residence time of bound Na^+ (ref. 73). Although AMPAR gating does not require Na^+ binding for channel activation[97], a binding site for smaller cations, such as Li^+, is nevertheless present that, when occupied, promotes channel activation[74] (Supplementary Fig. 1). This suggests that, during evolution, minor modifications to the architecture of the D1–D1 interface were all that was needed to determine the specific gating requirements of different iGluRs. In keeping with this, orphan class δ2 iGluRs are also capable of binding cations, in this case, the divalent Ca^{2+} (refs. 99,100) (Supplementary Fig. 1). In these receptors, Ca^{2+} binding stabilizes the LBD dimer interface[99], reminiscent of the allosteric effects of Na^+ and Li^+ on KARs and AMPARs, respectively. Although δ2 iGluRs were originally considered to be non-conducting components of a trans-synaptic organizer complex[101], recent work shows that they can undergo channel gating, particularly at higher physiological temperatures in the presence of D-serine and GABA[9]. Whether extracellular Ca^{2+} contributes to channel gating has yet to be explored. Curiously, NMDA-type iGluRs lack these electrostatic interactions at the LBD dimer interface: instead, the time course of channel gating in these receptors relies, at least in part, on hydrophobic interactions that involve a tyrosine residue on the lower D2 lobe of GluN1 subunits[102] (Supplementary Fig. 1).

Further evidence supporting the importance of electrostatic interactions to gating in AMPARs comes from mutational analysis of the charged and polar residues that contribute to key electrostatic interactions along the D1–D1 interface[74] (Supplementary Fig. 1). Specifically, the replacement of three key amino acids in this region with alanine almost eliminates the robust response to glutamate observed in wild-type homomeric GluA2 AMPARs. Remarkably, however, co-expression of the mutant AMPAR with either TARP γ2 or CNIH3 restores the agonist response[74], demonstrating that activation of native AMPAR complexes can be supported by multiple, partially redundant structural mechanisms. In this context, electrostatic interactions at the LBD dimer interface represent one mechanism for promoting gating, whereas association with auxiliary subunits provides an alternative means of stabilizing active conformations[74].

Auxiliary subunits as allosteric regulators

Both GSG1L and TARPs exhibit an archetypical claudin membrane topology, being composed of cytoplasmic N and C termini, four transmembrane domains and two extracellular loops, the large β1–β4–TM2 loop and a smaller β5 loop. The length of the first of these loops (that is, the β1–β4–TM2 loop) is the primary distinction between these auxiliary subunits[5,56] (Fig. 1c). Despite their similar overall topology, TARPs and GSG1L exert, in most cases, opposing effects on AMPAR entry into and recovery from desensitization[7,103], ion permeation[104] and synaptic transmission[105–107].

Recent work has been able to explain these marked functional differences[20,74,108] in structural terms. First, it was shown that TARP modulation of channel gating is due to an electrostatic interaction between the large extracellular loop (specifically the β4–TM2 portion) of the TARP and the evolutionarily conserved KGK site on the lower D2 lobe of the AMPAR LBD[52,74,109] (Fig. 3a–c). The absence of this site from the D2 lobe of KARs and NMDARs accounts for the selective effect of TARPs on AMPARs. An initial functional study on AMPAR homomers showed that changing the positively charged lysine residues in the KGK motif to negatively charged aspartate residues on all subunits was sufficient to eliminate the ability of TARPs to slow entry into desensitization[74]. This finding was subsequently supported by cryo-EM studies showing that the TARP β4–TM2 loop interacts with the LBD KGK motif[51,52] (Fig. 3a–c). Importantly, structural analysis of the GluA2–TARPγ2 complex revealed that TARPs occupy four positions around the receptor but engage the LBDs of different subunits asymmetrically, with the B/D subunit-associated TARPs positioned closer to the LBD lower lobes than those associated with A/C subunits[5,51]. This suggests that interactions between the TARP extracellular loops and the KGK motif are more likely to occur at the B/D subunits.

In line with this, subsequent functional studies of GluA1/A2 heteromeric AMPARs revealed an asymmetrical contribution of TARPs across the different subunits. Mutation of the KGK motif on GluA1 subunits (the A/C subunits) alone did not abolish the effects of TARP on gating, whereas mutation of the site on GluA2 (B/D) subunits produced a partial loss of TARP modulation[20]. The apparent lack of effect of the GluA1 KGK mutation could be explained by structural work showing that the TARP β1 loop selectively engages a different site on the LBD of GluA1, known as helix K and located adjacent to the M4 gating linker, rather than the KGK motif itself[5,110]. A similar asymmetry was also reported from structural and functional analysis of homomeric GluA4 channels[111]. However, when all four KGK motifs in the GluA1/A2 heteromer are mutated, TARP modulation is completely abolished[20], indicating that KGK sites on both GluA1 and GluA2 subunits contribute to the overall effect of TARPs. It is possible that TARPs do not engage the GluA1 KGK motif in the resting or desensitized state[110], but instead interact with this region during gating-associated conformational changes, which would reconcile these functional and structural observations. Such transient interactions would probably escape detection in cryo-EM structures, which predominantly capture relatively stable receptor conformations. Elucidating the dynamic interplay between the KGK motif and TARP extracellular loops will therefore be essential for understanding how TARPs regulate the millisecond switching between high-open-probability and low-open-probability modal gating behaviour of AMPARs[74,112], a phenomenon observed in single-channel recordings in which individual channels transition between distinct low-open-probability and high-open-probability gating modes over time, which probably shapes the time course of fast synaptic events at glutamatergic synapses[112].

A similar functional asymmetry accounts for the slow recovery from desensitization[103] characteristic of GSG1L-containing AMPAR heteromers[20]. However, the GSG1L-related slowing of recovery from desensitization is mediated by a separate evolutionarily conserved electrostatic site that is functionally distinct from the KGK site but nevertheless located on the lower D2 lobe of the AMPAR LBD[20] (Fig. 3d–f).

Although GSG1L can, in principle, regulate AMPARs via the KGK site, the allosteric effect is very weak and probably represents a vestige of its evolutionary past[20], given that GSG1L is thought to have emerged later as an auxiliary subunit during evolution[32,113]. In keeping with its unique effects on slowing recovery from AMPAR desensitization, GSG1L expression is region specific and developmentally regulated in the rodent brain, with cell-type-specific expression observed in the cerebellum, caudate putamen and cerebral cortex[20]. Interestingly, GSG1L is often expressed in cells that regulate information flow through circuits, such as granule cells of the dentate gyrus or cerebellum[20,106,107], suggesting that its impact on AMPAR recovery rates may act to filter synaptic transmission. Furthermore, GSG1L's regulation of AMPARs appears to occur in an input-specific manner, as GSG1L-containing and TARP γ2-containing AMPARs have been shown to be segregated to different glutamatergic synapses on the same nerve cell in the anterior thalamus[106]. Whether this apparent solitary role of GSG1L at central synapses can be extended to cerebellar granule cells, where GSG1L is confined to lobules of the anterior lobe[20], awaits future investigation. AMPAR–GSG1L complexes uniquely associate with the synapse organizer protein LRRTM4[20], which may act as a trans-synaptic scaffold to guide the axons of presynaptic cells in establishing GSG1L-specific glutamatergic synapses[22,114]. These findings thus highlight recovery from desensitization as a key parameter of AMPAR signalling that can be tuned by auxiliary proteins in a cell-specific and synapse-specific manner.

CNIHs can also act as allosteric modulators of AMPAR gating. In native and synaptic receptors, CNIHs prolong channel deactivation and thus increase the duration of single-channel bursts and slow the time course of AMPAR responses[10,68]. Although the structural basis for CNIH-mediated gating modulation remains less well understood, structural and mutational studies suggest that these auxiliary proteins influence receptor gating through distinct interactions with the transmembrane components of the AMPAR complex[64,108]. However, like the TARPs, CNIHs can reinstate channel gating when the electrostatic dimer interface is disrupted[74] (Supplementary Fig. 1). Whether CNIHs promote the modal gating behaviour commonly associated with TARP-containing AMPARs remains unclear[112]. In addition to modifying gating, CNIHs can modulate AMPAR pharmacology and ion permeation properties[10,115–117]. They may also interact differentially with TARP isoforms[118], and are expressed in both neuronal and glial cells, suggesting that they have broad roles in regulating glutamatergic signalling in the brain[119], much like the TARPs.

Recently, two members of the claudin family have emerged as potential AMPAR auxiliary subunits. Claudin-24 (CLDN24), and possibly the closely related claudin-22, associate with AMPAR complexes and accelerate recovery from desensitization, with prominent expression of CLDN24 in cerebellar granule cells[61]. Interestingly, CLDN24 counterbalances the gating modulation imposed by TARP γ2 and GSG1L in cerebellar granule cells, which is reminiscent of the counterbalancing effects of TARP γ8 and another auxiliary subunit, CKAMP44, in hippocampal granule cells[120]. Exactly how this newly identified modulatory mechanism integrates with the established modes of AMPAR regulation by TARPs, CNIHs and GSG1L remains to be determined.

Splice variants modulate AMPAR properties

The transcripts of all AMPAR subunit genes are subject to alternative splicing of a segment encoding a 38 amino acid cassette that precedes the M4 region and can exist as either a flip or flop version[17] (Fig. 3g). The alternative splicing of this region regulates a number of important properties of AMPARs, including the time course of the onset of desensitization[8,17,30] (Fig. 2) and short-term depression[10], sensitivity to positive allosteric modulators[121,122] and allosteric anions[8], AMPAR biogenesis and trafficking from the ER[123,124] as well as AMPAR sensitivity to TARP regulation[8]. Recent work has suggested that the flip/flop cassette regulates these apparently disparate properties of the AMPAR by controlling the nanoscale mobility of the receptor in its apo (resting) state[8] (Fig. 3h). In support of this, the differences in channel gating[29], ER trafficking[124,125] and positive allosteric modulator[121], anion[8] and TARP regulation[8] that arise as a result of alternative splicing are all controlled by the residue at position 775 within the flip/flop cassette, which differs between isoforms, with a serine in the flip variant (Ser775) and an asparagine in the flop variant (Asn775). Notably, Ser775 stabilizes the receptor in conformations associated with reduced nanoscale mobility of the N-terminal domain (NTD), whereas Asn775 promotes increased NTD mobility in the apo state[8] (Fig. 2h). Thus, there is a causal link between the mobility of the apo state and channel gating, allosteric modulation and ER trafficking[8].

Interestingly, the flip/flop cassette's differential regulation of the influence of auxiliary proteins on AMPARs is unique to the TARP protein family and is not observed with other auxiliary subunits, such as the CNIH or CKAMP families and even the other claudin-like protein, GSG1L (ref. 10). This is because both alternative splicing and TARP binding to the KGK site selectively regulate the rate of onset of AMPAR desensitization. Flip variants of the AMPAR promote a moderate resting mobility of the apo state, which favours the formation of electrostatic interactions at the D1–D1 interface as well as allowing the extracellular β4–TM2 loop of TARPs to lock on to the lower D2 lobe of the LBD, where the KGK site is located[52,73]. By contrast, the greater mobility induced in the apo state by the flop cassette overrides the electrostatic interactions at the D1–D1 interface as well as disfavouring allosteric coupling between the TARP and KGK site, that is, the transmission of conformational and energetic changes between the TARP extracellular loops and the LBD that stabilizes gating-relevant interactions[8,10]. As most native AMPRs assemble in complex with a TARP family member, we can conclude that allosteric coupling between the flip/flop cassette and TARPs represents a common design element and the starting point for the assembly of almost all AMPAR complexes of the mammalian brain. The exception to this, of course, would be GSG1L-containing AMPARs, which appear to be able to assemble in the absence of TARPs[20].

Mechanisms of AMPAR Ca^{2+} transport

AMPARs are non-selective cation channels that are permeable to monovalent ions such as Na^+ and K^+ ions[28]. The presence or absence of the GluA2 subunit in the AMPAR tetramer has long been thought to determine Ca^{2+} permeability, owing to RNA editing at the Q/R site[14,15], with GluA2-containing receptors being largely impermeable to Ca^{2+} (refs. 1,28). Ca^{2+} entry through AMPARs can act as an important intracellular signalling messenger. Consistent with this, AMPAR responses in Bergmann glia — one of the few cells in the mammalian CNS that do not express the GluA2 subunit[44,126] — exhibit a doubly rectifying current–voltage (I–V) relationship (Fig. 4a) when exposed to external solutions rich in Na^+ ions, due to voltage-dependent block of the ion channel by cytoplasmic polyamines[40,127–129]. This polyamine block is a hallmark of Ca^{2+}-permeable AMPARs and arises because the absence of the edited GluA2 subunit leaves a neutral residue at the Q/R site, allowing both Ca^{2+} permeation and access of intracellular polyamines to the pore. In solutions rich in external Ca^{2+}, however, the I–V relationship is linear and reverses close to 0 mV (Fig. 4a), corresponding to a relative permeability ($P_{Ca2+/Na+}$) of Ca^{2+} with respect to Na^+ of 3–5 (refs. 43,44).

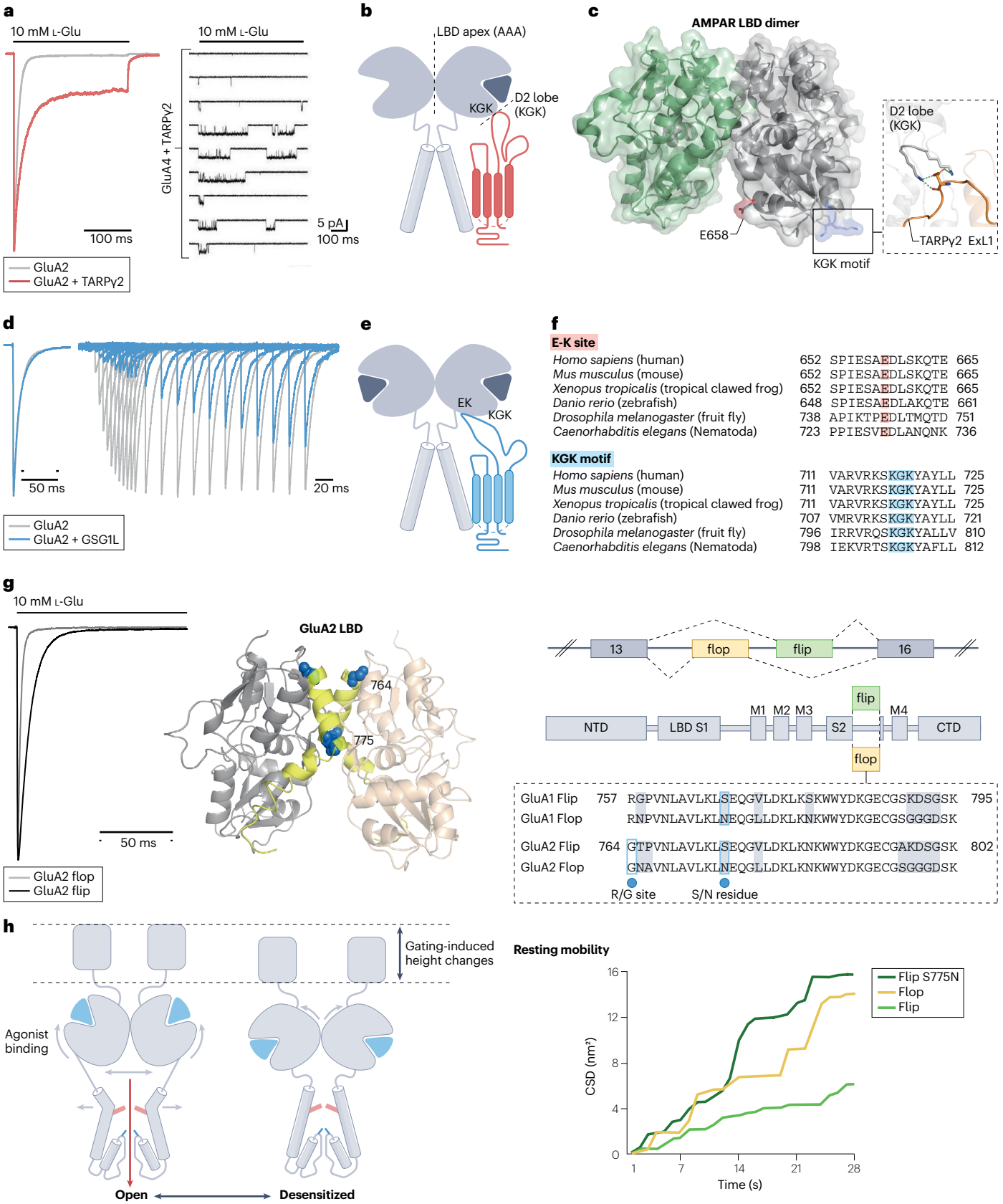

a
10 mM L-Glu
100 ms
GluA2
GluA2 + TARPγ2
10 mM L-Glu
GluA4 + TARPγ2
5 pA
100 ms
b
LBD apex (AAA)
KGK
D2 lobe (KGK)
c
AMPAR LBD dimer
D2 lobe (KGK)
E658
KGK motif
TARPγ2 ExL1
d
50 ms
20 ms
GluA2
GluA2 + GSG1L
e
EK
KGK
f
E-K site
Homo sapiens (human) 652 SPIESAEDLSKQTE 665
Mus musculus (mouse) 652 SPIESAEDLSKQTE 665
Xenopus tropicalis (tropical clawed frog) 652 SPIESAEDLSKQTE 665
Danio rerio (zebrafish) 648 SPIESAEDLAKQTE 661
Drosophila melanogaster (fruit fly) 738 APIKTPEDLTMQTD 751
Caenorhabditis elegans (Nematoda) 723 PPIESVEDLANQNK 736
KGK motif
Homo sapiens (human) 711 VARVRKSKGKYAYLL 725
Mus musculus (mouse) 711 VARVRKSKGKYAYLL 725
Xenopus tropicalis (tropical clawed frog) 711 VARVRKSKGKYAYLL 725
Danio rerio (zebrafish) 707 VMRVRKSKGKYAYLL 721
Drosophila melanogaster (fruit fly) 796 IRRVRQSKGKYALLV 810
Caenorhabditis elegans (Nematoda) 798 IEKVRTSKGKYAFLL 812
g
10 mM L-Glu
50 ms
GluA2 flop
GluA2 flip
GluA2 LBD
764
775
13
flop
flip
16
NTD
LBD S1
M1 M2 M3
S2
M4
CTD
GluA1 Flip 757 RGPVNLAVLKLSEQGVLDKLKSKWWYDKGECGSKDSGSK 795
GluA1 Flop RNPVNLAVLKLNEQGLLDKLKNKWWYDKGECGSGGGDSK
GluA2 Flip 764 GTPVNLAVLKLSEQGVLDKLKNKWWYDKGECGAKDSGSK 802
GluA2 Flop GNAVNLAVLKLNEQGLLDKLKNKWWYDKGECGSGGGDSK
R/G site
S/N residue
h
Agonist binding
Gating-induced height changes
Open
Desensitized
Resting mobility
CSD (nm²)
Time (s)
Flip S775N
Flop
Flip

Fig. 3 | Structural and post-transcriptional regulation of AMPAR gating and desensitization. a, Claudin-type auxiliary subunits engage discrete electrostatic sites on the lower D2 lobe of the AMPA receptor (AMPAR) ligand-binding domain (LBD) to modulate gating. The left panel shows a typical AMPAR response to glutamate when GluA2 subunits are co-expressed with or without transmembrane AMPA receptor regulatory protein (TARP) γ2. Co-expression with TARP γ2 slows entry into desensitization and produces a large steady-state agonist response. Data adapted from ref. 20. The right panel shows nine contiguous sweeps of GluA4/γ2 AMPAR single channel activity. When TARP γ2 engages and disengages with GluA4, it induces modal gating behaviour, where the channels alternate between high and low open channel probability, respectively[112]. The first three sweeps exhibit low open probability, presumably owing to the lack of TARP engagement, whereas the latter six sweeps show high-open probability, consistent with TARPs interacting with the KGK site[74]. **b**, A cartoon showing how the large extracellular TARP loop interacts with the lower D2 lobe of the AMPAR LBD to slow desensitization and increase the agonist-induced steady-state response[74]. **c**, The position of the conserved KGK motif that forms the principal electrostatic interface through which TARPs engage with AMPARs to slow desensitization[74]. Structural studies suggest direct contacts between the positively charged KGK motif and acidic residues within the TARP large extracellular loop (ExL1) (inset)[52]. The position of the E658 residue, which mediates germ cell-specific gene 1-like protein (GSG1L) gating effects, is also shown[20]. **d**, GSG1L exerts a distinct form of regulation of AMPARs by stabilizing the desensitized state. Representative ensemble electrophysiological traces comparing the rates of recovery from desensitization for GluA2 AMPARs co-expressed with or without GSG1L are shown. Co-expression with GSG1L markedly slows the rate of recovery from desensitization[20,103]. **e**, A cartoon highlighting primary effect of the E-K residue and the lesser effect of the KGK site that contribute to the gating effects of GSG1L[20]. **f**, Sequence alignments of representative AMPAR subunits from different vertebrate and invertebrate species showing that both the E-K and KGK sites are highly conserved. **g**, Alternative splicing of the flip/flop cassette introduces nanoscale mobility differences in the resting LBD dimer interface that exert large long-range effects on receptor motion[8] and therefore channel desensitization rates. The left panel shows representative electrophysiological traces of AMPAR responses to glutamate, revealing that desensitization is much faster for flop versus flip variants[10]. The right panel shows the GluA2 LBD dimer structure, highlighting the position of the flip/flop cassette (yellow), the R/G site (blue, 764) and the Ser/Asn residue (blue, 775) that is largely responsible for the distinct effects of flip versus flop isoforms[8]. **h**, Nanoscale mobility analyses reveal that flop-containing AMPARs exhibit increased resting amino-terminal domain mobility and greater vertical compression of the extracellular domains upon activation[8]. This elevated intrinsic mobility in the apo (resting) state is proposed to lower the energetic barrier for subsequent conformational rearrangements, thereby facilitating the transition into desensitized states and modulating responsiveness to agonists, allosteric modulators and auxiliary subunits[8]. The cartoon depicts the gating-induced reduction in receptor height during desensitization, referenced to the resting configuration (dashed outline) to illustrate how enhanced basal mobility predisposes receptors to greater vertical compression. The inset shows the differing resting mobilities of flip and flop variants of GluA2 homomers; a single amino acid substitution in GluA2 flip (S775N) shifts the resting mobility to that characteristic of flop receptors[8]. CSD, cumulative squared displacement; CTD, C-terminal domain; NTD, N-terminal domain. The left panel in part **a**, and parts **c**,**d** and **e** are adapted from ref. 20, CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/). The right panel in part **a** is adapted with permission from ref. 112, Wiley. Part **b** is adapted from ref. 74, CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/). Part **g** is adapted from ref. 10, CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/). The inset in part **h** is adapted with permission from ref. 8, Elsevier.

By contrast, the AMPARs expressed by cerebellar Purkinje cells exhibit typical characteristics of divalent cation-impermeable receptors, including linear *I*–*V* relationships in solutions rich in external Na^+ (due to their insensitivity to cytoplasmic polyamine block, conferred by the presence of the edited GluA2 subunit, in which a positively charged arginine at the Q/R site reduces both Ca^{2+} permeability and polyamine access to the pore[28]) (Fig. 4a) and a negative reversal potential in solutions rich in external Ca^{2+}, corresponding to a weak relative Ca^{2+} permeability[9,130,131] (Fig. 4a).

Dual binding sites define Ca^{2+} permeation

A long-standing question has been how GluA2-lacking AMPARs are able to transport extracellular Ca^{2+} three to five times better than Na^+, despite the fact that the extracellular milieu of nervous tissue contains almost 70 times more Na^+ than Ca^{2+} (150 mM Na^+ versus 2 mM Ca^{2+}). A recent study addressed this question by investigating putative ion and water densities in the open pore of a Ca^{2+}-permeable AMPARs[75]. Unexpectedly, this uncovered an extracellular Ca^{2+} binding site (Site-G) within the ion-permeation pathway of these receptors that is present only when the channel gate moves into the open configuration (Fig. 4b,c). Site-G is constituted by the Thr and Ala residues of the SYTANLAAF motif that contributes to the upper channel gate (Fig. 4b,c). The binding site is highly selective for Ca^{2+} over Na^+ and, as it lies outside the membrane's electric field, its occupancy by Ca^{2+} is insensitive to voltage[75]. Once Ca^{2+} is bound to Site-G, this favours the movement of Ca^{2+} towards another Ca^{2+} binding site: the Q/R site within the selectivity filter of the AMPAR pore (Fig. 4b,c). Evidence in support of this mechanism came from experiments studying the seizure-related N619K mutation, which is adjacent to Site-G. This mutation promotes channel opening but, in doing so, attenuates Ca^{2+} binding to Site-G, greatly diminishing overall Ca^{2+} transport through the pore[75]. Thus, Site-G coordinates with the Q/R-site to ensure the preferential transport of Ca^{2+} through the AMPAR channel pore[75]. Given that the SYTANLAAF motif is conserved across all iGluRs (Fig. 4b) and that Ca^{2+} permeability is a shared feature of many iGluRs, including ancestral receptors[32,132], it is plausible that NMDARs and KARs also transport Ca^{2+} via a Site-G-like mechanism, although this remains to be formally demonstrated.

Ca^{2+} permeability in GluA2-containing receptors

Although the conventional understanding has long been that GluA2-containing AMPARs are Ca^{2+} impermeable[1,28], a recent comparison of GluA2-containing AMPARs co-assembled with different stoichiometric arrangements of TARP and CNIH auxiliary subunits revealed that, in fact, they form a continuum of polyamine-insensitive ion channels with varying degrees of Ca^{2+} permeability[9] (Fig. 4a).

This study showed that TARPs offer the first level of regulation of Ca^{2+} permeability: the ability of GluA2-containing AMPARs to transport Ca^{2+} is shaped by the type of TARP, which AMPAR subunit the TARP associates with and which heteromer is being regulated (Fig. 5a). For example, GluA1/A2 heteromers alone or when co-assembled with four TARP γ2 subunits exhibit weak Ca^{2+} permeability ($P_{Ca2+/Na+}$ in the range of 0.06–0.3)[9] (Fig. 5a). However, untethering TARP γ2 from the GluA1 subunit renders a significantly higher Ca^{2+} permeability ($P_{Ca2+/Na+}$ of 0.42). A similar boosting effect was not observed when TARP γ2 was untethered from the GluA2 subunit. The discovery that asymmetry in the arrangement of auxiliary subunits impacts divalent transport is

consistent with findings in heteromeric GluK2/K5 KARs, where asymmetry in the architecture of the pore has been proposed to account for the facilitated transport of larger cations, such as Ca^{2+} or polyamines, compared with GluK2 homomers[133]. Interestingly, tethering TARP γ8 selectively to either GluA1 or GluA2 to form AMPAR heteromers did not boost Ca^{2+} permeability, demonstrating that not only is the position within the tetramer important but also the type of TARP involved. Finally, TARP γ2 did not affect Ca^{2+} permeability when co-assembled with GluA2/A3 heteromers, regardless of the subunit to which it was tethered (Fig. 5a). Taken together, these observations reveal that GluA1/A2 heteromers are preferentially targeted by TARPs for regulation of Ca^{2+} permeability over GluA2/A3 heteromers. However, Ca^{2+} permeability is increased only when the TARP is γ2, TARP stoichiometry is 2:4, and the TARP co-assembles at the GluA2 or B/D position.

CNIHs offer the next level of regulation of Ca^{2+} permeability in GluA2-containing receptors. The ability of TARP-bound GluA2

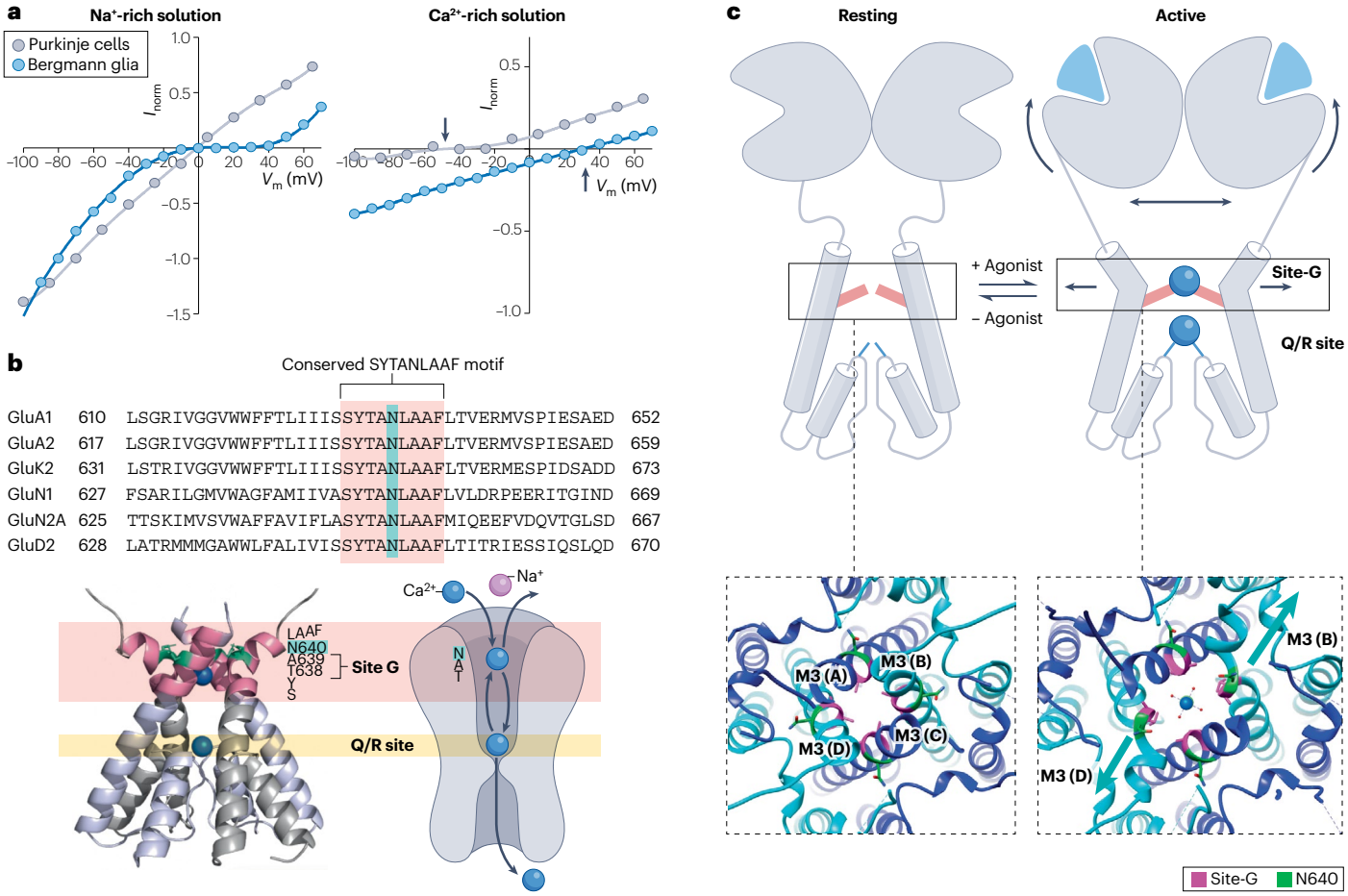


Fig. 4 | Structural basis for divalent-ion permeation through AMPARs. **a**, AMPA receptors (AMPARs) differ markedly in their Ca^{2+} permeability across cerebellar cell types. Purkinje cells, which predominantly express GluA2-containing receptors, show a linear relationship between the ionic current across the cell membrane (I_{norm}) and the membrane potential (V_m) when tested in a Na^+-rich external solution, but minimal Ca^{2+} influx in a Ca^{2+}-rich external solution, reflected in a strongly negative reversal potential (arrow)[9]. By contrast, Bergmann glia express GluA2-lacking AMPARs that display polyamine-mediated inward rectification in a Na^+-rich solution and robust Ca^{2+} permeation in a Ca^{2+}-rich solution, yielding positive reversal potentials (arrow)[9]. These contrasting profiles illustrate how subunit composition and pore architecture govern divalent permeation. **b**, Two discrete Ca^{2+}-binding sites shape permeation through the AMPAR pore. The upper panel shows a conserved SYTANLAAF motif within the M3 helix that contributes to 'Site-G', located at the extracellular mouth of the channel. Within Site-G, two residues, Thr638 (T638) and Ala639 (A639; GluA2 numbering) form part of a divalent cation-binding site that coordinates with Ca^{2+} when the channel is in the open state[75]. The lower-left image is a structural view of the AMPAR channel pore (RCSB PDB identifier: 7OCA)[181], where the two discrete Ca^{2+} binding sites that shape ion permeation have been highlighted[75]. The lower-right image is a cartoon of the pore showing that Site-G is positioned to funnel Ca^{2+} ions towards the apex of the selectivity filter, where the Q/R site forms a second divalent-binding site and determines Ca^{2+} permeability[9,75]. Notably, Ca^{2+} can occupy Site-G irrespective of whether a glutamine (Q) or arginine (R) is present at the Q/R site (for example, in homomeric GluA2(R) AMPARs[75]). However, when a Q is present, Ca^{2+} can proceed through the selectivity filter, whereas substitution with a positively charged R introduces electrostatic repulsion that prevents Ca^{2+} permeation[9]. Occupation of Site-G therefore enables Ca^{2+} entry into the pore, but permeability is ultimately determined by the Q/R site. **c**, Ca^{2+} binding to Site-G is tightly coupled to channel activation, as illustrated in the cartoon. Opening of the pore involves pronounced kinking of the M3 helices and reorientation of the asparagine residue adjacent to Site-G (N640, green) in subunits located in the B and D positions within the tetramer. Together, this creates the coordinated geometry necessary for Ca^{2+} capture and subsequent divalent-ion permeation[75]. Structure adapted from PDB: identifier 8FQB[75]. Parts **a** and **b** are adapted from ref. 9, Springer Nature Ltd. Part **c** is adapted from ref. 75, Springer Nature Ltd.

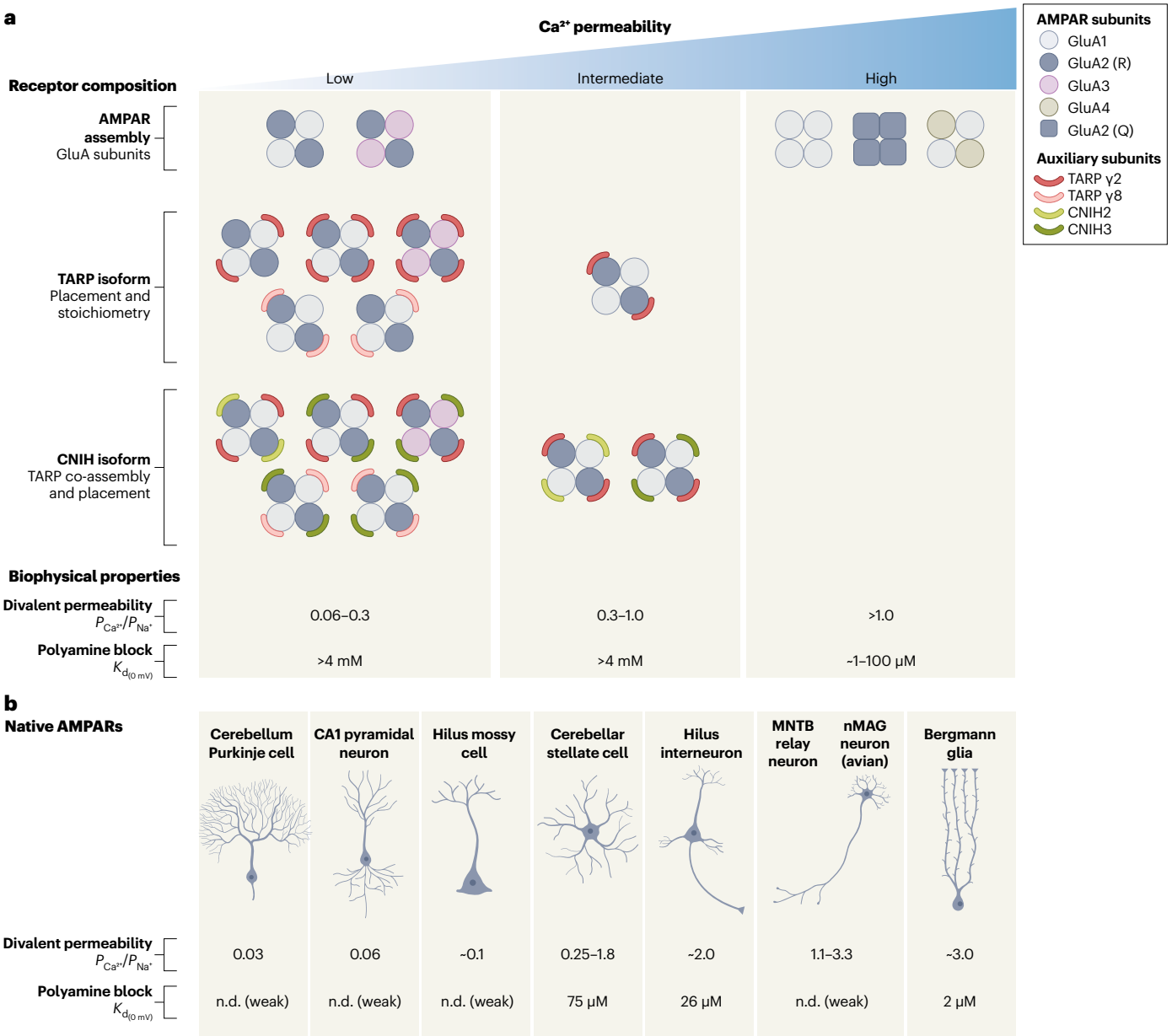


Fig. 5 | GluA2-containing AMPARs form a continuum of Ca^{2+} permeable ion channels. a, AMPA receptor (AMPAR) permeability to Ca^{2+} is not binary but spans a broad continuum shaped by subunit assembly and auxiliary-subunit stoichiometry[9]. Heteromers composed of GluA1 and GluA2 can exhibit modest Ca^{2+} permeability, whereas GluA2/3 assemblies remain essentially Ca^{2+} impermeable. Incorporation of two transmembrane AMPA receptor regulatory protein (TARP) γ2 molecules tethered to GluA2 into GluA1/A2 heteromers markedly enhances their Ca^{2+} permeation, increasing the relative permeability of Ca^{2+} with respect to Na^+ ($P_{Ca2+/Na+}$) approximately sixfold[9]. Association with cornichon (CNIH) proteins provides an additional modulatory layer: co-assembly of GluA1/A2-γ2 heteromers with CNIH2 or CNIH3 further elevates Ca^{2+} permeability in a subunit-specific manner, approaching values near unity for CNIH3-containing complexes[9]. Notably, these highly Ca^{2+}-permeable GluA1/A2 receptors remain insensitive to intracellular polyamines, as indicated by a low apparent spermine affinity (K_d > 4 mM at 0 mV), demonstrating that Ca^{2+} permeability and polyamine block can be mechanistically dissociated. By contrast, GluA2-lacking AMPARs, such as GluA1/A4 heteromers in Bergmann glia, exhibit high Ca^{2+} permeability and micromolar affinity for spermine. **b**, Native AMPARs across neuronal and glial populations likewise display cell-type-specific Ca^{2+} permeability profiles, reflecting differences in subunit composition, editing state and associated auxiliary proteins. Purkinje cells, CA1 pyramidal neurons, cerebellar stellate cells, hilar mossy cells, interneurons, medial nucleus of the trapezoid body (MNTB) relay neurons, avian nucleus magnocellularis (nMAG) neurons and Bergmann glia each express AMPAR complexes with characteristic Ca^{2+} selectivity and polyamine sensitivity. Reported $P_{Ca2+/Na+}$ and K_d (0 mV) values illustrate how the molecular rules defined in recombinant systems extend to physiological settings, generating diverse functional phenotypes tuned to the computational demands of each circuit. n.d., not detected. Data from refs. 9,43.

receptors to transport Ca^{2+} is regulated by the type of CNIH that is bound, which AMPAR subunit the CNIH associates with and which heteromers are being regulated (Fig. 5a). For example, co-assembly of GluA1/GluA2-γ2 AMPAR heteromers with CNIH3 doubles $P_{Ca2+/Na+}$ to 0.95 (ref. 9), demonstrating that TARP and CNIH family members can have an additive effect. The ability of CNIH3 to boost Ca^{2+} permeability is shared by the closely related, CNIH2, which when co-assembled with the GluA1/GluA2-γ2 AMPAR, increased $P_{Ca2+/Na+}$ to 0.55 (ref. 9). Co-assembly of the other abundant AMPAR heteromer, GluA2/A3, with either TARP γ2 or CNIH3 produced much smaller increases in Ca^{2+} transport, indicating that this effect is more pronounced in GluA1/A2 heteromers.

There is an apparent upper limit on the ability of TARP γ2 and/or CNIH3 to increase the Ca^{2+} permeability of GluA2-containing AMPARs while allowing these receptors to remain insensitive to block by cytoplasmic polyamines (Fig. 5a). So far, the upper limit occurs when $P_{Ca2+/Na+}$ is close to 1.0 (that is, when Ca^{2+} and Na^{+} permeabilities are equivalent)[9] (Fig. 4a). It is possible to further increase Ca^{2+} transport in these receptors; however, this requires notable alterations to the chemical and electrostatic nature of the pore's selectivity filter. For example, substitution of the Arg at the Q/R site of the GluA2 subunit with either a Gly or Glu increases $P_{Ca2+/Na+}$ of GluA1/A2 AMPARs to 1.5 and 3.5 respectively[9], similar to GluA2-lacking AMPARs expressed by Bergmann glia (Fig. 5b). Of note, these specific amino acid substitutions have been identified as missense mutations in patients with intellectual disability and neurodevelopmental abnormalities[134] suggesting that such alterations to the nature of the Q/R site have detrimental effects on the developing brain. As a result, it is likely that the upper limit on the Ca^{2+} permeability of GluA2-containing AMPARs is tightly regulated to avoid detrimental effects. From a structural perspective, there is also a cost to augmenting Ca^{2+} permeability in this way: once the selectivity filter lacks the repulsive electrostatic effect of the Arg residue on the GluA2 subunit, polyvalent ions, such as Ca^{2+} and spermine, can enter the pore, block it and pass through.

Exactly where in the permeation pathway do TARPs and CNIHs act to regulate Ca^{2+} flow? Although auxiliary subunits could, in principle, modify the architecture of Site-G to favour or disfavour divalent transport, recent modelling firmly demonstrates that TARPs and CNIHs augment Ca^{2+} transport in GluA2-containing AMPARs by lowering the affinity of Ca^{2+} for the selectivity filter[9]. Importantly, the position of the selectivity filter within the membrane electric field does not change as the voltage dependence of Ca^{2+} binding is unchanged. TARP γ2 and CNIH3 therefore promote Ca^{2+} permeation by altering the architectural properties of the Q/R site, in much the same way auxiliary subunits have been proposed to facilitate polyamine permeation[117]. Given that the estimated ionic radius of Ca^{2+} is about 2 Å (which fits into a pore with a radius of 8 Å (ref. 135)), measurable changes in Ca^{2+} permeation might be detectable even with sub-angstrom modifications to the architecture of the selectivity filter.

In summary, Ca^{2+} passes through AMPARs by binding to Site-G, an extracellular site that selectively captures and guides divalent ions into the membrane electric field and towards the Q/R selectivity filter of the pore. TARPs and CNIHs alter Ca^{2+} permeability mainly by acting at the Q/R site, where they regulate the ion's transit time through the pore.

Exactly which neuronal cell types in the mammalian brain express Ca^{2+}-permeable GluA2-containing AMPARs remains to be formally established, although several candidate populations have been proposed[136]. Notably, early studies examining native AMPARs across different brain regions reported substantial variation in Ca^{2+} permeability[43] (Fig. 5b). At the time, this variability was attributed to differences in the number of GluA2 subunits incorporated into individual receptors. We now know that this interpretation was limited by the understanding of AMPAR architecture at the time: iAMPARs were then widely thought to assemble as pentamers and the polyamine block characteristic of GluA2-lacking AMPARs had not yet been identified, which would have enabled clearer distinction between receptor classes[28]. Furthermore, the existence of AMPAR auxiliary subunits had yet to be established. In light of more recent findings[9,137], these earlier data are consistent with the existence of a continuum of GluA2-containing AMPARs exhibiting varying degrees of Ca^{2+} permeability (Fig. 5b). From an evolutionary perspective, this diversity is perhaps unsurprising, since before the emergence of vertebrates, ancestral AMPARs were highly permeable to Ca^{2+} (ref. 32). Thus, it might be expected that many native AMPARs in the mammalian brain retain some capacity for Ca^{2+} permeation.

Developmental regulation of AMPARs

Developmental changes in AMPARs

RNA editing of the R/G site, alternative splicing of the flip/flop cassette, and the expression of TARP and GSG1L auxiliary proteins all undergo dynamic changes during brain development[16,18,19,138] (Fig. 6). At embryonic stages in rodents (embryonic day (E)14–E18), R/G editing is low in all AMPAR subunits that can be edited (GluA2–4)[16]. Embryonic and early postnatal brains are also dominated by the flip isoforms of AMPAR subunits, whereas flop variants are nearly undetectable (Fig. 6b). During this same period, TARP expression remains generally similar across family members, with the notable exception of TARP γ4, which shows higher expression than the others[19] (Fig. 6c).

Between postnatal day (P)7 and P14, an important developmental period in rodents[139], flop isoform expression rises for all AMPAR subunit genes, such that by adulthood, flip and flop transcripts are present in comparable amounts for GluA1–3, and flop predominates slightly for GluA3 and GluA4[16,18] (Fig. 6b). Thus, CNS development corresponds to a gradual shift from a flip-rich embryonic AMPAR repertoire to a more balanced or flop-biased adult repertoire. RNA editing of the R/G site in AMPARs also increases steeply over the first two postnatal weeks. By P7–P14, GluA2 and GluA3 flip and flop isoforms and GluA4 flop isoforms are already heavily edited, whereas GluA4 flip lags behind[16] (Fig. 6a). In the adult brain, most GluA2/3 flip and flop transcripts, as well as GluA4 flop transcripts, are edited at the R/G site at levels approaching ~80–100%, whereas GluA4 flip reaches only ~50–60% editing[16]. This pattern is consistent with the persistent expression of unedited the GluA4 flip isoform in Bergmann glia and other specialized cell types[140].

In terms of auxiliary proteins, TARP γ4 expression peaks around birth and during the first postnatal week and then gradually declines[19]. By contrast, from P7 to P14 and into the adult brain, TARPs γ2, γ3 and γ8, as well as γ7 and γ5, are strongly upregulated[19]. GSG1L emerges much later: its expression is almost absent from the embryonic brain and increases only during the second postnatal week, eventually becoming abundant in select regions of the adult brain[20,106] (Fig. 6c). Overall, the TARP and GSG1L landscape thus transitions from a γ4-dominated embryonic state to a diverse adult ensemble in which γ2 and γ8 predominate, with contributions from γ3, γ5, γ7 and GSG1L. CNIHs also show notable developmental regulation. In the forebrain, CNIH expression displays a dynamic postnatal profile, including a transient reduction during early postnatal development before a later increasing as excitatory circuits mature[6]. Biochemical and functional studies further indicate that AMPAR populations can exist both with and without associated CNIHs, suggesting that developmental changes

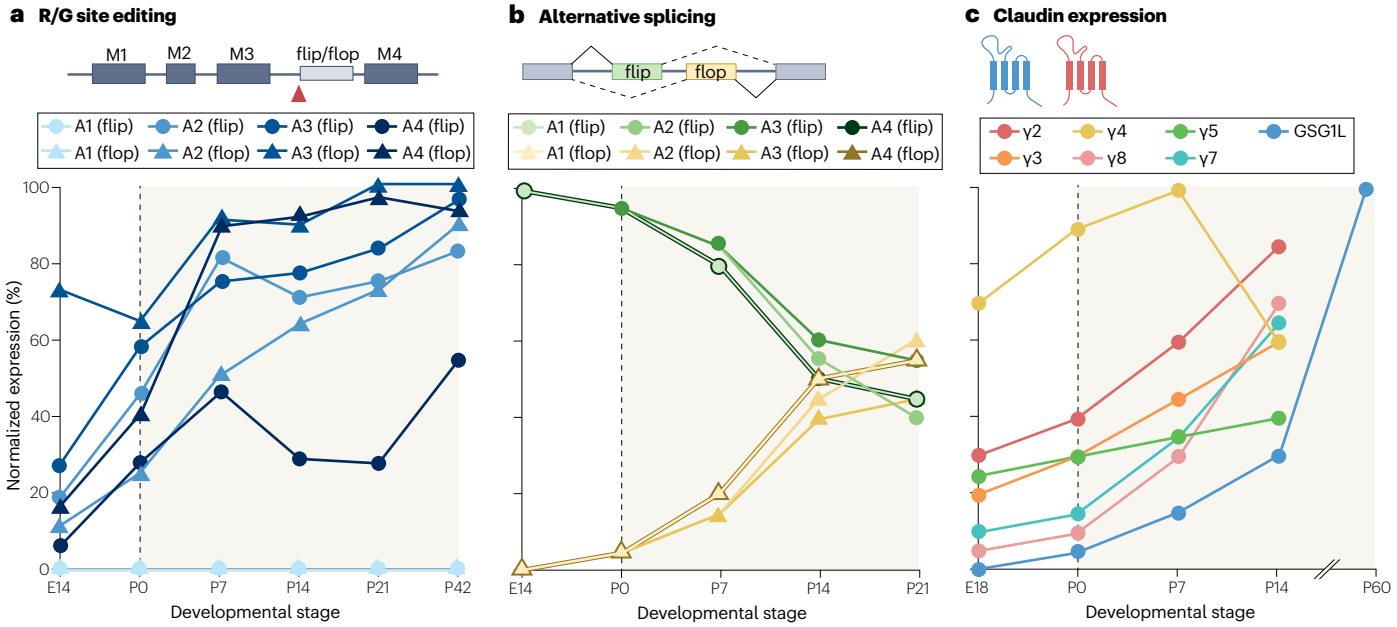


Fig. 6 | Developmental tuning of AMPAR regulation. A schematic summary of coordinated changes in AMPA receptor (AMPAR) subunit processing and auxiliary protein expression across rodent brain development. The curves are semi-quantitative, literature-constrained reconstructions intended to capture directionality and relative timing (rather than exact abundances) and were generated from data relating to a small set of representative developmental stages (from embryonic stages (E) to adult (postnatal; P)) to facilitate comparison. A more detailed description of the method used to construct the curves is provided in the Supplementary Methods. **a**, The top image is a schematic of the AMPAR subunit transmembrane topology highlighting the position of the R/G editing site within the extracellular loop (S2 segment within LBD) between TM3 and TM4; 'flip' and 'flop' denote the flip and flop variants. The bottom image shows that there is a developmental increase in the expression of R/G site edited GluA2, GluA3 and GluA4 subunits, whereas GluA1 remains unedited. This maturation of R/G editing might be expected to enhance the kinetic precision of AMPAR signalling as synapses and circuits undergo refinement. Values were approximated from the developmental trajectories reported in ref. 16 by binning reported stages into a reduced set of timepoints and rounding to avoid false precision. **b**, The top image is a schematic of the alternatively spliced flip/flop cassette within AMPAR subunits, highlighting the mutually exclusive inclusion of flip or flop exons in the extracellular loop (S2 segment within LBD) preceding the fourth transmembrane domain. The bottom image shows that there is a developmental transition from a predominant expression of AMPAR subunit transcripts that include the flip version of the alternatively spliced flip/flop cassette in the embryonic and early postnatal brain to an increase in the inclusion of flop variants with CNS maturation. As the brain matures, this produces receptors with faster kinetics and regulatable transmembrane AMPA receptor regulatory protein (TARP) engagement. Values were estimated from ref. 18 and related summaries of the flip to flop shift and are plotted as normalized within-subunit proportions (flip + flop = 100%) to visualize the switch rather than absolute transcript levels (Supplementary Methods). **c**, The expression of claudin-type auxiliary subunits also undergoes substantial developmental remodelling[19,20]. The expression of TARPs and germ cell-specific gene 1-like protein (GSG1L) generally increases from embryonic to adult stages, contributing to the stabilization, trafficking and gating behaviour of mature AMPAR complexes, although GSG1L expression is more prominent later in development[20]. An exception is TARP γ4, which is highly enriched during early development but declines postnatally[19], suggesting a possible specialized role in establishing immature synapses. TARP γ5 exhibits a distinct profile, being expressed from embryonic stages and having relatively stable, regionally restricted expression rather than strong global upregulation. This TARP is also enriched in specific cell types such as hippocampal CA2 neurons and cerebellar Bergmann glia, consistent with a non-canonical role compared with other TARPs[46,47]. The curves show within-gene normalized trajectories (each gene is scaled to its own maximum across the plotted period) derived from reported developmental and regional expression patterns in refs. 19,20,46. Together, these transcriptional, post-transcriptional and auxiliary-protein changes generate a dynamic landscape of AMPAR signalling that is tightly aligned with the functional maturation of excitatory circuits.

in CNIH expression may alter the proportion of CNIH-containing versus CNIH-free receptors during synaptic maturation[62].

Taken together, these findings show that developing brain exhibits a coordinated developmental programme in which low R/G editing, flip-biased transcripts and a restricted auxiliary-protein repertoire in embryos gives way to highly edited receptors, increased flop usage and a rich array of auxiliary subunits in the adult brain.

Ancestral features in embryonic AMPARs

Our understanding of the evolutionary origins of AMPARs suggests that the transcriptional regulation of these receptors and the emergence of auxiliary subunits first appeared in early vertebrates[32,113,141]. Thus, it is reasonable to ask whether AMPARs in the embryonic brain mimic, in some sense, early vertebrate or even invertebrate AMPAR-like receptors, in which these regulatory features were essentially absent[32]. There are indeed meaningful parallels between embryonic AMPAR biology and the AMPAR systems of early-diverging animals. The features of the embryonic brain resemble a regulatory landscape in which AMPARs are less 'processed', and may therefore be considered similar to the simpler AMPAR architectures found in early animals[32,113,141]. Both embryos and early animals use AMPARs with fewer regulatory layers and, as a result, exhibit higher Ca^{2+} permeability, less finely tuned gating and reduced

auxiliary subunit modulation. However, embryos do not mimic a true invertebrate state because they already possess vertebrate gene duplicates (GluA1–4), and TARPs and CNIHs are present in the genome. Editing and splicing are thus suppressed by developmental programmes, not absent owing to ancestral simplicity.

In summary, the AMPARs expressed during early postnatal development functionally resemble the simpler, more Ca^{2+}-permeable AMPAR-like channels found in early-diverging metazoans. However, this resemblance reflects a transient developmental state rather than a recapitulation of evolutionary history. Thus, the embryonic brain reveals how AMPAR signalling operates in the temporary absence of the vertebrate-specific regulatory layers that later refine synaptic transmission in the adult.

The AMPAR life cycle

Together, these studies have established how the intrinsic structural features of AMPARs determine their functional properties. In addition to this receptor-intrinsic layer, a second layer of diversity arises from processes such as receptor assembly (Fig. 7), NTD interactions and synaptic targeting (Fig. 8). These mechanisms, which operate across the receptor life cycle, are discussed in the following sections.

ER-based assembly and quality control

Following translation, AMPARs require a specialized ER-based assembly pathway, involving a series of interacting biogenesis complexes, for their correct formation[13]. This pathway, which is dominated by the actions of the DOMON domain-containing protein FRRS1L, palmitoyl thioesterase CPT1C, monoacylglycerol lipase ABHD6, lysophosphatidylserine lipase ABHD12, protein-serine *O*-palmitoleoyltransferase porcupine (PORCN) and phosphatidylinositol-3-phosphatase SAC1, is essential for producing functional synaptic receptors[11,142–144] (Fig. 7). AMPAR biogenesis proceeds through a series of discrete, ordered states within the ER[12]. In early stages, ABHD6 stabilizes AMPAR monomers and prevents their premature dimerization[12,13]. FRRS1L–CPT1C complexes then promote dimerization and tetramerization, crucial checkpoints for generating functional receptors[11,13]. Mature tetramers exit the ER only when TARPs

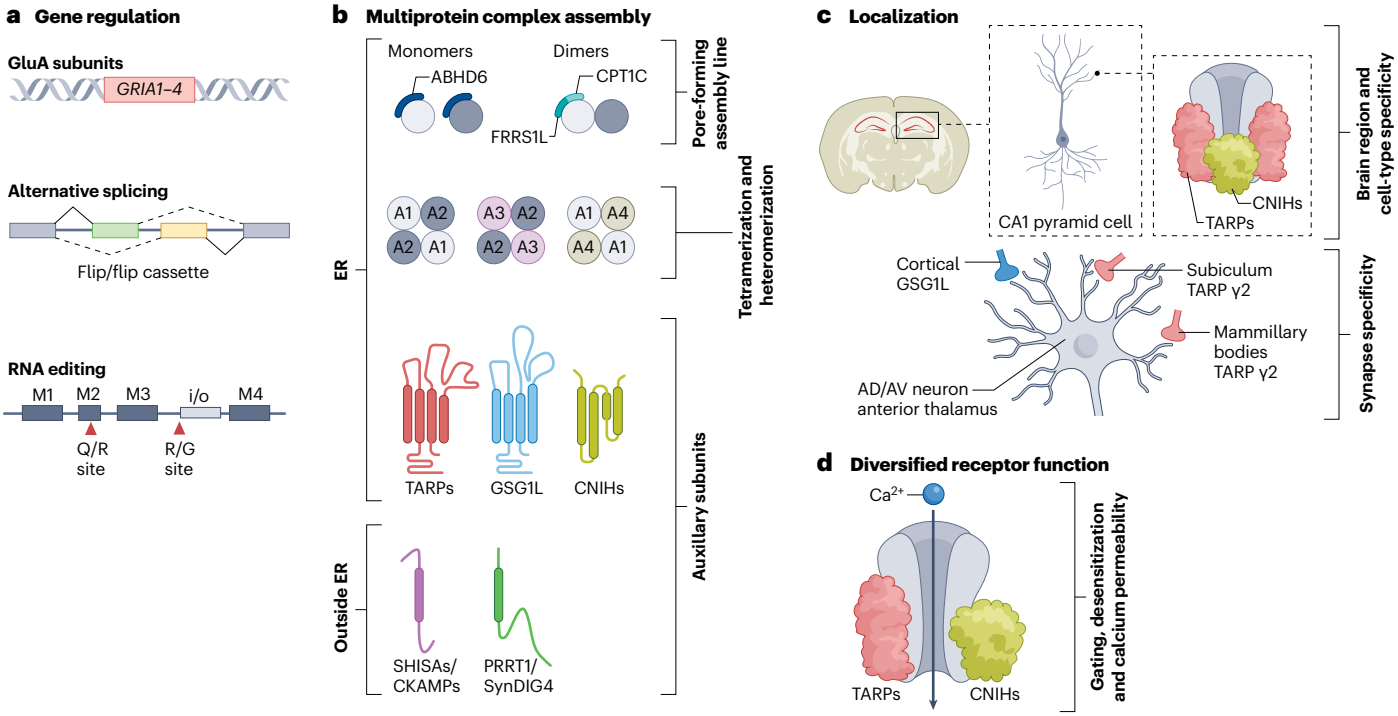


Fig. 7 | A multilayered framework for AMPAR diversity and synaptic specialization. Schematics summarizing major conceptual advances in our understanding of AMPA receptor (AMPAR) biology, organized into four thematic domains that have emerged over successive decades. **a**, Early molecular studies in the 1990s identified the four *GRIA* genes encoding AMPAR pore-forming subunits and revealed the post-transcriptional mechanisms that diversify receptor function[182,183]. Mutually exclusive flip/flop splicing within the ligand-binding domain[17] and RNA editing at the Q/R[15] and R/G[16] sites were shown to fine-tune channel gating, desensitization and Ca^{2+} permeability, establishing gene regulation as the primary source of AMPAR heterogeneity. **b**, Work from the early 2000s to 2015 defined AMPARs as modular multiprotein assemblies. Receptors are built through a stepwise assembly line in the endoplasmic reticulum (ER)[11,12], beginning with monomer translation and progressing through dimer formation, tetramerization and subunit-specific heteromerization[13]. During this period, claudin-type transmembrane AMPA receptor regulatory proteins (TARPs)[19,170] and germ cell-specific gene 1-like protein (GSG1L)[7,103], cornichon homologues (CNIHs)[63], cystine-knot AMPAR-modulating proteins (CKAMPs)[72,184] and synapse differentiation-induced gene (SynDIG)[69,70,185] proteins, were identified as auxiliary subunits that associate with AMPARs and profoundly influence trafficking, gating, pharmacology and synaptic targeting. **c**, Recent work has highlighted the importance of AMPAR composition in determining their cellular and synaptic destinations. Distinct auxiliary-subunit combinations and splice variants are enriched in specific brain regions, cell types and synapse classes, contributing to the precise allocation of AMPARs with different signalling properties (such as the TARP γ2 and GSG1L-specific corticothalamic synapses illustrated)[106]. **d**, The molecular differences between receptors influence not only their synaptic efficacy but also Ca^{2+} transport, allowing diverse AMPAR complexes to support the functional specialization of excitatory circuits. AD, anterdorsal nucleus; AV, anteroventral nucleus; i/o, flip (i) and flop (o).

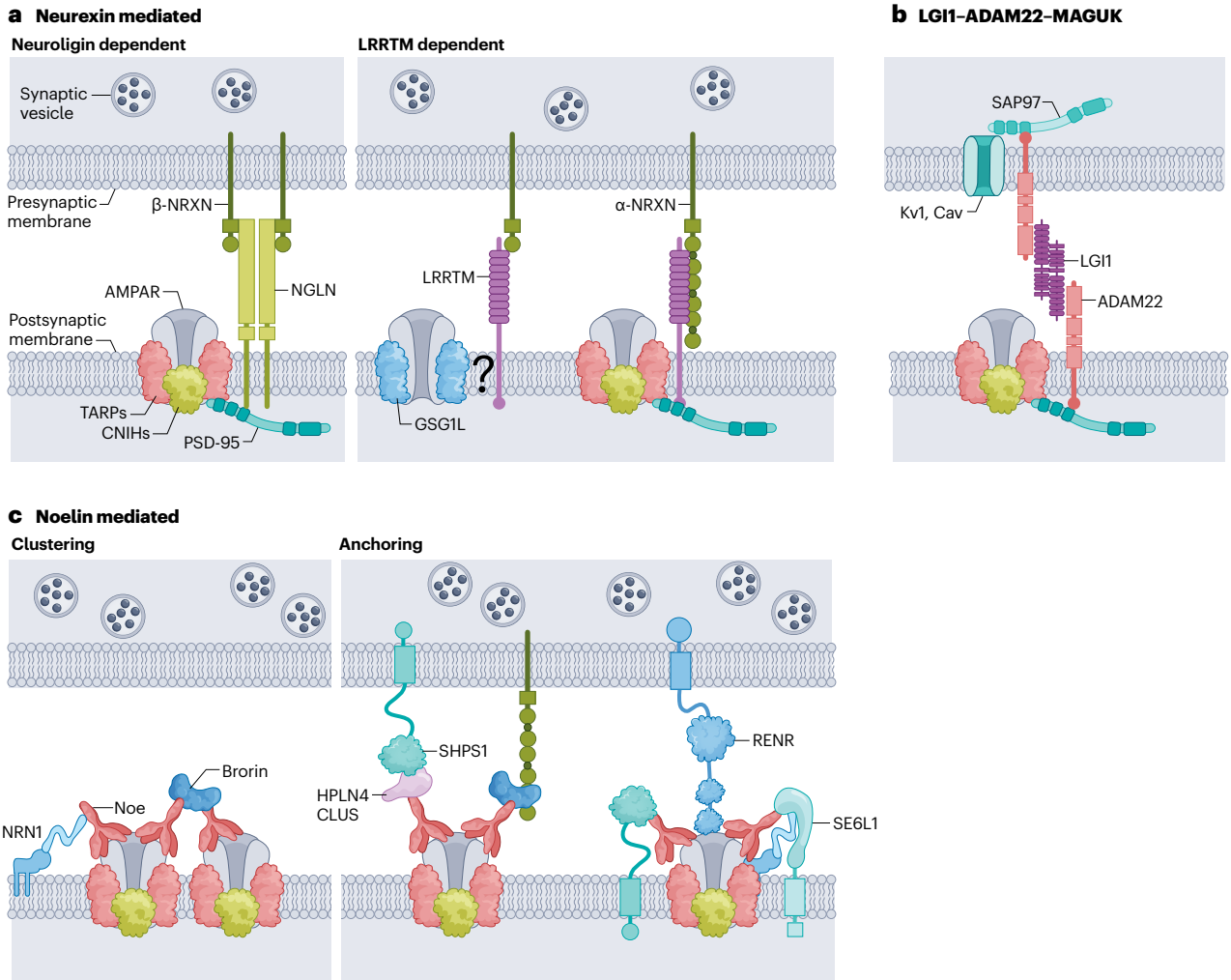


Fig. 8 | Synaptic adhesion molecules organize AMPARs at synapses. Schematics illustrating several major families of cell-adhesion molecules that shape the molecular architecture of glutamatergic synapses. Although not shown, the extracellular N-terminal domain of AMPA receptors (AMPARs) can also engage adhesion molecules which may regulate receptor stabilization and mobility at synapses. Therefore, although it is not exhaustive, the figure highlights trans-synaptic complexes with established roles in aligning presynaptic release machinery with AMPAR-rich postsynaptic sites, as well as emerging pathways whose organizational logic is only beginning to be understood. The spatial arrangements shown should be considered schematic working models; although several ectodomain interactions have been structurally characterized in vitro, the full trans-synaptic assemblies remain incompletely resolved. **a**, Neurexin–neuroligin complexes form a canonical adhesion axis linking presynaptic neurexins to postsynaptic neuroligins, thereby coupling vesicle release probability to AMPAR anchoring[21,159,160,186]. Neurexins also interact with leucine-rich repeat transmembrane protein (LRRTM) family members, creating an additional set of parallel adhesion pathways that recruit AMPARs, often through postsynaptic density protein 95 (PSD-95) and related membrane-associated guanylate kinase (MAGUK) scaffold proteins, and contribute to synapse maturation and subtype-specific signalling[22,161,162]. Structural studies have resolved several ectodomain interactions within these systems, but their organization across the synaptic cleft in intact synapses remains an active area of investigation. **b**, A second major cell-adhesion complex defined by LGI1–ADAM22–MAGUK assemblies is now recognized as a predominant organizing principle across forebrain excitatory synapses[23,163]. Leucine-rich glioma-inactivated 1 (LGI1) secreted from postsynaptic neurons binds a disintegrin and metalloproteinase domain-containing protein 22 (ADAM22) on the membrane, which in turn associates with PSD-95 family proteins. These complexes influence postsynaptic organization and synaptic stability, although direct structural information for the full membrane-associated assemblies is still limited. **c**, Recent work has identified noelin (Noe, also called olfactomedin-like) proteins as additional AMPAR-interacting factors that bind the receptor N-terminal domain. The arrangement shown here represents a provisional model based on available biochemical and functional data from ref. 24 and should be considered hypothetical pending further high-resolution structural analysis. Together, these cell adhesion complexes and noelins may contribute to the layered molecular logic through which glutamatergic synapses may specify AMPAR subtype, auxiliary-subunit composition and postsynaptic nanodomain organization. Cav, voltage-gated calcium channel; CLUS, clusterin; CNIHs, cornichon homologues; GSG1L, germ cell-specific gene 1-like protein; HPLN4, hyaluronan and proteoglycan link protein 4; Kv1, voltage-gated potassium channel subfamily 1; NRN1, neuritin 1; NRXN, neurexin; RENR, renin receptor protein; SAP97, synapse-associated protein 97; SE6L1, seizure related 6 homolog like 1; SHPS1, Src homology 2 domain-containing phosphatase substrate 1; TARPS, transmembrane AMPA receptor regulatory proteins.

or CNIHs associate with the tetramers, displacing the FRRS1L–CPT1C complex and thereby clearing the receptors for ER export[12]. Loss of FRRS1L in mice disrupts AMPAR assembly, reduces surface receptor levels, impairs excitatory transmission, and leads to major behavioural and cognitive deficits[16]. In humans, FRRS1L mutations cause severe intellectual disability[13,145,146]. Thus, AMPAR biogenesis is not merely a precursor step but a major regulatory process that determines excitatory synapse strength and function. Although nearly all existing data on this biogenesis pathway concern the assembly of GluA2-containing AMPARs, it is assumed that GluA2-lacking receptors follow a similar pathway, although additional work is required to confirm this.

Studies show that intrinsic structural features of AMPAR subunits themselves can also influence receptor assembly. For example, interactions between the extracellular NTDs of subunits contribute to the earliest steps of their association, helping to specify preferred heteromeric partnerships and guiding the formation of tetrameric receptors as dimers of dimers[147,148]. In parallel, the RNA-processing events that diversify AMPAR subunits also modulate assembly. Alternative splicing of the flip/flop cassette within the LBD can alter receptor maturation and ER export[123,149], whereas RNA editing of the GluA2 Q/R site affects oligomerization efficiency and ER retention (with edited GluA2(R) subunits assembling less efficiently than the unedited Q form)[150]. As these RNA processing mechanisms also strongly shape the functional properties of AMPAR–TARP complexes, their influence on receptor assembly raises the intriguing possibility that post-transcriptional diversification of AMPARs and their auxiliary proteins may have co-evolved in early vertebrates not only to tune receptor gating, as noted recently[32], but also to ensure efficient receptor assembly and biogenesis. Consistent with the idea that receptor-intrinsic determinants intersect with ER quality-control pathways during assembly, the ER-resident protein PORCN, best known for its role in Wnt processing[151,152] and identified in a proteomic screen of AMPAR-associated proteins[7], has been shown to promote receptor maturation and surface expression independently of its canonical enzymatic activity[153].

Together, these findings indicate that AMPAR assembly is shaped not only by dedicated ER biogenesis factors but also by structural determinants encoded within the receptor subunits themselves. It is, however, still unclear where other auxiliary subunits, such as members of the CKAMP and/or SynDIG families, are added to the AMPAR complex. Finally, whether the insertion of pre-assembled AMPARs into the plasma membrane occurs through the action of the ER membrane protein complex, as recently shown for voltage-gated calcium channels[154], awaits future study.

NTD in synaptic regulation

The NTD of AMPAR subunits plays a central role not only in receptor biogenesis but also in the regulation of receptor function at synapses. Functional studies show that the NTD is required for efficient synaptic anchoring of AMPARs and synaptic plasticity, through a role in supporting activity-dependent stabilization of AMPARs at postsynaptic sites[155,156]. Consistent with this, the NTD forms a prominent extracellular layer that can interact with binding partners within the synaptic cleft. These include adhesion molecules such as neuroplastin-65, which was recently reported to interact with the NTD of GluA1[157], as well as secreted factors such as the noelins[24], which can organize extracellular protein networks that influence AMPAR localization and stability. Structural differences among NTDs contribute to synapse-specific regulation. For example, cryo-EM studies show that in GluA2-containing AMPARs a stabilizing tetrameric interface forms between NTD dimers, whereas GluA2-lacking receptors such as GluA1 homomers exhibit a more dynamic NTD architecture that permits rearrangements of the extracellular domain[110]. These structural distinctions influence receptor gating and signalling and are linked to differences in short-term synaptic plasticity that are associated with GluA2-containing versus GluA2-lacking receptors[158].

Synaptic targeting

Given that the AMPAR NTD provides an extracellular interface for synaptic interactions, and with so many possible combinations of AMPAR pore-forming and auxiliary subunits, it is tempting to speculate that receptor assemblies may be matched to different synapse organizer proteins, thus forming a molecular barcode that targets receptors to specific glutamatergic synapse types. This concept is rooted in classic work on neurexins and neuroligins[21,159,160] that has been extended by more recent studies linking neurexins to the LRRTM family[22,161,162] (Fig. 8). The discovery of additional synaptic pathways that interact with AMPARs, such as those involving the LGI1–ADAM22–MAGUK complexes, has further highlighted how extracellular organizers contribute to synapse identity and to the molecular architecture of excitatory synapses[23,163] (Fig. 8). As noted above, very recent work suggests that noelins can bind to the AMPAR NTD and may contribute to the stabilization or positioning of receptors at postsynaptic sites, potentially in a synapse-specific manner[24,58]. Whether AMPAR subunit composition directly determines interactions with specific synapse-organizing proteins, including secreted noelins, and thereby influences synaptic targeting, remains unknown. However, early evidence from corticothalamic synapses indicates that the short-term plasticity associated with GSG1L-containing AMPARs is unique to cortical inputs and distinct from other converging pathways[106]. Whether this molecular segregation reflects interactions between specific auxiliary subunits and synapse-organizing proteins — such as the potential association of GSG1L with LRRTM4 identified in recent proteomic analyses[20] — remains an intriguing question for future work.

Conclusion and perspectives

Over the past decade, multidisciplinary approaches, from structural biology and proteomics to single-cell transcriptomics and super-resolution imaging, have notably sharpened our view of the glutamatergic synapse. With the advent of large-scale whole-genome sequencing applied to patient cohorts, it is now clear that missense mutations and truncating variants across multiple iGluR genes contribute substantially to a range of neurodevelopmental disorders[134,164–166]. The central role of glutamatergic synapses in neurodevelopmental disorders is further emphasized by the many other synaptic proteins now linked to these debilitating conditions, including ionotropic and metabotropic glutamate receptors, postsynaptic density proteins, cell adhesion molecules and proteins involved in presynaptic vesicular release (Supplementary Tables 2–5). Yet, despite this progress, several foundational questions about AMPAR signalling and synapse diversity remain unanswered.

A striking example of the continuous revision of textbook assumptions concerns the long-held view that Q/R site editing in GluA2 operates as a binary molecular switch controlling Ca^{2+} permeability[167,168]. Whereas seminal genetic studies established that loss of Q/R editing leads to severe neurological dysfunction due to excessive Ca^{2+} influx[167,168], the recent work described in this Review has shown that Ca^{2+} permeability is more nuanced than previously believed. Rather than being impermeable, GluA2 AMPARs display tightly regulated

Glossary

Auxiliary subunits
Non-pore-forming proteins that stably associate with AMPAR tetramers to tune receptor trafficking, gating, pharmacology and synaptic localization.

Cryo-electron microscopy
(Cryo-EM). A structural biology technique in which biological samples are rapidly frozen to preserve native states and imaged with an electron beam, enabling high-resolution, three-dimensional reconstruction of macromolecules without the need for crystallization.

Desensitization
A rapid conformational transition of AMPARs into a non-conducting state despite continued agonist binding, profoundly shaping synaptic transmission. The process by which AMPARs return from the desensitized to the activatable state determines how they respond during repetitive synaptic activity.

Flip/flop cassette
A 38–39-amino acid alternatively spliced module in the ligand binding domain that tunes AMPAR desensitization, recovery kinetics and coupling to auxiliary subunits.

Molecular barcode
A proposed concept in which combinations of AMPAR subunits, splice variants and auxiliary proteins specify their synaptic destinations and functional roles.

Native receptors
Receptors expressed in their natural cellular or tissue context, retaining endogenous composition, regulation and interactions, and thus reflecting physiological function more accurately than recombinant systems.

Pore-forming subunits
In vertebrate AMPARs, the four proteins (GluA1–GluA4 (also known as GRIA1–4)) whose combinatorial assembly determines channel kinetics, ion permeability and regulation by auxiliary proteins.

Q/R site
A genomically encoded glutamine (Q) residue within the pore loop of GluA2 that is RNA-edited to arginine (R), controlling Ca^{2+} permeability and polyamine block.

R/G site
A developmentally regulated RNA editing site in AMPAR subunits GluA2–GluA4, located immediately upstream of the flip/flop cassette, that modulates recovery from desensitization. GluA1 is not edited at this site.

Recombinant AMPARs
AMPARs produced by introducing engineered DNA into host cells, enabling controlled expression of specific receptor variants for functional, pharmacological or structural studies.

Selectivity filter
The narrow ion-selective region of the AMPAR pore, centred on the Q/R site, that determines cation permeability and polyamine sensitivity.

Site-G
A Ca^{2+}-selective extracellular binding pocket formed by the SYTANLAAF motif of the open channel gate that funnels Ca^{2+} towards the selectivity filter.

Stoichiometry
The number and arrangement of auxiliary proteins bound to an AMPAR tetramer, which strongly influences gating kinetics and ion permeability.

Synapse organizer
A trans-synaptic adhesion molecule, such as neurexins, neuroligins, leucine-rich repeat transmembrane proteins or LGI1–ADAM22, that positions AMPARs within postsynaptic nanodomains to shape synapse identity and strength.

Tetramerization
The assembly of four AMPAR subunits into a functional channel, an important biogenesis step that is tightly regulated within the ER.

Ca^{2+} flux[9], suggesting additional modes of physiological modulation[137]. In retrospect, this regulatory flexibility aligns well with an evolutionary perspective, as invertebrate AMPARs, which lack Q/R editing, are inherently Ca^{2+} permeable and subject to strong polyamine block[32,169]. Thus, stringent control of Ca^{2+} permeability appears to be a key innovation of the vertebrate lineage. How this regulatory layer integrates into Hebbian and homeostatic plasticity, and whether subtle shifts in Ca^{2+} flux serve as instructive synaptic signals, remains an open and compelling question for future research.

The functional contribution of the second editing site in AMPAR subunits, the R/G site, also remains incompletely understood. Positioned immediately upstream of the flip/flop cassette, the R/G site is ideally located to influence the kinetics of alternatively spliced isoforms, and early and more recent work continue to demonstrate effects on receptor recovery from desensitization[16,110]. Yet the pronounced developmental and activity-dependent dynamics of R/G editing[16] suggest that its role extends beyond simple kinetic tuning. An important unresolved question is whether R/G editing modulates the engagement of auxiliary subunits, analogous to how flip/flop variants influence TARP association[8] and/or how TARPs regulate the Q/R site[9]. If so, this would strengthen the emerging concept that RNA editing and auxiliary subunit families co-evolved in early vertebrates to encode a modular post-transcriptional logic that underpins the diversification of AMPAR signalling[32].

The AMPAR auxiliary subunits provide another major axis of complexity. Although six TARP family members share structural homology, their divergent expression profiles suggest specialized functions[170]. For example, γ4 is most abundant during early CNS development, whereas γ5 appears enriched in glial lineages[19]. Among neuronal subtypes, regional specificity is striking, with γ3 and γ8 predominating in cortex and hippocampus and γ2 and γ7 strongly expressed in cerebellar circuits[6]. Whether such diversity equips different brain regions with tailored AMPAR subtypes, or whether TARPs diversify AMPAR signalling across distinct synapse types within a single neuron, remains unknown. High-resolution single-cell and spatial proteomic approaches across developmental stages and disease states will be essential to disentangle these layers of biological specialization.

Growing evidence implicates glutamatergic synapse dysfunction as a central feature of neurodevelopmental disorders associated with intellectual disability[166,171]. Despite this, therapeutic progress has been limited, with most pharmacological efforts having arisen through serendipity rather than mechanistic insight. A more rational strategy, guided by developmental regulation of AMPAR subunits, auxiliary proteins and RNA processing events, could identify more precise molecular targets. Although gene therapies hold promise for correcting pathogenic variants or restoring disrupted synaptic signalling networks, appreciable technical barriers remain[172]. In the near term, small-molecule strategies that leverage our expanding knowledge of synaptic organization, AMPAR allosteric regulation and circuit-level dysfunction may offer more immediately tractable therapeutic pathways[166].

Finally, the structural landscape of glutamatergic synapses is undergoing a transformative shift. Near native-state cryo-EM

and cryo-electron tomography now reveal AMPAR assemblies, auxiliary-subunit complexes and associated extracellular partners at unprecedented resolution[42,58,59,71,173]. Concurrently, advances in super-resolution microscopy demonstrate that synapses possess intricate nanoscale architectures, with receptor nanodomains that probably encode functional specificity[174,175]. Together, these advances raise the possibility of achieving atomic-resolution maps of individual synapses across defined circuit contexts. Such maps may ultimately reveal whether AMPAR composition functions as a molecular barcode governing synapse identity, stability and plasticity.

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Author contributions
D.B. conceived the organization and structure, designed the figures and drafted the manuscript. X.-T.W. contributed to the discussions, manuscript editing and figure construction. F.M.-C. contributed to the discussions and editing of the manuscript. A.M.P. contributed to the discussions, manuscript editing and figure construction.

Competing interests
The authors declare no competing interests.

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