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Review

Heterodimers Revolutionize the Field of Metabotropic Glutamate Receptors

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ABSTRACT

Identified 40 years ago, the metabotropic glutamate (mGlu) receptors play key roles in modulating many synapses in the brain, and are still considered as important drug targets to treat various brain diseases. Eight genes encoding mGlu subunits have been identified. They code for complex receptors composed of a large extracellular domain where glutamate binds, connected to a G protein activating membrane domain. They are covalently linked dimers, a quaternary structure needed for their activation by glutamate. For many years they have only been considered as homodimers, then limiting the number of mGlu receptors to 8 subtypes composed of twice the same subunit. Twelve years ago, mGlu subunits were shown to also form heterodimers with specific subunit combinations, increasing the family up to 19 different potential dimeric receptors. Since then, a number of studies brought evidence for the existence of such heterodimers in the brain, through various approaches. Structural and molecular dynamic studies helped understand their peculiar activation process. The present review summarizes the approaches used to study their activation process and their pharmacological properties and to demonstrate their existence in vivo. We will highlight how the existence of mGlu heterodimers revolutionizes the mGlu receptor field, opening new possibilities for therapeutic intervention for brain diseases. As illustrated by the number of possible mGlu heterodimers, this study will highlight the need for further research to fully understand their role in physiological and pathological conditions, and to develop more specific therapeutic tools.

Introduction

Glutamate was eventually recognized as the main excitatory neurotransmitter in the early 80's, acting on the ionotropic AMPA, kainate and NMDA receptors. In the mid 80's, glutamate was found to also activate the phospholipase C pathway, a response that displays a specific pharmacological profile, leading to the recognition that glutamate also activates G protein coupled receptors (GPCRs) called mGlu receptors (Sladeczek et al., 1985; Nicoletti et., 1986; Sugiyama et al., 1987). This was firmly demonstrated by the cloning of the first mGlu receptors (Houamed et al., 1991; Masu et al., 1991) followed by 7 other cloned cDNA, revealing a new group of GPCRs (Pin and Duvoisin, 1995), the so-called class C, due to their peculiar features and sequences. Thus, 8 genes (GRM1-8) encode these receptors that have been classified into three distinct groups based on their sequence homology, G-protein coupling and pharmacological properties. Group I mGlu receptors (mGlu1 and mGlu5) are coupled to Gq proteins and activate phospholipase C (PLC) signaling pathways. They are mainly reported as post-synaptic receptors

regulating the excitatory action of glutamate. Group II (mGlu2 and mGlu3) and group-III (mGlu4, mGlu6, mGlu7, and mGlu8) mGlu receptors are coupled to Gi/Go proteins, inhibiting adenylyl cyclase (AC) and regulating Ca²⁺ and K⁺ channels. Both Group II and III are mainly pre-synaptically located, but can also be found at post-synapses. Of note, alternative splicing processes of the mGlu receptor encoding mRNA can generate variants that mainly differ in their intracellular C-termini (Ferraguti and Shigemoto, 2006).

mGlu receptors are mainly found in the brain, but each subtype (mGlu1 to 8) has a specific distribution (Niswender et al., 2010). mGlu receptors have also been reported in other tissues including heart, lungs, and immune system (Skerry and Genever, 2001; Du et al., 2016). Roles of these receptors in the control of heart activity, immune tolerance of cancer development have been reported (Stepulak et al., 2014; Yi et al., 2020). The mGlu receptor proteins are much more complex than the rhodopsin-like GPCRs (Class A, GPCRs), being composed of four main domains (Pin and Bettler, 2016), an N-terminal extracellular domain called Venus FlyTrap (VFT domain) that contains the glutamate binding

Abbreviations: mGlu, metabotropic glutamate; AC, adenylyl cyclase; CRD, cysteine-rich domain.

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site (defining the orthosteric site), connected through a cysteine-rich domain (CRD) to a typical 7 transmembrane (7TM) domain, followed by an intracellular domain variable in length and sequence that allows association with protein partners (Enz, 2012). The VFT domain is composed of two lobes in an open form in the absence of ligand that closes upon glutamate binding in the hinge region (Stern-Bach et al., 1994).

Initially thought to be monomeric like the other GPCRs, mGlu receptors were proposed to be homodimeric in 1996 (Romano et al., 1996). Indeed, mGlu5 proteins migrated at a molecular weight twice as high as that of a monomer, and co-immunoprecipitation experiments showed that these were homodimers. The absence of immunoprecipitation of mGlu1 by mGlu5 further set the general opinion that mGlu

receptors are strict homodimers. Monomers could only be observed after treatment with a reducing agent, indicating that a disulfide bridge was involved in the stability of the dimers, possibly through an inter-subunit linkage, as demonstrated later (Romano et al., 2001). The dimeric organization of mGlu receptors has proven to be essential for their activation mechanism and for agonists to induce receptor active state (El Moustaine et al., 2012). This was then firmly demonstrated by the structural understanding of the mechanism of action, shown to result from a relative movement of the VFTs upon closure, leading to a change in the dimeric interface of the two 7TM domains leading to the activation of one of them (Pin and Bettler, 2016; Seven et al., 2021; Lin et al., 2022).

The specific distribution of each of the 8 mGlu subtypes, was used for

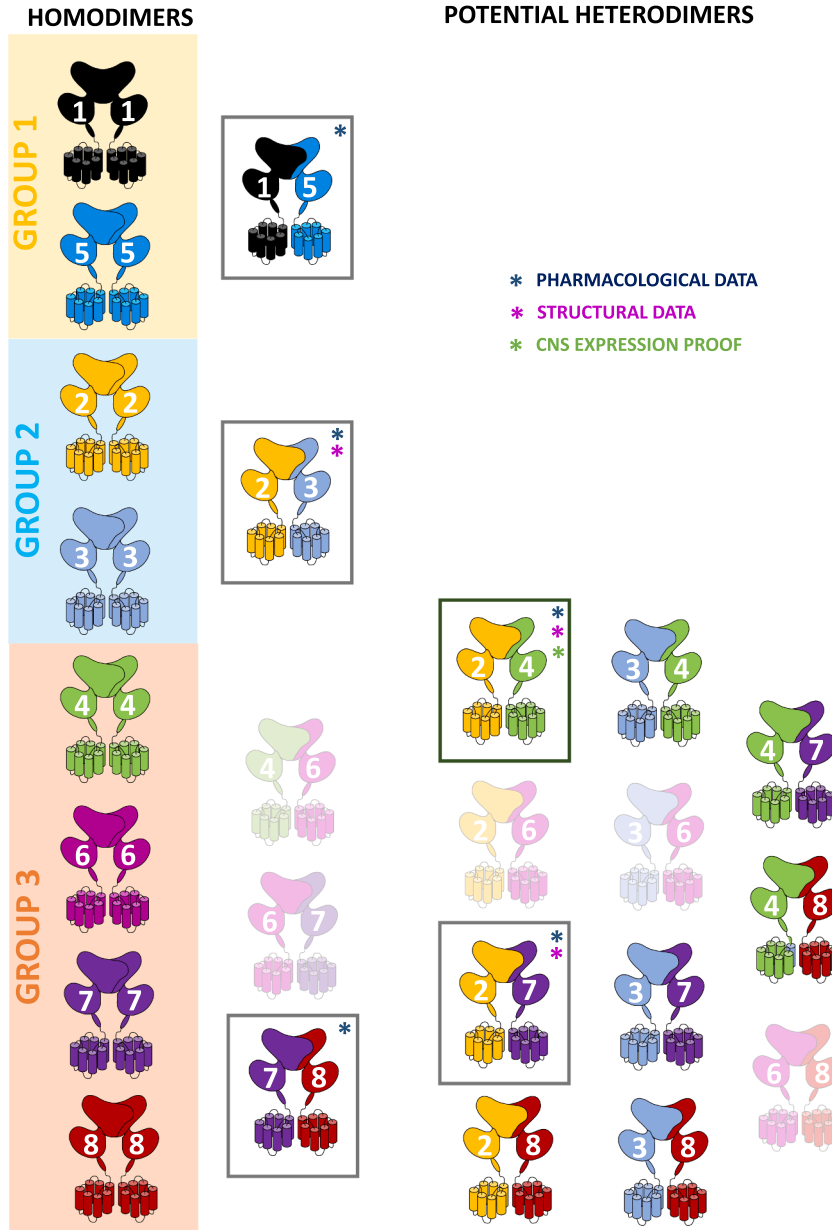


Fig. 1. Schematic representation of mGlu Homo- and Heterodimers. Illustration of the 8 mGlu homodimers, classified into three distinct groups and the 16 potential heterodimers. Heterodimers that include mGlu6 are depicted in transparency to indicate their limited probability as mGlu6 is predominantly expressed in the retina. Framed heterodimers represent those that have been subjects of pharmacological or structural studies, or the expression of which in the central nervous system has been evidenced. mGlu1-5 heterodimer: Pharmacological data (Werthmann et al., 2021); mGlu2-3 heterodimer: Pharmacological data (Lee et al., 2020), Structural data (Wang et al., 2023); mGlu2-4 heterodimer: Pharmacological data (Liu et al., 2017; Moreno Delgado et al., 2017; Haubrich et al., 2021; Yin et al., 2014; Xiang et al., 2021), Structural data (Wang et al., 2023), CNS expression (Meng et al., 2022); mGlu2-7 heterodimer: Pharmacological data (Du et al., 2021), Structural data (Du et al., 2021); mGlu7-8 heterodimer: Pharmacological data (Lin et al., 2022).

many years as an argument against the possible existence of mGlu heterodimers. However, further analysis revealed the expression of different subunits in the same cells (Shigemoto et al., 1997; Ferraguti and Shigemoto, 2006; Lee et al., 2020), raising the possibility of heterodimeric assemblies. Moreover, the finding that the related GABA_B receptor, that belongs to the same class C GPCR family, was a mandatory heterodimer composed of two similar but different subunits (Jones et al., 1998; Kaupmann et al., 1998; Evenseth et al., 2020), as well as the sweet and umami receptors (Nelson et al., 2001, 2002; Li et al., 2002), raised the question of the strict homodimeric quaternary structure of mGlu receptors.

In 2011, it has been reported that transfected mGlu subunits were able to form heterodimers with specific subunit composition at the cell surface as revealed using an innovative approach based on the use of specific tags inserted upstream of the VFT that could be orthogonally labelled with time resolved FRET fluorophores (Doumazane et al., 2011). Such finding revealed 11 possible additional heterodimers, not including mGlu6 containing ones as mGlu6 is expressed in very specialized cells in the retina (Agosto et al., 2021), increasing the potential number of mGlu receptors from 8 to 19 (Doumazane et al., 2011). Indeed, the group I subunits (mGlu1 and mGlu5) were able to form heterodimers, while not being able to associate with groups II and III subunits. In contrast, all Group II and III subunits (not including mGlu6 not tested in this study) were able to associate with any other subunit from these groups. This structural ability to heterodimerize opened a completely new field of investigation to determine whether such heterodimers exist in the brain and in which areas and cells, whether they have specific localization, pharmacological, functional and internalization properties. Thus, we expect 19 mGlu receptors subtypes from the 8 mGlu subunits: 8 homodimers (two identical subunits) and 11 heterodimers (two different subunits) (Fig. 1).

Of much importance, what are the physiological roles of these receptors and can they represent interesting specific targets for some brain diseases? The present review will discuss these issues, and illustrate how some of these have already been clarified with specific tools and approaches. We will see that these recent observations led to a reinterpretation of findings and reports that only considered mGlu receptors as homodimer.

We will first report on the recent understanding of the activation mechanism of mGlu receptors, with a special emphasis on their asymmetric activation. We will then describe the different approaches that can be used to specifically study the properties of mGlu heterodimers, and how their identified properties will help exploring their *in vivo* relevance. We will then summarize on the studies addressing their physiology and highlight the perspectives opened for therapeutic application.

The asymmetric activation of dimeric mGlu receptors

Much has been clarified over the last years on the activation mechanism of mGlu receptors, highlighting the need for a dimeric structure for agonist activation, leading to an asymmetric active state with only one subunit coupling to a G protein at a time (Goudet et al., 2005; Hlavackova et al., 2005, 2012). Indeed, even though a monomeric subunit could be activated using a positive allosteric modulator (PAM) acting in the 7TM domain, a dimeric quaternary structure is needed for the activation of the receptor by agonists acting in the VFT (El Moustaine et al., 2012). Thanks to cross-linking experiments inter-subunit FRET measurements (Doumazane et al., 2013; Xue et al., 2015; Lecat-Guillet et al., 2023), and the resolution of the 3D structures of mGlu receptors in various states (Koehl et al., 2019; Seven et al., 2021; Lin et al., 2022), much is known on the various steps involved in the activation of mGlu receptors.

At the resting state, mGlu receptors are mainly associated via a hydrophobic interface at the level of the lobe-I of their VFTs, an association stabilized by the inter-subunit disulfide bridge connecting the flexible

loops located on top of each VFT. This resting state with both VFTs open (Roo) maintains the lobes-II apart, leading to a distant location of both CRDs, maintaining apart the two 7TM domains that face each other through TM4 and TM5.

The first step occurring upon agonist binding in the VFT is the closure of this domain (Kunishima et al., 2000), with most VFTs being closed at saturating agonist concentration (Lecat-Guillet et al., 2023). The closure of both VFTs in the dimer leads to an important change in their relative orientation, leading to a close apposition of the lobes-II (the Acc state). This second step is not efficient, as only a fraction of the dimers reaches this Acc state, while the others remain in a Rcc state, indicating that saturating concentration of glutamate cannot stabilize every dimer in an active conformation (Lecat-Guillet et al., 2023). Under condition when only one VFT is closed, probably even less receptors reach the active VFT orientation (Aco), leading to a partial activation of the receptor (Kniazeff et al., 2004) (Fig. 2).

The Acc state of the VFTs brings together the two CRDs, able to contact each other, leading to a decrease in distance between the top of the TM1 from the 7TM domains. This results in a major rotation of one 7TM domains relative to the other, going from facing each other through TM4 and 5, to contacting each other via their TM6. Of major importance, this TM6-TM6 interface is not symmetric, resulting in different conformational changes in each 7TM domain of the dimer. Such an asymmetric 7TM dimer can then activate a single G protein, as originally proposed (Goudet et al., 2005; Hlavackova et al., 2005, 2012) and confirmed by cryo-EM studies (Du et al., 2021; Seven et al., 2021; Liu et al., 2023). This has been confirmed by structural analysis of mGlu2 coupled to a G-protein in the presence of its agonist glutamate or an ago-PAM showing that the ICL2 and the C terminal part are ordered only in the protomer coupled to Gi (Seven et al., 2021) (Liu et al., 2023).

However, this new conformation of the dimer, through a contact between the CRD and the extracellular loop 2 (ECL2), results in a slight change in conformation of the intracellular loops, including Intracellular loop 2 and 3 (ICL2 and ICL3), in one subunit only, creating a G protein binding site located on the intracellular side of one 7TM domain. This is then very different from what is commonly observed in class A and B GPCRs, where the activation leads to the formation of a central intracellular cavity resulting from an outward movement of TM6, where the G protein interacts.

These observations clarify the need for mGlu receptors dimer formation for their activation by glutamate, and reveal a unique G protein activation mode by only one subunit due to the asymmetric conformational changes occurring within the 7TM dimer.

Demonstration of formation of mGlu heterodimers

The mGlu receptors are part of the class C GPCRs that also includes the GABA_B and the sweet and umami taste receptors. The GABA_B was the first mandatory heterodimeric GPCR to be characterized (Jones et al., 1998; Kaupmann et al., 1998), being composed of two subunits, GABA_B1 that contains the agonist binding site, and GABA_B2 responsible for G protein activation (Galvez, 2001; Evenseth et al., 2020). Similarly, the TAS1Rs were also shown to be mandatory heterodimers, with the TAR1 + T1R3 combination being responsible for the umami detection, while the T1R2 + T1R3 heterodimer being involved in the sweet detection (Nelson et al., 2001, 2002; Li et al., 2002). Such finding suggested that mGlu receptors could also potentially form heterodimers, a notion that was against the dogma in this field at that time. To test this possibility, we used a technology developed by the group of Kai Johnsson, based on the use of the covalent labeling of the suicide enzymes SNAP and CLIP (O'Hare et al., 2007; Gautier et al., 2008). These tags can be covalently labelled with their selective substrates carrying either a FRET donor or a FRET acceptor. Using such non-cell permeant substrate, and introducing these tags at the N-terminal extracellular end of the mGlu subunits allow their selective labeling at the cell surface exclusively. Co-expressing mGlu subunits carrying such tags at their N

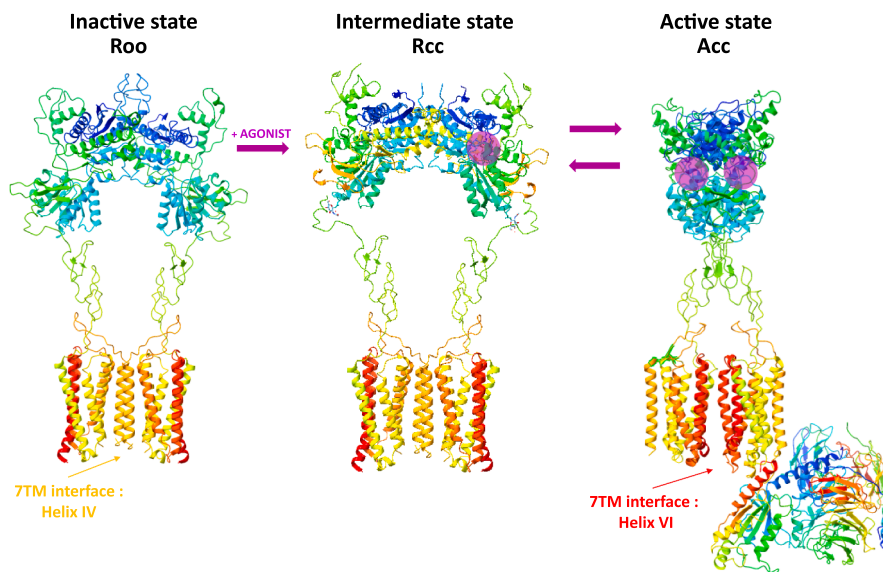


Fig. 2. Structural representation of conformational changes in mGlu2 from active to inactive states. Conformational transitions of mGlu2 homodimer between its active and inactive states are shown. The inactive state is characterized by a ‘Resting open open’ conformation (Roo), where both lobes of the Venus Flytrap (VFT) modules are open (in blue). The intermediate activation state shows the closed VFT lobes upon agonist LY354740 binding, however remaining in a resting state (‘Resting close close’ or Rcc, no relative movement of the two VFT domains) and then without movement in the 7-transmembrane (7TM) domains. The active state (‘Active close close’ Acc), following stimulation by LY354740, is depicted with the VFT lobes closed and the relative movement of the VFT domains. This state also illustrates the rearrangement of the cysteine-rich domains (in green) and the rotation of the 7TM domains (in orange), alongside the recruitment of the G-protein structure. Drown and modified from the PDB file 7EPA (Roo), 2E4U (Rcc) and 7E9G (Acc) using chimeraX.

terminus, we could orthogonally label each subunit subtype with either a FRET donor or a FRET acceptor. This approach revealed the capacity of group-I mGlu subunits on one side, and both group-II and -III subunits on the other side to associate into any possible heterodimers (Doumazane et al., 2011). The real formation of such heterodimers was further validated by western blotting of the mGlu2-4 heterodimer made of two subunits modified to have different molecular weight (Doumazane et al., 2011). Since then, multiple approaches confirm the capacity of these heterodimers to form in recombinant systems, including the pull-down of single molecules (Levitz et al., 2016) and single particles photobleaching experiments (Levitz et al., 2016; Lee et al., 2020).

These data were suggesting that heterodimers may exist, but to be formed in the brain, the two subunits had to be expressed in the same cells, and possibly at the same time as their covalent linkage likely prevent any exchange of subunits between preformed dimers. It was then necessary to develop specific assays to study the functional and pharmacological properties and to develop tools to specifically detect such heterodimers in native tissue.

Approaches to study the properties of mGlu heterodimers

The observation that heterodimeric mGlu receptors can be detected in transfected cells raised a number of issues, the most important being whether such heterodimers exist in native cells. Another issue is of course to know what could be the physiological advantage of such heterodimers and whether they have specific properties. Identifying their pharmacological properties will certainly help identify their roles at specific synapses. However, studying such heterodimers need the development of approaches that allow their analysis specifically. Indeed, when two different mGlu subunits are co-expressed in the same cell, three types of receptors are expected: both homodimers and the heterodimer, the later representing half of the receptors if both subunits are expressed at the same level, and if both subunits have the same capacity to either homodimerize, or to associate with each other. Several approaches have been used to specifically examine the functional and

pharmacological properties of mGlu heterodimers (Fig. 3).

a) Functional complementation assay

The functional complementation between two non-functional subunits has been used for many years to bring evidence for the formation of dimeric GPCRs, not only in transfected cells (Werthmann et al., 2021), but also in vivo (Rivero-Müller et al., 2010). This approach consists at co-expressing a receptor in which the binding site is mutated, together with a receptor made incapable of activating G proteins. Upon dimerization of these two different mutants, an agonist acting in one subunit can potentially activate a G protein if a *trans*-complementation process occurs between the two subunits. Within the class C GPCRs, such a process already exists naturally in the case of the GABA_B and TAS1 receptors (Galvez, 2001; Kniazeff et al., 2002; Monnier et al., 2011), and it has been clearly demonstrated using CaSR and mGlu5 mutants (Bai et al., 1999; Brock et al., 2007; Jacobsen et al., 2017; Werthmann et al., 2021), indicating that it can be used to study mGlu heterodimers. Indeed, such complementation has been observed between any group-II and group-III mGlu receptors (Liu et al., 2017), as well as between mGlu1 and mGlu5 (Werthmann et al., 2021). Such an approach can be used to study the activation process of these receptors and the inter-subunit allosteric processes. However, it cannot be used to study the pharmacological properties of such heterodimers as one binding site is mutated.

b) Inter-subunit biosensors

As indicated above, a major change in the relative orientation of the VFTs occurs upon activation. Such a large movement can easily be detected by FRET, after labeling the subunits with a pair of fluorophores compatible with FRET measurements. This was well demonstrated with the random labeling of the N terminal SNAP-tagged version of the mGlu homodimers with a combination of two fluorophores (Doumazane et al., 2011). As such, if two different labeling approaches are being used, one to label one subunit, and another to label the other subunit, one can

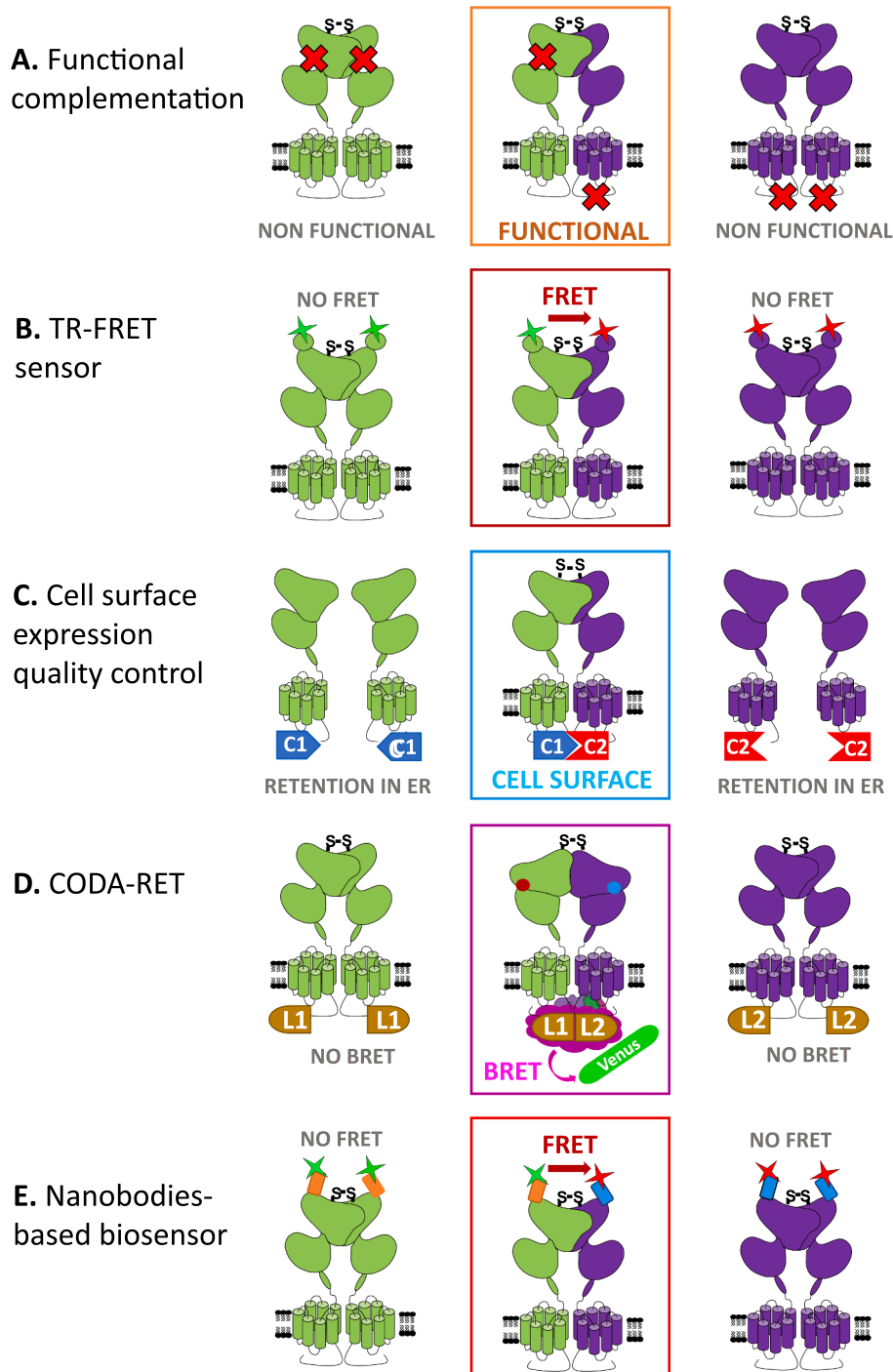


Fig. 3. Overview of methods for studying heterodimers. **(A).** The functional complementation approach needs two subunits bearing mutations (indicated by a red cross) that suppress the ligand binding of one subunit (green subunit) and the protein coupling of the other subunit (violet subunit). The framed cartoon highlights transactivation. **(B).** VFT Sensors based on TR-FRET require a subunit carrying a FRET donor (green subunit) and a subunit carrying a FRET acceptor (violet subunit). The framed cartoon emphasizes that FRET occurs exclusively in the heterodimer. **(C).** Quality control for cell surface expression requires a subunit containing a C1 domain in its C-terminal domain (green subunit), and a subunit containing a C2 domain in its C-terminal domain (violet subunit). The framed cartoon shows that only surface expression of the heterodimer occurs because of the interaction of both C1 and C2 domains that hide the ER retention signals. **(D).** CODA-RET approach requires a BRET donor Rluc split into two parts (L1 and L2, on dark yellow), each being inserted in one subunit (L1 in green subunit and L2 in violet subunit). The protein partner, like a G protein, is then fused to the BRET acceptor YFP (or its Venus variant). The framed cartoon underlines that BRET with Venus is possible only in the case of the heterodimer which reconstitutes a functional Rluc (L1:L2). **(E).** Nanobody-based biosensors require a nanobody (in orange) specific for one subunit (green subunit) labeled with a FRET donor fluorophore (in green), and another nanobody (in blue) specific for the other subunit (violet subunit) labeled with a FRET acceptor fluorophore (in red). The framed cartoon highlights that FRET between the two labeled nanobodies can occur exclusively in the case of the heterodimer.

specifically measure the conformational change occurring in a heterodimer, even though both homodimers are at the cell surface. Indeed, these homodimers carry the same labeling tool in each subunit and are then labelled either only with the donor, or only with the acceptor, then not generating any FRET signal. Such an approach has been validated using the SNAP and CLIP-tag labelling approach (Levitz et al., 2016; Liu et al., 2017; Moreno Delgado et al., 2017; Scholler et al., 2017; Habrian et al., 2019) or using a SNAP in one subunit and an ACP tag in the other subunit as illustrated with the GABA_B heterodimer (Scholler et al., 2017a), or using unnatural amino acids inserted at specific positions in one subunit or the other (Lecat-Guillet et al., 2023). Such FRET based biosensors can measure the re-orientation of the VFT₅ either at their N-terminal level (Scholler et al., 2017), or at the level of their bottom lobes (Lecat-Guillet et al., 2023), the repositioning of the CRDs (Liauuw et al., 2021) or even the closure of one VFT within a dimer (Lecat-Guillet et al., 2023). This approach can even be used at the single molecule level, making sure the measured FRET signal originates from an heterodimer (Levitz et al., 2016; Habrian et al., 2019, 2023) and not from a dimer of homodimers (Cao et al., 2021; Lecat-Guillet et al., 2023). Thus, such an approach allows the analysis of the structural dynamics of the receptors at the single molecule level, providing key information on the mode of action of specific ligands, such as partial agonists or allosteric modulators (Moreno Delgado et al., 2017; Cao et al., 2021; Lecat-Guillet et al., 2023).

c) Control of cell surface targeting

To guarantee a specific targeting to the cell surface of functional GABA_B receptors that need the correct association of the two subunits, evolution selected a natural quality system that prevents the over-expressed GABA_B1 subunit to reach the cell surface due to the presence of a signal retaining the subunit in the endoplasmic reticulum (ER) (Couve et al., 1998). This signal interacts with the ER gatekeeper PRAF2, preventing it from reaching the Golgi and the plasma membrane unless associated with GABA_B2 (Margeta-Mitrovic et al., 2000). Indeed, thanks to a coiled-coil interaction between the GABA_B1 and GABA_B2 C terminal domains, the ER retention signal is no longer able to interact with PRAF2, allowing the complex to reach the cell surface. This system has been optimized and used to control the cell surface expression of mGlu subunit, through the exchange of their C-terminal domains by those of GABA_B1 and GABA_B2 (Kniazeff et al., 2004). In addition, an ER retention motif has been added in the GABA_B2 C-terminal domain (C2 domain) such that neither GABA_B1 nor GABA_B2 C- domains (C1 and C2 domains, respectively) containing mGlu subunits could reach the cell surface alone, but only when co-assembled (Brock et al., 2007). Using such a system, only the heterodimer can reach the cell surface and be functional, allowing its pharmacological analysis. However, one must consider that the presence of the GABA_B C terminal domains may affect the signaling properties of the receptor, limiting the use of this approach for signaling analysis.

d) CODA-RET approach

GPCR activation of G proteins can be followed through a BRET transfer between the receptor and the G protein. Such an approach has been optimized to selectively detect the G protein recruitment to a GPCR dimer. For this, each subunit within the dimer is fused to one part of the luciferase, such that a complemented and active luciferase is formed only within the heterodimer. This technique, that has been called CODA-RET (Complemented Donor Acceptor Resonance Energy Transfer) (Urizar et al., 2011) can be used to measure the activity of GPCR dimers composed of specific subunits, including mGlu heterodimers (Lin et al., 2022). This technology allows the pharmacological and functional analysis of such heterodimers selectively (Niswender et al., 2016; Xiang et al., 2021; Lin et al., 2022).

e) Nanobody-based biosensors

The development of single domain antibodies, called nanobodies (Hamers-Casterman et al., 1993; Muyldermans, 2013) offered exciting new possibilities to study GPCR dimers. These small antibodies can be as specific as the conventional IgGs, but, because of their small paratopes, can recognize cavities in proteins that can be different depending on its conformational state. Such nanobodies have been selected that recognize specific mGlu subunits, and can be either neutral (Scholler et al., 2017b; Meng et al., 2022), recognizing both basal and active states, or specific of the active state then being either agonists (Steyaert and Kobilka, 2011), ago-PAMs or PAMs (Staus et al., 2016; Scholler et al., 2017). Such tools offer fantastic possibilities to study mGlu heterodimers. First, after being labelled with FRET compatible fluorophores, they can be used to detect specific heterodimer combinations in native tissues, as already reported for the mGlu2-4 heterodimer (Meng et al., 2022). Most importantly, they can also be used to developed biosensors of native receptors, using one nanobody that recognizes the active state of one subunit, and a nanobody selective of the other subunit, such that FRET signals can be measured only from an active heterodimer, allowing the characterization of the pharmacological properties of native heterodimers (Meng et al., 2022). Most importantly, nanobodies contacting both subunits interface have already been found, being specific of the homodimers (Scholler et al., 2017; Lin et al., 2021), then revealing the possibility to identify heterodimer specific ones. Such tools will definitely be essential to delineate the localization and function of specific mGlu heterodimers in the brain.

Agonist action at mGlu heterodimers

Today, the main known natural ligand for mGlu receptors is glutamate, and each subunit in a mGlu5 or mGlu2 homodimers needs to bind glutamate for an efficient signaling (Kniazeff et al., 2004; Levitz et al., 2016). Glutamate potency at the eight mGlu homodimers range from the sub-micromolar (mGlu3), to tens of millimolar (mGlu7), with the other mGlu receptors being in the tens of micromolar range (Pin et al., 1999; Schoepp et al., 1999; Scholler et al., 2017; Acher et al., 2022). Then what could be the consequence of heterodimerization for the action of glutamate? The first observation is that heterodimerization can increase glutamate efficacy relative to its action at one homodimer. This has been well demonstrated for the mGlu2-4 and mGlu2-7 heterodimers, for which the glutamate efficacy is close to that of mGlu2, and more than 2 times higher than that observed with mGlu4 (Liu et al., 2017; Moreno Delgado et al., 2017) or 10 times that of mGlu7 (Habrian et al., 2019) homodimers. Similarly, at the mGlu1-5 heterodimer, glutamate efficacy is intermediate to that of mGlu1 and mGlu5 homodimers (Werthmann et al., 2021).

The glutamate potency can also be largely increased, as observed with mGlu2-7, for which the glutamate potency is close to that of mGlu2, and then much higher than that of mGlu7 (Habrian et al., 2019). On the mGlu2-3 heterodimer, both a conformational FRET sensor and the signaling analysis revealed that the agonist potency at mGlu2-3 is intermediate between the high potency at mGlu3 and the lower one at mGlu2 (Lee et al., 2020). Such observation already highlights the influence of heterodimer formation on the action of glutamate, and illustrates the influence of one VFT on the other within the heterodimer. Indeed, such increase in glutamate potency and efficacy at mGlu2-7 relative to those at mGlu7 homodimers, can well be explained if the closed state of the mGlu2 VFT better stabilizes the closed state of mGlu7 bound to glutamate, then stabilizing the active Acc state of the VFT dimer.

To better understand the action of agonists on heterodimers, and possibly find a pharmacological signature that could be used to study the physiological roles of such receptors in synaptic physiology, the effect of ligands selective for each subunit has been examined. On mGlu2-4, the mGlu2 and mGlu4 agonists are partial, with the mGlu2 ligands being

more efficient. A clear cooperativity between both VFTs within the heterodimer is also observed, with a low, almost inactive concentration of one ligand, largely increasing the potency of the other, as observed both in transfected cells (Moreno Delgado et al., 2017; Scholler et al., 2017) and in native brain dissociated cells (Meng et al., 2022). Such a property of the mGlu2-4 heterodimer has been used to bring evidence for such a receptor acting at the terminals of the lateral perforant path in the hippocampus (Moreno Delgado et al., 2017), and to show its existence in brain samples (Meng et al., 2022).

The agonist action at other mGlu heterodimer may be different. At mGlu2-3, despite the absence of highly selective agonists for each subunit, the role of each binding site has been examined using the photochemical activation of one or the other subunit, and the use of mutated subunits (Levitz et al., 2016). In this heterodimer, activation of the mGlu2 VFT leads to a better activity than the activation of mGlu3, revealing an asymmetric activation at the level of the VFTs, which appears stronger than that observed with mGlu2-4. A higher basal activity of mGlu2-3 is also observed, as measured by the VFT dynamics by FRET. This can be due either to an action of Ca^{2+} ions at the mGlu3 VFT (Kubo et al., 1998; Vafabakhsh et al., 2015; Levitz et al., 2016) or to the high affinity of glutamate at this subunit due to Cl^- ions (Tora et al., 2018), allowing the activation by ambient glutamate.

Oriented asymmetric signaling by mGlu heterodimers

As mentioned above, mGlu receptors are known to activate a single G protein at a time, and this can obviously come from either subunit in a mGlu homodimer (Goudet et al JBC 2005 et Hlavackova EMBO J 2005). Indeed, the recent cryo-EM studies revealed a differential structural rearrangement of the two 7TM such that only one can bind a G protein (Lin et al., 2021; Seven et al., 2021). The coupling to G proteins of mGlu homodimers is dependent, among other things, on the rearrangement of hydrophobic residues in ICL3 facilitating the interactions with ICL2, the C terminal part and the G protein helix. The main residue responsible of this hydrophobic interaction (Phe in 756 position), crucial for G-protein coupling and activation, is therefore highly conserved among Class C GPCRs (Seven et al., 2021).

The asymmetric G protein coupling and activation is also likely the case for the heterodimeric receptors, raising the question whether both subunits have an equivalent role in G protein signaling or not (Liu et al., 2017; Lin et al., 2021; Du et al., 2021; Wang et al., 2023). A first analysis was reported studying mGlu2-4 heterodimers, as well as any heterodimers composed of a group-II and a group-III subunit (Liu et al., 2017). In all these cases, it was found that the group-III subunit was indeed responsible for the G protein activation by such heterodimers. This was quite surprising, knowing that mGlu4 and mGlu7 homodimers for example, display a much lower coupling efficacy than mGlu2, as estimated by the maximal response obtained in functional assays (Liu et al., 2017), indicating that the 7TM domain of these group-III subunit is capable of an efficient G protein activation providing they are stabilized in their active state by a group-II 7TM. Such information adds to the evidence that such heterodimers likely exist naturally, as such situation would not have been selected during evolution if these heterodimers did not exist in native cells.

In the case of the group-I mGlu heterodimer, the mGlu1-5 receptor, the situation appears different, as both subunits can couple to G proteins, though either one at a time (Werthmann et al., 2021), highlighting important differences among these class C GPCRs. For mGlu2-3, the selective photo-activation of the mGlu2 subunit, can indeed transactivate the mGlu3 7TM, arguing in favor of a transactivation mechanism, but whether this can be achieved in the other direction is not known, and likely difficult to test due to the low coupling efficacy of this heterodimer when mGlu3 is activated (Levitz et al., 2016). Indeed, it was reported that the mGlu2 subunit is mainly responsible for G protein activation by this heterodimer (Wang et al., 2023). In the mGlu2-7 heterodimer, activation of mGlu2 can transactivate the 7TM of mGlu7,

but the selective activation of mGlu7 cis-activate the 7TM of this subunit (Du et al., 2021) (Table 1).

The oriented asymmetry observed in group-II-group-III heterodimers can still be observed if only one or the other VFT is being activated (Liu et al., 2017). Moreover, the orientation can be controlled by allosteric modulators acting in either 7TM domain of the dimer. In the case of the mGlu2-4 heterodimer, the presence of the mGlu2 PAM LY487379, reorients the asymmetry toward the mGlu2 subunit being responsible for the coupling. The asymmetric change is also observed if the 7TM of mGlu4 is locked in an inactive conformation with the mGlu4 NAM optogluNAM4.1 (Liu et al., 2017). Such data indicate that an action at either 7TM can orient the asymmetry of the heterodimeric mGlu. However, such an observation is not general, as in the case of the mGlu2-7 heterodimer, the mGlu7 NAMs MMPiP, ADX71743 or MDiP did not promote G protein coupling by the mGlu2 subunit (Du et al., 2021), while the mGlu2 PAM JNJ-40411813 does. Such data illustrate how important the conformation stabilized by the allosteric modulator can impact the asymmetry in such heterodimer.

Specific residues within key position in the 7TMs of each subunit may be involved in the oriented asymmetry. Indeed, as the change in conformation of the Tryptophane at position 6.50 is important for the activation of the 7TM, any neighboring residues stabilizing one or another conformation of Trp6.50 may stabilize either the active or inactive state. This is the case in the mGlu3 7TM where residues stabilizing the conformation of this residue corresponding to the inactive state may limit its activation, and then favor the activity via the mGlu2 subunit in an mGlu2-3 heterodimer (Wang et al., 2023). Conversely, Trp6.50 in mGlu4 is not stabilized in the inactive conformation, making this subunit more prone to reach its active state in the mGlu2-4 heterodimer (Lebon, 2023).






G protein coupling by mGlu heterodimers

The group-I mGlu1-5 heterodimer is composed of two subunits known to activate Gq, and because either subunit can signal within this complex, it is not surprising that this heterodimer also activates Gq. However, group-I mGlu receptors are also known to activate Gs (Nasrallah et al., 2018) and Gi/o proteins (Garcia-Marcos, 2021; McCulloch et al., 2024). The influence of the heterodimer formation on the G protein coupling profiles of the group-I mGlu receptors is however not known. Group-II and -III mGlu receptors couple exclusively to Gi/o proteins and do not interact with Gs or Gq proteins. Therefore, it is not expected that group-II-group-III heterodimers possess a distinct G protein coupling profile, but further investigation is necessary to clarify this. Coupling to Gz should be examined as only group-II mGlu receptors have been reported to couple to this G protein subtype (McCulloch et al., 2024) despite the fact that only group-III mGlu receptors activate Gqz (Blahos et al., 1998). The observation that one protomer can largely increase coupling efficacy by the associated subunit and the modulation of the oriented asymmetry, raises important questions regarding the G protein coupling selectivity of mGlu heterodimers.

Effect of allosteric modulators on mGlu heterodimers

mGlu receptors have been among the first GPCRs for which high throughput screening campaigns have been carried out, leading to the discovery of the first mGlu5 NAMs by SIBIA and Novartis, followed by the discovery of PAMs as well as silent allosteric ligands (SAL). Most of these compounds have been shown to bind within a cavity of the 7TM domains, equivalent to the ligand binding site of many rhodopsin-like (Pin et al., 2003; Seven et al., 2021; Wang et al., 2023). Because this site is unlikely under pressure during development, it diverged during mGlu receptors evolution, leading to the easy identification of highly selective allosteric modulators for any mGlu subunit. It was therefore of much interest to examine their effect of mGlu heterodimers, targeting either one or the other subunit.

Table 1
Summary of the structure, pharmacology and brain localisation of mGlu heterodimers.

STRUCTURE	PHARMACOLOGY	BRAIN DETECTION	REFERENCE	
	<ul style="list-style-type: none"> • Agonist binding to both subunits for full receptor activation • Both mGlu1 and mGlu5 NAMs required for inhibition • Either subunit couple to G protein 	Hippocampus Cortex	Kammermeier and Yun, 2005 Werthmann et al., 2021Pandya et al., 2016	
	<ul style="list-style-type: none"> • Inactive I: pdb 8JCU, 8JCW, 8JCY, 8JD0 • Inactive II: 8JCV, 8JCX • Inactive III: pdb 8J CZ • Intermediate: pdb 8JD1 • Active: pdb 8JD2 • Active + G: pdb 8JD3 	Frontal cortex Co-expression exclusively in pyramidal neurons (L2/3 and L5 IT)	Lee et al., 2020Levitz et al., 2016Wang et al., 2023	
	<ul style="list-style-type: none"> • Active: pdb 8JD4 • Active + G: pdb 8JD5 	<ul style="list-style-type: none"> • Some compound are not active on mGlu2-4 (See Fig. 4) • Dominance of mGlu4 subunit for the G protein coupling. Trans-activation by mGlu2 if mGlu4 TMD is inhibited by a NAM 	Cortico-striatal synapses and dentate gyrus Thalamo mPFC synapses Olfactory bulb and the PFC	Liu et al., 2017Fulton et al., 2020Niswender et al., 2016Moreno Delgado et al., 2017Yin et al., 2014 Xiang, Z. et al. 2021 Meng et al., 2022Wang et al., 2023
	<ul style="list-style-type: none"> • Inactive: pdb 7EPD 	<ul style="list-style-type: none"> • mGlu7 NAM MMPIP failed to block these responses in brain slices 	Hippocampus	Du et al., 2021Lin et al., 2022Habrian et al., 2019
	<ul style="list-style-type: none"> • NAM activity on both mGlu7 and mGlu7-8 for ADX71743 • NAM activity on mGlu7 not on mGlu7-8 for MMPIP 	Hippocampal (Schaffet collateral CA1 synapse) Inner hair cell ribbon synapse (cochlea)	Lin et al., 2022 Klotz and Enz, 2021	

We already mentioned that these allosteric modulators could reorient the asymmetric activation of heterodimers, but it is also interesting to examine their allosteric action on the activity and signaling of heterodimers. Indeed, due to their ability to influence the allosteric relationships between both protomers, allosteric modulators could act differently in a specific homodimer vs a heterodimer containing one subunit of the homodimer (Lin et al. JBC 2022). For example, the mGlu7 homodimer NAM, MMPIP is inactive at the mGlu7/8 heterodimer (Lin et al. JBC 2022), providing explanation for its context selectivity (Niswender et al., 2010). Moreover, when considering that the 7TMs of both protomers in heterodimers do not interact the same way than in homodimers, the binding of any ligand (PAM, NAM, or SAL) in these 7TMs could then impact the interaction and the allosteric influence of the 7TMs on each other, modifying the activation mechanism (Wang et al., 2023). We can then even speculate that heterodimeric specific allosteric modulators could be identified.

At mGlu2-4, the mGlu2 PAMs LY487379 and BINA have almost no allosteric effect, while the mGlu4 PAM VU0155041, ADX88178 and Lu-AF21934 conserved their potentiating effects in contrast to VU0415374, VU418506, VU0361737 (ML-128) or PHCCC (Kammermeier, 2012; Yin et al., 2014; Niswender et al., 2016; Liu et al., 2017; Wang et al., 2023). Surprisingly, while neither BINA nor VU0415374 had no potentiating effect, their co-application largely enhances the mGlu2-4 activity (Moreno Delgado et al., 2017). In addition, the mGlu2 NAM MRK-8-29 is capable of blocking the effect mediated by L-AP4 at mGlu2-4

heterodimers (Xiang et al., 2021) (Fig. 4).

As for the mGlu1-5 heterodimer, blocking mGlu5 with MPEP leads to a very partial inhibition, while blocking mGlu1 with JNJ1625968 produces a stronger, but not total inhibition. Only when both NAMs are co-applied is the mGlu1-5 signaling fully blocked, in agreement with both subunits being capable of signaling (Werthmann et al., 2021).

Regarding the mGlu7 containing heterodimers, it was early on surprising to observed that the mGlu7 NAM MMPIP had no effect at some synapses (Niswender et al., 2010). Indeed, MMPIP was found to have no effect on the mGlu7-8 heterodimer, and this may not be unique to this specific heterodimer but may be possibly also true for other mGlu7 containing heterodimers (Lin et al., 2022).

Already, these observations indicate that allosteric modulators may be used in combination or not with selective agonists to identify mGlu heterodimer function in the brain. However, not every combination of heterodimers has been examined, and only when this will be clarified, could conclusion be reached regarding the activity of the specific mGlu dimer at a specific synapse.

Native mGlu heterodimers, evidence and physiopathological implications

The main argument used against the possible existence of mGlu heterodimers was that the 8 mGlu proteins are differentially expressed in the brain. However, a more detailed examination of the distribution of

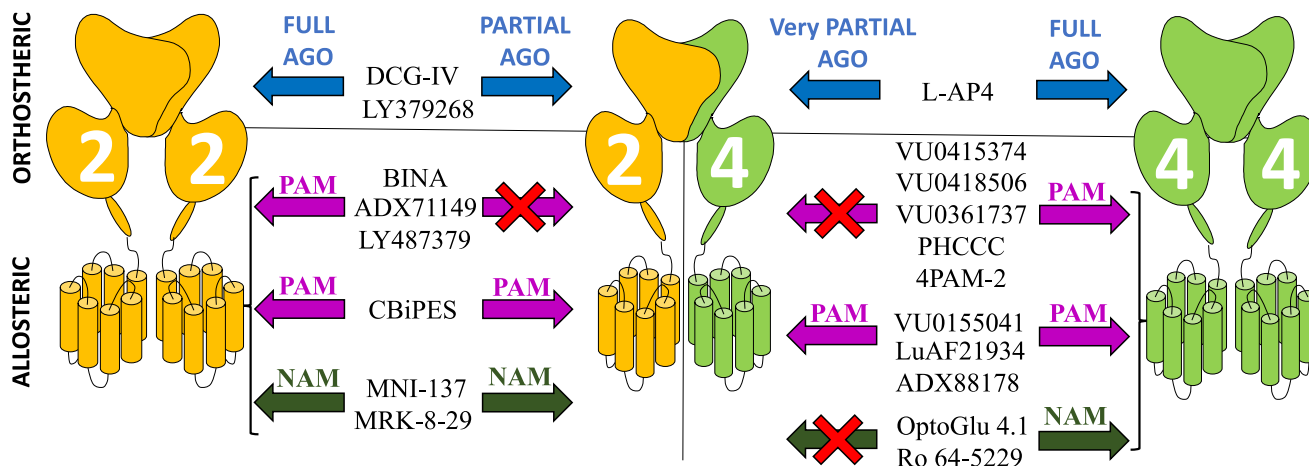


Fig. 4. Comparison of the effect of orthosteric and allosteric compounds on mGlu2 and 4 homo and heterodimers.

these proteins in the brain reveals regions of overlap. For example, while mGlu1 is highly expressed in the cerebellum, and mGlu5 in the forebrain, including the cortex, both proteins can be detected in the hippocampus for example (Shigemoto et al., 1997; Ferraguti and Shigemoto, 2006; Lee et al., 2020). mGlu4 is highly expressed in the cerebellum, with a large expression in the molecular layer where mGlu4 is found in the terminals of the parallel fibers (Mateos et al., 1998). However, mGlu4 is found in lower levels in many brain regions including the olfactory bulb and the cortex (Wada et al., 1998; Benitez et al., 2000). mGlu7 is also found in most brain regions, therefore in many cells co-expressing other mGlu receptors. Even at the subcellular level, mGlu7 and mGlu8 are found in the same terminals (Ferraguti and Shigemoto, 2006). A systematic analysis of the co-expression of the eight mGlu genes was recently performed, and revealed that many neurons in the mouse cortex co-express at least 2 different mGlu receptors (Lee et al., 2020), bringing further evidence for the possible formation of mGlu heterodimers. The same study also shows that some mGlu subunits, such as mGlu2 can have a better tendency to associate with other subunits, such as mGlu3 or even mGlu4 than with itself. Co-immunoprecipitation and co-pull down experiments have been used to argue in favor of GPCR association into functional heterodimers (Yin et al., 2014; Levitz et al., 2016; Pandya et al., 2016; Werthmann et al., 2021), although they do not excluded that these proteins may only be part of a large protein complex stabilized by scaffolding proteins (Pin et al., 2007). Due to the 200 nm resolution of common imaging techniques, co-localization data also do not prove the existence of heterodimers. Despite the proposal of functional heterodimers, this approach doesn't exclude the possibility of the two proteins being part of a large protein complex stabilized by scaffolding proteins, and co-localization data, limited by the 200 nm resolution of common imaging techniques, fails to confirm the existence of heterodimers (Pin et al., 2007).

We did use an original approach to examine whether mGlu2-4 heterodimers could be detected in freshly dissociated brain cells (Meng et al., 2022). We took advantage of the high affinity nanobodies recognizing specifically either mGlu2 or mGlu4, and observed a significant FRET between these two nanobodies labelled with tr-FRET compatible fluorophores on brain cells, a signal lost in either mGlu2 or mGlu4 knock-out brain samples. Of much interest, although both mGlu2 and mGlu4 are highly expressed in the cerebellum, no FRET signal could be detected, in agreement with the known expression of these two subunits in different neurons (granule neurons for mGlu4, and Golgi cells for mGlu2), demonstrating the specificity of the approach. However, such data only indicate a close proximity between these two subunits in some brain cells, and this could even be the consequence of a proximity between mGlu2 and mGlu4 homodimers. We then use a nanobody-based biosensor to examine the pharmacological profile of

the receptor complex co-labelled with both mGlu2 and mGlu4 nanobodies. This biosensor is based on the use of an mGlu2 specific nanobody that only binds on the active receptor, such that the binding of both nanobodies required for FRET can only occur in the presence of agonists. As such, the pharmacological profile of the native complex could be determined, and found to be identical to that of the mGlu2-4 heterodimer, with a very partial activity of L-AP4, and a large increase of its potency in the presence of low concentration of an mGlu2 agonist (Meng et al., 2022). Such data bring a clear demonstration that the two nanobodies recognize an mGlu2-4 heterodimer. This approach also allowed the relative quantification of the mGlu2-4 heterodimer relative to mGlu4 homodimers, and revealed a non-expected higher expression of the heterodimer in the brain outside the cerebellum (Meng et al., 2022).

The use of the pharmacological signature of specific heterodimers was also used to reveal the presence of mGlu heterodimers at specific synapses using electrophysiological recordings. These data brought evidence for mGlu2-4 at the cortico-striatal terminals (Yin et al., 2014), as illustrated by the lack of PHCCC potentiation of the L-AP4 effect, while VU0155041 was effective, and the mGlu2 NAM MNI-137 blocks the response. The mGlu2-4 is also proposed to be present at the lateral perforant path terminals in the hippocampus, as illustrated by the strong synergy between the mGlu2 and mGlu4 agonists in inhibiting these synapses (Moreno Delgado et al., 2017). In contrast, mGlu4 homodimers are reported at the striatal GABAergic terminals in the globus pallidus (GP) where PHCCC is effective (Marino et al., 2003), and considered as important for the anti-Parkinsonian effect of an mGlu4 PAM inactive at the mGlu2-4 heterodimer (Niswender et al., 2016). Using a similar pharmacological approach, evidence for mGlu2-4 heterodimers were found at the thalamo-cortical terminals in the medial prefrontal cortex where the L-AP4 effect could be inhibited by the mGlu2 NAM MRK-8-29, but not at the hippocampo-cortical terminals in this same area (Xiang et al., 2021).

Evidence for the existence of the group-III mGlu7-8 heterodimers was also provided using pharmacological tools at the hippocampal Schaffer collateral-CA1 synapses. Indeed, the evoked responses could be inhibited by one of the mGlu7 NAM, ADX71743, but not by another MMPiP (Lin et al., 2022). In a CODARET approach, the authors show that indeed, MMPiP is unable to antagonize the mGlu7-8 heterodimer while ADX71743 can, providing an explanation for the context dependent inhibition of mGlu7-mediated responses by MMPiP in brain slices (Niswender et al., 2010).

The mGlu1-5 presence has also been proposed in the hippocampus as these two subunits are part of the same protein complex (Pandya et al., 2016; Werthmann et al., 2021). Its existence was proposed earlier as the blockade of both mGlu1 and mGlu5 was necessary to prevent LTD

induction in the CA1 hippocampal area (Volk et al., 2006), none of the selective inhibitor having an effect applied alone.

These data bring further evidence for the existence of such mGlu heterodimers and provide important information on their action at specific synapses. However, only a few possible heterodimers were examined, and more have to be identified *in vivo* and localized at specific synapses. Already, all the information gained on the mGlu2-4 heterodimer already highlight the possible therapeutic application of drugs targeting mGlu receptors with specific subunit composition, as mGlu4 homodimers may be still a target for Parkinson disease (Niswender et al., 2016) while the mGlu2-4 may constitute an interesting target for schizophrenia treatment (Xiang et al., 2021).

Perspectives and future work

Ignored for many years and put in the frontline thirteen years ago, mGlu heterodimers changed the perspective of the mGlu receptors field. Their existence is now well documented, not only in transfected and *in vitro* assays, but most importantly in the brain (Table 1). However, the actual data are still not sufficient to propose specific roles for these receptors, nor possible therapeutic applications of drugs that could selectively target them.

These data raised a number of questions regarding the proportion of such heterodimers relative to the homodimers. The question of their cell trafficking and targeting is also essential as one subunit may affect the trafficking of another when they are co-assembled. As illustrations, the C terminal domain of mGlu3, involved in its capacity to be internalized, can drive mGlu2 subunit internalization due to heterodimerization, and mGlu8 rules the β -arrestin-induced trafficking of the heterodimers mGlu2-8 and mGlu3-8 (Abreu et al., 2021; Lee et al., 2023). As such, all other combinations need to be examined for their intracellular localization, trafficking to specific sites within the neurons, desensitization properties, etc... The signaling properties must also be further studied, not only in terms of G protein signaling, but also considering the role of specific interacting signaling proteins. Extracellular proteins have also been essential for the correct localization of mGlu subtypes at specific synapses (Dunn et al., 2019, 2018; Matsunaga and Aruga, 2021). Their respective roles for homo- versus heterodimers have to be clarified. One key information also coming from these studies, is the possibility that the mGlu7 receptor, displaying extremely low activity and affinity for glutamate, may indeed be part of more effective and potent receptors if associated with another subunit such as mGlu2 or mGlu8.

For all these studies, tools enabling the specific study of mGlu heterodimers have to be developed, and selective activators and inhibitors identified. Our actual knowledge already offers a number of pharmacological possibilities. Bifunctional ligands composed of molecules targeting each subunit of a heterodimer is an interesting approach (Fulton et al., 2020), although each ligand component of the bifunctional molecule may still be active on homodimers. Antibodies, and especially nanobodies interacting at the interface of heterodimers, may be able to selectively act on these receptors stabilizing either their inactive or active states (Scholler et al., 2017b; Lin et al., 2021). The studies show that GABA_B receptor PAMs act at the active interface of the 7TM domains of the two subunits (Shaye et al., 2020; Liu et al., 2021; Gao et al., 2022) because their binding requiring residues from both subunits. Such information suggests that it might be possible to identify mGlu modulators interacting at this site, being then selective for mGlu dimers composed of specific and different subunits. All these possibilities should stimulate the research in this field and open new possibilities to treat a number of brain disease.

The hope is that such precise knowledge of any mGlu receptors subtypes, homo and heterodimers, will provide ways to further explore the potential therapeutic effects of drugs targeting specific mGlu dimer combinations. Besides, the endogenous presence of heterodimers could explain why drugs targeting mGlu receptors failed in clinical trials. For instance, Pomaglumetad methionil, a non-selective mGlu2 and mGlu3

agonist, was tested in patients with schizophrenia already receiving second-generation antipsychotics (SGA) to reduce negative symptoms (Stauffer et al., 2013). The study revealed no significant improvement in the symptoms with pomaglumetad methionil compared to patients receiving placebo. However, this compound is active at many different mGlu subtypes, not only mGlu2 and mGlu3 homodimers, but also any possible mGlu2 or mGlu3 containing heterodimers, making its action less precise and so variable, with possible side effect. Identifying which dimeric combination, among all these group-II subunits containing receptors, is then crucial for the treatment of Schizophrenia related disorders.

Recently, a new compound, the selective mGlu2 PAM ADX71149 (also called JNJ-40411813), entered phase 2 clinical study to treat epilepsy (Bialer et al., 2020). The multicenter phase 2 study was designed to evaluate the administration of ADX71149 in patients suffering from focal epileptic seizures compared to traditional anti-epileptic drugs (levetiracetam or brivaracetam). Considering the different action of certain mGlu2 PAMs on either homo- or heterodimers, and the large proportion of mGlu2-4 heterodimers in the rat brain (Meng et al., 2022), a clear analysis of the effect of this compound on all mGlu2-containing dimers would be of great interest for analyzing the clinical results. As such, improvement of the therapeutic effects are expected with drugs that may be more selective for the receptor mainly involved in this effect, either mGlu2 homo, or heterodimers.

In summary, the discovery of mGlu heterodimers has opened up new avenues for drug development and treatment of neurological disorders. Further research into the structure and function of these heterodimers, as well as the development of more specific pharmacological tools, is crucial to fully clarify their therapeutic potential.

CRediT authorship contribution statement

Kawthar Belkacemi: Conceptualization, Writing – original draft, Writing – review & editing. **Philippe Rondard:** Conceptualization, Funding acquisition, Supervision. **Jean-Philippe Pin:** Conceptualization, Funding acquisition, Investigation, Project administration, Supervision, Writing – original draft, Writing – review & editing. **Laurent Prézeau:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing.

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