Functional Cross-Talk between Adenosine and Metabotropic Glutamate Receptors

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Abstract: G-protein coupled receptors are transmembrane proteins widely expressed in cells and their transduction pathways are mediated by controlling second messenger levels through different G-protein interactions. Many of these receptors have been described as involved in the physiopathology of neurodegenerative diseases and even considered as potential targets for the design of novel therapeutic strategies. Endogenous and synthetic allosteric and orthosteric selective ligands are able to modulate GPCRs at both gene and protein expression levels and can also modify their physiological function. GPCRs that coexist in the same cells can homo- and heteromerize, therefore, modulating their function. Adenosine receptors are GPCRs which stimulate or inhibit adenylyl cyclase activity through G\textsubscript{i}/G\textsubscript{o} protein and are involved in the control of neurotransmitter release as glutamate. In turn, metabotropic glutamate receptors are also GPCRs which inhibit adenylyl cyclase or stimulate phospholipase C activities through G\textsubscript{i} or G\textsubscript{o} proteins, respectively. In recent years, evidence of crosstalk mechanisms between different GPCRs have been described. The aim of the present review was to summarize the described mechanisms of interaction and crosstalking between adenosine and metabotropic glutamate receptors, mainly of group I, in both in vitro and in vivo systems, and their possible use for the design of novel ligands for the treatment of neurodegenerative diseases.

Keywords: Metabotropic glutamate receptors, adenosine receptors, crosstalk, oligomerization, protein interaction, signalling.

1. INTRODUCTION

Cellular communication is a key feature of evolution. The multitude of stimuli that can reach a cell may elicit different cellular responses according to the signalling pathways present in the cell. In addition, there may exist a cross-communication between these signalling pathways which is crucial for the fine-tuning of cellular response. The first interaction or crosstalk process can take place at the surface receptor level [1]. The crosstalk between two given pathways can modify not only the response to their corresponding specific signals (ligands) but also their regulation (e.g. desensitization). This crosstalk can be only functional (no direct contact exists between signalling components) or can involve a direct association, mainly at the receptor level through di/oligomerization [2]. G protein-coupled receptors (GPCRs) transduce a diversity of extracellular signals and regulate virtually every aspect of physiology, however, their overstimulation can be harmful, resulting in cellular toxicity or uncontrolled cellular growth. Therefore, different desensitization (decrease in response to repeated or continuous stimulation) mechanisms have been developed to avoid such overstimulation, involving a network of kinases, ubiquitin ligases, and adaptor proteins acting on GPCRs [3]. GPCR-mediated transduction pathways can be modulated by the interaction between different receptors. In fact, oligomerization exerts a significant impact on receptor function and physiology which contributes to increasing the diversification of receptor signalling, pharmacology, regulation, crosstalk, internalization and trafficking [4, 5].

GPCR signalling can be modulated not only by ligand binding to orthosteric sites of receptors but also by binding to allosteric sites, which are involved in the affinity and selectivity of ligands for GPCRs and represent a promising target for the design of new drugs to treat disorders of the central nervous system through GPCR activation/blockade [6]. Moreover, the formation of hetero-oligomers may provide novel allosteric binding pockets for ligand binding and new targeting possibilities [7].

Adenosine is an endogenous purinergic nucleoside present in all cells where forms part of the nucleic acids and the energy currency of life, ATP. Furthermore, adenosine is also
able to act as signalling molecule through binding adenosine receptors (ARs) which belong to class A GPCR superfamily being linked, therefore, to different transduction mechanisms including adenylate cyclase (AC), phospholipase C (PLC), phospholipase A2, phospholipase D and G protein-coupled inwardly-rectifying potassium channels (GIRK). ARs have been further subdivided into four main types (A1, A2A, A2B and A3) according to pharmacological profile, functional coupling to adenyl cyclase activity and tissue distribution. Thus, A1 and A2 receptors inhibit AC through Gqi proteins whereas A2A and A2B receptors stimulate AC through Gs proteins. ARs are widely expressed throughout the body where they participate in physiological processes such as immune function, heart rate and circulation, lipolysis, sleep regulation, and angiogenesis [8]. GPCRs dimerization is also an important research field in adenosine receptors [9, 10] and can be involved in physiological and pathological processes by homo- and heteromerization processes. Thus, adenosine receptors can interact with each other and with different GPCRs such as A1/A2A, A2A/β1, A2A/β2/β3/D2, A2A/mGlu5, or A2A/D2 [4]. This heterodimerization can be modulated by purines and xanthis like caffeine and theophylline [11]. Even A1/A2A, A2A/D2, A2A/mGlu5 heterodimers and A2A/mGlu5/D2 heterotrimers have been related to Alzheimer disease [12, 13].

L-glutamate is a naturally occurring aminoacid widely known to be the main excitatory neurotransmitter in the vertebrate Central Nervous System although its actions reach peripheral and non-neural tissues such as adrenal gland, pancreas, immune cells and a large etcetera [14]. Glutamate actions are mediated by a group of membrane receptors named glutamate receptors which include metabotropic (mGlu) and ionotropic (iGlu) glutamate receptors. mGlu receptors also belong to class C GPCR superfamily and have been subclassified into three groups: Group I (mGlu1 and mGlu5) which stimulate PLC activity through a Gq11 protein, Group II (mGlu2 and mGlu3) and Group III (mGlu4, mGlu6, mGlu7 and mGlu8) which inhibit AC activity through a Gi/o protein [15]. iGlu receptors are ligand-gated ion channels involved in fast excitatory neurotransmission and which include AMPA, NMDA and Kainate receptors according to the response to agonist.

One of the most relevant functions of adenosine in Nervous System is to regulate synaptic transmission. This neuromodulatory action is possible thanks to a combination of effects at presynaptic and postsynaptic levels that permits adenosine to influence both the release of several neurotransmitters, including L-glutamate, and also regulate neurotransmitter action [16]. The neuromodulatory role of adenosine is mainly linked to A1 and A2A receptors since both are predominant in brain. In fact, A1 receptor is the most abundant and widespread in the brain whereas A2A receptors are concentrated in the basal ganglia although they can also be found throughout the brain at lower densities. Concerning synaptic localization, both receptors are mainly distributed at presynaptic regions but they have been also detected at postsynaptic membranes [17]. Thus, ultrastructural analysis has demonstrated the A2A receptors postsynaptic localization in the dendrites and dendritic spines of GABAergic neurons in rat striatum [18]. In contrast, hippocampal A2A receptors are mostly located in nerve terminals and particularly in the presynaptic active zone [19]. Striatal A2A immunoreactivity has been observed primarily at glutamatergic synapses, predominantly at the postsynaptic but also at the presynaptic site [18, 20]. This localization of A2A receptors is in line with its suggested role in the fine-tuning modulation of glutamatergic neurotransmission in striatal GABAergic neurons both at the postsynaptic and presynaptic level [18, 20-21]. Moreover, A2A, mGlu5 and D2 receptors do oligomerize in GABAergic striatopallidal neurons, and these receptors are distributed in postsynaptic structures along the extrasynaptic and perisynaptic plasma membrane of spines, establishing asymmetrical, putative glutamatergic, synapses with axon terminals [22]. Thus, although striatal A2A receptors are mostly located postsynaptically, they are also present in the presynaptic active zone. Conversely, although A2A receptors are mostly related postsynaptically, they are also present in the postsynaptic density. Accordingly, A2A receptors are able to modulate the release of neurotransmitters (GABA, acetylcholine and glutamate) in the striatum, whereas they are able to control postsynaptic responsiveness in the hippocampus [19]. This pre- and postsynaptic presence A2A receptors has been also reported in monkey basal ganglia, with a high degree of colocalization between A2A and mGlu5 in dendrites and spines of striatal neurons where they functionally interact [23].

The excitatory neurotransmitter L-glutamate can also act as neuromodulator through binding mGlu receptors which are widely distributed throughout the CNS where they specifically localize in both synaptic and extrasynaptic regions. In general, group I-mGlu receptors are frequently found at postsynaptic regions, but also located presynaptically [24, 25], and their activation often increases neuronal excitability whereas group II and group III mGlu receptors are mainly distributed on presynaptic terminals where they can inhibit neurotransmitter release at both inhibitory (GABAergic) and excitatory (glutamergic) synapses [26, 27].

2. FUNCTIONAL INTERACTION BETWEEN ADENOSINE AND MGLU RECEPTORS

In recent decades, the functional interaction between adenosine and metabotropic glutamate receptors has received a great deal of attention in the GPCR field. Most of these studies have been carried out in cells from Central Nervous System using both in vivo and in vitro approaches. The results of these studies have indicated that the crosstalk between the two groups of receptors took place at multiple levels including second messenger production, synaptic transmission, neurotransmitter release and behavioural effects. In the present review, we will focus on group I mGlu/adenosine receptor interactions, although there are also data showing the functional interaction between group II/III mGlu and adenosine receptors [28, 29].

2.1. Adenosine A1 and Metabotropic Glutamate Receptors Cross-talk

Ogata and coworkers showed the first evidence of a crosstalk process between both groups of receptors in 1994 using cultured type 1 astrocyte from the hippocampus. They observed that the activation of A1 receptor potentiated group I
mGlu receptor-mediated calcium responses. Two years later, the same research group also described the opposite crosstalk showing that the accumulation of cAMP induced by the non-selective adenosine receptor agonist, 2-chloroadenosine, was potentiated by different agonists of group I mGlu receptors [28, 30]. The utilization of more selective receptor ligands allowed the study of the functional interaction with more precision. Thus, Toms and Roberts (1999) [31], by using DHPG, a selective group I mGlu receptor agonist, described how A1 receptor activation potentiated DHPG-evoked intracellular calcium responses in cultured cortical type 2 astrocytes. In line with this, Ciruela and coworkers (2001) [32] by using co-transfected HEK-293 cells with the cDNAs for mGlu1a and A1 receptors showed that the activation of mGlu1a potentiated the calcium peak obtained in response to A1 activation. Similarly, they also found that A1 receptor activation increased the calcium responses induced by mGlu1 activation. Inhibitory interactions between the two groups of receptors have also been reported. Thus, the measurement of intracellular cAMP in several clones with inducible expression of A1 and mGlu1 receptors (HEK293am cells) revealed that mGlu1 activation decreased A1 receptor signalling measured as adenylate cyclase inhibition [33] whereas the activation of the A1 receptor in Purkinje cells lead to the depression of mGlu1-evoked calcium responses.

In vivo experiments also revealed the functional interaction between mGlu1 and adenosine receptors in rodent models. Thus, our research group showed that chronic subcutaneous injection of R-N\(^{-}\) phenylisopropyladenosine (R-PIA), a selective A1 receptor agonist, for 6 days in male rats evoked an increase in group I-mGlu receptors functionality which was also accompanied by an up-regulation of mGlu receptor number [34]. Chronic oral consumption of R-PIA during gestation also altered group I-mGlu receptor functionality in plasma membrane from the maternal brain. However, in this case, we observed a significant decrease of mGlu1 and mGlu5 receptors and a corresponding desensitization of mGlu receptor/PLC signalling pathway [35] which suggest that crosstalk processes may be influenced by different parameters such as gender or routes of administration. In reciprocal experiments, continuous L-glutamate intake throughout gestation also decreased A1 receptor/AC signalling pathway in the maternal brain [36]. Finally, we have observed not only changes in group I-mGlu receptors functionality upon prolonged exposure to A1 receptor agonist but also we showed a loss of group I-mGlu receptors responsiveness following chronic caffeine exposure which suggests that the blockade of adenosine receptors might also alter mGlu receptor functionality [37].

There are multiple works showing a negative interaction between A1 and mGlu1 receptors at the level of synaptic transmission. Thus, Tabata and coworkers (2007) showed that the activation of A1 depressed mGlu1 signalling measured as mGlu1-coupled inward cation current and mGlu1-coupled calcium release from intracellular stores in Purkinje cells [38]. Similarly, Kamikubo and coworkers (2013) also demonstrated that the activation of A1 in cultured mouse Purkinje cells blocked mGlu1-mediated long-term depression of glutamate-responsiveness [39]. Reciprocal negative interaction between mGlu1 and A1 receptors has also been demonstrated in: (1) cerebrocortical synaposomes where an agonist of group I/II mGlu receptors suppressed the inhibitory action of A1 on glutamate release [40]; and (2) in rat hippocampal slices where electrophysiological recordings showed that the activation of mGlu1A suppressed A1-mediated responses [41].

2.2. Adenosine A2 and Metabotropic Glutamate Receptors Cross-talk

A positive interaction between A2A and mGlu5 receptors has been demonstrated in the ventral striatopallidal pathway, striatum and hippocampus where the activation of A2A in the nucleus accumbens and in glutamatergic nerve terminals of the rat striatum facilitated GABA and L-glutamate release, respectively [27, 42]. On the other hand, in neostriatal slices, both receptors acted in a synergistic way by phosphorylating DARPP-32 [43]. Interestingly, there are no data reporting functional interaction at the level of the second messenger between A2A and mGlu5 receptors although complexes containing A2A and mGlu5 have been described in heterologous systems and in native tissues and functional interaction has also been revealed on ERK 1/2 phosphorylation, c-fos expression and neurotransmitter release [44]. The synergy between A2A and mGlu5 also affect other receptors such as NMDA receptor or dopamine D2 receptor (D2R). In the case of NMDA receptor, it was described how adenosine A2A enhanced mGlu5-mediated potentiation of NMDA effects in the striatum and hippocampus [45, 46]. Concerning D2R, both receptors acted synergistically to reduce the affinity of D2R and to modulate D2R-mediated control of striatopallidal GABA neurons [47, 48].

Striatal region plays a fundamental role in motor and in reward, motivation and drug-seeking behaviour. As shown above, there are multiple works reporting a functional interaction between A2A and mGlu5 receptors in the striatum. It is reasonable that the interaction between the two groups of receptors at a behavioural level has been focused on animal models of Parkinson disease (PD) and in abuse drug-induced mechanisms. Thus, Cocculore and coworkers (2004) observed that perfusion of 6-hydroxydopamine in the striatum of rats produced akinetic deficits [49]. These deficits were not abolished when rats were treated with a non-effective dose of MPEP, a selective mGlu5 antagonist. Similar results were found when hydroxydopamine rats were exposed to a non-effective dose of CSC, a selective A2A antagonist. However, coadministration of both antagonists promoted a full recovery of akinesia demonstrating a behavioural interaction between A2A and mGlu5. Kachroo and coworkers (2005) also found that a simultaneous blockade of A2A and mGlu5 receptors acutely stimulates motor activity in a synergistic way in parkinsonian mice [50]. Interestingly, authors reinforced the importance of developing novel and efficient dual antagonist in order to avoid that the basal activity of mGlu5 may hamper the antiparkinsonian potential of A2A antagonist administered alone.

Concerning the role of A2A-mGlu5 interactions in drug-seeking behaviour, Brown and coworkers (2012) showed that this interaction participates in mediating the conditioned effects of cocaine but not cocaine-induced hyperactivity [51]. Thus, by using a combination of pharmacological and
genetic approaches, authors found that the selective mGlu₅ antagonist MTEP prevented cocaine-conditioned place preference in wild-type mice but failed in A₂A KO mice. The functional interactions between A₂A and mGlu₅ have also been shown to regulate alcohol-seeking in a rodent model of alcohol use. Thus, a combination of subthreshold doses of MTEP, mGlu₅ antagonist, and SCH 58261, A₂A antagonist, induced a higher reduction in alcohol-self administration compared to vehicle or either antagonist alone [52]. Finally, the interaction A₂A-mGlu₅ has also been shown to modulate the locomotor, stereotypic and drug-seeking effect of methamphetamine. In this case, authors also used a combination of subthreshold doses of MTEP and SCH 58261 and found that antagonist combination prevented methamphetamine-induced locomotor and stereotypic rearing responses and methamphetamine-seeking behaviors [53].

3. POSSIBLE MECHANISMS OF CROSSTALK BEHIND FUNCTIONAL INTERACTION

In general, the mechanisms through which adenosine and mGlu receptors interact are poorly investigated. We present here a range of mechanisms suggested to participate in some of the functional interactions described in the above paragraphs, including direct association of receptors (di/oligomerization), phosphorylation by second messengers dependent protein kinases (e.g. protein kinase A or PKA, protein kinase C or PKC), and sharing of G proteins or other cytoskeletal and/or adaptor proteins.

A signalling pathway can be considered as a succession of interactions between proteins that result in the generation of a cellular response. Such interactions must occur in an orderly and coordinated way, otherwise the normal functioning of the cellular signalling would be lost, probably leading to diseases such as cancer or Alzheimer’s. These interactions are part of the so-called interactomics of the cell. Protein–protein interactions are the physical contacts of high specificity established between two or more proteins as a result of biochemical events steered by electrostatic forces including the hydrophobic effect [54]. These interactions may last a short time or a long time, or be the basis of the formation of macromolecular complexes.

It is easy to imagine that thousands of interactions between different proteins can occur in the interior of a cell and that to generate a list of all of them can be a huge work. The combination of the available data about these interactions, not only between proteins but between the different components of a cell, generates such a large quantity of data that it becomes necessary (essential) to develop large databases and the appropriate tools to access meaningful data. In fact, availability and accessibility of these databases to the scientific community are increasing. Sometimes the information is redundant, that is, there is not a single database in which all the information is stored in an organized way.

Table 1. List of consulted databases with corresponding webpage links (URL) and other available information such as number of interactions and version of database.

| Name                  | Interactions | Version | URLs                                      |
|-----------------------|--------------|---------|-------------------------------------------|
| APID Interactomes     | 1,225,163    | 1.1.5   | http://apid.dep.usal.es/                  |
| BAR                   | 115,325      | 1.3.13  | http://bar.utoronto.ca/                   |
| BindingDB             | 1,011,029    | v1.3    | http://www.bindingdb.org                  |
| BioGrid               | 1,384,042    | 1.3.9   | http://www.thebiogrid.org                 |
| ChEMBL                | 628,504      | 1.2.7   | https://www.ebi.ac.uk/chembl/             |
| DIP                   | 104,965      | 1.3     | http://dip.doe-mbi.ucla.edu/dip/Main.cgi  |
| GPCR-HetNet           | 537          | -       | http://gpcr-hetnet.com/                   |
| HPIDb                 | 4,763        | 1.3.13  | http://hpidb.igbb.msstate.edu/main.html   |
| I2D                   | 817,915      | 1.1.6   | http://ophid.utoronto.ca/ophidv2.204/    |
| IntAct                | 589,538      | 1.3.13  | https://www.ebi.ac.uk/intact/             |
| iRefWeb               | -            | 13.0    | http://wodaklab.org/iRefWeb/search/index  |
| MatrixDB              | 1,342        | 1.3.13  | http://matrixdb.univ-lyon1.fr/            |
| Mentha                | 1,144,545    | -       | http://mentha.uniroma2.it/                |
| MINT                  | 123,717      | 1.3.13  | http://mint.bio.uniroma2.it/              |
| Reactome-FIs          | 209,988      | 1.2.7   | http://reactome.org/                      |
| STRING                | 1,380,838,440| 10.5    | https://string-db.org/                    |
| UniProt               | 14,968       | 1.3.13  | http://www.uniprot.org/                   |
| ZINC                  | 19,811       | 1.3.13  | http://zinc15.docking.org/                |
these interactions are collected, but at least several of them can be combined to obtain the most relevant information available. In line with this, The Proteomics Standard Initiative Common QUery InterfaCe (PSICQUIC) specification was created by the Human Proteome Organization Proteomics Standards Initiative (HUPO-PSI) in 2008. Its web service provides computational access to molecular interaction data resources. The PSICQUIC protein interaction datasets are accessible directly from the Cytoscape molecular interaction network tool, allowing users to select the interaction data from all or a subset of the datasets available. In the present review, we used Cytoscape 3.5.1 tool, an open source software platform for visualizing molecular interaction networks and biological pathways and integrating these networks with annotations, gene expression profiles and other state data [55]. Through its Universal Interaction Database Client searching tool, we collected data about interactions where any of the receptors reviewed here were involved. Other database sources of protein interactions were manually browsed. All of them have been summarized in Table 1.

It must be noted that many of adenosine and mGlu receptor proteins reviewed here can be identified with more than one name (Table 2). Therefore, searching started with the ‘official’ proposed name (UniProt ID) but its synonyms (see www.uniprot.org) were also employed to browse databases, including Worldwide Protein Data Bank (https://www.wwpdb.org) [56].

| Description | Homo sapiens | Rattus norvegicus |
|-------------|--------------|------------------|
| Gene Name   | Uniprot ID   | PDB              |
| Adenosine receptor A1 | ADORA1 | P30542 5N2S | Adora1 P25099 |
| Adenosine receptor A2A | ADORA2A | P29274 3EML | Adora2a P30543 |
| Adenosine receptor A2B | ADORA2B | P29275 | Adora2b P29276 |
| Adenosine receptor A3 | ADORA3 | P0DMS8 | Adora3 P28647 |
| Metabotropic glutamate receptor 1 | GRM1 | Q13255 4OR2 | Grm1 P23385 1EWK |
| Metabotropic glutamate receptor 5 | GRM5 | P41594 4O9 | Grm5 P31424 |

3.1. Direct Interaction between Adenosine and Metabotropic Glutamate Receptors

The protein-protein interactions have been studied by different methodological approaches and many of them can be recovered from the public online databases cited above. Fig. (1) shows a direct interaction network established between adenosine and group I metabotropic glutamate receptors in Homo sapiens, according to available scientific data, that could contribute to the crosstalk modulation reviewed herein (Table 3). These direct interactions have also been reported in Rattus norvegicus; even a direct interaction between rat mGLU5 and human A2A has been detected, probably due to a conserved structure in the latter receptor with respect to their homologue in the rat.

GPCRs function can be the result of their individual (monomer state) and/or aggregated activation (oligomerized state). This oligomerization with themselves (homomers) or different receptors (heteromers) can modulate its pharmacology and functionality [5]. Different experimental techniques have been devised to study GPCR oligomerization, which was deeply reviewed elsewhere [57]. GPCR homomers, mainly dimers, seems to be a more generalized feature for GPCR than expected [58]. In fact, several adenosine and metabotropic glutamate receptors are now known to form homodimers [59, 60]. Moreover, such dimerization processes could be a key determinant of their activation and function. It has been proposed that mGlur receptors must be in a homodimer state to be activated by glutamate, as reported when a full-length mGlur receptor was purified, reconstituted, and stabilized in a native-like environment [61]. This preferential formation of mGlur receptors homodimers at the surface of cells has been also reported by using a time-resolved Förster resonance energy transfer (trFRET) combined with SNAP-tag technology [62, 63] and by photobleaching experiments using GFP labeled mGlur receptors [64]. Higher order structures (e.g. dimers of dimers) seems to be very transient, as they have only been detected in the case of mGlur when their interaction was stabilized by a disulfide bridge [65].

In turn, the formation of heteromers probably represents the easiest way through which different GPCRs can interact with them. Heterologous expression studies revealed that group I mGlur receptors on one hand, and group II and III mGlur receptors, on the other hand, could form heterodimers [63], increasing to 16 the number of additional mGlur receptors with likely specific pharmacological and functional properties.

The combination of co-immunoprecipitation, Western-blotting and immunocytochemical assays revealed that mGlur2α and A1 assembled into functionally interacting complexes in rat cerebellum and in HEK-293 cells [32]. Similarly, co-immunoprecipitation, immunofluorescence and FRET assays also showed that A1 and mGlur1 receptors formed a complex in HEK293am cells [33]. The formation of this complex has been suggested to participate in the crosstalk process in which the activation of mGlur inhibited A1 functionality, measured as inhibition of forskolin-stimulated
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A₁ heteromerizes with mGluR₁a, resulting in the modulation of glutamate cytotoxicity, while A₂A functionally interacts with mGlu₅ in the striatum, as supported by studies in cotransfected cells and knockout mice [9, 53, 66] but also in native rat striatum [22, 27].

3.2. Functional Cross-talk through the G-proteins

Guanine nucleotide-binding proteins (G proteins) are involved as modulators or transducers in various transmembrane signalling systems. Ga-subunits are divided into four families: Gαs, Gαi/o, Gαq/11 and Gα₁₂/₁₃. The Gαs and Gαi/o families activate or inhibit the activity of adenylate cyclase, respectively. The Gαq/₁₁ family stimulates phospholipase C (PLC). Finally, the Gα₁₂/₁₃ family activates small GTPases including the Rho family (e.g., RhoA) [67]. Thus, mGlu₂ and mGlu₄, two receptors negatively coupled to adenylate cyclase, its canonical pathway, are able to activate PLC, its noncanonical pathway, when coexpressed with the G proteins Gα₁₅ or Gαq [68]. In ON bipolar neurons from retina Gα₁₅ mediates mGlu₄ signalling [69]. Adenosine A₁ receptor also needs Gαo₁ binding to form the ternary complex between agonist, receptor, and G protein [70]. Another mechanism proposed that involves G protein was described in retinotectal transmission where both A₁ receptor and group II mGlu receptors mediated presynaptic inhibition at the retinotectal synapse of goldfish by sharing a Gαi protein. For that reason, when both groups of receptors were simultaneously activated the inhibitory effects on synaptic transmission were not additive but occluded each other [29]. The interaction with and the activation of these G proteins by GPCRs is a regulated process. Thus, a regulator of G-protein signalling 12 (RGS12).
Table 3. Experimentally determined protein-protein interactions in both *Homo sapiens* and *Rattus norvegicus* models. mGluN: metabotropic glutamate receptor N. AN: adenosine AN receptor. Empty cells: no data available.

| Protein A | Protein B | Method | Interaction Type | Source | Publication |
|-----------|-----------|--------|-----------------|--------|-------------|
| A<sub>2A</sub> | A<sub>2A</sub> | anti tag coimmunoprecipitation | within the same physical complex | IntAct (EBI-2904802) | Ciruela et al., 2006 (PMID:16481441) |
| | | anti tag coimmunoprecipitation | within the same physical complex | IntAct (EBI-2904883) | |
| | | bioluminescence resonance energy transfer | physical association | IntAct (EBI-2904906) | Schicker et al., 2009 (PMID:18973777) |
| | | confocal microscopy | colocalization | IntAct (EBI-2904959) | |
| | mGlu<sub>1</sub> | fluorescent resonance energy transfer | oligomerization | GPCR-HetNet | |
| | | bioluminescence resonance energy transfer | oligomerization, in transfected HEK-293 cells | GPCR-HetNet | Cristóvão-Ferreira et al., 2013 (PMID:23657626) |
| | | experimental knowledge based | | | |
| A<sub>1</sub> | | radioligand binding | oligomerization | GPCR-HetNet | Hill et al., 2014 (PMID:24024783) |
| | | experimental knowledge based | | | Wu et al., 2010 (PMID:20482850) |
| | | affinity chromatography technology | physical association | BioGRID (302761) | Ciruela et al., 2001 (PMID:11278325) |
| mGlu<sub>1</sub> | A<sub>2A</sub> | fluorescent resonance energy transfer | physical association | IntAct (EBI-8491775) | Vidi et al., 2008 (PMID:1903155) |
| | | bimolecular fluorescence complementation | physical association | IntAct (EBI-8491974) | |
| | | bimolecular fluorescence complementation | physical association | IntAct (EBI-8492130) | |
| | | fluorescent resonance energy transfer | physical association | BioGRID (258280) | Kamiya et al., 2003 (PMID:12804599) |
| | | bioluminescence resonance energy transfer | physical association | IntAct (EBI-8041827) | |
| | | bioluminescence resonance energy transfer | physical association | IntAct (EBI-8041845) | Gandia et al., 2008 (PMID:18675812) |
| | | bimolecular fluorescence complementation | physical association | IntAct (EBI-8041898) | |
| | | affinity chromatography technology | physical association | BioGRID (616424) | Burgueño et al., 2003 (PMID:12837758) |
| | mGlu<sub>1</sub> | fluorescent resonance energy transfer | | | |
| | | bimolecular fluorescence complementation | | | |
| | | bioluminescence resonance energy transfer | oligomerization, in transfected HEK-293 cells | GPCR-HetNet | Cabello et al., 2009 (PMID:19344374) |
| | | pull down | | | |
| A<sub>2A</sub> | A<sub>3</sub> | radioligand binding | allostERIC interaction | PubMed | May et al., 2011 (PMID:21715680) |
| mGlu<sub>1</sub> | mGlu<sub>1</sub> | affinity chromatography technology | physical association | BioGRID (316750) | Robbins et al., 1999 (PMID:10349865) |
| | | pull down | physical association | BioGRID (280937) | Ray et al., 2000 (PMID:10945991) |
| | | x-ray crystallography | physical association | IntAct (EBI-9636453) | Wu et al., 2014 (PMID:24603153) |
| | | two hybrid prey pooling approach | molecular interaction | HPRD (Q13255) | Kitano et al., 2002 (PMID:11850456) |

(Table 3 contd....)
inhibits signal transduction by increasing the GTPase activity of Ga subunits, thereby driving them into their inactive GDP-bound form. RGS12 interacts with Ga1 [71], and surface plasmon resonance analysis of RGS12 PDZ domain binding specificity has revealed its interaction with mGlu1, mGlu2 and mGlu3 receptors [72].

Not only can Ga-subunits mediate crosstalk processes between GPCRs. G protein βγ subunits have been also proposed as mediators of the enhanced group I mGlu-evoked [Ca2+] responses observed in type 2 astrocytes when adenosine A1 receptors are co-activated [31], suggesting that phospholipase C activity could be increased by G protein βγ subunits although the precise mechanism was not investigated. However, other reported crosstalk processes seem to be G protein-independent. Thus, Tabata and coworkers (2007) by using a perforated-patch voltage-clamp technique in cerebellar Purkinje cells showed that A1 activation induced a continuous depression of mGlu1 [38]. This interaction did not require Ga10 proteins. Finally, it has also been reported that mGlu2 potentiated A2 receptor signalling in neostriatal slices in a process in which mitogen-activated protein kinase (ERK) pathway seems to participate but not a synergism at the second messenger level [43, 44].

3.3. Functional Cross-talk through other Intracellular Proteins

The interaction of several GPCRs with specific proteins ('accessory proteins') can completely change their expression, function and fate. GPCRs themselves can also be accessory proteins when considering their homo- or heterodimers. Some of the main interacting proteins of GPCRs reviewed elsewhere [73]. Adenosine and metabotropic glutamate receptors are also examples of GPCRs modulated by these interactions, and Table 4 summarizes several proposed linkers between the different receptors reviewed here. These linkers have been grouped according to their possible roles in receptor’s crosstalk: cell surface expression, cytoskeletal linkage, G-protein coupling, GPCR regulation and GPCR degradation.
Table 4. Possible protein-linkers mediating adenosine and metabotropic glutamate receptors crosstalk at different levels, according to their possible roles in relation to GPCR function.

| Role                  | Possible Linker | Linked Proteins | Authors                                  | PubMed ID |
|-----------------------|-----------------|-----------------|------------------------------------------|-----------|
|                       |                 |                 |                                          |           |
| Cytoeskeletal adapters| ADA             |                 |                                          |           |
| Cell surface expression| A<sub>1</sub> | Torvinen et al. (2002) | 12150791                                |           |
|                       |                 |                 | Franco et al. (1997)                     | 9247966   |
|                       |                 |                 |                                          |           |
|                       | A<sub>2A</sub> | Herrera et al. (2001) | 11125033                                |           |
|                       | A<sub>2B</sub> |                 |                                          |           |
|                       |                 |                 |                                          |           |
|                       |                 |                 | Málaga-Díéquez et al. (2010) | 20219842  |
|                       | mGlu<sub>5</sub>|                 |                                          |           |
|                       |                 |                 | Ango et al. (2001)                       | 11418862  |
|                       |                 |                 | Brekeman et al. (1997)                   | 9069287   |
|                       | mGlu<sub>6</sub>|                 |                                          |           |
|                       |                 |                 | Ango et al. (2001)                       | 11418862  |
|                       |                 |                 | Brekeman et al. (1997)                   | 9069287   |
|                       |                 |                 |                                          |           |
|                       | Cyth2           |                 |                                          |           |
|                       | A<sub>1</sub>   | Guandner et al. (2005) | 16027149                                |           |
|                       |                 |                 |                                          |           |
|                       |                 | mGlu<sub>1</sub>| Kitano et al. (2002)                     | 11850456  |
|                       |                 |                 |                                          |           |
|                       | Efnh2           |                 |                                          |           |
|                       | mGlu<sub>1</sub>|                 |                                          |           |
|                       | mGlu<sub>6</sub>|                 |                                          |           |
|                       |                 |                 | Calò et al. (2005)                       | 15745950  |
|                       |                 |                 |                                          |           |
|                       | FLNA            |                 |                                          |           |
|                       | mGlu<sub>1</sub>|                 |                                          |           |
|                       | mGlu<sub>6</sub>|                 |                                          |           |
|                       |                 |                 | Enz et al. (2002)                        | 11943148  |
|                       |                 |                 |                                          |           |
|                       | Necab2          |                 |                                          |           |
|                       | A<sub>2A</sub>  | Canela et al. (2009) | 19694902                                |           |
|                       | mGlu<sub>1</sub>|                 |                                          |           |
|                       |                 |                 | Canela et al. (2007)                     | 17689978  |
|                       |                 |                 |                                          |           |
|                       |                 | mGlu<sub>6</sub>| Canela et al. (2009)                     | 19694902  |
|                       | NHERF1-2        |                 |                                          |           |
|                       | A<sub>2B</sub>  | Paquet et al. (2006) | 16891310                                |           |
|                       |                 |                 | Sitaraman et al. (2002)                  | 12080047  |
|                       |                 |                 |                                          |           |
|                       | G<sub>1</sub>α  | Waldhoer et al. (1999) | 10521440                                |           |
|                       |                 | mGlu<sub>1</sub>| Dholra et al. (2000)                     | 11124982  |
|                       |                 | mGlu<sub>6</sub>|                                          |           |
|                       |                 |                 |                                          |           |
|                       | RGS12           |                 |                                          |           |
|                       | mGlu<sub>1</sub>|                 |                                          |           |
|                       | mGlu<sub>6</sub>|                 |                                          |           |
|                       |                 |                 | Snow et al. (1998)                        | 9651375   |
|                       | G<sub>3</sub>α  | Toms and Roberts, (1999) | 10530813                                |           |
|                       |                 | mGlu<sub>1</sub>|                                          |           |
|                       |                 | mGlu<sub>6</sub>|                                          |           |
|                       |                 |                 |                                          |           |
|                       | CaM             |                 |                                          |           |
|                       | A<sub>1</sub>α  | Navarro et al. (2009) | 19632986                                |           |
|                       |                 | mGlu<sub>1</sub>| Ko et al. (2012)                         | 23152621  |
|                       |                 | mGlu<sub>6</sub>| Saugstad et al. (2002)                   | 11953448  |
|                       |                 |                 | Minakami et al. (1997)                   | 9242710   |
|                       |                 |                 |                                          |           |
|                       | GRASP           |                 |                                          |           |
|                       | mGlu<sub>1</sub>|                 |                                          |           |
|                       | mGlu<sub>6</sub>|                 |                                          |           |
|                       |                 |                 | Kitano et al. (2002)                     | 11850456  |

Adenosine deaminase (ADA) enzyme acts as a positive modulator of adenosine A<sub>1</sub> and A<sub>2A</sub> receptors, by enhancing their ligand affinity via conformational change [74]. Interestingly, ADA can interact with, and mediate the interaction between, A<sub>1</sub> [75, 76], A<sub>2A</sub> and A<sub>2B</sub> receptors at the cell surface [77]. Moreover, binding of ADA to A<sub>2B</sub> receptors increases the affinity of the agonist 5'-N-ethylcarboxamidoadenosine and cAMP production [77]. In turn, the ER-associated 40-kDa heat shock protein family member D<sub>1</sub> receptor interacting protein 78 (DRIP78) physically interacts and retain adenosine A<sub>1</sub> receptor in ER, and negatively regulates the cell-surface expression of this adenosine receptor [78] avoiding its interaction with other GPCRs at the cell surface.

Homer protein homolog 1 (Homer 1) is a postsynaptic density scaffolding protein which binds and cross-links cytoplasmic regions of mGlu<sub>1</sub> and mGlu<sub>5</sub> [79, 80] and regulates the trafficking and surface expression of these receptors. By physically linking mGlu<sub>1</sub> and mGlu<sub>5</sub> with ER-associated ITPR1 receptors, Homer 1 aids the coupling of surface receptors to intracellular calcium release [81].

NHERF1-2 can bind G<sub>α</sub><sub>q</sub>-subunit, PLC and PKC, suggesting that NHERFs act as a scaffold protein bringing the effectors of the pathway together to facilitate the signal transmission. NHERF2 has also been shown to bind to the
mGlu$_3$ [82], where NHERF2 also potentiates the receptor-induced PLC activation, prolonging the intracellular Ca$^{2+}$ mobilization. Moreover, NHERF1 and NHERF2 contribute to regulating the distribution of mGlu$_2$ and mGlu$_3$ in the murine brain [83].

Filamin-A (FLNA) promotes orthogonal branching of actin filaments and links actin filaments to membrane glycoproteins. Anchors various transmembrane proteins to the actin cytoskeleton and serves as a scaffold for a wide range of cytoplasmic signalling proteins. Some of these proteins are mGlu$_4$, mGlu$_5$, mGlu$_7$, mGlu$_9$, and mGlu$_{10}$, where a conserved tyrosine within mGlu receptor carboxi termini mediates the binding to FLNA [84].

N-terminal EF-hand calcium-binding protein 2 (Necab2) may act as a signalling scaffold protein that senses intracellular calcium. The interaction of Necab2 with adenosine A$_2A$ receptor modulates the cell surface expression, the ligand-dependent internalization and the receptor-mediated activation of the MAPK pathway [85]. Moreover, Necab2 regulates receptor function and coupling efficiency of mGlu$_5$ by its physical interaction with this receptor [86].

Crosstalk between adenosine and metabotropic glutamate receptors can involve second-messengers dependent protein kinases. Thus, it is well known that in Central Nervous System, A$_2A$ is coupled to G$_i$ proteins, and stimulates the activity of adenylate cyclase which increases cyclic AMP production leading consequently to an increase in protein kinase A activity (PKA). It has also been described how PKA, by phosphorylating the aminoacid residue Ser870 located within the calmodulin binding site I in mGlu$_5$, regulated the function of mGlu$_5$ assayed as such as extracellular signal-regulated kinase phosphorylation and intracellular Ca$^{2+}$ oscillations [87]. Finally, the participation of PKA in the crosstalk process between A$_2A$ and mGlu$_5$ has been shown in striatal slices where the facilitatory role of A$_2A$ receptors on mGlu$_5$-mediated responses was abolished by PKA inhibitor [46]. Interestingly, the same work also revealed that the participation of PKA seemed to be brain region-dependent since the synergism between the two receptors in hippocampal slices was resistant to PKA inhibitor [46].

Concerning group I mGluR, both receptor subtypes mGlu$_1$ and mGlu$_3$ are coupled in a positive way to phospholipase C through G$_q$ proteins leading to triggering of inositol-1,4,5-trisphosphate (IP$_3$)-mediated calcium release and to activation of protein kinase C (PKC). The ability of PKC to phosphorylate A$_2A$ receptor has been shown in rat C6 glioma cells where the phosphorylation of receptors on three different aminoacid residues (Thr298, Ser320, and Ser335) was increased by pretreatment with PKC activator although no variations in A$_2A$ receptor signalling were detected [88]. However, PKC has been suggested as participating in the synergism observed between group I mGlu and adenosine receptors in primary cultures of rat striatal neurons. Thus, in these cells, the potentiation of adenosine receptor responses observed upon group I mGlu receptors activation was abolished by a PKC inhibitor [89]. On the other hand, several works using rat hippocampal slices found that mGlu$_{1a}$ receptor abolished A$_1$ receptor-mediated responses in a process in which PKC seemed to be involved [41, 90, 91].

The functional effects cited above could be mediated by physical interactions with proteins such as calmodulin, GRP1 or Pick1, which together with PKC and PKA would contribute to GPCR regulation. Calmodulin (CaM) mediates the control of a large number of enzymes, ion channels, aquaporins and other proteins through calcium-binding. The carboxi terminal domain of mGlu$_7$ and mGlu$_9$ has been shown to bind to CaM [92, 93] and immobilized-CaM beads pulled down mGlu$_5$, mGlu$_6A$, and mGlu$_{10}$ immunoreactivity from solubilized rat brain membranes in a Ca$^{2+}$-dependent manner [94]. On mGlu$_7$ only one CaM-binding site exists, whereas mGlu$_9$ binds CaM at two distinct sites [95]. Moreover, CaM competes with the E3 ligase seven in absentia homolog (Siah)-1A for mGlu$_7$ binding in a phosphorylation-dependent manner in rat hippocampal neurons, dynamically regulating mGlu$_7$ trafficking [96]. In turn, CaM-binding to mGlu$_7$ [93] overlays the PKC phosphorylation site; thus calcium/CaM binding to the receptor inhibits PKC phosphorylation of mGlu$_7$ [97-99]. It has been reported that CaM binds preferentially to a proximal C-terminus epitope of the adenosine A$_2A$ receptor [100]. Thus, Ca$^{2+}$ seems to induce conformational changes in the CaM-A$_2A$-D$_2$ receptor oligomer which leads to a selective modulation of MAPK signalling mediated by the A$_2A$/D$_2$ receptor heteromer [100]. A similar interaction could occur between A$_2A$ and other mGlu receptors such as mGlu$_5$.

General receptor for phosphoinositides 1-associated scaffold protein (GRP1-associated scaffold protein or GRASP) interacts with group I metabotropic glutamate receptors (mGlu$_1$ and mGlu$_3$). The yeast two-hybrid system and in vitro pull-down assays indicated that the PDZ (PSD-95/Dlg1/ZO-1) domain-containing, amino-terminal half of GRASP directly binds to the class I PDZ-binding motif of group I mGlu receptors [101]. The C-terminal half of GRASP also bound to cytohesins, the members of guanine nucleotide exchange factors (GEFs) specific for the ADP-ribosylation factor (ARF) family of small GTP-binding proteins. In turn, cytohesin-2 (Cynh2) interacts with the adenosine A$_2A$ receptor [102] leading to the activation of mitogen-activated protein (MAP) kinase, via a pathway that is independent of heterotrimeric G proteins. Native mGlu$_{1a}$/GRASP/cytohesin-2 complexes have also been communo-precipitated from rat brain tissue [101].

MGlut$_{1a}$ receptor signalling via InsP$_3$ production induces the activation of PKC which, in turn, phosphorylates a threonine residue involved in receptor coupling to G$_{q/11}$, resulting in the desensitization of mGlu$_{1a}$ [103]. PKC is also involved in phosphorylation-dependent trafficking of mGlu$_5$ [92, 96] and the phosphorylation of mGlu$_7$ [104]. PKC$\alpha$ type (PKC-A) is a classical (or conventional) PKC activated by calcium and diacylglycerol in the presence of phosphatidylinerine, and interacts with protein interacting with C-kinase (Pick1) via PDZ domain [105]. In addition, Pick1, a synaptic organizer protein, binds to the C terminus of mGlu$_{1a}$ [104, 106] and mGlu$_3$ [106]. Pick1 probably acts as an adapter protein that binds to and organizes the subcellular localization of a variety of membrane proteins containing some PDZ recognition sequence. In the agreement, Pick1 modulates PKC$\alpha$-evoked phosphorylation of mGlu$_{1a}$ and is involved in
the clustering of various receptors, including mGlu7, at pre-synaptic release sites [107], possibly by acting at the receptor internalization level [104].

Apart from interactions with proteins that modulate GPCR cell surface expression, G-protein coupling, cell localization and regulation, other partners could mediate its trafficking and final degradation. Many GPCRs require post-translational modification with ubiquitin and interaction with ubiquitin-binding domains of the endosomal-sorting complex required for transport (ESCRT) machinery for lysosomal sorting. In line with this, ubiquitin-C exists either covalently attached to another protein, or free (unanchored). When covalently bound, it is conjugated to target proteins via an isopeptide bond either as a monomer (monoubiquitin), a polymer linked via different Lys residues of the ubiquitin (polyubiquitin chains) or a linear polymer linked via the initiator Met of the ubiquitin (linear polyubiquitin chains). This binding of ubiquitin to an Lys residue determines different protein consequences on function (e.g. protein degradation). Quantitative Lys-c-Gly-Gly (diGly) proteomics aimed to identify ubiquitin ligases substrates revealed the interaction of adenosine A2a receptor with ubiquitin [108]. Similar proteomic approaches also revealed the interaction of ubiquitin with different plasma membrane receptors, including mGluS [109] and mGlu7 [110]. However, not all GPCRs require direct ubiquitination or all components of the ESCRT machinery for degradation in the lysosome, suggesting that alternate sorting pathways exist [111]. One of them is GPCR lysosomal sorting that is regulated by the G protein-coupled receptor associated sorting protein-1 (Gasp-1), which modulates lysosomal sorting and functional down-regulation of a variety of GPCRs. Gasp-1 targets receptors for degradation in lysosomes via its interaction with BECN2 [112] and interacts with cytoplasmic tails of a variety of GPCRs such as D2 and D4 dopamine receptors, delta opioid receptor 1 and beta-2 adrenergic receptor. Interestingly, Gasp-1 also interacts with carboxy terminal domains from mGlu1A, mGlu5A, and mGlu1 [113], probably contributing to the fate of these receptors in the cell.

Taken together, these functional and physical interactions with proteins cited above comprise the participation of cytoskeletal proteins and/or adaptor proteins that modify receptor localization, activation, trafficking and regulation, and participate in the crosstalk between these receptors.

4. NEW DRUG DESIGN POSSIBILITIES: TARGETING GPCR OLIGOMERS

According to previous paragraphs, GPCRs function is modulated through oligomer formation and protein-protein interactions. Therefore, a deeper knowledge about all these molecular interactions would be very helpful for the design of new, selective and therapeutically potent active drugs targeting these receptors, especially in the field of neurodegenerative disease [6]. Fine tuning of GPCR function through protein-protein interactions (e.g. homomerization or heteromerization) would comprise ligand interactions, cellular trafficking, and mobility [114, 115].

4.1. GPCR Homomerization can Modulate or Create new Binding Sites

Besides the orthosteric site in a monomeric GPCR, the assembly of two monomers could result in new binding sites in the dimeric form of the receptor. In addition, when a ligand binds to the second protomer in a homodimer, it will often act as an allosteric modulator of the intrinsic efficacy of the ligand when binding to the first protomer [115]. Accordingly, it has been reported that adenosine A1 receptors form homomers not only in cell cultures but also in bovine brain cortex and that caffeine binding to one protomer increases the agonist affinity for the other protomer, a pharmacological characteristic that correlates with the low caffeine concentrations-induced activation of agonist-promoted A1 receptor signalling [116].

4.2. GPCR Heteromerization-mediated Transactivation and Transantagonism

In the heteromeric form, the activation (or blockade) of a GPCR could modulate the function of its partner. For instance, the stimulation of adenosine A2A receptors decreases the affinity of dopamine D2 receptors in rat striatal membranes, where they heteromerize. This A2A/D2 heteromer may underlie the neuroleptic-like actions of adenosine agonists and the enhancing effects of adenosine antagonists on locomotor activity [117]. This heteromer represents the new concept of 'transantagonism' when an oligomer interaction can occur in such a way that the activation of one receptor inhibits the signalling activity of the other. Another mechanism of crosstalk between GPCR heteromers would be 'transactivation', which occurs when the signalling cascade of one receptor is initiated upon an agonist binding to the other protomer [115]. These oligomers have been discussed as potential therapeutic targets for schizophrenia and/or Parkinson's disease as more specific alternatives to existing pharmaceuticals that target only the D2 monomer [118]. A2A/mGlu5 heteromer function also depends on the activation of one or both protomers. Thus, the stimulation of the mGlu5 receptor potentiates A2A signalling in a MAPK-dependent manner in striatal slices [43] and could be mediated by the formation of A2A/mGlu5/D2 receptor heteromers [119]. An interesting possibility is the simultaneous activation of two different GPCRs, when heteromerized, through bivalent ligands, as has previously been evaluated in the case of A1/D1 [120], A2A/D2 [121] or µ opioid receptor/mGlu3 [122] heteromers.

4.3. GPCR Oligomerization-modulated G-protein Coupling

Finally, a potential result of GPCR oligomerization is the activation of different pathways from that activated by the monomeric state, for instance by novel G-protein coupling [123]. In line with this, the primary coupling of the group I mGluRs is with Goαq; however, these receptors can couple with Goα and Goiα as well when overexpressed, probably forming receptor mosaics, in HEK293 and CHO cells [124]. Although the binding of two G proteins to a heterodimer seems not feasible due to steric clashes [125], the α1-α2A heterotetramer (one α1 homodimer plus one α2A homodimer)
may bind to both Gq and G13 proteins [126], suggesting receptor oligomerization dynamics as a crucial point in the determination of G-protein coupling and activation.

CONCLUSION
From the previous paragraphs, we may draw some conclusions and lines of future research. Related to the envisaged work to do in the GPCR field, it seems mandatory: i) to develop new tools for the analysis of protein-protein interactions in the context of cell signalling, and ii) to improve the availability and accessibility of meaningful data through curated non-overlapping databases. To this end, it would be desirable to reduce the multiplicity of names used for the same protein/gene or for the same kind of data, and also to promote global initiatives similar to the above mentioned PSICQUIC. Finally, it is clear that signalling possibilities of a GPCR are not only limited to classical and linear signalling pathway activation (or blockade) but are impressively increased and diversified as a result of the variety of partners with which that GPCR could interact. The immediate consequence is the higher complexity that research on GPCR signalling deserves. But paradoxically, this difficulty should be viewed as an optimistic future as there would also be newer possibilities to target this GPCR in pathological status and disease.

CONSENT FOR PUBLICATION
Not applicable.

CONFLICT OF INTEREST
The authors declare no conflict of interest, financial or otherwise.

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