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► **To cite this version:**

Jean-Philippe Pin, Julie Kniazeff, Laurent Prézeau, Jiang-Feng Liu, Philippe Rondard. GPCR interaction as a possible way for allosteric control between receptors. *Molecular and Cellular Endocrinology*, 2019, 486, pp.89-95. 10.1016/j.mce.2019.02.019 . hal-02396289

HAL Id: hal-02396289

<https://hal.science/hal-02396289v1>

Submitted on 5 Dec 2019

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GPCR interaction as a possible way for allosteric control between receptors

Jean-Philippe Pin, Julie kniazeff, Laurent Prézeau, Jiang-Feng Liu, Philippe Rondard

Abstract

For more than twenty years now, GPCR dimers and larger oligomers have been the subject of intense debate in the field. Evidence for a role of such complexes in receptor trafficking to and from the plasma membrane have been provided, but one of the main issue is of course to determine whether or not such a phenomenon can be responsible for reciprocal control (allosteric control) of the subunits. Such a possibility would indeed add to the possible ways a cell can integrate various signals targeting GPCRs. Among the large GPCR family, those of the class C that include mGlu and GABA_B receptors, represent excellent models to examine such a possibility as they are mandatory dimers. In the present review, we will report on the observed allosteric interaction between the subunits of class C GPCRs, both mGluRs and GABA_BRs, and on the structural bases of these interactions. We will then discuss these findings for other GPCR types such as the rhodopsin-like class A receptors. We will show that many of the observations made with class C receptors have also been reported with class A receptors, providing interesting evidence that such allosteric interactions can be of physiological relevance.

Introduction

G protein-coupled receptors are essential elements involved in intercellular communication as well as communication with the environment making them ideal targets for drug development (Overington, Al-Lazikani and Hopkins, 2006, Santos, Ursu, Gaulton et al., 2017). These receptors encoded by 3% of our genes (more than 800 genes in humans) are expressed in every cell, and each cell express ten's of different types of receptors (Moore-Morris, Varrault, Mangoni et al., 2009). The GPCR expression pattern in a given cell type is even dependent on its developmental and physiopathological status, then providing a way to target a specific set of cells in a given situation for an optimal therapeutic effect (Moore-Morris et al., 2009). These GPCRs are activated by a large variety of signals including external signals, various metabolites, hormones and transmitters as well as proteins involved in cell adhesion. These multiple types of signals are being transduced inside the cell through a limited set of signaling pathways involving or not G proteins of 4 major types, Gs, Gi/o, Gq and G12. An important question is then raised: how can all the GPCR-mediated signals reaching a given cell be integrated? Are there mechanisms that can control one GPCR pathway when another is or as been activated? As for many other receptor-mediated signaling, multiple possible cross-talks exist that allow the cell to respond differently whether one or two pathways are activated simultaneously or consequently. One best example is the synergy reported between the Gq and Gi-mediated responses (Prezeau, Rives, Comps-Agrar et al., 2010). When both pathways are activated simultaneously, either through one or two different GPCRs, the Ca^{2+} signal is largely amplified, due in part to the action of the G protein $\beta\gamma$ dimer on $PLC\beta 3$. Such potentiation of the Gq-mediated response is even larger when the Gi pathway is activated first, few minutes ahead (Rives, Vol, Fukazawa et al., 2009). Moreover, the Gi-coupled receptor can generate a Ca^{2+} signal if activated few minutes after the Gq cascade is activated (Rives et al., 2009). This illustrates a complex signaling synergy between these two pathways that can not only integrate co-activation of both pathways, but also provide specific information when activated sequentially. Cross talks between other G protein signaling pathways have also been reported.

Although such signal integration through signaling crosstalk is of main interest, the observation that GPCR can interact with each other, leading to the formation of dimers and larger oligomers, has generated a lot of interest as this may represent another possible way for a receptor to control the activity of the other (Angers, Salahpour and Bouvier, 2002, Bouvier, 2001, Ferre, Baler, Bouvier et al., 2009, Ferré, Casadó, Devi et al., 2014, Gomes, Ayoub, Fujita et al., 2016, Smith and Milligan, 2010, White, Grodnitzky, Louis et al., 2007). The initial observation using biophysical methods illustrated the possible interaction between different GPCRs expressed in heterologous cells, likely at higher densities than the physiological ranges (Angers et al., 2002, Bouvier, 2001). As such, functional cross-talk between different GPCRs has also been often used as an argument to support the formation of such GPCR heterodimers (Gomes et al., 2016, Pin, Neubig, Bouvier et al., 2007). However, most of the reported studies could not bring strong enough data illustrating a crosstalk resulting from a direct interaction between the receptors, or from a functional interaction between the signaling cascades not requiring GPCR interaction (Gomes et al., 2016, Pin et al., 2007). This, plus the more recent studies that question the existence of GPCR dimers in native cells due to their low density, and the probable instability of the GPCR dimers (Calebiro, Rieken, Wagner et al., 2013, Felce, Davis and Klenerman, 2018, Hern, Baig,

Mashanov et al., 2010, Kasai and Kusumi, 2014) largely limited the enthusiasm in better understanding the possible bases of GPCR crosstalk through dimerization (Bouvier and Hebert, 2014, Bouvier and Hebert, 2014, Lambert and Javitch, 2014).

Among the various types of GPCRs, those of the class C, composed of the eight mGlu receptors, the GABA_B receptor as well as the CaSR and the sweet and umami taste receptors are mandatory dimers, either homodimers or heterodimers (Kniazeff, Rovira, Rondard et al., 2016, Pin and Bettler, 2016, Rondard and Pin, 2015). In contrast to most other GPCRs in which agonists bind to the seven transmembrane (7TM) domain, class C agonists interact on a venus flytrap (VFT) domain that is part of their large extracellular domain (Pin and Bettler, 2016, Kunishima, Shimada, Tsuji et al., 2000). Dimerization has been shown to be mandatory for the agonist to activate the 7TM domain leading to G protein activation (El Moustaine, Granier, Doumazane et al., 2012). Such a process involves clear allosteric interactions between the subunits, at the level of both their VFT and their 7TM domains (Pin and Bettler, 2016).

In the present review, we will report on what it is known on the allosteric interactions between the 7TMs of class C dimers, and the structural bases for these. We will then discuss these observations in relation to what has been observed with class A GPCRs

1- Class C GPCRs as interesting models of other GPCRs

Class C GPCRs diverged from the other GPCRs early during evolution (Nordstrom, Sallman Almen, Edstam et al., 2011). Their structure, stoichiometry and activation mechanism is quite different from the other GPCRs (Pin and Bettler, 2016). These receptors have a large extracellular domain composed of a VFT domain where agonists bind, linked to the 7TM domain via a cysteine-rich rigid segment. These receptors are mandatory dimers associated via a hydrophobic surface on one side of the N-terminal lobe of the VFT. Agonist bind in the cleft that separates both lobes of the VFT, leading to the stabilization of a closed conformation of this domain. This closed conformation allows the VFTs of the class C dimer to not only interact via their N-terminal lobe-I, but also through their lobe-II, an interaction made possible by a 70° rotation of one VFT compared to the other (Kunishima et al., 2000, Doumazane, Scholler, Fabre et al., 2013, Muto, Tsuchiya, Morikawa et al., 2007, Tsuchiya, Kunishima, Kamiya et al., 2002). This important movement of the two VFTs during activation, is expected to affect the relative position of both 7TM domains (Hlavackova, Zabel, Frankova et al., 2012, Tateyama, Abe, Nakata et al., 2004), resulting in the generation of the intracellular signal.

Such a divergent general structure and activation mechanism raised the question whether or not these receptors can bring information useful for our understanding of specific properties of receptors from the other classes.

Despite their low sequence similarity, class C and class A 7TM domains share a number of important similarities. First, their general structure is similar, although the class C 7TM domain is more compact, still the 3D repartition of the seven transmembrane helices is very similar. The ionic interaction between transmembrane segment 3 (TM3) and TM6 that maintains class A GPCRs in an inactive state is also found in class C receptors (Dore, Okrasa, Patel et al., 2014, Wu, Wang, Gregory et al., 2014). Although not in the same environment, this ionic lock seems to play the same role in class C and class A GPCRs, consistent with a movement of TM6 being critical for the activation of either class A and class C receptors (Dore et al., 2014, Binet, Duthey, Lecaillon et al., 2007). Indeed, as for class A GPCRs,

conformational changes in class C 7TMs is important to control signaling. This is best illustrated by the discovery of a number of negative allosteric modulators that bind in the 7TM domain of class C GPCRs and prevent their activation (Gregory, Noetzel and Niswender, 2013, Urwyler, 2011). This is also supported by the discovery of a number of positive allosteric modulators that also bind in the 7TM domain at a site similar to that occupied by class A agonists, and that stabilize the active conformation of the receptor. In addition, when truncated of their large extra and intracellular domain, class C 7TM domain can be expressed in cells where they conserved their basal activity. Most importantly, these isolated class C 7TM domains can be directly activated by PAMs further demonstrating their structural similarity with class A receptors (Goudet, Gaven, Kniazeff et al., 2004).

Taken together, these observations bring confidence that, although they diverged very early during evolution, class C 7TM domain still conserved a similar mode of activation as that of the other GPCRs, consistent with their action on the same effectors including heterotrimeric G proteins.

2- Negative interaction between the 7TM domains of class C GPCRs

The analysis of the allosteric interaction between the subunits in class C GPCRs first concentrated on their VFT domains. It was found that agonist binding in one VFT increases affinity in the associated VFT, then facilitating the double occupancy of the homodimeric receptors which is the most effective state for the mGlu receptors (Kniazeff, Bessis, Maurel et al., 2004). In the case of the GABA_B receptor composed of two distinct subunits, GABA_{B1} and GABA_{B2}, agonist affinity in GABA_{B1} is largely increased by its interaction with GABA_{B2} VFT due to additional contacts between the two VFTs in the ligand occupied active state of the dimer (Geng, Bush, Mosyak et al., 2013). It was therefore soon considered that such interaction between the VFTs would favor the activation of both 7TM domains likely in a symmetrical way, as proposed for allosteric proteins (Changeux and Christopoulos, 2016, Monod, Wyman and Changeux, 1965).

Thanks to the development of an optimized quality system allowing the perfect control of the subunit composition of an mGlu dimer at the cell surface (Brock, Oueslati, Soler et al., 2007), it has been possible to clarify the role of each 7TM domain in the coupling properties of the mGlu homodimeric receptors. By using PAMs and NAMs acting in a single 7TM within the dimer, associated with a point mutation introduced in only one subunit and preventing G protein activation, it was found that only one 7TM domain could be active at a time (Goudet, Kniazeff, Hlavackova et al., 2005, Hlavackova, Goudet, Kniazeff et al., 2005). This is best illustrated by the full inhibition of G protein activation by a mGlu5 dimer in which the mutated subunit unable to activate G proteins is stabilized in an active conformation by a PAM acting exclusively on this subunit (Goudet et al., 2005). Such a conclusion was confirmed using FRET-based sensor technologies (Hlavackova et al., 2012). This illustrates that, if this subunit is in an active conformation, the associated subunit cannot couple to G proteins (Hlavackova et al., 2012, Goudet et al., 2005).

Although surprising, these findings were consistent with the observation that in the heterodimeric GABA_B receptor, one subunit only is responsible for G protein activation, the GABA_{B2} subunit (Galvez, Duthey, Kniazeff et al., 2001). Similarly, in the heterodimeric taste receptors, data also suggest that T1R3 is the only subunit responsible for G protein activation (Xu, Staszewski, Tang et al., 2004).

Whereas mGlu receptors were thought to only form homodimers, we and others brought evidence for the existence of mGlu heterodimers, not only in heterologous cells (Doumazane, Scholler, Zwier et al., 2011, Moreno Delgado, Møller, Ster et al., 2017), but also in native neurons (Moreno Delgado et al., 2017, Yin, Noetzel, Johnson et al., 2014). We then examined whether both subunits could activate G protein in the mGlu2-4 heterodimer. We found that G protein coupling was assumed by the mGlu4 7TM only in this receptor (Liu, Zhang, Moreno-Delgado et al., 2017), and not mGlu2 even though the mGlu2 7TM is capable of activating G protein when assembled in a homodimeric form.

Taken together, these data indicate that in class C dimers only one 7TM domain activates G protein at a time. It can be either one in a homodimer, but the allosteric interaction is such that only a specific one does it in a heterodimeric entity (Galvez et al., 2001, Xu et al., 2004, Liu et al., 2017). Such an asymmetry can be explained in two ways. It can be either due to the inability, resulting from steric hindrance for example, of both subunits to activate two G proteins at a time. The second possibility is that the physical interaction of the 7TM domains in the active state is such that only one domain can reach the active conformation as a result of an allosteric interaction. This second possibility is more likely than the first one, as the former cannot explain that a given subunit is responsible for G protein activation in the heterodimeric receptors.

3- Positive interaction between the 7TM domains of class C GPCRs

We have shown above that in class C dimers, one subunit, when in the active form, prevents the associated one from being active. Does this mean the associated, not functional subunit, remains in a basal state? Is the inactive associated subunit able to influence the activity of the other? The analysis of the role of each subunit in heterodimeric receptor function brought important information along these lines. The analysis of the role of the GABA_{B1} subunit in the GABA_B heterodimer early indicated that it was playing an important role in the activation of the GABA_{B2} 7TM, with a positive action (Monnier, Tu, Bourrier et al., 2011). It was also found that a change in conformation in the GABA_{B1} 7TM was likely essential in this effect as introducing a disulfide bridge within this domain prevented activation of the GABA_{B2} 7TM (Monnier et al., 2011). Such a conclusion was further supported by our recent data using the mGlu2-4 heterodimer. Indeed, stabilizing the mGlu2 7TM in an inactive state with a NAM known to interact within this domain, largely diminished the ability of the mGlu4 7TM to activate G proteins (Liu et al., 2017). This was observed not only with the mGlu2-4 heterodimer, but indeed with any mGlu heterodimeric receptor composed of a group-II (mGlu2 or 3) and a group-III (mGlu4, 7 or 8) subunits, with the group-II 7TM domain facilitating the G protein coupling efficiency of the group-III 7TM domain.

These findings then show that, even though only one subunit in a class C dimer is responsible for G protein activation, the other subunit is far from being neutral, and is indeed involved in the process, facilitating the activation of the active subunit through a conformational change that remains to be determined. Such observations further support a clear allosteric interaction between both 7TM domains with class C GPCR dimers.

4- Class C as dimers or larger oligomers ?

If class C receptors are commonly considered as dimers, the GABA_B receptor was soon found to associate into larger entities composed of at least two heterodimeric entities associated via the GABA_{B1} subunit (Maurel, Comps-Agrar, Brock et al., 2008). Such complexes were not only observed in transfected cells at physiological densities, but also in brain membranes (Comps-Agrar, Kniazeff, Norskov-Lauritsen et al., 2011, Schwenk, Metz, Zolles et al., 2010). In addition, these complexes appear stable at least in transfected cells as heterodimers are poorly exchangeable between these complexes (Comps-Agrar, Kniazeff, Brock et al., 2012). These data strongly suggest that such complexes are real in neurons and then may be of physiological importance. The obvious next question was to examine whether one GABA_B heterodimer could affect the signaling of the other in such complexes. It was soon observed that within a tetrameric entity, only one heterodimer was functional (Maurel et al., 2008, Comps-Agrar et al., 2011). The selective coupling of only one GABA_{B2} subunit is unlikely due to steric hindrance as the GABA_{B2} subunits are distant from each other, separated by the GABA_{B1} subunits (Maurel et al., 2008, Comps-Agrar et al., 2011). These data reveal a negative interaction between the GABA_B heterodimers within such tetrameric entities. Although we cannot exclude a direct negative interaction via the 7TM domains within the complex, it was found that a negative interaction already occurs at the level of the VFTs, with a single ligand being able to bind in a tetrameric GABA_B receptor (Stewart, Comps-Agrar, Norskov-Lauritsen et al., 2018).

Although GABA_B receptors were found to associate into large entities, mGlu receptors were instead soon reported as strict dimers both in cell lines (Maurel et al., 2008) and in xenopus oocytes (Levitz, Habrian, Bharill et al., 2016). This was confirmed in cultured hippocampal neurons at expression levels close to the native conditions (Moller, Hottin, Clerte et al., 2018). Surprisingly, co-diffusion analysis using the number and brightness analysis of labeled snap-tagged mGlu2 receptors reveal that they associate into larger entities, mostly tetramers when their conformation is stabilized either in the inactive state or in the active state with ligands (Moller et al., 2018). Because mGlu2 receptors were found to oscillate between various conformations under their basal state (Olofsson, Felekyan, Doumazane et al., 2014, Vafabakhsh, Levitz and Isacoff, 2015), we proposed that the stabilization into a given state, either active or inactive, favors the association between receptors through specific interfaces, likely due to the increase in the number of receptors in a conformation compatible with such oligomerization. However, so far, there is no direct indication that allosteric interactions can occur between mGlu dimers within such complexes.

5- Concerted movements between the 7TM domains of class C GPCRs

How can one 7TM domain control the activity of its associated subunit? A clear answer to this question definitively needs the determination of the 3D structure of the dimeric complex in various states, and eventually with the associated G protein. So far, the only structure of a truncated class C GPCR 7TM domain showing a possible dimeric entity is that of mGlu1 (Wu et al., 2014). In that case, the two domains are found in contact via TM1. However, for several reasons, this is unlikely the case in the full-length receptor. First, this dimer interface is unlikely affected upon receptor activation making it very unlikely involved in an allosteric coupling between the two subunits. Moreover, the distance between the TM1s of both subunits is expected to be driven by the distance between the C-terminal ends of the CRDs as the latter are directly linked to TM1, and these are not expected to be in close

contact. Cys crosslinking experiments were then conducted to identify the possible interface of mGlu 7TM domains. The replacement of many lipid exposed residues of most TM helices in the mGlu2 receptor led to the crosslinking of the two subunits when introduced into TM4, TM5 or TM6 (Xue, Rovira, Scholler et al., 2015). It was also found that stabilizing the receptor in its inactive state using competitive antagonists and NAMs increases the proportion of receptors crosslinked through TM4 or TM5, while stabilizing the receptor in its active state favors TM6 crosslinking. Moreover, crosslinking through TM4-5 results in a receptor that can no longer be activated by glutamate, while TM6 crosslinking generate a receptor almost fully active in the absence of agonist (Xue et al., 2015). Accordingly, it was proposed that both 7TM domains may contact each other mainly through TM4-5 under basal conditions, while via TM6 in the active state. Such a proposal nicely fit with the expected distance between the N terminal ends of the TM1 helices deduced from the distances of the C terminal ends of the CRDs in the active and inactive states determined in the 3D structures of the entire extracellular domain (Muto et al., 2007).

Such a proposal also provides possible explanation for the asymmetric activation of the class C 7TM domains, with one being active only per dimer. Because of the large movement of TM6 needed to fit the C terminal end of the G protein alpha subunit within the 7TM domain, a dimer interface involving TM6 may allow only one subunit to reach the active state, while the other may require to change conformation to allow this TM6 to move. Such a model nicely explains the allosteric interaction observed between class C 7TM domains.

Such a model has been further validated when analyzing the 7TM domain association and movements in a tetrameric GABA_B receptor. In that case it was observed that the helices corresponding to TM6 of the GABA_{B1} subunits are facing each other in the inactive tetramer, while both contact those of the GABA_{B2} subunits in the active state, allowing the later to reach the active conformation. This concerted movement between the 4 subunits of a possible GABA_B tetramer also provides possible explanation for the control of a class C dimer by another GPCR, as observed with the 5HT_{2a} receptor regulation of the mGlu2 signaling possibly involved in the action of anti-psychotics (Fribourg, Moreno, Holloway et al., 2011, Gonzalez-Maeso, Ang, Yuen et al., 2008). Indeed, the interaction of the 5HT_{2a} receptor with the TM4-5 of mGlu2 is expected to favor mGlu2 subunit interaction through their TM6, then influencing their ability to be active. Work is however necessary to bring experimental evidence for this model.

6- Comparison with what has been reported for other GPCRs

Although class C GPCRs represent an excellent model to analyze the possible allosteric interaction between 7TM domains, the question remains to know whether the observations made on class C GPCRs can be applied to other GPCRs, especially class A receptors.

Interestingly, when possible allosteric interactions between subunits within GPCR dimers were examined, a negative interaction was very often observed, with one subunit active preventing the other from being active. This has been well demonstrated with the D2 and 5HT_{2C} receptor dimers (Han, Moreira, Urizar et al., 2009, Herrick-Davis, Grinde, Harrigan et al., 2005), as well as with the alpha_{2A} adrenergic – mu opioid receptor heterodimer using FRET-based receptor biosensors (Villardaga, Nikolaev, Lorenz et al., 2008). This is also consistent with a number of studies reporting a negative cooperativity between agonist binding on various GPCRs (Springael, Urizar, Costagliola et al., 2007). Indeed, if only one subunit can reach an active state stabilized by a G protein, then only one subunit can reach

the high agonist affinity state, and not the other. Such a proposal help explain the increased off rate of bound agonist upon addition of high concentration of unlabeled ligands (Springael et al., 2007, Urizar, Montanelli, Loy et al., 2005). This was further confirmed with the oxytocin receptor for which both positive and negative cooperativity for ligand binding were reported (Albizu, Balestre, Breton et al., 2006), and for which a single high affinity site for agonist per dimer was clearly demonstrated by measuring FRET between ligands (Albizu, Cottet, Kralikova et al., 2010). Moreover, when studied with recombinant purified leukotriene B4 receptor, a dimer was found to interact with a single G protein (Baneres and Parello, 2003), and clear allosteric interaction were observed between the subunits leading to the activation of only one subunit per dimer, consistent with what is proposed for class C GPCRs (Damian, Martin, Mesnier et al., 2006). Such a property of GPCR dimers does not appear to be general as in some cases it has been proposed that both subunits in a dimer can simultaneously activate G protein (Pellissier, Barthet, Gaven et al., 2011).

Several studies also examined the possible dimer interfaces in class A GPCR dimers. Most studies pointed toward TM4 and TM5 as a possible interface (Mancia, Assur, Herman et al., 2008, Guo, Shi, Filizola et al., 2005, Liu, Chun, Thompson et al., 2012, Wu, Chien, Mol et al., 2010, Manglik, Kruse, Kobilka et al., 2012), with TM6 being also reported (Baneres and Parello, 2003, Damian et al., 2006, Hebert, Moffett, Morello et al., 1996). Of interest, and consistent with the observation that class A GPCR dimers may not be very stable, such a dimer interface appears to be dynamic, with changes of the interface being observed between active and inactive states, as revealed by Cys cross-linking experiments (Mancia et al., 2008, Guo et al., 2005). Moreover, several crystal structures revealed possible dimer assembly via TM4-5 (adenosine A₂A receptor) (Liu et al., 2012), TM5 (CXCR4 receptor) (Wu et al., 2010), or via TM5-6 (Mu opioid receptor) (Manglik et al., 2012). More recently, the dimer interface of the neurotensin receptor was reported to be dynamic, possibly resulting from a possible change in the interface over time (Dijkman, Castell, Goddard et al., 2018), as suggested for the class C GPCRs.

Class A GPCR dimers have also been proposed to associate via TM1 and helix 8. In the case of the Mu Opioid receptor, this interface has been proposed to be responsible for a possible oligomerization of the receptor. If this can be the case, because such an interface is not expected to undergo conformational changes upon ligand binding, it is unlikely that this interface be involved in allosteric interaction between the subunits. This may explain why, in some cases, no evidence for cooperativity between subunits of a GPCR dimer has been reported (Pellissier et al., 2011).

Conclusion

Class C GPCRs represent an interesting model to examine the allosteric interactions between 7TM domains. The recent studies point to important possible interactions, both positive and negative leading to an asymmetric activation of these dimeric receptors, with one subunit facilitating the activation of the other, such that a single subunit activate G proteins. Further studies pointed to a dynamic dimer interface involving TM4-5 in the inactive state, and TM6 in the active conformation of the dimer. Because major conformational changes are expected at this level of a 7TM protein upon G protein activation, such a TM6 interface appear ideal to explain the important allosteric interactions observed. While the debate is still active regarding class A GPCR dimers, it is puzzling to observe that many of the characteristics of the class C GPCR dimers were also reported for many class A dimers. These include the control of one subunit by the other leading to the

activation of only one subunit in a dimer, as well as the likely involvement of TM4-5 and 6 as a possible dimer interface. One main issue will be to elucidate when and how such a dimeric process can occur for class A GPCRs that appear not to be stable in contrast to class C receptors. Could such class A dimers be stabilized in some way with scaffolding proteins? Whatever, even if such interactions are transient, this does not exclude a possible physiological relevance. Indeed, GPCR and G protein interaction is also not stable, and everyone won't question its physiological implications.

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