Structural insights into promiscuous GPCR-G protein coupling

Ángela Carrión-Antolí, Jorge Mallor-Franco, Sandra Arroyo-Urea and Javier García-Nafría

Institute for Biocomputation and Physics of Complex Systems (BIFI) and Laboratorio de Microscopías Avanzadas (LMA), University of Zaragoza, 50018, Zaragoza, Spain

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Corresponding author:
Javier García-Nafría
Email: jgarcianafria@unizar.es
Telephone: +34 876 55 5494

Abstract

G protein-coupled receptors (GPCRs) transduce extracellular signals across biological membranes by activating heterotrimeric Gαβγ proteins. There are 16 different human Gα proteins grouped into four families (G_S, $G_{i/O}$, $G_{q/11}$ and $G_{12/13}$), each one activating different signaling cascades. Around 50% of non-olfactory GPCRs activate more than one type of Gα proteins with different efficacy and kinetics, triggering a fingerprint-like signaling profile. In this chapter we review the GPCR-G protein promiscuity landscape and discuss recent structures of GPCRs coupled to different Gα proteins. Overall, the size and shape of the intracellular cavity (determined by the extent of outward movement of TM6) is maintained when the receptor is coupled to different Gα proteins, and is determined by the type of primary Gα coupling. The "sub-optimal" secondary Gα coupling is further supported by interactions with the intracellular loops, with ICL2 and ICL3 having a relevant role in promiscuous couplings.

Keywords

Cryo-electron microscopy, G protein-coupling specificity, G protein-coupling promiscuity, GPCR, signaling, biased agonism.

Introduction

G protein-coupled receptors (GPCRs) form the largest family of membrane receptors (>800 members in humans) and recognize a staggering amount of extracellular signals (~1,000) that range from subatomic particles (photons) to macromolecules[1]. Their high versatility in signal detection as well as their ubiquitous distribution involves GPCRs in a wide variety of (patho-)physiological processes as well as being highly prolific therapeutic targets[2,3]. Upon detection of stimuli GPCRs transduce the information across biological membranes into the intracellular milieu where they recruit and activate heterotrimeric Gαβγ proteins and arrestins[4]. Heterotrimeric Gαβγ proteins are the primary route for signal transduction which, upon coupling and activation by GPCRs, dissociate into the Gα and Gβγ subunits triggering an array of signaling cascades through various effectors (e.g. adenylate cyclase, phospholipase C…) and secondary messengers (cAMP, Ca^{2+}, DAG…) that lead to a cell-specific response[5,6]. The nature of the activated signaling cascade depends mainly on the type of Gα protein. In humans, there are 16 genes that code for distinct Gα proteins organized into four families: G_S (G_{olf} and G_S), $G_{i/O}$ (G_{i1}, G_{i2}, G_{i3}, G_O, G_z, G_{t1},G_{t2} and G_{gust}),$G_{q/11}$ (G_q, G_{11}, G_{14}, G_{15}) and $G_{12/13}$(G_{12} andG_{13}). The main signaling routes initiated from the different Gα proteins are well established: $G\alpha_S$ activates adenylate cyclase and promote the formation of cAMP, $G\alpha_{i/O}$ inhibits the formation of cAMP, $G\alpha_{q/11}$ activates phospholipase C and consequently calcium signaling, and $G\alpha_{12/13}$ activates Rho A GTPases. Although differential expression and sub-cellular compartmentalization can influence the ability of certain GPCRs to activate specific Gproteins[7–10], it is known that many GPCRs and G proteins can be highly expressed in a single cell-type[11,12]. Hence, a major contributor to GPCR-G protein selectivity is likely to be the set of specific interactions between GPCRs and G proteins. GPCRs can be specific, coupling to and activating a single type

of Gαβγ heterotrimer, or have different degrees of promiscuity, where additional primary and/or secondary couplings to other Gα proteins occur. Promiscuous couplings increase the complexity of GPCR signaling, activating different Gα proteins with different strengths (efficacies) and kinetics yielding a fingerprint-like profile[13,14]. Such a complex signaling is bound to be tunable by receptor environment or distinct endogenous/exogenous agonists through biased agonism/functional selectivity[15]. In this chapter we review the advances in characterizing promiscuity within the GPCR family and analyze recent structures of GPCRs coupled to different Gα proteins.

On the search for the GPCR – G protein *couplome*

A map of the GPCR *couplome* that includes detailed information of which Gα proteins are activated by which GPCRs (with associated efficacy/kinetic information when activated by different agonists) would be of great value, and efforts are directed towards that goal. Information about individual GPCR-G protein couplings is recorded, in a qualitative manner (as primary/secondary couplings), in the IUPHAR/BPS Guide to Pharmacology[16]. This information originates from the literature and is expert-curated. Additionally, the development of robust cellular Bioluminescence Resonance Energy Transfer (BRET) assays that monitor Gαβγ activation has allowed more systematic comparisons of GPCR-G protein couplings[17–20]. Two recent large-scale studies and their quantitative merging and normalization have enhanced our knowledge on the GPCR *couplome*[21–23]. First, Inoue *et al*. used a TGF-α shedding assay in HEK293 cells to study the coupling of the 16 human Gα proteins to 148 non-olfactory receptors. In this case the wild-type G_q was used together with chimeric Gα proteins where the six C-terminal residues were replaced by their corresponding counterparts in other Gα proteins. Additionally, Avet *et al*. used a G protein Effector Membrane Translocator assay

98 (GEMTA) where BRET sensors were used to monitor Gαβγ activation by measuring
99 the translocation of downstream effectors to the plasma membrane. Such an
100 approximation enabled the use of wild-type G proteins and receptors and it was used to
101 determine the GPCR-G protein couplings of 100 GPCRs to the most ubiquitous Gα
102 proteins (G_S, G_{i1}, G_{i2}, G_z, G_{OA}, G_{OB}, G_q, G_{11}, G_{14}, G_{15}, G_{12} andG_{13} and excluding the
103 specific G_{olf}, G_{i3}, G_{t1}, G_{t2} and G_{gust}). A final study merged and normalized the GPCR-G
104 protein couplings from both datasets and joined it to the information in the
105 IUPHAR/BPS Guide to Pharmacology[22]. The final consensus map of GPCR-G protein
106 couplings(deposited in the GPCRdb[24,25]) includes coupling information of 265 non-
107 odorant receptors (67% coverage of non-olfactory GPCRs). Several insights about
108 GPCR-G protein promiscuity can be learnt from this data. First, Gα protein promiscuity
109 is a common feature in GPCRs, with ~50% of the receptors (130/256) coupling to two
110 or more types of Gα proteins (G_S, $G_{i/O}$, $G_{q/11}$ and $G_{12/13}$). Such magnitude of promiscuity
111 is in agreement with previous estimations using the IUPHAR/BPS Guide to
112 Pharmacology[12]. Within the promiscuous receptors, ~64% (83 receptors) have double
113 couplings, ~26% (34 receptors) have triple couplings and 10% (13 receptors) could
114 activate all families of Gα proteins (G_S, $G_{i/O}$, $G_{q/11}$ and $G_{12/13}$). All of the later highly
115 promiscuous receptors are Class A GPCRs. Second, there is generally little coupling
116 selectivity between Gα protein sub-types, i.e. GPCRs that couple to the $G_{i/O}$ family can
117 normally couple to all sub-types of $G_{i/O}$ proteins. This is somewhat expected due to the
118 high sequence similarity between Gα protein sub-types but some GPCRs showed
119 selectivity for a particular sub-type[22]. Since different Gα proteins sub-types have
120 differences in effector engagement selectivity and kinetic profiles[13], receptor sub-type
121 selectivity can yield relevant differences in functional outcomes[26,27]. Third, promiscuous
122 receptors showed a negative correlation for co-coupling of G_S and $G_{i/O}$ (this is expected

since G_S and $G_{i/O}$ have opposite functional effects) but showed a positive correlation for $G_{i/O}$ and $G_{q/}11$ co-coupling, i.e. promiscuous receptors that co-couple to $G_{i/O}$ and $G_{q/11}$ are much more frequent that receptors that co-couple to other Gα protein pairs. Finally, receptors that couple primarily to $G_{i/O}$ are more selective than receptors coupling to G_S and $G_{q/11}$ (in line with previous reports from the literature[18]), while receptors that couple to $G_{12/13}$ tend to couple to other Gα proteins frequently (i.e. selective $G_{12/13}$ coupling is uncommon). Overall, GPCR – G protein promiscuity is ubiquitous and thus, is an important element within GPCR signaling.

Structural studies of GPCRs coupled to G proteins

The selectivity mechanisms by which GPCRs couple to specific Gα proteins is a subject of intense research with high-resolution structural determination being a highly valuable tool. Although an initial X-ray crystal structure of a GPCR-G_S complex was determined in 2011[28], the high requirements of X-ray crystallography has made crystallization of GPCR-G protein complexes an arduous task and alternative approximations have been used[29,30]. The cryo-electron microscopy (cryo-EM) "resolution revolution"[31] made structure determination of GPCR-G protein complexes more accessible[32]. Initial cryo-EM structures of Class B and A GPCRs coupled to a G_S heterotrimer[33] were rapidly followed by structures of GPCRs coupled to $G_{i/O}$[34–37] and $G_{q/}11$ heterotrimers[38]. Since then, cryo-EM structures of GPCRs coupled to different G proteins, arrestins and kinases have been growing exponentially[39] and, as of April 2022, over 200 structures of GPCR-G protein complexes have been deposited in the Protein Data Bank[40]. In general, agonists binding at the extracellular orthosteric site triggers a conformational change in the conserved CWxP, PIF, NPxxY and E/DRY motifs in the receptor that converge at the intracellular cavity where there are rearrangements of TM3, TM6 and TM7that

allow to accommodate the C-terminal α5 of the Gα protein[41,42]. The selectivity barcode between GPCRs and G proteins is still not understood, although it is believed that a three-dimensional epitope presented by the Gα protein is read by the receptor determining successful coupling and activation[43]. The α5 of the Gα protein seems to be the major determinant of specificity since replacement of its outmost C-terminal residues are enough to modify its specificity[44]. However, elements outside the α5 have been shown to have differential contributions in a GPCR-G protein specific manner[18,45]. From the initial cryo-EM structures, distinct modes of engagement that are Gα protein dependent arose[46,47]. First, a trend in the magnitude of TM6 outward swing differentiates between G_S and $G_{i/O}$-$G_{q/11}$ coupling receptors which is wide for G_S coupling receptors (accommodating the bulkier G_Sα5) and narrower for $G_{i/O}$ and $G_{q/11}$ coupling receptors (Figure 1A). Such movement contributes majorly to the size and shape of the intracellular cavity for the α5 of the Gα protein. As usual with GPCRs, this is only a trend and exceptions to the rule have been reported[48,49]. Second, the angle of insertion of the G protein α5 with respect to the receptor TM3 is larger for $G_{i/O}$ coupled receptors than for G_S coupling receptors (i.e. $G_{i/O}$ inserts to the receptor more perpendicularly to the membrane than G_S) (Figure 1B), while an anti-clockwise rotation of the G proteins (as viewed from the extracellular side) tends to be more pronounced for $G_{q/11}$ coupling[38] than for $G_{i/O}$ and G_S coupling (Figure 1C). Lastly, the insertion and rotation angle is somewhat correlated with the amount of interactions between the intracellular loop 2 (ICL2) and the β1-αN of the Gα protein, with G_q and G_S displaying extensive interactions and $G_{i/O}$ having weaker or absent interactions[34,46,50].

Structures of GPCRs bound to multiple G proteins

There are currently seven receptors whose cryo-EM structures have been determined in the presence of more than one type of Gα proteins. Here we compare, for each receptor, the structures bound to the same agonist but coupled to different Gα proteins. These include: one receptor coupled to G_S and $G_{i/O}$(GCGR[51]), one receptor coupled to G_S and $G_{q/11}$ (NK_1R[52]), one receptor coupled to G_q, G_{i1} and G_S (CCK_AR[53,54]) and four receptors coupled to $G_{i/O}$ and $G_{q/11}$ (GSHR[50,53,55],CCK_BR[56], GPR139[57]and MRGPRX2[58]) (Table 1). Overall, there are six Class A receptors and one Class B receptor, with a large number of examples of receptors coupled to $G_{i/O}$ – $G_{q/11}$ (consistent with the increased frequency of this co-couplers[22]).

G_S-$G_{i/O}$ coupling: the GCGR. The GCGR structure has been determined when coupled to G_S (primary coupling) and G_{i1}(secondary coupling). The active GCGCR shows a large 19 Å swing of TM6 characteristic of Class B receptors when coupled to G_S[59], and is also maintained when coupled to G_{i1} (not characteristic in primary $G_{i/O}$ coupling receptors)(Figure 2A). Hence, the G_{i1} and G_S α5 share a similar cavity, although the G_{i1} α5 engages in less contacts with a smaller amount of buried surface area. The major differences in the receptor between the G_S and G_{i1} coupled structures are found within the ICLs. ICL2 in the G_{i1} complex swings away from the β1-αN losing the extensive interactions made during G_S coupling (Figure 2D). ICL1 and ICL3 also contribute with interactions to G_{i1} which upon mutation were found to be functionally important for G_{i1} and, to lesser extent, for G_S coupling[51].

$G_{q/11}$-G_S (and $G_{i/O}$) coupling: the NK_1R and CCK_AR. These receptors have marked differences in the degree of preference for the $G_{q/11}$ and G_S proteins, NK_1R has slight preference (or no preference depending on source) for G_q while CCK_AR has up to 1000

197 times preference for G_q[54].The structures of NK_1R coupled to G_S and G_q show a
198 conserved receptor conformation with a narrow opening of TM6 characteristic of G_q
199 coupling (Figure 2B). The angle of insertion and rotation of the Gα protein relative to
200 the receptor is conserved for both Gα proteins and is reminiscent of G_q coupling with
201 extensive interactions between ICL2 andβ1-αN. Overall the NK_1R seems to achieve a
202 similar coupling for both Gα proteins by engaging G_S in a G_q-like manner (Figure
203 2G).G_qα5 binds slightly deeper in the intracellular crevice making just one more
204 interaction than the α5 of $G\alpha_S$. The CCK_AR can couple to all four families of Gα
205 proteins. CCK_AR structures coupled to G_S, G_q and G_{i1} (structures with Gα closest to
206 wild-type were chosen for analysis) show a receptor with small swing of TM6
207 characteristic of G_q and $G_{i/O}$ coupling (Figure 2B). In this case, G_q and G_S bind
208 differently (Figure 2G) with an angle of insertion and rotation that is characteristic of
209 each Gα protein (as so does G_{i1}). ICL2 appears more flexible in the G_q and G_i couplings
210 with less interactions at the ICL2- β1-αN interface than the G_S coupling. In this case,
211 the G_q-like TM6 limits the space available for the bulky G_S and hence, the outmost
212 residues within the "wavy hook" are forced to unwind protruding out of the receptor
213 between TM6 and TM7 (Figure 2G). ICL3 interacts with G_q and G_i but not with G_S. In
214 this case ICL3 is sandwiched between TM5 and the Gα protein and its modification
215 influences specifically the primary G_q coupling[53] (Figure 2F).
216
217 *$G_{q/11}$ – $G_{i/O}$ couplers: the GSGHR, MRGPRX2, CCK_BR and GPR139.*All of the$G_{q/11}$ –
218 $G_{i/O}$ coupling receptors are primary couplers to $G_{q/11}$ except for the MRGPRX2 to $G_{i/O}$
219 is as efficient as to $G_{q/11}$[58] .The angles of insertion and rotation for all $G_{q/11}$ and $G_{i/O}$
220 couplings are characteristic of each Gα protein, and they all display a conserved
221 receptor structure when coupled to $G_{q/11}$ and $G_{i/O}$ (Figures 2C). In all receptors there is a

minor extension/ordering of TM6 when coupled to $G_{i/O}$ in order to keep its conserved interaction with the final aromatic residue in $G_{i/O}$ (G.H5.26, common CGN numbering[5]). ICL2 makes interactions with theβ1-αN in all complexes except for the GSHR coupled to G_{O1} (Figure 2E). Finally, in the MRGPRX2-G_{i1} complex, ICL3 makes extensive interactions with G_i but it is disordered when coupled to G_q.

Insights from structures of GPCRs bound to multiple G proteins

In accordance with previous studies[46], there is not a simple correlation between selective or promiscuous couplings and sequence conservation. However, some overall trends arise from these structures of GPCRs coupled to different Gα proteins.

First, promiscuous GPCRs use a similar intracellular cavity for primary and secondary couplings, as determined by the movement (or lack thereof) of the receptor TM6. The outward swing of TM6 is a hallmark of GPCR activation and determines the size and shape of the intracellular cavity. The magnitude of the swing is correlated with the type of Gα coupling (larger for G_S and narrower for $G_{i/O}$ and $G_{q/11}$[38,46,47]). In the available structures of GPCRs coupled to different Gα proteins, TM6 does not change upon coupling to different G proteins, and therefore, primary and secondary Gα proteins are required to use a similar intracellular cavity.

Second, the magnitude of the TM6 outward swing in promiscuous GPCR-G protein pairs is determined by the primary coupler. As an example, the GCGR, uses a wide open TM6 characteristic of its primary G_S coupling, which the secondary G_{i1} is required to use. Conversely, the CCK_AR and NK_1RG_q which are primary G_q couplers adopt a narrower TM6 typical of its G_q coupling, while their secondary G_S is required to adapt to this narrow G_q-like pocket in both of them. It is tempting to speculate that promiscuous GPCRs regulate coupling preference by optimizing the conformation of

TM6 to its primary coupler while the secondary coupler will be required to bind "sub-optimally". Of relevance is the unwinding of the "wavy hook" in secondary G_S coupling when bound to the CCK_AR, a feature not present in the NK_1R when bound also to its secondary G_S protein. It is tempting to speculate that such a difference might be the base of their difference in secondary G_S coupling efficacy. In the case of $G_{i/O}$-$G_{q/11}$ co-couplers, all adopt a TM6 conformation that is narrower in comparison to receptors coupling to G_S. The fact that the TM6 outward swing is similar for $G_{i/O}$ and $G_{q/11}$ couplings might explain the fact that receptors that co-couple to $G_{i/O}$ and $G_{q/11}$ are much more abundant than receptors coupling to other pairs[22]. This would be in line with the hypothesis that receptors coupling to G_S are more promiscuous that receptors that couple primarily to $G_{i/O}$[18,21], however how $G_{q/11}$ primary couplers are more promiscuous than $G_{i/O}$ is unknown.

Third, the angle of insertion and rotation of the Gα protein in comparison to the receptor is normally maintained as is characteristic for each type of Gα protein, with the only exception of the NK_1R-G_S and G_q complexes. This has an impact on the interaction of Gα proteins with the ICLs of the receptors. The fact that the engagement mode is maintained using a different intracellular cavity might support the idea of this interaction to be "sub-optimal".

Finally, the ICLs are the structural elements in the receptors that most change when coupling to different Gα proteins. ICL3 contributes differential interactions between the Gα proteins in 5 out of 7 GPCR-G protein complexes (non in CCK_BR and NK_1R). In the MRGPRX2-G_{i1} and GCGR-G_S there is an ordering of ICL3 to make additional interactions with the Gα protein. In the GSHR-G_O (and not the G_q or G_{i1} complexes), an extension of TM6 contributes additional interactions with the Gα protein and, in the GPR139-$G_{i1}$the ICL3 rearranges to make a different set of interactions with the Gα

protein. Finally, in the CCK_AR there is an increasing ordering of ICL3 to make additional interactions to each Gα protein that correlates with their respective efficacies (G_q>G_i>G_S)(consensus efficacy ranking in both Inoue and Avet datasets) (Figure 2F). Hence, it could be that ICL3 takes a prominent role in regulating G protein coupling efficacy in CCK_AR. There is no correlation between ordering or type of interactions of ICL3 and primary/secondary couplings. Previous studies using chimeric receptors with exchanged ICL3s having a functional impact on G protein promiscuity support the role of ICL3 in promiscuous Gα couplings[45]. However, it seems that there are divergent modes of using ICL3 to regulate Gα protein promiscuity. ICL2 changes conformation or interactions (correlated to the different angle of insertion/rotation of the Gα protein) in most GPCR-G protein complexes. The most prominent conformational changes in ICL2 occur in the GCGR and the GSHR where it forms extensive interactions with the β1-αN in the G_S and G_q complexes respectively and loses all interaction when coupled with G_i and G_O respectively. The interactions between ICL2 and the β1-αN junction are important for G protein selectivity and hence, promiscuity[60,61], however no patterns can be extracted from the current dataset. Overall, they follow the engagement mode of each type of Gα protein where G_S and $G_{q/11}$ make extensive interactionswithβ1-αN and $G_{i/O}$ shows weaker interactions. Finally, ICL1 is seen to interact in a functional manner with G_{i1} and not G_S in the GCGR. However, no other receptor shows a differential interaction of ICL1 within their different Gα protein couplings. Based on these structures, care must be taken when using chimeric Gα proteins for structural studies so as not to distort interactions outside the α5 of the Gα protein taking place through the ICLs.

Summary and future perspectives

Overall, GPCR-G protein promiscuous couplings occur through the same intracellular cavity whose features seem to be dictated by the primary Gα coupling, while the ICLs take a prominent role in making differential interactions during promiscuous couplings. There seems to be divergent roles for GPCRs using ICLs in promiscuous couplings, which seem to be specific for each GPCR-G protein pairs (at least with current data). This information could guide drug development, e.g. regulation of promiscuous GPCR-G protein activation through modulation of ICLs. Additional structural information as well as more established GPCR-G protein couplings will aid in the determination of the selectivity barcode and mechanisms of GPCR-G protein promiscuity. A better chance of finding a more defined sequence barcode for GPCR-G protein selectivity might be to search in more segregated groups such as selective GPCRs of a particular type or promiscuous GPCRs with the same primary coupler. However, given the seeming complexity of GPCR selectivity, where promiscuous GPCRs activate differentially, in efficacy and kinetics, different families and sub-types of Gα protein, aunique selectivity barcode for each GPCR and Gα protein set might be possible.

Author contributions

ACA, JMF, SAU and JGN contributed to all aspects of this chapter.

Conflicts of interest

ACA, JMF, SAU and JGN declare no conflicts of interest.

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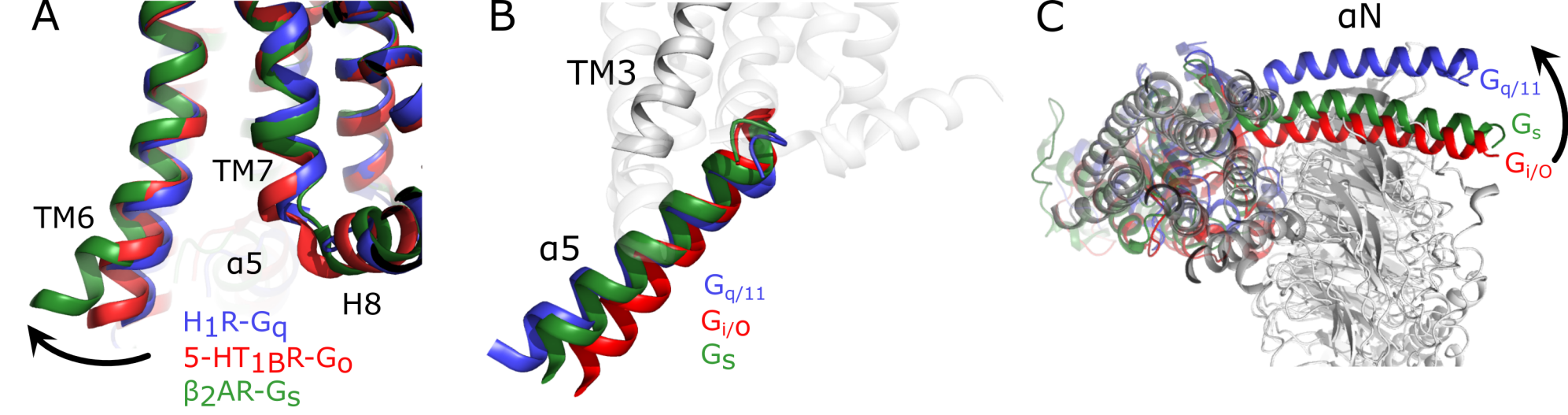
Table 1. Summary of GPCR structures coupled to different Gα proteins.

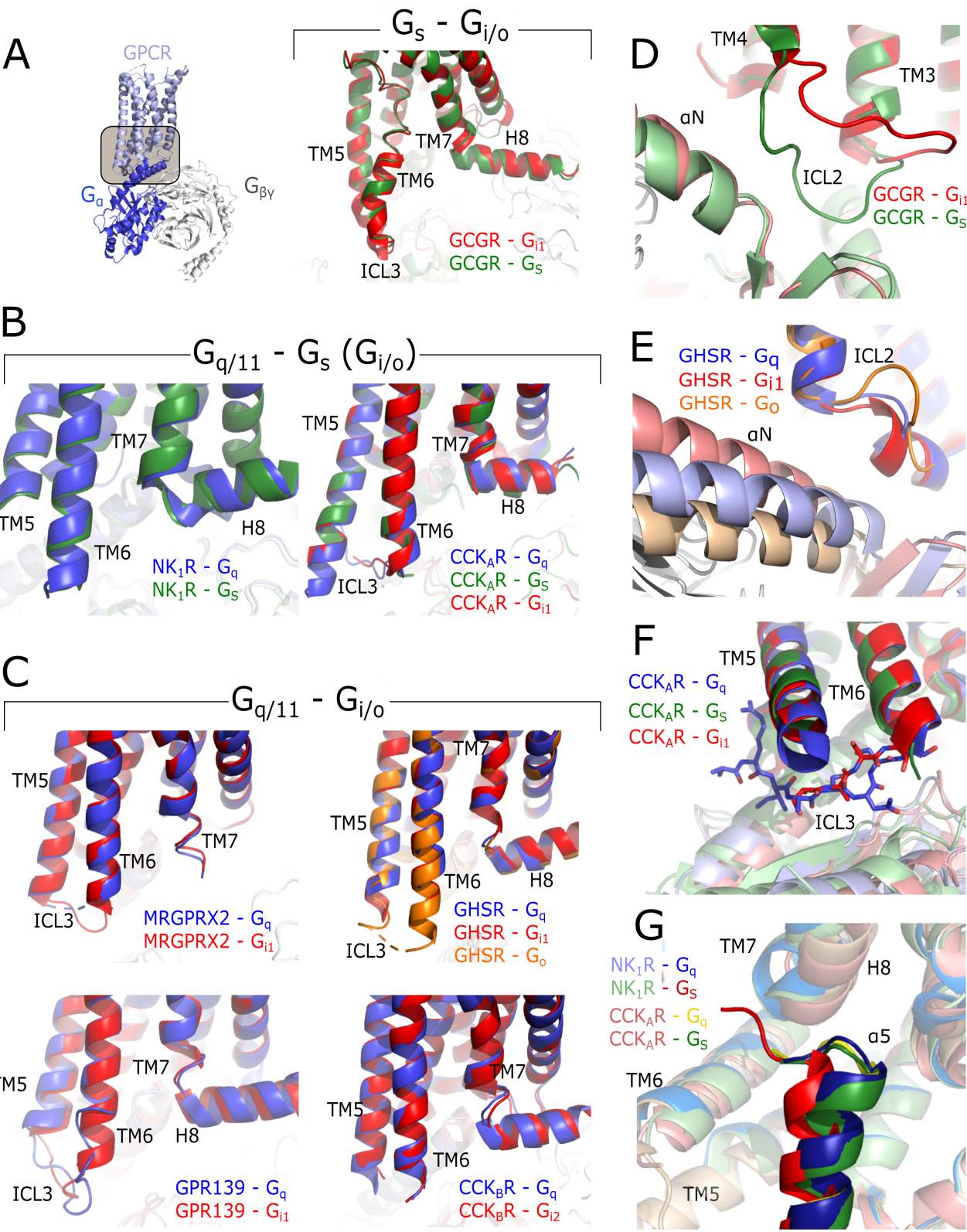
DN: Dominant Negative Gα protein.

Figure 1. Engagement modes of different Gα protein. Structures of model GPCRs coupled to G_S (β_2 adrenergic receptor, β_2AR, PDB 3SN6), $G_{i/O}$ (serotonin 5-HT_{1B} receptor, 5-HT_{1B}R, PDB 6G79) and $G_{q/11}$ (Histamine 1 receptor, H_1R, PDB 7DFL). Structures are aligned on the receptor and depicted as green (β_2AR), blue (H_1R) and red (5-HT_{1B}R) cartoons. **(A)** TM6 outward swing in the G_S, G_O and G_{11} coupled receptors. **(B)** Insertion angle of G_S, $G_{i/O}$ and $G_{q/11}$ α5 into the receptor. **(C)** Rotation angle of the G_S, $G_{i/O}$ and $G_{q/11}$ with respect to the receptor when view from the extracellular side.

Figure 2: Structural comparison of receptors coupled to different Gα proteins. Structures aligned on the receptor are shown as blue ($G_{q/11}$), red (G_i), orange (G_O) and green (G_S) cartoons with receptors and G proteins shown in bright and pale colors respectively. Structures of GPCRs bound to different Gα proteins are arranged depending on their co-coupling: G_S-$G_{i/O}$ **(A)**; $G_{q/11}$-G_S (and $G_{i/O}$) **(B)** and $G_{q/11}$–$G_{i/O}$ **(C)**. Conformational changes of receptor ICL2 are shown for GCGR **(D)** and GSHR **(E)** and

in ICL3 in CCK_AR. ICL3 residues are depicted as sticks of their respective colours **(F)**. **(G)**The differential engagement of the G_q and G_S α5 between the NK_1R (blue and red respectively) and CCK_AR (yellow and green respectively) is shown in **(G)** with the Gα and receptors shown as dark and pale colors respectively.


A
TM6
TM7
α5
H8
H1R-Gq
5-HT1BR-Go
β2AR-Gs
B
TM3
α5
Gq/11
Gi/o
Gs
C
αN
Gq/11
Gs
Gi/o


A
GPCR
G_α
$G_{\beta\gamma}$
G_s - $G_{i/o}$
TM5
TM7
H8
TM6
ICL3
GCGR - G_{i1}
GCGR - G_S
B
$G_{q/11}$ - G_s ($G_{i/o}$)
TM7
TM5
H8
TM6
NK_1R - G_q
NK_1R - G_S
TM5
TM7
H8
TM6
ICL3
CCK_AR - G_q
CCK_AR - G_S
CCK_AR - G_{i1}
C
$G_{q/11}$ - $G_{i/o}$
TM5
TM6
TM7
ICL3
MRGPRX2 - G_q
MRGPRX2 - G_{i1}
TM7
TM5
TM6
H8
ICL3
GHSR - G_q
GHSR - G_{i1}
GHSR - G_o
TM7
TM6
H8
ICL3
GPR139 - G_q
GPR139 - G_{i1}
TM7
TM5
TM6
CCK$_B$R - G_q
CCK$_B$R - G_{i2}
D
TM4
TM3
αN
ICL2
GCGR - G_{i1}
GCGR - G_S
E
GHSR - G_q
GHSR - G_{i1}
GHSR - G_o
ICL2
αN
F
TM5
TM6
CCK_AR - G_q
CCK_AR - G_S
CCK_AR - G_{i1}
ICL3
G
TM7
H8
NK_1R - G_q
NK_1R - G_S
CCK_AR - G_q
CCK_AR - G_S
α5
TM6
TM5